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USE OF LABORATORY TOXICITY TESTS WITH BIVALVE AND ECHINODERM EMBRYOS TO EVALUATE THE BIOAVAILABILITY OF COPPER IN SAN DIEGO BAY, CALIFORNIA, USA

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Abstract—Copper concentrations in parts of San Diego Bay (CA, USA) exceed ambient water quality criteria (WQC; currently 3.1 µg/L dissolved, U.S. Environmental Protection Agency [U.S. EPA]). In order to better understand the bioavailability of copper to water-column organisms in the bay, toxicity tests were performed with copper added to surface water collected from various sites in the estuary over a three-year period. The species and endpoints used, bivalve and echinoderm embryo-larval development, are among the most sensitive in the U.S. EPA's national toxicity dataset, which is used to derive WQC. No toxicity was observed in ambient bay water samples, as indicated by high proportions of normally developed larvae in control treatments, averaging 93 ± 5% across all sites and all sampling events. Median effects concentrations (EC50), obtained by copper spiking of ambient water samples, ranged from 1.7 to 3.4 times lower at sites located near the mouth compared to sites near the back of the bay. These data indicate a gradient in complexation capacity increasing from the mouth to the back of the bay, which is consistent with similar trends in dissolved organic carbon and total suspended solids. For the bay as a whole, estimates for total recoverable and dissolved water-effect ratios (WER) ranged from 2.07 to 2.27 and 1.54 to 1.67, respectively. Water-effect ratios of this magnitude suggest that adoption of a somewhat higher site-specific WQC for San Diego Bay still would achieve the level of protection that is intended by the WQC guidelines.

Keywords—Copper   Bioavailability   Embryo-larval development   Water-effect ratio

INTRODUCTION

Copper is a contaminant of concern in estuarine and coastal environments because of its potential to impact the biota at very low concentrations. Sources of copper in San Diego Bay include industrial discharges, storm water and nonpoint-source runoff, and leaching from copper-based ship hull coatings. The metal is regulated at the state level by imposing strict concentration limits on discharges. These limits are based on water quality criteria (WQC) derived by the U.S. Environmental Protection Agency (U.S. EPA) from laboratory toxicity data for over 25 fish, invertebrate, and plant species. The current national acute criterion for dissolved copper in saltwater is 4.8 µg/L, and the chronic criterion is 3.1 µg/L [1]. The U.S. EPA's criteria, however, do not accurately predict real-world exposure and effects, because the data used to derive WQC were generated with laboratory water possessing little to no potential for sequestering copper. A large number of studies have shown that copper in treated wastewater effluents and receiving waters rapidly binds with organic and inorganic matter, rendering it less bioavailable to both freshwater [2–9] and marine organisms [10–13]. Furthermore, it has become apparent that free copper-ion activity is a better predictor of biological effects than the total or dissolved concentration [6,12–15]. Without taking these factors into account, water quality standards may not be representative of bioavailability due to site-specific water quality characteristics (i.e., pH, temperature, alkalinity, hardness, suspended solids, and dissolved organic carbon).

The U.S. EPA recognizes the need for regulation based on

the bioavailable fraction of metal [3]. Although a biotic ligand model (BLM) has been proposed [16] that considers concentrations and binding strength of cations that compete with free-metal ion at the biotic ligand (e.g., fish gill), the currently accepted regulatory approach is the water-effect ratio (WER) [17]. Water-effect ratio studies indirectly address speciation by comparing toxicity of metal added to effluents or receiving waters with identical exposures in laboratory water similar to that used in WQC development. The ratio of the median effects concentration (EC50) or median lethal concentration (LC50) values between the exposures is then multiplied by the national WQC criterion to derive a site-specific criterion, which can be used by states in development of permit limits and/or water quality standards.

Bivalve and echinoderm embryos are among the most-sensitive indicators of copper toxicity in the marine environment, based on laboratory test data, and commonly are used in ecotoxicological testing. The current species mean acute value, based on 48-h EC50s, for blue mussel (*Mytilus edulis*) embryos is 9.63 µg/L in laboratory water, making it the most-sensitive species in the national saltwater toxicity dataset for copper [1]. Sea urchin and sand dollar embryos are nearly as sensitive, with reported EC50 values ranging from 6 to 24 µg/ L [1,18–20].

In order to assess the potential for surface waters from San Diego Bay to buffer against copper-associated toxicity, numerous sites within the bay were sampled over a three-year period. Bivalve (*Mytilus galloprovincialis*) and/or echinoderm (*Strongylocentrotus purpuratus* or *Dendraster excentricus*) embryos were exposed to bay water samples with and without addition of copper in laboratory toxicity tests. The EC50s from
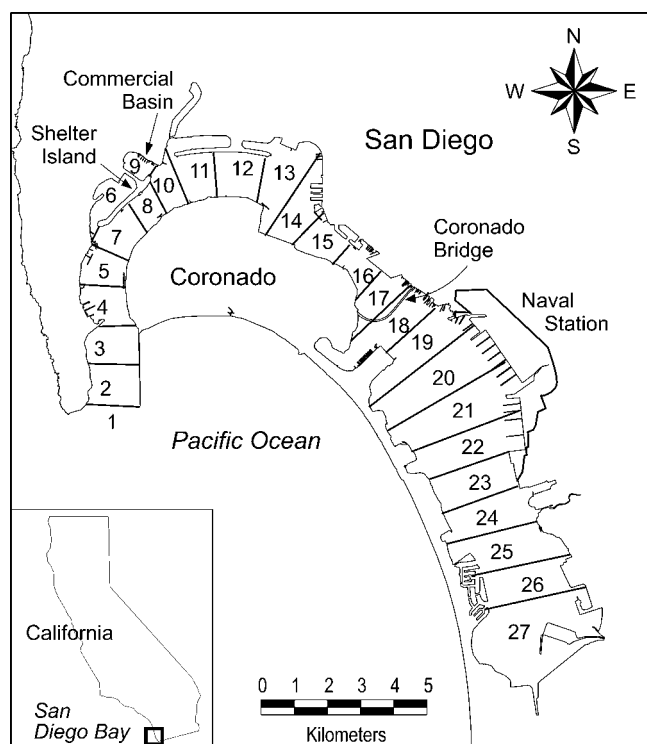
Fig. 1. Map of San Diego Bay (CA, USA) including locations of boxes (1–27) from which surface water samples were collected for toxicity testing.

the tests were compared to water quality characteristics (i.e., dissolved organic carbon [DOC] and total suspended solids [TSS]) measured at respective sampling locations to determine if such characteristics might be associated with copper bioavailability in the bay. In addition, the data were used to estimate WERs to approximate a site-specific criterion for copper for San Diego Bay.

MATERIALS AND METHODS

Study site

San Diego Bay is a semienclosed embayment that borders the city of San Diego (CA, USA; Fig. 1). The bay is classified as a low-inflow estuary, with little natural freshwater input throughout the year [21]. The region south of the Coronado Bridge frequently is referred to as South Bay, and the area north towards the bay mouth is known as North Bay (Fig. 1). Copper loading largely is associated with the use of recreational, commercial, and naval watercraft within the bay [22].

Sample collection and preparation

Water samples were collected in two ways: composites and grabs. Composite samples were collected as part of a whole-basin approach to measure and model physical, biological, chemical, and toxicological characteristics associated with copper in San Diego Bay [23]. Toxicity tests presented here are from four sampling events, which occurred on August 30, 2000, September 19, 2001, February 27, 2002, and May 14, 2002. Samples were collected on board the U.S. Navy's research vessel, *RV/ECOS,* using the Marine Environmental Survey Capability, which measures an array of physico-chemical parameters in real time and provides a continuous stream of seawater for sampling. Composite samples were collected from a series of transects along the axis of the bay, which was

divided into 27 boxes, or segments (Fig. 1). Two side basin boxes (Shelter Island [6] and Commercial Basin [9]) were designated for sampling purposes, but generally were not evaluated for toxicity. Samples were collected from each box at a vessel speed of 6 kts by continuous pumping of water from approximately 2 m below the surface into a 20-L, high-density, polyethylene carboy. Roughly 2 L was subsampled from the carboy for the toxicity tests. The remaining water was used for other measurements, including total recoverable copper and dissolved copper. Trace-metal clean techniques were used for sampling and analysis throughout the surveys.

Additional toxicity tests were conducted with grab samples obtained and tested in April 1999 and July 2001. Grabs were collected in precleaned, 20-L polycarbonate carboys from a depth of approximately 1 m near the shoreline. In order to compare results from grab samples to the rest of the study, each grab was labeled with a box number designating the location to which it was most closely associated.

All samples, composites and grabs, were held at 4°C following return from the field, and tests were initiated as soon as possible, generally within 24 h. Samples were not manipulated in any way prior to testing because physical parameters (i.e., salinity, dissolved oxygen, and pH) consistently were within the range tolerated by the test organisms.

Toxicity tests

One bivalve, *M. galloprovincialis* (Mediterranean mussel), and two echinoderms, *S. purpuratus* (purple sea urchin) and *D. excentricus* (sand dollar), were evaluated for larval development success following short-term exposures. The urchin and sand dollar were used interchangeably, depending on the availability of gravid adults. Both echinoderm species were collected from Mission Bay in San Diego. Mussels were purchased from Carlsbad Aquafarm (Carlsbad, CA, USA).

Toxicity tests followed guidance provided by the U.S. EPA for conducting static, nonrenewal tests with bivalve and echinoderm embryos [24]. In brief, echinoderms were induced to spawn by injection of 0.5 M KCl into the gonad, and mussel spawning was induced by temperature shock. Once spawning began, individuals were segregated and their gametes assessed for quality (highly motile sperm and eggs free of germinal vesicles or signs of deterioration). Within 4 h of fertilization, embryos were added to unfiltered seawater samples that had been dosed with eight or nine copper concentrations as follows: 0, 3, 6, 9, 12, 15, 18, 24, 35, and 50 μg/L. Copper was added in the form of copper sulfate, which was first dissolved in distilled water to create a 1,000-mg/L stock solution. On the testing date, a 1-mg/L working solution was made from the stock solution by dilution with filtered laboratory seawater. The concentration of this working solution was confirmed by graphite furnace atomic absorption spectrometry (GFAAS). Copper was allowed to equilibrate in the test solutions for 1 to 3 h prior to addition of organisms [17].

Tests were conducted in 20-ml glass scintillation vials, in triplicate, and held in a temperature-controlled light chamber at 15°C with a 16:8-h light:dark photoperiod. Bivalve exposures were 48 h, and echinoderm exposures were 96 h. Water quality (temperature, pH, dissolved oxygen, salinity) was monitored daily. Reference toxicant tests were conducted alongside bay water samples under identical conditions to assess batch sensitivity. Dilution water for reference toxicant tests was filtered (0.45 μm) coastal seawater collected from the research pier at Scripps Institute of Oceanography (San Diego, CA,

USA). The reference toxicant test also served as the laboratory water test for the WER calculation.

Tests were terminated with 10% buffered formalin following verification that controls had achieved the desired stage of larval development. 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 (prodissoconch). Approximately 100 larvae were counted in each replicate with $\times 40$ magnification, and scored as either normal or abnormal. Proportions of normal larvae were used to calculate EC50 values, which are expressed in terms of total recoverable copper. The total recoverable concentration for each dilution was estimated by summing the nominal concentration and the measured ambient concentration determined for the bay water sample. Stock solutions and ambient seawater concentrations were confirmed by GFAAS, as described below.

Total and dissolved copper

Dissolved (i.e., 0.45-μm filtered) and total recoverable (i.e., unfiltered) copper samples were obtained from the composites. Trace-metal clean techniques were used throughout the sampling, handling, and analysis of these samples. Acid-cleaned polytetrafluoroethylene and Masterflex® tubing (Cole-Parmer Instruments, Vernon Hills, IL, USA) and a peristaltic pump were used for the subsampling. Dissolved samples were filtered through in-line, acid-cleaned, all-polypropylene, high-volume filters. Filtered and unfiltered samples were collected in 1-L, acid-cleaned, low-density polyethylene bottles, which were kept double-bagged. Samples were 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 following a 1-pyrrolidinedithiocarbamate and diethylammonium diethyldithiocarbamate (APDC/DDDC) liquid/liquid procedure [25]. The efficiency of the concentration procedure for copper averaged $99 \pm 8.7\%$ (average $\pm$ one standard deviation), $n = 18$, for National Research Council of Canada Standards CASS-3 and CASS-4 (http://inms-ienm.nrc-cnrc.gc.ca/calserv/new_certifcates_pdf/CASS-4%20certificate.pdf), and $102 \pm 8.6\%$, $n = 4$, for Standard Reference Material 1643d of the National Institute of Standards and Technology (https://srmors.nist.gov/contactSRM.cfm).

Copper in the concentrated samples 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 $\leq 5\%$. Procedural blanks of 250 ml of high-purity (18 MΩ/cm) water had a mean and a standard deviation of 1.05 ± 0.47 ng of copper. The method limit of detection, defined as three times the standard deviation of the procedural blanks, was 0.004 ± 0.002 μg/L for copper.

DOC and TSS analysis

Dissolved organic carbon and TSS were quantified for all composite samples, but not for grab samples. For DOC, subsamples were filtered through a 0.7-μm, nominal pore-size, precombusted glass–fiber filter and immediately transferred in triplicate to 5-ml amber ampules containing 8 μl 85% phosphoric acid. The ampules were sealed quickly and frozen for shipment and storage. MilliQ blanks (Bedford, MA, USA) and method blanks (rinsed through syringe and filter unit) were conducted at the beginning, middle, and end of each survey.

Blanks generally were low (<25 μM), but were as high as 35 μM. The DOC was measured using an MQ1001 high-temperature combustion TOC analyzer [26]. Sodium phthalate was used to make standards that were run for each 30 samples. Standard curves for all DOC analyses had >0.98 correlation coefficients.

Composite subsamples were quantified for TSS by filtering approximately 900 ml of sample through predried and preweighed glass-fiber filters (1.2 μm nominal pore size). The filters were rinsed with deionized water to remove dissolved salts, then dried and weighed to determine the mass of the filtered solids.

Statistical analysis

Following arc–sine square root transformation of the toxicity data and confirmation of normal distributions and equal variances, EC50 values were calculated using Probit analyses with the assistance of ToxCalc™ (Tidepool Scientific, McKinleyville, CA, USA; Ver 5.0 [27]). Linear regression analysis was used to describe the relationship between toxicity and DOC or TSS, and between total and dissolved copper. Coefficients of determination (r^2) were reported where regressions were significant ($p < 0.05$). A t test was used to determine if there were significant differences ($p < 0.001$) between North and South Bay EC50 values.

RESULTS

Toxicity tests

An absence of ambient toxicity to bivalve and echinoderm embryos was observed for all boxes and sampling events for which tests were conducted, with normal development averaging $93 \pm 5\%$ (average $\pm$ 1 standard deviation [SD]) in control treatments.

Mussel embryos were approximately two times more sensitive to copper than embryos of both echinoderm species. Reference toxicant test EC50 values for three mussel embryo exposures (composites and grabs combined) averaged 6.43 ± 1.36 μg/L (Tables 1 and 2). Mean sand dollar reference toxicant EC50s were 14.8 ± 1.60 μg/L, and the sea urchin reference toxicant test EC50 was 13.1 μg/L (Table 1).

Copper bioavailability, quantified here by comparison of EC50 values, was greater in the reference toxicant tests compared to bay water for all sites and sampling events, except for a few samples located near the bay mouth in August 2000 (Table 1) and April 1999 (Table 2). Within the bay, it generally took larger copper additions to observe a dose response with increasing distance towards the back of the bay (Fig. 2). This resulted in a strong spatial trend upwards in EC50 values towards South Bay (Fig. 3). Within a sampling event, EC50 values through the bay differed by factors of 1.7, 1.8, 3.2, and 3.4, for May 2002, September 2001, August 2000, and February 2002 surveys, respectively. Typically, the lowest EC50 value was observed for box 1, and the highest EC50 was determined from water samples collected in boxes 26 or 27 (Fig. 3). The May 2002 sampling event deviated from the norm to some extent, with a relatively high EC50 observed at the bay mouth (box 1) as well as relatively low EC50 values for boxes 15 and 23 (Table 1). In August 2000, EC50 values also dipped after box 1, but then gradually rose again towards South Bay (Table 1).

For the four surveys, mean EC50s from South Bay averaged 1.65 ± 0.33 times higher than those from North Bay. The smallest difference (factor of 1.36) was observed for the May

Table 1. Median effects concentrations (EC50) and associated 95% confidence intervals from toxicity tests and water quality characteristics in composite samples. Water quality characteristics were measured in the unadjusted samples (e.g., no copper added). DOC = dissolved organic carbon; TSS = total suspended solids; Tot = total recoverable; Diss = dissolved. Dashed lines indicate measurement was not made

Survey date	Location (box no.)	Bivalve EC50 (μg/L)	Bivalve 95% CI (μg/L)	Echinoderm[a] EC50 (μg/L)	Echinoderm[a] 95% CI (μg/L)	DOC (mg/L)	TSS (mg/L)	Tot [Cu][b] (μg/L)	Diss [Cu][b] (μg/L)
May 2002	Lab[c]	5.3	5.1–5.5	—	—	—	—	0.4	—
	1	10.8	9.9–11.6	—	—	2.01	1.59	0.4	0.3
	3	9.2	8.8–9.5	—	—	1.94	2.77	0.5	0.3
	12	10.5	10.0–11.0	—	—	2.78	4.22	2.4	1.7
	15	7.9	7.2–8.7	—	—	3.58	4.43	2.7	2.1
	18	14.1	9.8–15.9	—	—	2.08	3.88	2.9	2.2
	21	13.1	11.1–14.4	—	—	2.38	4.83	3.4	2.6
	23	7.0	5.3–8.1	—	—	3.53	4.62	3.6	2.8
	25	13.6	13.3–13.8	—	—	2.49	4.19	3.9	2.5
	26	14.5	13.9–15.0	—	—	2.64	4.50	4.0	2.5
	27	15.8	15.2–19.5	—	—	3.15	4.76	2.7	2.4
Feb. 2002	Lab	6.0	5.7–6.3	13.1	12.1–14.1	—	—	0.2	—
	1	7.1	6.9–7.2	13.2	11.6–14.8	0.88	0.66	0.1	0.1
	2	12.0	11.3–12.6	16.2	13.6–19.6	1.00	1.26	0.2	0.1
	3	9.9	9.2–10.5	17.1	16.0–18.5	0.76	1.24	0.3	0.2
	4	9.9	9.6–10.1	20.3	17.7–23.3	1.14	2.25	0.6	0.4
	5	12.5	11.0–13.9	16.5	13.8–20.0	0.85	7.57	0.4	0.3
	9	13.1	12.6–13.6	24.7	22.5–34.2	1.16	2.80	3.7	2.7
	11	15.1	14.8–15.5	—	—	1.08	2.83	1.9	1.2
	12	12.3	12.0–12.5	23.0	18.7–28.8	1.26	3.58	2.3	1.7
	15	11.3	10.5–12.0	23.0	21.1–24.8	1.30	2.98	3.3	2.4
	18	16.8	16.4–17.3	29.5	28.8–30.0	1.36	2.25	3.2	2.7
	21	19.2	18.8–19.6	26.9	20.2–31.8	1.58	3.71	3.4	2.9
	23	16.9	16.0–17.7	30.0	28.0–35.8	1.57	6.57	3.0	2.3
	25	18.0	17.8–18.3	35.3	33.4–37.4	1.81	7.93	2.8	1.8
	26	15.5	15.3–15.8	27.6	25.3–31.1	1.58	4.12	3.1	2.2
	27	24.3	23.4–25.4	44.5	—	1.79	9.05	2.6	1.7
Sept. 2001	Lab	—	—	14.8	13.4–16.8	—	—	0.2	—
	1	—	—	19.2	13.8–25.6	2.99	1.93	0.3	.3
	3	—	—	23.3	22.8–23.8	2.66	5.72	0.4	0.2
	4	—	—	23.0	22.2–24.0	2.85	3.11	0.3	0.2
	5	—	—	26.2	22.4–30.0	2.49	3.10	0.5	0.3
	7	—	—	27.7	23.4–32.3	2.39	4.39	0.4	0.3
	9	—	—	22.2	20.2–23.8	2.31	5.00	2.9	2.3
	11	—	—	27.2	22.5–32.2	1.96	2.91	1.0	0.7
	12	—	—	27.9	24.0–36.3	3.16	2.50	2.0	1.5
	13	—	—	20.6	19.5–21.0	2.68	2.60	2.0	1.7
	15	—	—	22.3	20.0–30.8	2.29	4.41	2.3	1.8
	16	—	—	29.9	27.4–32.5	2.70	2.16	2.3	1.8
	21	—	—	28.9	25.7–59.6	3.80	2.68	3.1	2.6
	23	—	—	35.8	32.4–39.7	3.35	3.91	3.3	2.7
	25	—	—	34.3	32.9–36.6	3.39	9.12	3.5	2.4
	26	—	—	38.0	34.3–40.0	3.38	6.19	3.5	2.7
	27	—	—	34.6	33.7–35.2	3.79	6.56	2.7	2.2
Aug. 2000	Lab	—	—	16.4	14.8–18.9	—	—	0.2	—
	1	—	—	19.7	16.4–26.0	2.18	0.36	0.4	0.3
	5	—	—	9.8	8.8–11.0	1.92	1.28	0.5	0.3
	6	—	—	9.3	6.8–11.3	1.81	2.28	4.8	2.4
	13	—	—	13.4	9.0–21.4	2.11	2.44	2.2	1.3
	26	—	—	31.2	27.1–39.9	2.93	3.31	3.1	2.1

[a] *Strongylocentrotus purpuratus* (purple sea urchin) was used for the February 2002 survey and *Dendraster excentricus* (sand dollar) was used for the other surveys.
[b] Total recoverable or dissolved concentrations reported for ambient bay water samples, prior to copper additions for toxicity testing.
[c] Lab refers to laboratory water, which in this case was coastal seawater collected from the research pier at Scripps Institute of Oceanography, La Jolla (CA, USA).

2002 survey, while the largest difference was observed for the August 2000 survey (factor of 2.18). Differences between North and South Bay groupings were statistically significant ($p < 0.001$) for September 2001 and both February 2002 datasets, but not for the May 2002 sampling event ($p = 0.074$). The availability of only one sample tested for South Bay for the August 2001 survey prevented a statistical comparison, but also indicated lower bioavailability in South Bay.

Similar spatial trends were observed with grab samples, with both datasets resulting in higher EC50s at sampling locations towards the back of the bay compared to those near the bay mouth (Table 2). The EC50 values differed by a factor of 2.67 and 3.07 between the southernmost samples (equivalent to box 27) and samples collected near the bay mouth (equivalent to boxes 3 and 5), for the April 1999 and July 2001 sampling dates, respectively. Statistical analyses between

Table 2. Median effects concentrations (EC50) and associated 95% confidence interval (CI) from toxicity tests, and total recoverable copper concentrations (under ambient conditions) from grab samples

Sample date	Location (box no.)	EC50[a] (µg/L)	95% CI (µg/L)	Total [Cu] (µg/L)
July 2001	Lab[b]	13.2	11.1–15.8	0.2
	5	13.2	10.3–17.9	0.5
	18	22.2	19.3–24.2	2.5
	27	35.3	29.2–40.5	4.2
April 1999	Lab	8.0	7.5–8.4	0.2
	3	7.5	6.7–8.0	0.3
	14	14.6	14.3–14.8	2.5
	21	14.0	13.7–14.3	3.2
	27	23.0	22.1–24.0	3.1

[a] EC50 values from July 2001 are for exposures with *Strongylocentrotus purpuratus* (purple sea urchin) and values from April 1999 are for exposures with *Mytilus galloprovincialis* (Mediterranean mussel).
[b] Lab refers to laboratory water.

North and South Bay groups could not be determined due to insufficient data.

Water quality characteristics

Total suspended solids ranged from <1 to about 9 mg/L, and generally increased from the mouth towards the back of the bay (Table 1). The mean ± 1 SD concentration among North Bay samples used in toxicity testing was 2.96 ± 1.61 mg/L, and the mean for tested South Bay samples was 5.12 ± 2.04 mg/L. The bay-wide mean was 3.83 ± 2.07 mg/L. Statistically significant relationships ($p < 0.05$) between EC50 and TSS were observed for three of the five datasets (Table 3).

Dissolved organic carbon ranged from <1 to just under 4 mg/L for the samples tested, and also generally increased with increasing distance towards the back of the bay (Table 1). Mean concentrations were 2.05 ± 0.79 and 2.59 ± 0.85 mg/L for North Bay and South Bay, respectively. The bay-wide mean was 2.27 ± 0.85 mg/L. All but the May 2002 surveys indicated a statistically significant relationship between EC50 and DOC (Table 3).
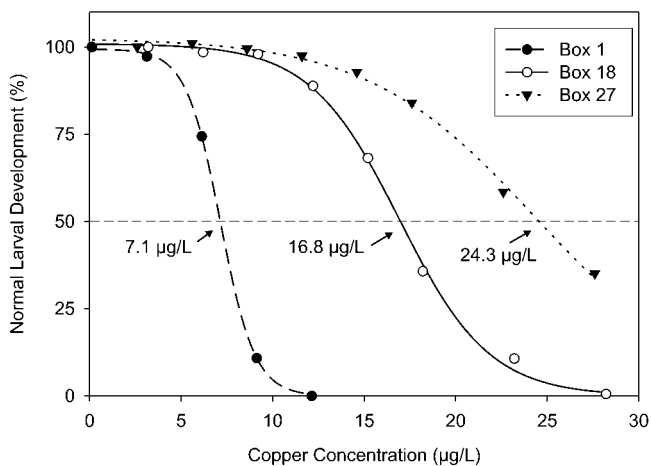

Fig. 2. Dose response of copper additions to surface water collected from three sites in San Diego Bay (CA, USA; February 2002) to *Mytilus galloprovincialis* (Mediterranean mussel) embryos. Box 1 is located at the mouth of the bay, while box 27 is at the back of the bay. Concentrations next to arrows represent the median effects concentration (EC50).
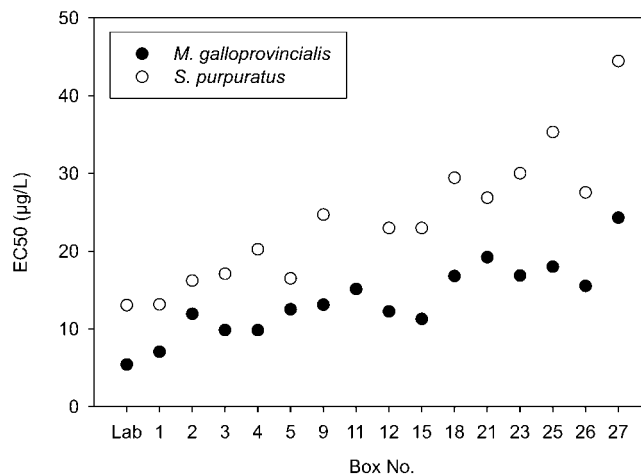

Fig. 3. Median effects concentrations (EC50) from embryo-larval development tests for two species from San Diego Bay (CA, USA) surface water spiked with copper. Data depicted are from the February 2002 sampling event. Box number increases with increasing distance towards the back of the bay.

Salinity, pH, dissolved oxygen, and temperature did not vary substantially among any of the exposures. Mean ± 1 SD values during the toxicity exposures were as follows: Salinity, 34.9 ± 0.8; pH, 7.95 ± 0.12; dissolved oxygen, 7.49 ± 0.58 mg/L; temperature, 17.0 ± 1.6°C.

Total and dissolved copper

For all surveys, a general upward trend in both total and dissolved copper was observed from the bay mouth towards the head (Table 1). Total recoverable concentrations ranged from 0.13 to 4.8 µg/L, and dissolved concentrations ranged from 0.09 to 2.9 µg/L. Dissolved copper accounted for 79 ± 11% (mean ± 1 SD) of the total recoverable copper in samples collected from 25 sites over the course of six surveys. A strong linear relationship existed between dissolved and total copper concentrations (overall $r^2 = 0.93$; Fig. 4). The upward trend in copper concentrations is consistent with previous studies for San Diego Bay [21,28,29], and a more detailed account of ambient copper concentrations from this specific set of surveys is available [23].

Water-effect ratio

Water-effect–ratio values were estimated for each of four data sets. The datasets chosen for the calculation were those from the May 2002, February 2002 (both mussel and urchin datasets), and September 2001 surveys because of the relatively large amount of toxicity data available compared to other datasets. Reference toxicant tests conducted alongside the bay water tests served as the laboratory water. Final total recoverable WER (geometric means of the four datasets) estimates were 1.70, 2.56, and 2.07 for North Bay, South Bay, and bay-wide groupings, respectively (Table 4).

DISCUSSION

Spatial, temporal, and species variability in toxicity

Though total and dissolved copper concentrations consistently trended higher heading into the bay, toxicity of copper added to bay water reliably decreased. For the four composite sampling events, EC50 values averaged 2.5 times higher at the bay's innermost sites compared to the outermost sites, in-

Table 3. Coefficient of determination (r^2) values comparing significant linear relationships (α = 0.05) between median effects concentration (EC50) (μg/L) and dissolved organic carbon (DOC) or total suspended solids (TSS) (mg/L). NS indicates no significant relationship

Sampling date	Test species	n	r^2 DOC	r^2 TSS
May 2002	*Mytilus galloprovincialis*	8	NS (p = 0.130)	NS (p = 0.071)
February 2002	*Strongylocentrotus purpuratus*	14	0.825	0.499
February 2002	*M. galloprovincialis*	15	0.686	0.503
September 2001	*Dendraster excentricus*	16	0.360	0.258
August 2000	*D. excentricus*	5	0.954	NS (p = 0.535)

dicating that neither total nor dissolved metal concentrations in ambient samples would have been good predictors of toxicity. This likely is a result of the well-documented notion that copper forms complexes with organic and inorganic ligands in aquatic environments, rendering >99.9% of the copper in a form that is relatively nonbioavailable [2,4,6–11,14].

Studies of tidal exchange within San Diego Bay indicate significantly greater flushing and shorter residence times between the ocean and the northern portion of the bay ($\leq$1 d) compared to the southernmost region (as high as 40 d) [22,23,29,30]. It was not surprising, therefore, that both DOC and TSS concentrations generally were higher for South Bay. The more significant relationship observed between EC50s and DOC compared to TSS suggests that binding of copper in San Diego Bay probably primarily is driven by DOC, which is a common characteristic among receiving environments [2,22,31].

Reduced copper toxicity in South Bay was observed for all four surveys of composite samples, but the magnitude of toxicity reduction varied to some extent. Though individual EC50 values within a survey varied 3.4-fold in February 2002, they only differed by a factor of 1.7 in May of the same year. The relatively high TSS concentrations measured for sites in the back of the bay in February may have contributed to the reduced toxicity observed in exposures from these sites (Table 1). The reason for the generally low EC50s observed for stations in the back of the bay in May 2002 is not readily apparent, as TSS and/or DOC were not particularly low for samples in this survey. It should be noted, however, that some of the variability observed among surveys is associated with differences in species sensitivity [32]. Mussels were nearly twice

as sensitive as sea urchins and sand dollars. The February 2002 dataset most clearly depicts these differences, where both mussels (box 1 EC50 = 7.06 μg/L) and sea urchins (box 1 EC50 = 13.16 μg/L) were tested simultaneously.

It also is important to note that ambient conditions in the bay were not toxic, with normal larval development averaging 93 $\pm$ 5% over the course of the study. Although all of the toxicity tests conducted in this study involved a series of copper additions, unadjusted bay water samples served as negative controls, representing ambient conditions in the bay. Even though the copper concentrations in some of these samples approached the WQC, this was not problematic to mussel, sea urchin, or sand dollar embryos in laboratory exposures. This is significant because mussel (*Mytilus* spp.) embryo tests are the driver of the saltwater WQC, due to their high sensitivity to copper and status as an economically important species [1]. Unless a more sensitive endpoint (acute or chronic) is identified for San Diego Bay, the lack of ambient toxicity provides additional evidence that the current WQC for copper may more than adequately protect aquatic species present at the site.

Copper toxicity in reference toxicant tests (see Lab results, Table 1) compared well with other studies. The EC50s based on total copper concentration averaged 5.8 [33], 6.9 ([34]; http://www.ci.san-jose.ca.us/esd/pub_res.htm), and 7.8 μg/L for *M. galloprovincialis* [20]. The U.S. EPA's species mean acute value of 9.6 μg/L for *M. edulis* [1] likely is a result of data collected from both *M. edulis* and *M. galloprovincialis* [35]. *Mytilus edulis*, which does not occur on the west coast of the United States, appears to be less sensitive, with reported EC50 values of 12.4 and 21.5 μg/L dissolved for two separate WER studies [35,36]. EC50 values for *S. purpuratus* range from 6.3 μg/L [18] to 15.3 μg/L [20], and values for *D. excentricus* embryos range from less than 20 (G. Rosen, unpublished data) to 33 μg/L [18].

Regulatory significance

Because WQC are based on dissolved concentrations in laboratory waters that are not representative of the natural buffering capacity of a water body, National Pollutant Discharge Elimination System permit limits for copper typically are overprotective, making compliance sometimes difficult or not attainable. Development of a site-specific water quality criterion for copper helps ensure that WQC for a body of water are neither over- nor underprotective, but are consistent with the level of protection intended by the guidelines [37]. Data from this study were used to estimate a site-specific criterion by use of the WER procedure [17]. It should be noted, however, that the objective of the study that produced this dataset was not originally intended to develop such a criterion. Therefore, the WER Guidance was not followed strictly, and WERs generated should be considered as estimates only. Perhaps the
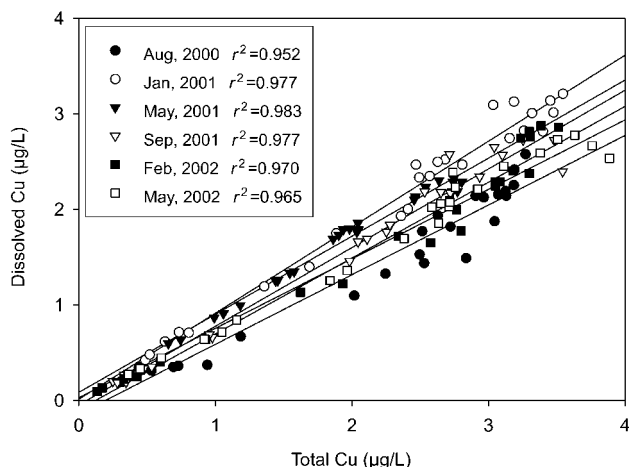

Fig. 4. Relationship between dissolved and total copper for six field surveys of San Diego Bay (CA, USA). The mean $\pm$ 1 standard deviation dissolve/total ratio was 0.79 $\pm$ 0.11.

Table 4. Total recoverable and dissolved water-effect ratios (WER) calculated from four toxicity datasets. A WER of 1 would be indicative of no reduction in toxicity compared to laboratory water

Sample date	Test species	Total recoverable WER			Dissolved WER (estimated)		
		North Bay	South Bay	Bay-wide	North Bay	South Bay	Bay-wide
May 2002	*Mytilus galloprovincialis*	1.81	2.45	2.19	1.34	1.81	1.62
February 2002	*M. galloprovincialis*	1.90	3.06	2.36	1.38	2.23	1.72
February 2002	*Srongylocentrotus pur-puratus*	1.47	2.47	1.90	1.08	1.80	1.39
September 2001	*Dendraster excentricus*	1.66	2.32	1.86	1.27	1.79	1.43
Geometric mean		1.70	2.56	2.07	1.26	1.90	1.54

most significant deviation from the Guidance is that total recoverable and dissolved concentrations were not measured in each of the test solutions employed in each toxicity test. Ambient site water concentrations, however, were measured and incorporated into EC50 calculations, as described previously.

Total recoverable WERs (geometric mean of four datasets) estimated from the toxicity data are 1.70, 2.56, and 2.07 for North, South, and Bay-wide groupings, respectively (Table 4). The positive relationships often observed between EC50 and DOC or TSS concentrations indicate that differences in water quality characteristics between the two regions could explain the spatial differences in WERs. The whole bay could be considered one site, because WERs among sampling locations were within a factor of three [17]. The statistical differences between North and South Bay groupings for three of the four datasets used in the WER, however, indicates that it might be prudent to develop a criterion for both North and South Bay, or even multiple criteria for smaller regions of the bay.

The total recoverable WER may be applicable to National Pollutant Discharge Elimination System permits, because discharge limits tend to be expressed in terms of total recoverable metal. Water quality criteria, however, are expressed in terms of dissolved metal. Although dissolved concentrations were not measured in the test solutions themselves, the ratio between dissolved and total copper in the bay is predicted very reliably over space and time ($r^2 = 0.93$; Fig. 4), with 79 ± 11% of the total recoverable copper being dissolved. To estimate the dissolved EC50 values, the average dissolved-to-total ratio measured from each survey was used as a conversion factor, to take into account seasonal differences. The dissolved bay water EC50s were then divided by the laboratory water EC50s to generate an estimate of the dissolved WER. Reference toxicant tests employed filtered (0.45 µm) seawater, so no conversion was performed for the laboratory water data. Using the conversion, dissolved WERs were 1.26, 1.90, and 1.54 for North, South, and bay-wide groupings (Table 4). Using the bay-wide dissolved WER, a site-specific chronic criterion for the bay would be 4.8 µg/L, as compared to the national criterion of 3.1 µg/L. Because ambient concentrations in the majority of sites in the bay do not approach this concentration, adoption of this criterion could be effective towards both providing protection for sensitive species in the bay and minimizing issues associated with compliance.

It should be noted that enclosed marinas such as Shelter Island Yacht Basin, where dissolved concentrations have been known to exceed 4.8 µg/L, were not evaluated thoroughly in this study (only one data point for box 6, representing Shelter Island, is available). It is possible that WERs for the enclosed marinas might differ from the main channels of the bay due to differences in both inputs and the rate of flushing. Use of the very sensitive embryo tests also may not be appropriate

if ambient concentrations in such areas exceed the toxic threshold for the species.

Water-effect ratios were quite consistent across the four datasets, indicating little overall seasonal variability. Some variability, however, likely is associated with differences in sensitivity among the species used than temporal differences in copper bioavailability. Both of the mussel datasets yielded higher WERs than did the echinoderms. In addition, when both mussels and sea urchins were tested simultaneously (February 2002), we observed 24 to 30% higher WERs with mussels (Table 4). This is expected, as more sensitive species generally yield higher WERs because of changes in metal speciation as the concentration of total metal increases in site water [32]. Ordinarily, final WERs would be calculated using only one species, but we decided that combining data from multiple sampling events would be preferable to using fewer datasets. If only the two mussel datasets had been considered, however, the bay-wide total recoverable and dissolved WERs would have been 2.27 and 1.67, respectively, about 10% higher than those observed with all four datasets.

Water-effect ratios from this study are quite comparable to those determined for other estuaries. For four naval bases in the Hampton Roads, Virginia (USA) area, total recoverable and dissolved WERs were 2.30 and 1.76, respectively [38]. The species used in the Hampton Roads study was *Acartia tonsa*, a marine copepod. A New York Harbor (New York, NY, USA) WER study resulted in a dissolved WER of 1.5, using a combination of three species, including *M. edulis* as well as the sea urchin *Arbacia punctulata* [36]. San Francisco Bay (CA, USA) has been the focus of several WER studies. A bay-wide total recoverable WER of 1.7 was obtained in 1991, though a subsequent study of South San Francisco Bay resulted in a total WER of 3.66 and a dissolved WER of 2.77 [34]. South San Francisco Bay is similar hydraulically to San Diego Bay and has been reported to have comparable levels of trace-metal concentrations [21]. The San Francisco Bay WER, however, focused on sites adjacent to the Dumbarton Bridge, where DOC (~4.1 mg/L) and TSS concentrations (averaging 28 mg/L) were higher than those typically observed in San Diego's South Bay.

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