Marine Pollution Bulletin 85 (2014) 225–234

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Copper bioavailability and toxicity to *Mytilus galloprovincialis* in Shelter Island Yacht Basin, San Diego, CA

Casey Bosse [a], Gunther Rosen [b], Marienne Colvin [b], Patrick Earley [b], Robert Santore [c], Ignacio Rivera-Duarte [b,*]

[a] University of San Diego, 5998 Alcalá Park, San Diego, CA 92110, United States
[b] Space and Naval Warfare (SPAWAR) Systems Center Pacific, 53475 Strothe Rd., San Diego, CA 92152, United States
[c] HDR|HydroQual, 1304 Buckley Road, Suite 202, Syracuse, NY 13212-4311, United States

ARTICLE INFO

Article history:
Available online 18 June 2014

Keywords:
Copper bioavailability
Mytilus sp.
Biotic Ligand Model
Copper complexation
Water effect ratio

ABSTRACT

The bioavailability and toxicity of copper (Cu) in Shelter Island Yacht Basin (SIYB), San Diego, CA, USA, was assessed with simultaneous toxicological, chemical, and modeling approaches. Toxicological measurements included laboratory toxicity testing with *Mytilus galloprovincialis* (Mediterranean mussel) embryos added to both site water (ambient) and site water spiked with multiple Cu concentrations. Chemical assessment of ambient samples included total and dissolved Cu concentrations, and Cu complexation capacity measurements. Modeling was based on chemical speciation and predictions of bioavailability and toxicity using a marine Biotic Ligand Model (BLM). Cumulatively, these methods assessed the natural buffering capacity of Cu in SIYB during singular wet and dry season sampling events. Overall, the three approaches suggested negligible bioavailability, and isolated observed or predicted toxicity, despite an observed gradient of increasing Cu concentration, both horizontally and vertically within the water body, exceeding current water quality criteria for saltwater.

Published by Elsevier Ltd.

1. Introduction

Shelter Island Yacht Basin (SIYB), a marina in San Diego Bay (Fig. 1), exhibits consistently elevated copper (Cu) concentration and has been the subject of several studies on bioavailability and/or toxicity to relevant marine organisms (Zirino et al., 1998; Rivera-Duarte et al., 2005; Neira et al., 2009, 2011). Elevated Cu concentration in SIYB is due to the combined effect of restricted hydrological conditions and the presence of approximately 2300 recreational vessels, most of which have copper-based antifouling coatings on their hulls (CRWQCB, 2005). Copper leaching from these vessel's hulls has been estimated to contribute 93% of the Cu entering SIYB (CRWQCB, 2005).

The elevated Cu concentration in SIYB has become a subject of regulatory concern, not only because it exceeds the United States Environmental Protection Agency (USEPA) national saltwater quality criterion of 3.1 μg dissolved Cu L^{-1} (USEPA, 1995a), but because elevated Cu can be detrimental to aquatic communities (Rygg, 1985; Chang and Reinfelder, 2000; Neira et al., 2011). Observed toxicity, however, is related to bioavailability, which is not sufficiently predicted by total or dissolved Cu. Rather, in accordance with the free ion model, toxicity is more closely related to the concentration of free ionic Cu^{2+} (Morel, 1983). Because bioavailable metal is a better predictor of toxicity to aquatic organisms than dissolved or total metal (Anderson and Morel, 1978; Kim et al., 1999), it is critical to quantify Cu bioavailability in order to make meaningful predictions of its propensity for adverse effects.

This study examined the toxicological effects and bioavailability of Cu in SIYB, and whether or not dissolved Cu, the form currently monitored for regulatory compliance (e.g. USEPA, 1995a), poses a threat to sensitive organisms inhabiting the site. Independent toxicological, chemical, and modeling lines of evidence were used to evaluate metal bioavailability in seawater samples collected from SIYB. Toxicological evidence was assessed using two approaches to empirically quantify toxicity, both of which employed 48 h embryo-larval development toxicity tests using the economically important Mediterranean mussel, *Mytilus galloprovincialis* (USEPA, 1995b), which is the most sensitive genera used in the calculation of the water quality criterion for Cu in saltwater (USEPA, 1995a; HDR|HydroQual, 2012). In one approach, embryos were exposed to surface and near-bottom seawater from each of the stations to establish any presence and/or gradient in ambient toxicity. In the

* Corresponding author. Tel.: +1 (619) 553 2373; fax: +1 (619) 553 6305.
E-mail addresses: gunther.rosen@navy.mil (G. Rosen), ignacio.rivera@navy.mil (I. Rivera-Duarte).

http://dx.doi.org/10.1016/j.marpolbul.2014.05.045
0025-326X/Published by Elsevier Ltd.

second, surface seawater samples from four stations (and relevant laboratory seawater) were spiked with multiple Cu concentrations to derive median effective concentrations (EC50s) and compared using water effect ratios (WER) as an indicator of Cu bioavailability (USEPA, 1994a).

Chemical evidence was obtained from complexometric titrations (Zirino et al., 1998; Rivera-Duarte et al., 2005). These titrations were based on the response of a Cu ion selective electrode (CuISE) to additions of Cu to the sampled water and were used to estimate the complexation capacity (i.e. buffering capacity) of the water to Cu additions. This measurement is used to verify whether total Cu concentration might result in adverse effects, and identify how much total Cu can be added to a water sample prior to eliciting an adverse biological response.

The third, model-based, line of evidence was obtained by use of the marine Biotic Ligand Model (BLM) for Cu. The BLM is a chemical speciation model that uses site-specific water chemistry parameters (e.g. temperature, salinity, pH and dissolved organic carbon [DOC]) to predict the dissolved Cu concentration that results in toxicity (HDR|HydroQual, 2012). The BLM and empirical toxicity data were both used to estimate maximum dissolved Cu concentrations (at each of up to 16 stations, and for the marina as a whole) that would be protective of *M. galloprovincialis*. These maximum safe concentrations were expressed as site-specific criteria, which integrated national water quality criteria with adjustments for Cu bioavailability at the site.

2. Methods

2.1. Study site

Shelter Island Yacht Basin (SIYB) is located at the northern end, close to the mouth, of San Diego Bay. It is a semi-enclosed man-made basin with an average depth of 6 m (CRWQCB, 2005). The climate of this region can be described as Mediterranean with a low average rainfall of about 28 cm yr^{-1} (Largier, 1995). Nearly all of this rainfall occurs during the months of November through April, which are considered the wet season, while the months of May through October exhibit very little to no rainfall, and are considered the dry season (NOAA, 2006). There are no municipal or industrial discharges into SIYB; however, normally there are about 2300 boats berthed in SIYB, and the Cu released by passive leaching and cleaning of the copper-containing antifouling coatings on the boat hulls is considered the primary source of Cu to the marina (CRWQCB, 2005).

2.2. Water collection

Two sampling events were conducted in SIYB to represent the wet and dry seasons. Seawater samples were collected from 1 m below the surface (surface water) and 1 m above the sediment (bottom water) from 15 stations during the wet season on March 22, 2011, and from 16 stations during the dry season on July 5, 2011 (Fig. 1). The wet season sampling occurred after a relatively substantial rain event for San Diego, where 63% (2.0 cm) of the total rainfall (3.7 cm) in March occurred during the two days prior to the sampling day (NOAA, 2011). Rainfall in March 2011 was very similar to the average rainfall for that month in San Diego, which averaged 4.1 cm from 1914 to 2011 (WRCC, 2012). The dry season sampling attempted to represent a worst case scenario for Cu discharge from boat hulls, by sampling when a substantial number of boat hulls were expected to be cleaned in preparation for a holiday weekend. Hull cleaning results in the release of particles to surrounding water, and an associated increase of the Cu leach rate from boat hulls (Earley et al., 2014).

Trace metal clean techniques (USEPA, 1995c) were used throughout preparation of sampling equipment, and during sampling, manipulation, and analysis of the samples. These included acid-soaking of sampling material and equipment, collection of water using clean hands-dirty hands techniques, using a peristaltic pump with Teflon® pump-head and weighted Teflon® tubing, and the option for in-line filtration with acid-cleaned high-volume polypropylene 0.45 μm Calix® filters. Most of the parameters were sampled at all the stations, with the exception of those for the WER study, which were only collected from the surface at Stations 1, 4, 10, and 12 (Fig. 1).

2.3. Ancillary parameters

Environmental parameters were monitored in the laboratory during the toxicity testing for quality control and test acceptability purposes. Parameters included temperature, pH, salinity, dissolved oxygen (DO), total suspended solids (TSS), and DOC. The temperature, pH, salinity and DOC, were also required for calculation of chemical speciation and toxicity with the marine BLM (HDR|HydroQual, 2012). The temperature, pH, salinity and DO were measured using standard probes and corresponding meters. The TSS was quantified by filtration and dry mass. For the wet season, Whatman® glass microfiber 0.4 μm pore filters were used, while Whatman® Nuclepore® 0.45 μm polycarbonate membranes were used for the dry season.

Samples for DOC were collected in 500 mL polyethylene bottles containing 2 mL concentrated sulfuric acid. These bottles were provided by a private laboratory (Columbia Analytical Services, Kelso, WA), where the samples were analyzed following the persulfate-ultraviolet oxidation procedure of Standard Method 5310C (Standard Methods for the Examination of Water and Wastewater, 1996), with a detection limit of 0.2 mg L^{-1}.

2.4. Total and dissolved copper concentrations

Total (TCu) and dissolved (DCu) copper concentrations in ambient water samples were measured using in-line preconcentration flow injection analysis into an inductively coupled plasma mass spectrometer (ICP-MS) (Ndung'u et al., 2003). Each sample first ran through a Perkin–Elmer Flow Injection Analysis System (FIAS) 400 containing a TOYOPEARL AF-Chelate-650M resin to remove salts from the sample. The sample was then transferred into a Perkin-Elmer ELAN DRCII ICP-MS where the Cu concentration was quantified. Each run included three duplicates (average recovery ± standard deviation, 100.7 ± 2.8%) and three spiked samples (99.4 ± 3.1%). For every 5 samples, a blank was analyzed to ensure cleanliness of the system and to give a reference point for the background level of metals. The blank was the Standard Reference Material (SRM) NASS 2 (open ocean sea water) from the National Research Council of Canada, which had an average concentration of 0.188 ± 0.030 μg L^{-1}, or 172% of the certified concentration of 0.109 ± 0.011 μg L^{-1}. The SRM CASS 4 (coastal seawater), from the National Research Council of Canada, was also quantified every 5 samples to ensure that the instrument was measuring accurately and precisely, and had an average recovery of 94.2 ± 2.3% of the certified concentration of 0.592 ± 0.055 μg L^{-1}. Reported concentrations are not corrected for blank or SRM recoveries.

2.5. Copper complexation capacity

Complexation capacity is a measure of the natural buffering capability of marine water to control adverse effects on biota. In this case, copper complexation capacity (CuCC), a chemical measurement, was measured with an ion selective electrode. This endpoint has previously been correlated with the normal development

of *M. galloprovincialis* larvae exposed to Cu (Rivera-Duarte et al., 2005). For each sample, an Orion 94-29 Cu (II) Cu-ISE in combination with an Orion Ag/AgCl double-junction reference electrode were used to measure the free divalent Cu ion concentration (Cu^{2+}) that resulted from the addition (titration) of Cu with a Radiometer TIM860 titrator. The responses of the Cu-ISE and reference electrode were calibrated with two Cu^{2+} activity buffers, ethylenediamine and glycine (Zirino et al., 1998; Belli and Zirino, 1993). The electrodes were allowed to equilibrate in an aliquot of the sample for at least 1 h before they were transferred to an acid washed Teflon® beaker containing approximately 300 g of the seawater sample. The sample was placed in a constant temperature water bath at 25 ± 0.1 °C, with constant stirring. The titration solution was made with 200 μL of 1000 ± 3 μg mL^{-1} high purity copper standard and 32 g NaCl in 1 L of 18 MΩ/cm water. The automated titration was programmed to add 10 μL of titrant up to 20 mL, and then to add 200 μL of titrant up to 80 mL, for a total of 100 mL of titrant added. The change in potential (mV) between the two electrodes was recorded by the automated titrator. The data from the titration curves were analyzed using MATLAB® to estimate the inflection point which indicates the CuCC.

2.6. Toxicity tests

Two approaches were used to assess toxicity, including (1) exposures with site (ambient) water samples, and (2) site water samples spiked with multiple concentrations of Cu for WER determination (USEPA, 1994a). Ambient toxicity was investigated by exposing *M. galloprovincialis* embryos to seawater collected from the surface and bottom of each station in SIYB (Fig. 1). In addition, copper was added to unfiltered surface seawater from the four WER stations (Stations 1, 4, 10 and 12) and at least one laboratory control seawater at 10 nominal Cu concentrations (0, 2.9, 4.2, 5.8, 8.4, 12, 17.2, 24, 35 and 50 μg L^{-1}) to derive median effective concentrations (EC50), required for calculating a site specific water quality criterion (SSC) for Cu (USEPA, 1994a). Filtered (0.45 μm) seawater from the research pier at Scripps Institution of Oceanography (SIO) in La Jolla, California or from the Marine Pollution Studies Laboratory at Granite Canyon (GC), Monterey, California, served as laboratory control waters due to their previously reported acceptability for embryo-larval development tests and relatively low DOC concentration (Rosen et al., 2008).

Both ambient and WER toxicity testing employed similar methodology (USEPA, 1995b). Water samples were stored at 4 °C and the bioassays were initiated within 36 h of sampling. Exposures were conducted in 20 mL glass scintillation vials that were presoaked in uncontaminated filtered seawater (FSW) for 24 h, rinsed with deionized (DI, 16 MΩ cm^{-1}) water, and dried prior to use. Each water sample and laboratory control was replicated 5 times, with each vial consisting of 10 mL sample. Spiked Cu solutions were prepared in 125 mL Erlenmeyer flasks, which were acid cleaned and pre-soaked in FSW for 24 h, then rinsed with DI water. All spiked and unspiked samples associated with the WER exposures were allowed to equilibrate in an incubator for 1 to 3 h at 15 °C before addition of embryos.

Adult gravid *M. galloprovincialis* were purchased from Carlsbad Aquafarm (Carlsbad, CA, USA) and induced to spawn by thermal shock. Within 4 h of fertilization, approximately 150 embryos, at or beyond the two-cell stage, were transferred to each test vial. Vials were incubated at 15 °C for 48 h under a 16 h light:8 h dark photoperiod. At 0, 24 and 48 h, water quality measurements including pH, temperature, DO, and salinity were recorded. Once the successful development of embryos in the control treatments was established (within 46–50 h of initiation), the tests were terminated by adding 10% buffered formalin to each vial. Evaluation of the number of surviving larvae that developed normally (D-shaped, prodissoconch I stage) relative to the number of total embryos initially added to each vial was assessed under an inverted compound microscope at 40–60× magnification. This endpoint (% normal alive) represents the percentage of surviving larvae that developed normally, integrating both normal development and survival endpoints.

Ambient toxicity was evaluated by comparing larval development success from SIYB samples with laboratory controls using the test of significant toxicity (TST; USEPA, 2010; Denton et al., 2011) which incorporates the bioequivalence approach (Erickson and McDonald, 1995; Shukla et al., 2000).

The% normal alive larvae from the multi-concentration tests were used to calculate EC50s using measured TCu and DCu with the toxicity statistics program CETIS™ v1.8.7.16 (Tidepool Scientific). The site-specific criterion (SSC) for each station was calculated using the EC50s, WERs and current national WQC (3.1 μg L^{-1}; USEPA, 1995a). The WER served as a multiplier to adjust the national WQC to a more relevant SSC, and was used as follows (USEPA, 1994a):

$$\mathrm{WER} = \frac{\mathrm{EC50_{site}}}{\mathrm{EC50_{lab}}}$$

and

$$\mathrm{SSC} = 3.1\ \mu g\ L^{-1} \times \mathrm{WER}$$

where $EC50_{site}$ is the site water EC50, and $EC50_{lab}$ is the laboratory water EC50 (SIO or GC seawater).

2.7. Biotic Ligand Model

The BLM is a chemical speciation model that can be used to predict the equivalent of a SSC for a given body of water (Santore et al., 2001), defined here as the estimated chronic limit (ECL). For this study, the draft BLM for Cu in saltwater (HDR|HydroQual, 2012), currently under review for publication by the USEPA, was used to calculate a SSC for SIYB. Application of the marine BLM requires only water chemistry data (i.e. salinity, temperature, pH and DOC) from the site. Using chemical speciation data of the different components in seawater, the BLM predicts the DCu that would provide enough binding at the biotic ligand to reach the EC50 for that species ($EC50_{BLM}$), or other effective concentration, as desired. From the $EC50_{BLM}$, additional calculations can be made to derive a SSC (HDR|HydroQual, 2012).

3. Results

3.1. Ancillary parameters

Ancillary parameters were within acceptable ranges for the bioassays (USEPA, 1995b). Salinity measurements averaged 32.7 ± 0.2 and 33.5 ± 0.1 ppt for the wet and dry seasons, respectively. Temperature averaged 14.7 ± 1.2 and 15.3 ± 0.3 °C for the wet and dry seasons, respectively. Mean DO measurements were 8.3 ± 0.1 and 7.4 ± 0.1 mg L^{-1}, for the wet and dry seasons, respectively, while pH measurements averaged 8.1 ± 0.1 and 8.0 ± 0.1, for the wet and dry seasons, respectively. Table 1 summarizes the DOC and TSS data for each ambient sample.

3.2. Total and dissolved copper concentrations

3.2.1. Wet season

Spatial Cu distributions in SIYB were dominated by a gradient of increasing TCu and DCu from the mouth (Station 1) to the head of the basin (Station 15; Fig. 2a and b). For the wet season, Cu concentrations in surface water increased from 2.3 μg L^{-1} TCu (1.4 μg L^{-1}

Table 1
Dissolved organic carbon (DOC) and total suspended solids (TSS) for ambient sites within the Shelter Island Yacht Basin collected in March and July 2011. Dashes indicate not sampled. N/A indicates not analyzed.

Station ID	Wet season		Dry season	
	DOC ($mg\ L^{-1}$)	TSS ($mg\ L^{-1}$)	DOC ($mg\ L^{-1}$)	TSS ($mg\ L^{-1}$)
1 Surface	1.4	0.8	2.0	2.2
2 Surface	2.1	0.3	1.8	1.2
3 Surface	1.1	N/A	1.6	1.2
4 Surface	1.3	0.9	1.5	1.7
5 Surface	1.5	1.2	1.6	1.3
6 Surface	1.8	0.8	1.6	2.3
7 Surface	1.9	0.6	1.4	1.7
8 Surface	1.8	0.7	1.6	2.0
9 Surface	1.5	0.8	1.4	2.3
10 Surface	1.8	0.5	1.4	2.0
11 Surface	1.7	1.0	1.5	2.3
12 Surface	0.9	0.6	1.5	1.0
13 Surface	2.0	0.5	1.5	0.7
14 Surface	2.2	0.3	1.5	0.7
15 Surface	1.9	0.4	1.6	1.0
16 Surface	–	–	1.6	2.3
1 Bottom	1.7	2.3	1.7	4.4
2 Bottom	1.4	1.6	1.3	5.3
3 Bottom	2.2	0.9	1.3	2.7
4 Bottom	2.2	1.1	1.2	7.0
5 Bottom	1.8	2.3	1.3	3.7
6 Bottom	1.4	1.5	1.6	4.3
7 Bottom	2.0	1.9	1.3	6.7
8 Bottom	1.7	3.9	1.5	4.0
9 Bottom	1.3	4.6	1.5	5.0
10 Bottom	1.9	1.9	1.7	4.3
11 Bottom	1.6	2.0	1.8	2.0
12 Bottom	1.2	2.2	1.6	4.3
13 Bottom	0.8	7.0	1.8	6.3
14 Bottom	0.9	2.6	1.5	2.7
15 Bottom	1.6	2.9	1.5	5.0
16 Bottom	–	–	1.7	1.5
Average	1.6	1.7	1.5	3.0
Std. Dev.	0.4	1.5	0.2	1.8

DCu) in the mouth of SIYB, to a maximum of 8.2 $\mu g\ L^{-1}$ TCu (5.7 $\mu g\ L^{-1}$ DCu) at the head. Within this main gradient, the spatial distribution of Cu in surface waters could be visually divided into three areas demarcated in Fig. 2a; (1) the mouth, Stations 1–7 (excluding Station 3), with concentrations similar to those outside the basin (average and range TCu 3.1 ± 0.6, 2.3–4.2 $\mu g\ L^{-1}$; DCu 2.1 ± 0.4, 1.4–2.7 $\mu g\ L^{-1}$); (2) an area with intermediate Cu from Station 8–12, (TCu 5.8 ± 0.6, 5.1–6.7 $\mu g\ L^{-1}$; DCu 4.2 ± 0.4, 3.6–4.8 $\mu g\ L^{-1}$); and (3) the head which displayed a sharp increasing Cu gradient, Stations 13–15 (TCu 7.7 ± 0.4, 7.5–8.2 $\mu g\ L^{-1}$; DCu 5.7 ± 0.1, 5.7–5.9 $\mu g\ L^{-1}$). A radial gradient of Cu increasing from the main channel toward the boats was also apparent, and indicated by elevated levels of Cu in surface water at Stations 3 and 8 (Fig. 2a), which are surrounded by more boats than proximal stations; however, there is the possibility for water circulation to result in this effect as well, a possibility that was not addressed in this work.

The SIYB also exhibited a vertical gradient of Cu increasing from the bottom to the surface. Total Cu ranged from 2.3 to 8.2 $\mu g\ L^{-1}$ in surface water (Fig. 2a) and from 0.5 to 2.7 $\mu g\ L^{-1}$ in bottom water (Fig. 2b). Dissolved Cu ranged from 1.4 to 5.7 $\mu g\ L^{-1}$ in surface water (Fig. 2a) and from 0.2 to 2.0 $\mu g\ L^{-1}$ in bottom water (Fig. 2b). Surface water Cu was greater than bottom water Cu by an average of 3.3 and 2.7 $\mu g\ L^{-1}$ for TCu and DCu, respectively.

3.2.2. Dry season

Surface water Cu in the dry season ranged from 3.2 to 12.8 $\mu g\ L^{-1}$ TCu (1.6–8.9 $\mu g\ L^{-1}$ DCu) from the mouth to the head. On average, total and dissolved Cu were 2.1 $\mu g\ L^{-1}$ (41%) and 1.2 $\mu g\ L^{-1}$ (66%) higher than concentrations measured in the wet season, respectively. The surface water displayed a spatial distribution of Cu in the dry season that is similar to the spatial distribution in the wet season, but with an increase in concentration, and can also be visually divided into three areas demarcated in Fig. 2c; (1) the mouth, Stations 1–6 (excluding Station 3) (average and range TCu 3.7 ± 0.4, 3.2–4.1 $\mu g\ L^{-1}$, DCu 2.1 ± 0.3, 1.6–2.5 $\mu g\ L^{-1}$); (2) an area of intermediate Cu, Stations 7 to 10 (TCu 7.2 ± 0.2, 7.0–7.4 $\mu g\ L^{-1}$, DCu 4.3 ± 0.6, 3.6–5.1 $\mu g\ L^{-1}$); and (3) a sharp increasing gradient of Cu, Stations 11–16 (TCu 11.2 ± 1.4, 8.8–12.8 $\mu g\ L^{-1}$, DCu 6.5 ± 1.7, 4.7–8.9 $\mu g\ L^{-1}$). The dry season still exhibited elevated Cu compared to nearby stations at Station 3, but not at Station 8 (Fig. 2c). Similar to the wet season, surface water in the dry season exhibited higher Cu than bottom water. Total Cu

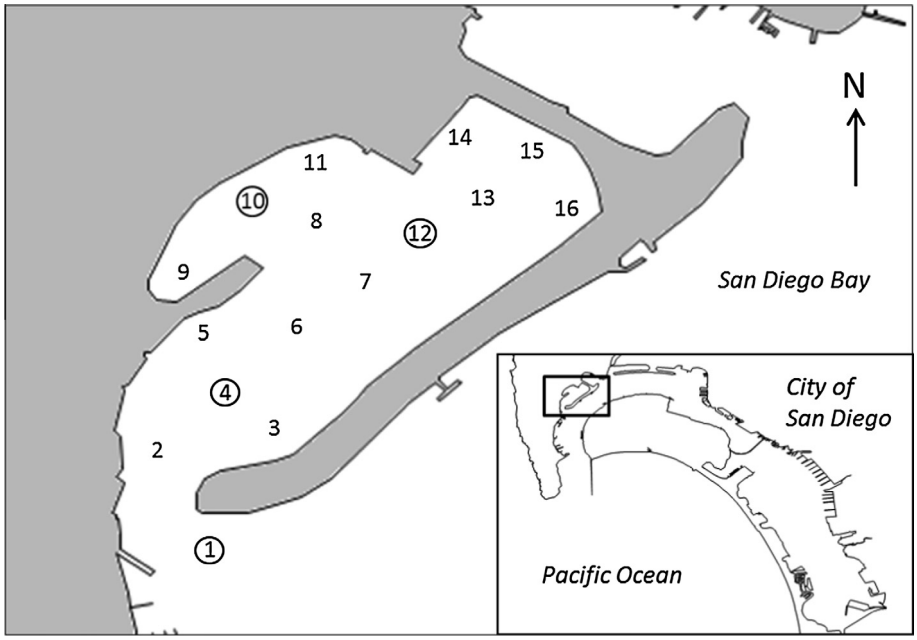

Fig. 1. Map of Shelter Island Yacht Basin (SIYB), in San Diego Bay, California. Approximate locations for each of 16 sampling stations are shown. Station numbers in circles were selected for water effect ratio (WER) experiments. Inset: San Diego Bay with location of Shelter Island Yacht Basin near mouth of the bay highlighted (black box).

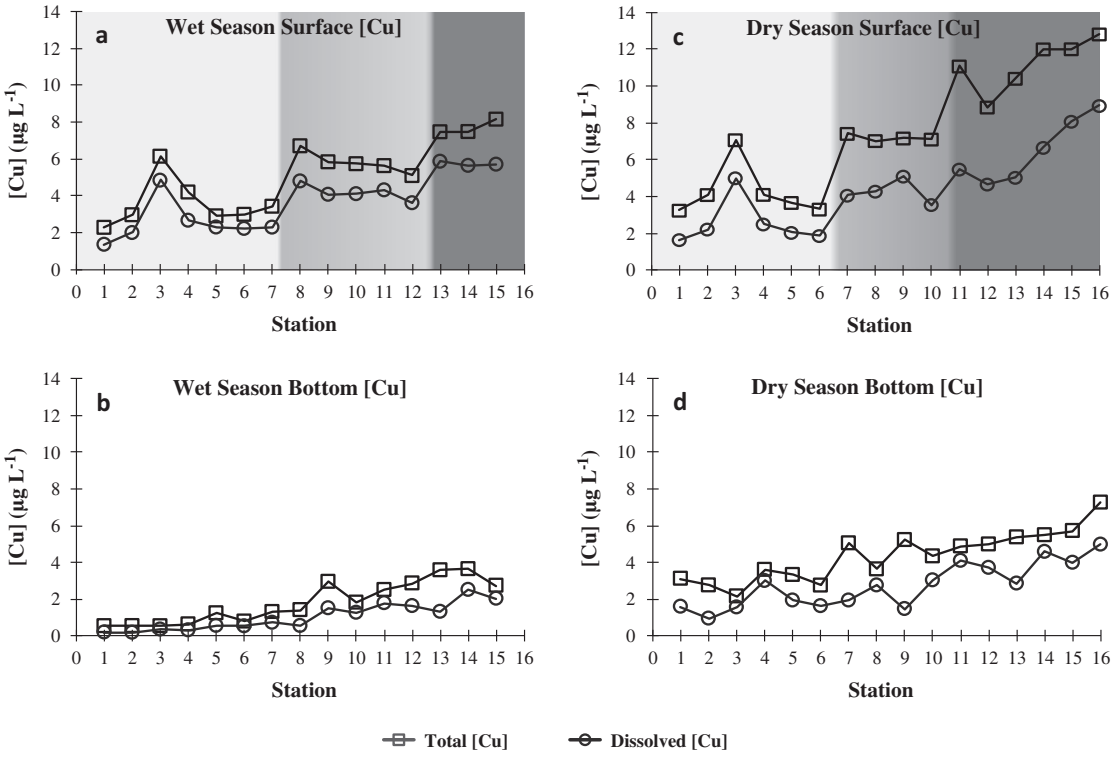

Fig. 2. Total (□) and dissolved (○) copper (Cu) concentrations (µg L^{-1}) in surface and bottom water of 15 stations sampled during the wet season (a and b), and 16 stations during the dry season (c and d). Surface water is visually divided into three sections based on Cu concentrations.

ranged from 3.2 to 12.8 µg L^{-1} in surface water and from 3.1 to 7.3 µg L^{-1} in bottom water. Dissolved Cu ranged from 1.6 to 8.9 µg L^{-1} in surface water (Fig. 2c) and from 1.6 to 5.0 µg L^{-1} in bottom water (Fig. 2d). Surface water Cu was greater than bottom water Cu by an average of 3.1 ± 2.2 µg L^{-1} and 1.6 ± 1.3 µg L^{-1} for TCu and DCu, respectively.

3.3. Copper complexation capacity

Copper complexation capacity (CuCC) was measured in surface water samples for both the wet and dry seasons, with the latter having the highest concentration range. The CuCC ranged from 5.7 to 12.1 µg L^{-1} (average 8.7 ± 1.7 µg L^{-1}) for the wet season, and 10.0–15.1 µg L^{-1} (average 12.0 ± 2.5 µg L^{-1}) for the dry season. The mean CuCC for both seasons combined was 9.8 ± 2.4 µg L^{-1}. In general, the CuCC increased from the mouth of the basin to the head in both the wet and dry seasons (with the exception of Station 16 dry season) (Fig. 3). The measured CuCC was higher than the TCu at every station during both seasons (Fig. 3) except at Station 16 in the dry season, where there was no detected CuCC. The mean differences between TCu and CuCC (CuCC being the higher of the two measurements) for the wet and dry seasons were 3.4 and 5.2 µg L^{-1}, respectively, with an average of 3.9 ± 2.0 µg L^{-1}.

3.4. Ambient toxicity tests

3.4.1. Wet season

There was no evidence of significant toxicity in ambient water from SIYB (n = 30) for the wet season event compared to the laboratory controls. The mean% normal alive was ⩾87% for the surface and bottom water at each station (Fig. 4), and the overall mean% normal alive ±1 SD for all stations was 93 ± 2.7%. Compared to the mean of 92 ± 3.3% normal alive for the laboratory control water (SIO and/or GC), the unspiked water from SIYB was not significantly different, using the TST.

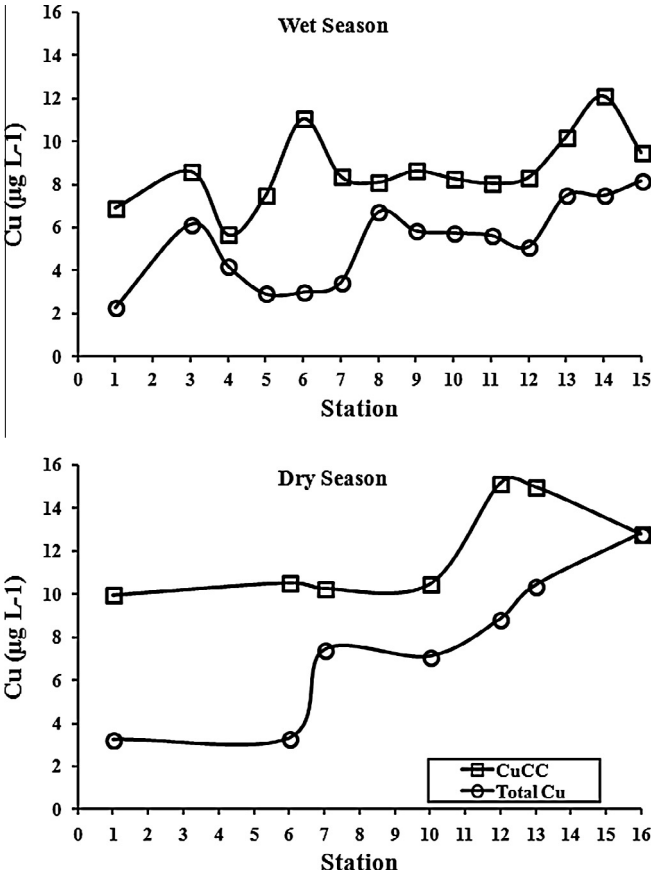

Fig. 3. Copper complexation capacity (CuCC) compared to total Cu concentrations at each station for the wet season (top) and the dry season (bottom) in Shelter Island Yacht Basin.

3.4.2. Dry season

No significant toxicity was observed for 31 of the 32 samples obtained during the dry season sampling. An adverse response was observed following exposure to only the surface water sample at Station 16 (the station furthest from the mouth of SIYB), where the mean% normal alive was 74.4 ± 6.2%, which was significantly lower than the laboratory control (93 ± 3.0%; Fig. 4). For the remainder of the stations where no toxicity was observed, the mean% normal alive exceeded 85%, except at Station 11 (83.5%; Fig. 4), and the overall mean% normal alive was 90 ± 4.0%.

3.5. Water effect ratio

The EC50, WER, and SSC were each calculated for surface water at Stations 1, 4, 10 and 12 (Table 2). For the wet season, EC50s (DCu) ranged from 8.00 to 10.0 μg L^{-1}, with no obvious spatial gradient. In contrast, dry season EC50s (DCu) ranged from 9.21 to 11.4 μg L^{-1}, with the stations located closest to the mouth (Stations 1 and 4) having the lowest values.

The SSC based on the WER study (SSC_{WER}) ranged from 3.31 to 4.13 μg L^{-1} Cu (geometric mean 3.68 ± 0.41 μg L^{-1}) for the wet season, and 4.12 to 5.08 μg L^{-1} Cu (geometric mean 4.53 ± 0.47 μg L^{-1}) for the dry season, with an overall geometric mean of 4.11 ± 0.44 μg L^{-1}. The geometric means were 18% and 46% higher than the USEPA (1995a) national criterion for DCu (3.1 μg L^{-1}) for the wet and dry seasons, respectively.

3.6. Biotic Ligand Model

Following a theoretical approach, the BLM was used to calculate BLM-predicted EC50s ($EC50_{BLM}$) and an estimated chronic limit (ECL_{BLM}), a value analogous to the SSC_{WER}, for each station (Table 3). The average ECL_{BLM} was 8.83 and 8.50 μg L^{-1} DCu for surface and bottom waters, respectively, for the wet season; and 8.56 and 8.34 μg L^{-1} DCu, for the surface and bottom water, respectively, for the dry season. The ECL_{BLM} for each station and event are shown in comparison to measured DCu in Fig. 5. Geometric means for surface water were calculated for $EC50_{BLM}$, $EC50_{WER}$, ECL_{BLM}, SSC_{WER}, and the DCu for each season and for both seasons combined (Fig. 6).

4. Discussion

The three lines of evidence (CuCC, WER, and the BLM) used in this study to predict toxicity associated with bioavailable copper indicated that the current national WQC for Cu (3.1 μg DCu L^{-1}; USEPA, 1995a) is more than sufficiently protective of *M. galloprovincialis* embryo-larval development, as DCu at the site exceeded the criterion by nearly a factor of three. The results suggest that CuCC and the marine BLM for Cu may be logical alternatives to current standardized approaches such as the use of resource intensive WER studies for development of site-specific criteria for copper, and provide substantial evidence that conditions during the two sampling events were not expected to impair mussel embryogenesis. The development of a site-specific criterion for copper could use the current EPA-recommended WER approach, but the BLM incorporates the latest science and a substantially updated toxicity dataset in the criterion calculation (HDR|HydroQual, 2012), and was designed to be sufficiently protective.

4.1. Ancillary parameters, total and dissolved copper concentrations and spatial distribution

The ancillary parameters (temperature, salinity, pH, DOC, DO and TSS) fell within normal ranges for seawater in San Diego Bay, with values that were similar to those from other studies (Blake et al., 2004: 33.5–36.0 ppt, 16.3–25.1 °C, ~pH 8; Chadwick et al., 2008: 34.1–35.8 ppt, 15.3–16.0 °C, pH 7.89–8.20, DOC 1.30–3.43 mg L^{-1}).

In comparison to the current national WQC for Cu, 25 of 62 samples (40%) exceeded recommended concentrations, the majority of which were at the surface and/or near the head of the basin. The range of DCu concentrations in the surface water for this study (1.3–8.9 μg L^{-1}, both seasons combined) was similar to those for previous studies in SIYB (Table 4). The SIYB exhibited a horizontal gradient of DCu and TCu increasing from the mouth to the head of the basin, a vertical gradient from the bottom water to the surface water and a radial gradient from the main channel towards the boats. These gradients are consistent with previous findings at this site (CRWQCB, 2005; Schiff et al., 2006; Neira et al., 2009), and as hypothesized previously, appear to be associated with density and distance from boats, and are sustained by poor tidal flushing

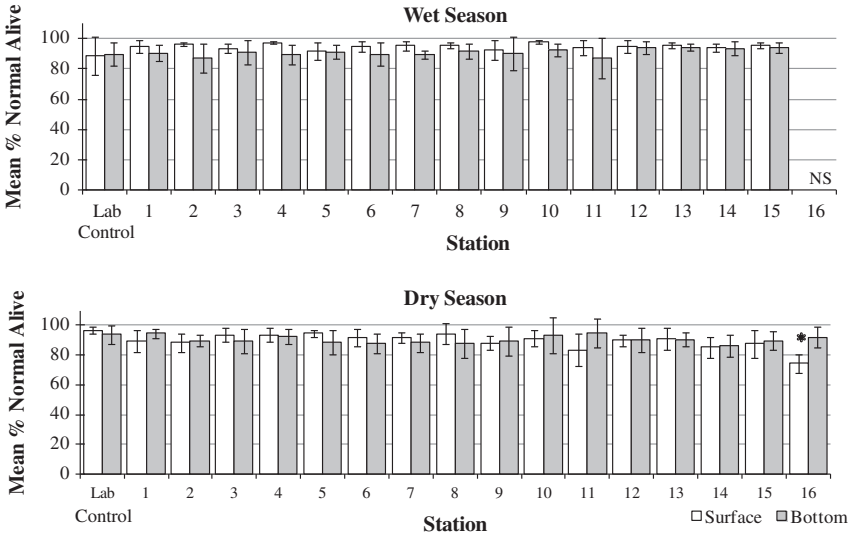

Fig. 4. Ambient *Mytilus galloprovincialis* embryo-larval development toxicity results for SIYB from the wet and dry season sampling events. *Significantly different from control using the test of significant toxicity (α = 0.05). NS = not sampled.

Table 2
Median effective concentrations (EC50), site-specific criteria (SSC), dissolved copper (DCu) concentrations and water effect ratios (WER) for the 4 WER stations and controls for the wet and dry seasons. All values are expressed as μg L^{-1}. The SSC_{WER} was calculated by multiplying the WER by USEPA (1995a) national criterion for DCu, 3.1 μg L^{-1}. Dashes indicate not relevant, so not calculated.

Station ID	Wet season				Dry season			
	$EC50_{WER}$	SSC_{WER}	DCu	WER	$EC50_{WER}$	SSC_{WER}	DCu	WER
SIO	7.5	–	–	–	6.9	–	–	–
GC	–	–	–	–	6.7	–	–	–
1	8.0	3.3	1.4	1.1	9.4	4.2	1.7	1.4
4	10.0	4.1	2.7	1.3	9.2	4.1	2.5	1.3
10	9.6	4.0	4.1	1.3	10.8	4.8	3.6	1.6
12	8.1	3.4	3.6	1.1	11.4	5.1	4.7	1.6
Geo Mean	8.9	3.7	2.7	1.2	10.1	4.5	2.9	1.5
Std. Dev.	1.0	0.4	1.2	0.1	1.1	0.5	1.3	0.2

Table 3
Biotic Ligand Model (BLM) calculated ECL and EC50 values for Cu (μg L^{-1}) in surface and bottom waters in SIYB during the wet and dry seasons. Dashed line indicates not tested.

Station ID	Wet Season				Dry Season			
	Surface		Bottom		Surface		Bottom	
	ECL_{BLM}	$EC50_{BLM}$	ECL_{BLM}	$EC50_{BLM}$	ECL_{BLM}	$EC50_{BLM}$	ECL_{BLM}	$EC50_{BLM}$
1	7.52	9.44	9.14	11.5	11	13.8	9.58	12
2	11.3	14.1	7.52	9.44	9.85	12.4	7.12	8.93
3	5.89	7.4	11.8	14.8	8.77	11	7.09	8.89
4	6.94	8.72	11.8	14.8	8.19	10.3	6.63	8.32
5	8.02	10.1	9.66	12.1	8.71	10.9	7.13	8.95
6	9.64	12.1	7.52	9.43	8.76	11	8.81	11.1
7	10.2	12.8	10.7	13.5	7.64	9.58	7.16	8.99
8	9.63	12.1	9.14	11.5	8.72	10.9	8.23	10.3
9	6.89	8.69	7	8.79	7.61	9.55	8.37	10.5
10	9.61	12.1	10.2	12.8	7.61	9.55	9.27	11.6
11	9.1	11.4	8.59	10.8	8.18	10.3	9.81	12.3
12	4.85	6.08	6.51	8.17	8.15	10.2	8.73	10.9
13	10.8	13.5	4.36	5.47	8.17	10.3	9.95	12.5
14	11.9	14.9	4.89	6.14	8.17	10.2	8.12	10.2
15	10.2	12.9	8.71	10.9	8.72	10.9	8.24	10.3
16	–	–	–	–	8.71	10.9	9.26	11.6
Average	8.83	11.1	8.5	10.7	8.56	10.7	8.34	10.5
Std. Dev.	2.05	2.57	2.23	2.8	0.87	1.09	1.07	1.34

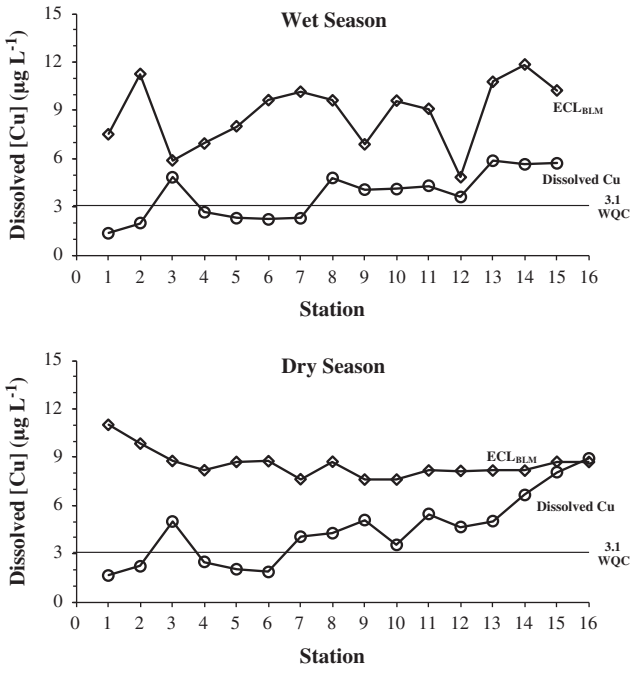

Fig. 5. Dissolved copper (DCu) and Biotic Ligand Model (BLM)-derived Estimated Chronic Limits (ECL) for each surface station in the wet and dry seasons. The national water quality criterion (WQC) of 3.1 μg L^{-1} DCu is shown for reference.

due to the configuration of the basin (CRWQCB, 2005; Neira et al., 2009). The vertical gradient of increasing Cu from the bottom to the surface may be sustained by thermohaline circulation. Cold water entering the basin flushes the bottom water keeping the Cu concentration low, then rises as it warms up and becomes concentrated with Cu near the surface where the source of Cu is present.

4.2. Temporal differences of total and dissolved copper concentrations

In general, both DCu and TCu were lower in the wet season in comparison to the dry season for both surface and bottom water. It is generally expected that these constituents will be higher in the wet season, when Cu is apportioned by urban runoff (CRWQCB, 2005). However, Blake et al. (2004) reported that total and dissolved Cu in San Diego Bay proper changed very little throughout the year, with runoff generally not affecting Cu concentrations. Our findings may suggest that hydrodynamic effects from runoff are controlled by the amount of precipitation and the size of receiving water body. For this study, a significant pluvial precipitation was able to flush out the SIYB basin to the point of decreasing Cu concentration, in comparison to the dry season. According to records from nearby Lindbergh Field, San Diego, CA, the rainfall in March 2011 (typically the 2nd wettest month of the year in San Diego) was 3.71 cm, 63% (2.33 cm) of which fell during the two days immediately prior to our sampling. The effect of this relatively high precipitation may have been a decrease of Cu in the

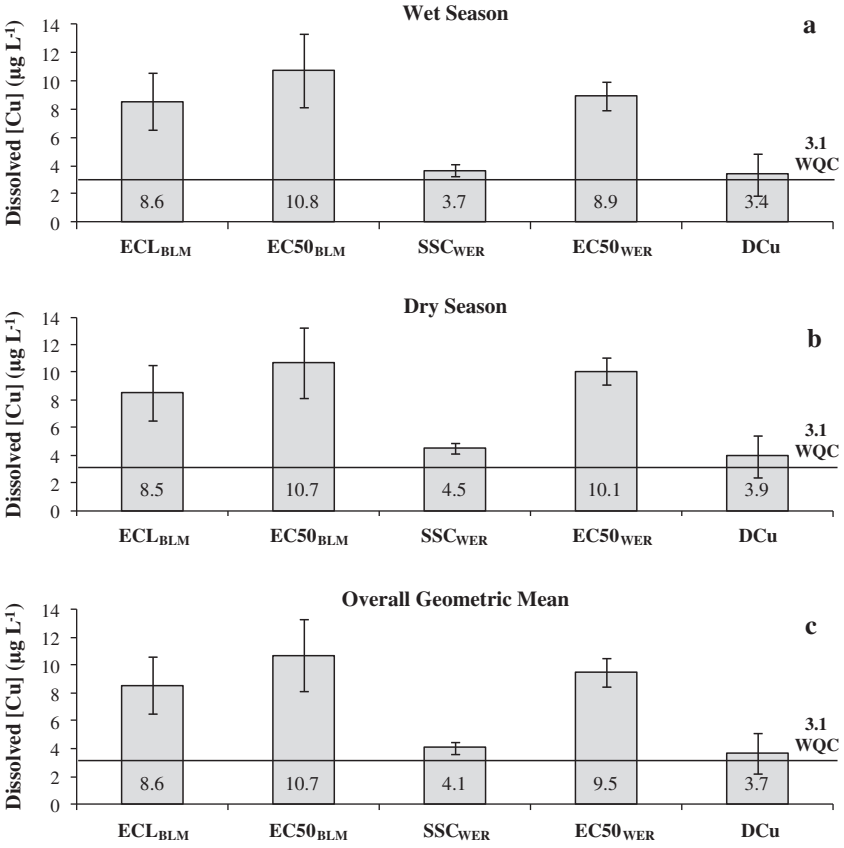

Fig. 6. Surface water geometric means (±1 standard deviation) for the site-specific criteria applied to the wet season (a), dry season (b), and combined (c). $N = 15$ (wet) or 16 (dry) for BLM-based criteria and 4 for WER-based criteria.

Table 4
Comparison of total (TCu) and dissolved (DCu) copper in Shelter Island Yacht Basin from this and historical studies.

Reference	Season/Year	Copper (µg L^{-1})	
		Dissolved	Total
Johnston (1989)	Summer 1978 to Summer 1979	0.9-17.9	1.7-19.1
Zirino et al. (1998)	Summer 1996	N/A	2.9-9.7
CRWQCB (2005)	Spring and Summer 2000	1.5–8.0	N/A
Schiff et al. (2006)	Summer 2005	6.7–13.4	N/A
Neira et al. (2009)	Spring 2006	1.3–14.6	N/A
This study	Spring 2011	1.4–5.7	2.3–8.2
This study	Summer 2011	1.6–8.9	3.2–12.8

water column, presumably by the combined effect of dilution and flushing.

4.3. Copper complexation capacity

The CuCC estimated from the titrations is a measure of the concentration of TCu that can be added to the site water before the Cu^{2+} becomes bioavailable to the point of producing a specific adverse effect. The data from the CuCC measurements show that the CuCC in SIYB is generally well above the concentration of TCu, by a combined average of 3.9 ± 2.0 µg L^{-1}, indicating that the amount of Cu^{2+} is below the level at which it would result in toxicity to organisms present at the site. An exception to this result was observed for the surface water sample at Station 16 during the dry season. The titration curve for Station 16 showed no CuCC, meaning that the level of Cu^{2+} was already at or above the concentration at which it would be expected to be toxic. This finding is consistent with the empirical toxicity observed and the prediction of toxicity from the BLM.

Complexation capacity is measured in unfiltered samples, providing total metal concentration. The CuCC combined for both seasons was measured at 9.8 ± 2.4 µg L^{-1} TCu. An estimate of the DCu representative of CuCC measurements could be approximated from the ratio of total to dissolved metal measured in those waters, however. For San Diego Bay, Blake et al. (2004) measured a ratio of 0.72 for TCu to DCu, which equates to a DCu of 7.1 ± 1.7 µg L^{-1}. This estimated mean CuCC for DCu is more than twice the WQC (3.1 µg L^{-1}), is almost double the WER-generated criterion (4.1 µg L^{-1}), and is similar to the BLM-derived criterion (ECL_{BLM}; 8.6 µg L^{-1}). The lack of toxicity observed in ambient waters for DCu concentrations at or below the mean estimated CuCC of 7.1 ± 1.7 µg L^{-1} indicates that this chemical approach is appropriately protective, while also not being overly conservative, as it predicted toxicity observed at Station 16.

4.4. Ambient toxicity

The concurrently derived lines of evidence (toxicological, chemical and modeled) suggest that Cu in SIYB, or other water bodies with similar physicochemical characteristics, is not bioavailable to a level that results in significant toxicity to *M. galloprovincialis* embryos. Despite elevated Cu at many of the test site locations relative to laboratory water control sites (SIO and GC), and to the National Recommended WQC for DCu (3.1 µg L^{-1}, USEPA, 1995a), even the most sensitive toxicity endpoint used in the calculation of the WQC (USEPA, 1995a; HDR|HydroQual, 2012) was protected. Only one sample (a surface water sample from Station 16 during the dry season) was statistically different from the controls and

defined as toxic. This station was selected specifically for its location in the back of the basin within a region of high boat density in an attempt to assess worst case Cu exposure, and potential for toxicity in SIYB. It also had the highest DCu measured in this study (8.9 μg L^{-1}). Although statistically significant, normal larval development was only moderately adversely affected at this station, with 74% of the embryos developing normally. The overall lack of adverse effects is attributed to site-specific characteristics of the water at the site, especially DOC which is well-known to sequester Cu, reducing its bioavailability in seawater in general (HDR|HydroQual, 2012, and references therein), as well as in San Diego Bay (Rosen et al., 2005, 2008). While the measured DCu at Station 16 was 2.9 times the nationally recommended WQC, the toxicity observed at this Station was an isolated occurrence that requires further assessment in order to firmly validate the presence of toxicity at this station over time.

4.5. Site specific criterion based on WER

The USEPA has long recognized that bioavailability-altering water chemistry (e.g. pH, salinity, and DOC) at marine sites differs, and various methods for the measurement and calculation of a SSC have been developed, including the WER procedure (USEPA, 1994a) used here. For this study, the ratio between the EC50s observed in site water and laboratory water (seawater representative of that used in WQC development; USEPA, 1985, 1994a) was always greater than one, indicating that the natural water complexation capacity (provided largely by DOC, total suspended solids, and inorganic and organic complexes) of water at the site was greater than the laboratory water. The final dissolved WER (geometric mean of all WER stations for both sampling events) of 1.33 was very similar to a dissolved WER generated for North San Diego Bay (1.26; Rosen et al. 2005). The geographical location of SIYB in North San Diego Bay (Fig. 1), and similar water chemistry between SIYB and North San Diego Bay, supports the highly comparable WERs between the two studies. The WERs for SIYB are also similar, but generally lower in magnitude, as compared to other WERs developed for multiple geographically distinct saltwater locations including Pearl Harbor, HI (1.40; Earley et al., 2007), Sinclair Inlet, WA (1.41; Rosen et al., 2009), Hampton Roads, VA (1.76; CH2 M Hill, 2000), New York, NY (1.5; USEPA, 1994b), San Diego Bay (bay-wide), CA (1.54; Rosen et al., 2005), and South San Francisco Bay, CA (2.77; ESD, 1998). The South San Francisco Bay study also reported the highest DOC concentrations in comparison to the other cited studies. Most of these studies indicated a positive relationship between DOC concentration and WER magnitude.

The resulting SSC derived from the WER (SSC_{WER}) of 4.11 μg L^{-1} DCu should be protective of 95% of the species represented in a species sensitivity distribution (USEPA, 1985). Similar WER studies at other sites that have utilized two or more species for corroborative purposes have revealed very similar responses when using endpoints of similar sensitivity (e.g. Rosen et al., 2005 and reference therein; Earley et al., 2007), therefore, the more streamlined approach used in this study is expected to be protective of other species. Further, even the most sensitive species (*M. galloprovincialis;* HDR|HydroQual, 2012) used in the calculation of the WQC, is protected at concentrations twice as large as the WER-derived SSC for SIYB.

4.6. Biotic Ligand Model estimation of site specific criterion

The BLM is a chemical speciation approach to predict Cu toxicity to aquatic organisms. The prediction is based on theoretical calculations of the chemical speciation of Cu, by only using site-specific measurements of DOC, salinity, temperature and pH, and assuming an expected strength of the different natural ligands in the seawater (DiToro et al., 2001; Santore et al., 2001; HDR|HydroQual, 2012). As a marine BLM for Cu is currently still under review by USEPA, SSC based on the BLM were tentatively expressed as an ECL, which is an analogous measurement for the empirically-derived SSC using the WER procedure. In this study, ECLs calculated with the BLM were in complete agreement with empirical toxicity data. Specifically, ECL_{BLM} values were higher than measured DCu at all stations where toxicity was not observed, suggesting the ECLs were sufficiently protective. Similarly, for the one ambient sample where toxicity to *M. galloprovincialis* was observed, Station 16, the ECL was exceeded, indicating that the model correctly predicted the potential for toxicity for that sample. This suggests that the BLM is sufficiently conservative to protect the biota in saltwater environments, but the low occurrence of adverse effects observed in this study precludes to ability to compare observed toxicity with modeled predictions of toxicity.

It should be noted that the draft BLM-based saltwater Cu criterion document (HDR|HydroQual, 2012) differs from the status quo criterion for Cu in saltwater (USEPA, 1995b) in multiple ways, including (1) a substantially more robust toxicity dataset; (2) normalization of toxicity data to DOC concentration; and (3) an adjustment of the calculation and magnitude of the final acute value (FAV) and the acute-chronic ratio (ACR) used in deriving acute and chronic criteria, respectively. In addition, SSC could be developed for all ambient stations evaluated in SIYB using the BLM, but SSC calculations were limited to only four stations for the WER study. Therefore, direct comparisons of the WER-derived SSC based on the 1995 criterion and the latest BLM-based criterion document (Fig. 6) are difficult. That said, should the EPA formally adopt the marine BLM for Cu in its current form (HDR|HydroQual, 2012), SSC calculations based on the BLM would be more relevant than those empirically derived in this study, as the criterion document was developed to be sufficiently protective of *Mytilus spp.*

5. Conclusions

This study examined the temporal and spatial variability of total and dissolved copper concentrations, direct measurements of toxicity, and measures of copper bioavailability (including complexation capacity (CuCC) and use of the marine Biotic Ligand Model (BLM)) for Shelter Island Yacht Basin (SIYB) in San Diego, CA. Despite a substantially greater concentration of Cu within SIYB (DCu 1.4 to 8.9 μg L^{-1}) compared to the rest of San Diego Bay (Blake et al., 2004: 0.4 to 3.3 μg L^{-1}), data from CuCC titrations, bioassays, and the application of the BLM provided multiple lines of evidence suggesting that Cu at the site was not bioavailable to the point of adversely affecting embryo-larval development of *M. galloprovincialis*, the most sensitive saltwater species used for Cu criterion development in saltwater. Moderate toxicity was observed at only one station, near the head of the marina, but response at that location was also measured or predicted using all three lines of evidence. Overall, results suggested that all of these measurements may be valuable in the development of site specific criteria for copper in saltwater environments, with the BLM being the most economical to use while still providing sufficient protection.

Acknowledgements

The authors gratefully acknowledge Naval Sea Systems Command (NAVSEA) for funding this effort. We also thank Drs. Ronald Kaufmann and Bethany O'Shea of the University of San Diego for guidance and technical support, and Brandon Swope for assistance in sample collection and ICP-MS support.

References

Anderson, D.M., Morel, F.M.M., 1978. Cu sensitivity of *Gonyaulax tamarensis*. Limnol. Oceanogr. 23, 283–295.

Belli, S.L., Zirino, A., 1993. Behavior and calibration of the Copper (II) ion-selective electrode in high chloride media and marine waters. Anal. Chem. 65, 2583–2589.

Blake, A.C., Chadwick, D.B., Zirino, A., Rivera-Duarte, I., 2004. Spatial and temporal variations in copper speciation in San Diego Bay. Estuaries 27 (3), 437–447.

California Regional Water Quality Control Board (CRWQCB) San Diego Region, 2005. Total maximum daily load for dissolved copper in Shelter Island Yacht Basin San Diego Bay. Resolution No. R9–2005-0019 Basin Plan Amendment and Technical Report, pp. 1–168.

Chadwick, D.B., Rivera-Duarte I., Rosen G., Wang P.-F., Santore R.C., Ryan A.C., Paquin P.R., Hafner S.D., Choi W.H., 2008. Demonstration of an integrated compliance model for predicting copper fate and effects in DoD harbors. Environmental Security Technology Certification Program, Project ER-0523. Technical Report 1973, SPAWAR Systems Center Pacific, pp. 118, <http://www.spawar.navy.mil/sti/publications/pubs/tr/1973/tr1973cond.pdf>.

Chang, S.I.L., Reinfelder, J.R., 2000. Bioaccumulation, subcellular distribution, and trophic transfer of copper in a coastal marine diatom. Environ. Sci. Technol. 34, 4931–4935.

CH2M Hill. 2000. Site-specific saltwater quality criteria for copper determined by the calculation procedure for the Hampton Roads/Elizabeth River Estuary. Final Report. Project 105020.A0, Contract N6247093D4014. Englewood, CO, USA.

Denton, D.L., Diamond, J., Zheng, L., 2011. Test of significant toxicity: A statistical application for assessing whether an effluent of site water is truly toxic. Environ. Toxicol. Chem. 30 (5), 1117–1126.

DiToro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environ. Toxicol. Chem. 20, 2383–2396.

Earley, P.J., Rosen G., Rivera-Duarte I., Gauthier R., Arias-Thode Y.M., Thompson J., Swope B., 2007. A comprehensive copper compliance strategy: Implementing regulatory guidance at Pearl Harbor Naval Shipyard & Intermediate Maintenance Facility. Technical Report 1952. SPAWAR Systems Center San Diego. pp. 93.

Earley, P.J., Swope, B.L., Barbeau, K., Bundy, R., McDonald, J.A., Rivera-Duarte, I., 2014. Life cycle contributions of copper from vessel painting and maintenance activities. Biofouling: J. Bioadhesion Biofilm Res. 30 (1), 51–68.

Erickson, W.P., McDonald, L.L., 1995. Tests for bioequivalence of control media and test media in studies of toxicity. Environ. Toxicol. Chem. 14 (7), 1247–1256.

Environmental Services Department (ESD). 1998. Development of a site-specific water quality criterion for copper in South San Francisco Bay. San Jose/Santa Clara Water Pollution Control Plant, San Jose, CA, USA.

HDR|HydroQual, 2012. Draft update of aquatic life ambient saltwater quality criteria for copper. pp. 1–43.

Johnston, R.K., 1989. The response of marine fouling communities to a pollution gradient in San Diego Bay, California. Masters thesis, San Diego State University, San Diego, CA, May 20, 1989, pp. 1–187.

Kim, S.D., Ma, H., Allen, H.E., Cha, D.K., 1999. Influence of dissolved organic matter on the toxicity of copper to *Ceriodaphnia dubia*: Effect of complexation kinetics. Environ. Toxicol. Chem. 18, 2433–2437.

Largier, J. L., 1995. San Diego Bay circulation, a study of the circulation of water in San Diego Bay for the purpose of assessing, monitoring and managing the transport and potential accumulation of pollutants and sediment in San Diego Bay. Center for Coastal Studies, Scripps Institution of Oceanography, University of California, San Diego, pp. 1–33.

Morel, F.M.M., 1983. Principles of Aquatic Chemistry. John Wiley and Sons Inc, New York, 456 pp.

Ndung'u, K., Franks, R.P., Bruland, K.W., Flegal, A.R., 2003. Organic complexation and total dissolved trace metal analysis in estuarine waters: comparison of solvent-extraction graphite furnace atomic absorption spectrometric and chelating resin flow injection inductively coupled plasma-mass spectrometric analysis. Anal. Chim. Acta. 481, 127–138.

Neira, C., Delgadillo-Hinojosa, F., Zirino, A., Mendosa, G., Levin, L.A., Porrachia, M., Deheyn, D.D., 2009. Spatial distribution of copper in relation to recreational boating in a California shallow-water basin. Chem. Ecol. 25, 417–433.

Neira, C., Mendoza, G., Levin, L.A., Zirino, A., Delgadillo-Hinojosa, F., Porrachia, M., Deheyn, D.D., 2011. Macrobenthic community response to copper in Shelter Island Yacht Basin, San Diego Bay, California. Mar. Pollut. Bull. 62, 701–717.

NOAA National Weather Service Forecast, 2006. Average Monthly Weather for San Diego Lindbergh Field, CA. <http://www.wrh.noaa.gov/sgx/climate/ san-san-month.htm> (accessed on 02.02.2012).

NOAA National Weather Service Climate Data, 2011. Preliminary Local Climatological Data for San Diego, Lindbergh Field, CA. http://www.nws.noaa.gov/climate/index.php?wfo=sgx (accessed on 02.02.2012).

Rivera-Duarte, I., Rosen, G., Lapota, D., Chadwick, D., Kear-Padilla, L., Zirino, A., 2005. Copper toxicity to larval stages of three marine invertebrates and copper complexation capacity in San Diego Bay, California. Environ. Sci. Technol. 39, 1542–1546.

Rosen, G., Rivera-Duarte, I., Kear-Padilla, L., Chadwick, D.B., 2005. Use of laboratory toxicity tests with bivalve and echinoderm embryos to evaluate the bioavailability of copper in San Diego Bay, California, USA. Environ. Toxicol. Chem. 24, 415–422.

Rosen, G., Rivera-Duarte, I., Chadwick, D.B., Ryan, A., Santore, R.C., Paquin, P.R., 2008. Critical tissue copper residues for marine bivalve (*Mytilus galloprovincialis*) and echinoderm (*Strongylocentrotus purpuratus*) embryonic development: Conceptual, regulatory and environmental implications. Mar. Environ. Res. 66 (3), 327–336.

Rosen, G., Rivera-Duarte I., Johnston R.K., Podegracz J., 2009. Sinclair and Dyes Inlets Toxicity Study: An Assessment of Copper Bioavailability and Toxicity in Surface Waters Adjacent to the Puget Sound Naval Shipyard and Intermediate Maintenance Facility, December 2009. SSC Pacific Technical Report 1985. pp. 43.

Rygg, B., 1985. Effect of sediment copper on benthic fauna. Mar. Ecol. Prog. Ser. 25, 83–89.

Santore, R.C., Di Toro, D.M., Paquin, P.R., Allen, H.E., Meyer, J.S., 2001. Biotic ligand model of the acute toxicity of metals. II. Application to acute copper toxicity in freshwater fish and daphnia. Environ. Toxicol. Chem. 20 (10), 2397–2402.

Schiff, K., Brown, J., Diehl, D., 2006. Extent and magnitude of copper contamination in marinas of the San Diego Region, California, Southern California Coastal Water Research Project. Tech. Rep. 483, 1–19.

Shukla, R., Wang, Q., Fulk, F., Deng, C., Denton, D., 2000. Bioequivalence approach for whole effluent toxicity testing. Environ. Toxicol. Chem. 19 (1), 169–174.

Standard Methods for the Examination of Water and Wastewater, 1996. 5310 Total Organic Carbon. 19th Edition Supplement, American Public Health Association, Washington, DC. pp. 9–14.

United States Environmental Protection Agency (USEPA), 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. EPA/822/R-85/100.

United States Environmental Protection Agency (USEPA), 1994a. Interim guidance on determination and use of water effect ratios for metals. Water Quality Standards Handbook Second Edition. EPA-823-B-94-001.

United States Environmental Protection Agency (USEPA). 1994b. Development of site-specific copper criteria for the NY/NJ Harbor Complex using the indicator species procedure. Surface Water Quality Branch, Region II, New York, NY.

United States Environmental Protection Agency (USEPA), 1995a. Ambient water quality criteria-saltwater copper addendum (Draft), April 14. Office of Water, Office of Science and Technology, Washington, DC.

United States Environmental Protection Agency (USEPA), 1995b. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. EPA-600-R-95-136.

United States Environmental Protection Agency (USEPA), 1995c. Method 1669, Sampling ambient water for determination of trace metals in environmental samples. EPA/600-R-94-111.

United States Environmental Protection Agency (USEPA), 2010. National pollutant discharge elimination system test of significant toxicity implementation document. EPA 833-R-10-003.

Western Regional Climate Center (WRCC), 2012. Cooperative Climatological Data Summaries San Diego WSO Airport, California <http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca7740> (accessed on 03.03.2010).

Zirino, A., VanderWeele, D.A., Belli, S.L., DeMarco, R., Mackey, D.J., 1998. Direct measurement of $Cu(II)_{aq}$ in seawater at pH 8 with the jalpaite ion-selective electrode. Mar. Chem. 61, 173–184.