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Alteration of benthic communities associated with copper contamination linked to boat moorings

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Abstract

Although copper (Cu) is an essential element for life, leaching from boat paint can cause excess environmental loading in enclosed marinas. The effects of copper contamination on benthic macrofaunal communities were examined in three San Diego Bay marinas (America's Cup, Harbor Island West and East) in Southern California, USA. The distribution of Cu concentration in sediments exhibited a clear spatial gradient, with hotspots created by the presence of boats, which in two marinas exceeded the effect range medium (ERM). Elevated sediment Cu was associated with differences in benthic assemblages, reduced species richness and enhanced dominance in America's Cup and Harbor Island West, whereas Harbor Island East did not appear to be affected. At sites without boats there were greater abundances of some amphipods such as the species *Desdimelita* sp., *Harpinia* sp., *Aoroides* sp., *Corophium* sp., *Podocerus* sp., bivalves such as *Lyonsia californica*, *Musculista senhousia*, *Macoma* sp., and polychaetes such as *Diplocirrus* sp. In contrast, at sites with boats, densities of *Pseudopolydora paucibranchiata*, *Polydora nuchalis*, *Euchone limnicola*, *Exogone lourei*, *Tubificoides* spp. were enhanced. The limited impact on Harbor Island East suggests not only lower Cu input rates and increased water flushing and mixing, but also the presence of adequate defense mechanisms that regulate availability and mitigate toxic impacts. At all three marinas, Cu in tissues of several macrobenthic species exhibited Cu bioaccumulation above levels found in the surrounding environment. The annelids *Lumbrineris* sp. and *Tubificoides* spp., and the amphipod *Desdimelita* sp. contained high levels of Cu, suggesting they function as Cu bioaccumulators. The spionid polychaetes *Polydora nuchalis* and *Pseudopolydora paucibranchiata* had much lower Cu concentrations than surrounding sediments, suggesting they function as Cu bioregulators. The macrobenthic invertebrates in San Diego Bay marinas that tolerate Cu pollution (*e.g. P. nuchalis, P. paucibranchiata, Euchone limnicola, Typosyllis* sp., *Tubificoides* sp.) may function as indicators of high-Cu conditions, whereas the presence of Cu-sensitive species (*e.g. Podocerus* sp., *Aoroides* sp., *Harpinia* sp., *Macoma* sp., *Lyonsia californica*) may indicate healthier conditions (less Cu-stressed). Parallel responses by faunas of Shelter Island Yacht Basin, also in San Diego Bay, suggest potential for development of regional Cu contamination assessment criteria, and call for functional comparisons with other marinas and coastal water bodies.

Introduction

Benthic infaunal communities are key components of estuarine and coastal ecosystems. Because of their importance to overall ecosystem structure and function, they have been considered effective indicators of habitat condition. Many sediment-dwelling species are sedentary and trophically diverse, and their communities can integrate the effects of water and sediment changes over time (Lenihan & Micheli 2001). Benthic fauna not only play an important role within the food web, as direct and indirect food sources for higher trophic levels (Feder & Jewett 1981), but they have also been linked to ecosystem functions such as resource availability, elemental cycling, nutrient exchange and production of biomass (Aller & Yingst 1985; Kuwabara *et al.* 2012). Hence, changes in community composition, abundances and diversity of benthic infauna can affect the functioning of the entire ecosystem (Levin *et al.* 2001; Danovaro *et al.* 2008).

Copper (Cu) input into aquatic ecosystems has increased sharply during the last century because the demand for Cu for multiple uses (*e.g.* for manufacture of electrical components, pipe and machinery, automobile brakes and pesticides) (Nriagu 1979; Rauch & Pacyna 2009). This has become a global environmental problem through disposal and industrial discharges into fresh and coastal waters, with subsequent impact on faunal communities (Lee & Correa 2007).

In coastal waters, particularly in industrialized harbors, bays and estuaries, Cu levels have increased due to its extensive use in antifouling paints (Salomons & Förstner 1984; Terlizzi *et al.* 2001; Carson *et al.* 2009). Since the ban of tributyltin (TBT), first by France in 1982 (Alzieu *et al.* 1986) and then worldwide by signatory countries of the International Maritime Organization (IMO 2001), including the USA in 1988, Cu use in antifouling paints has increased sharply (Dafforn *et al.* 2011). In the US harbors, there has been a steady decline in TBT concentrations in water and sediment (*e.g.* Seligman *et al.* 1990; Huggett *et al.* 1992; Sayer *et al.* 2001), in line with the 1988 ban of TBT.

Trace concentrations of Cu are essential for proper function of many biological systems (Weser *et al.* 1979) but Cu can be toxic at high levels (Buck *et al.* 2007). Anthropogenic release of Cu into the environment can adversely impact aquatic ecosystems because the effects of antifouling paints are not restricted to the boat hull surface but extend also to the surrounding water and sediments. Cu leached from paint ultimately deposits and accumulates in the sediment through binding and adsorption processes (Zirino *et al.* 2013). Hence, species living in soft-bottom communities are particularly exposed to Cu contamination. If the uptake of the contaminant exceeds the animal's ability for excretion and detoxification normal metabolic functioning can be affected (Chen *et al.* 2002).

Negative effects of Cu on sediment-dwelling fauna include disruption of reproductive processes, reduced feeding rates and survivorship (Reish 1993), reduced respiration, protein utilization, digestive enzyme inhibition (Chen *et al.* 2002), morphological abnormalities (Luoma & Carter 1991), impaired reaction to predation (Pyle & Mirza 2007), impaired habitat selection (Carreau & Pyle 2005) and inhibition of larval settlement (Reichelt-Brushett & Harrison 2000). Some organisms have evolved mechanisms for detoxifying and storing excess Cu within their bodies when exposed to elevated Cu levels (Betzer & Yevich 1975; Correia *et al.* 2002). Species vary in their Cu uptake responses at different background sediment Cu concentrations. Animal physiology, lifestyles, feeding habits, life stage, mobility, and routes of exposure may influence response to Cu (Calabrese *et al.* 1973; Beaumont *et al.* 1987; Coimbra & Carraca 1990; Mardsen & Rainbow 2004).

In the marine environment Cu occurs in many forms. Cu toxicity to aquatic organisms has been related primarily to the free cupric (Cu^{++}) ion (Campbell 1995), which typically constitutes <0.01% of the total dissolved Cu in seawater (Blake *et al.* 2004), as most of the dissolved Cu is complexed to organic ligands (Lucia *et al.* 1994; Buck *et al.* 2007). The amount of free Cu^{++} available in the water is dependent not only upon the total amount of Cu in the water but also upon the Cu complexation capacity (CuCC), *i.e.* the buffering capacity of the system. The complexation capacity is determined primarily (99%) by dissolved organic matter (ligands) that decreases the free ion form of the metal (Delgadillo-Hinojosa *et al.* 2008a). Excess CuCC provides a natural 'defense' mechanism against Cu toxicity (Zirino *et al.* 2013).

The San Diego region berths ~17,000 recreational boats, of which ~32% are moored in four marinas within San Diego Bay: Shelter Island Yacht Basin, America's Cup, Harbor Island West and Harbor Island East. The implementation of a total mean daily load (TMDL) program to gradually reduce Cu levels in Shelter Island Yacht Basin [California Regional Water Quality Control Board (CARWQCB) 2005] and the eventual phase out of Cu-based hull paints on recreational boats in San Diego Bay (Carson *et al.* 2009) have prompted the need to know the potential effects of Cu on sediment-dwelling faunal communities in highly contaminated marinas.

Analysis of Cu distribution in Shelter Island Yacht Basin (SIYB) revealed gradients and hotspots of Cu concentration in water and sediments that were associated with the number of boats and distance from them (Neira *et al.* 2009). At sites with elevated Cu levels in sediment, macrobenthic communities were less diverse (Neira *et al.* 2011). These observations have raised concerns about whether the impact of Cu on benthic communities living underneath and immediately adjacent to recreational boat docks of other marinas nearby SIYB [*e.g.* America's Cup (AC), Harbor Island West (HW) and Harbor Island East (HE)] is of the same extent and magnitude. These marinas have also been reported to have elevated levels of Cu, both in the water and in the sediment (Hayes & Phillips 1986, 1987; Stevens 1988) but until now they had not been studied at a fine scale spatial resolution.

In general, the effects of pollution on marine animals has usually been assessed and predicted from single-species studies under controlled conditions (Stark 1998; Clark *et al.* 2001). Although this approach is valid, it does not lead to predictions about the impacts of toxicants at the community level (Luoma 1996). Multispecies tests have been further applied to take into account the significance of biological interactions (Clements 2004; Luoma *et al.* 2010). Infauna are permanently in contact with sediments, which accumulate contaminants over long periods (Nixon *et al.* 1986). Thus macrofaunal community responses offer valuable insight into Cu pollution (Pearson & Rosenberg 1978; Gesteira & Dauvin 2000).

Here we examine relationships between boat distribution and sediment Cu concentration, and their effects on macrobenthic community structure and composition. Boat distribution at America's Cup, Harbor Island West and Harbor Island East provides a 'natural' experiment to address the following questions: (i) Are there 'hotspots' of high Cu concentrations in water and sediments within America's Cup and Harbor Island West basins? (ii) Are changes in macrofaunal (>300 μm) community structure associated with elevated sediment Cu concentration, or with specific Cu chemical species? (iii) Does Cu concentration in macrofaunal tissue reflect background Cu levels in sediment?

Material and Methods

Study sites

San Diego Bay (32°40′ N, 117°4′ W) in Southern California, USA, has elevated Cu levels both in the water column and sediments, associated with recreational boats (*e.g.* Zirino *et al.* 1998a; Schiff *et al.* 2004, 2007). In San Diego Bay more than 90% of boats have Cu-based antifouling paint (Nichols 1988; Johnson & Miller 2002) and nearly 48% of the Cu input reaches the sediment (Chadwick *et al.* 2004). San Diego Bay is a semi-enclosed embayment located in Southern California. The north section of the bay is characterized by high rates of flushing and strong tidal mixing, while the South Bay is shallow, with relatively slow currents and reduced mixing (Chadwick & Largier 2000). At the north section of the bay there are four man-made marinas: Shelter Island Yacht Basin, America's Cup, Harbor Island West and Harbor Island East, which together berth about 90% of the boats moored in San Diego Bay (Nichols 1988).

America's Cup (AC) is approximately rectangular in shape, with length of about 0.88 km North to South and 0.401 km East to West, and an area of ~0.35 km^2, average depth of 4.0 m. Approximately 820 boats are distributed in two groups, with docks arranged along the basin border facing the center, and in six rows at the center. The basin mouth leads to the main channel facing East (Fig. 1). Harbor Island consist of two embayments: Harbor Island West (HW) and Harbor Island East (HE). HW, which berths ~1700 boats, is about 1.46 km long and ~0.28 km wide (surface area of ~0.41 km^2 and depth of 4.2 m); its mouth connects with the main channel to the West. HE, with ~560 boats, is 0.73 km long and 0.167 wide (surface area of ~0.12 km^2 and depth of 4.1 m); its mouth faces to the East. In both Harbor Island marinas, all boats are moored on the south side, opposite to the adjacent water channel (Fig. 1). These marinas have relatively well-flushed mouths but restricted water mixing at the heads, as reported for SIYB (Zirino *et al.* 1998a). From the mouth of San Diego Bay, the hydrographic residence times estimated along the main channel are about 5–6 days for SIYB, 7 days for America's Cup and about 8–11 days for Harbor Island (Chadwick *et al.* 2004; F. Maicu, personal communication).

Sample collection and processing

Cu measurement

To examine the current concentrations of Cu in water (surface and bottom) and sediments (porewater and solids), we conducted a field sampling on 9–10 June 2009. Sampling in the spring–summer period was selected because previous studies indicated that during this phase, contaminant inputs may be accumulated rather than flushed from the system (Blake *et al.* 2004) and the residence time of water inside the system is longer (Largier *et al.* 1997; Delgadillo-Hinojosa *et al.* 2008b). In spring–summer, free Cu^{++} concentrations are highest in the marina water column and sediment porewater, and the contribution of particulate Cu to the total Cu pool is highest (Neira *et al.* 2009). Also, the warm season is when differences in benthic response between reference and degraded sites are greatest (Alden *et al.* 1997). The sampling design, with a total of 35 sites (15 in AC, 12 in HW and eight in HE), ensured that whole basins were sampled, allowing us to contrast multiple areas where (i) boats are docked densely concentrated (hereafter 'with

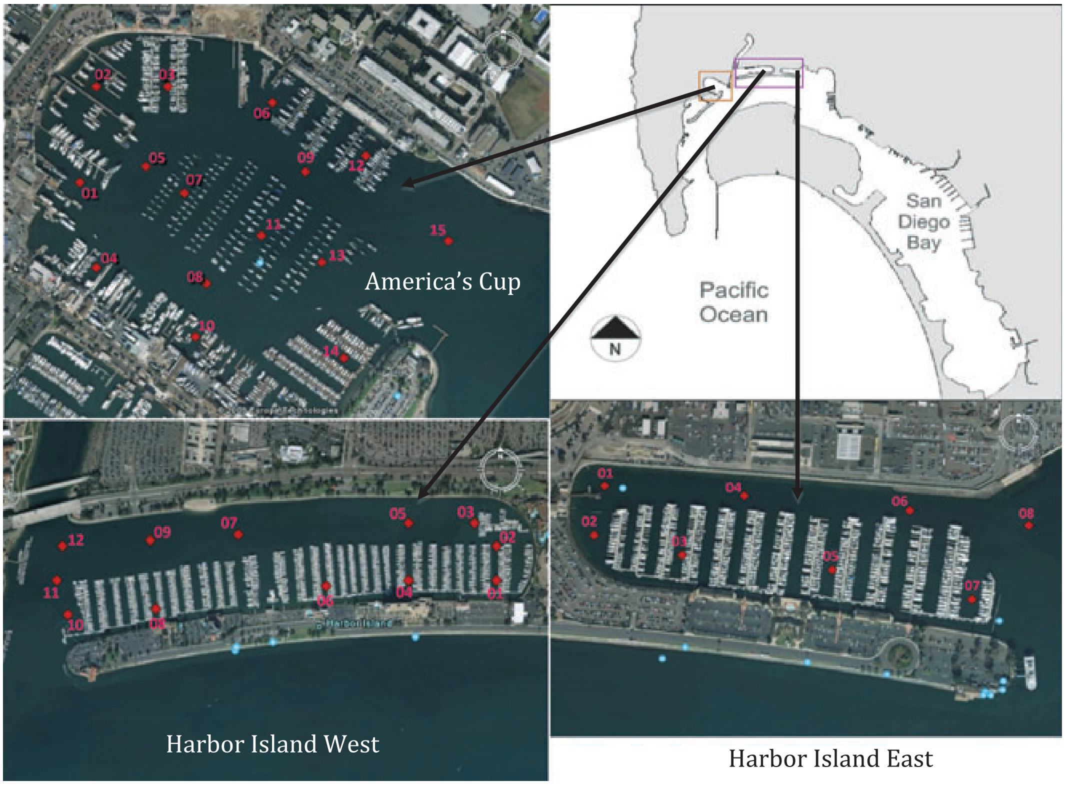

Fig. 1. Study sites at three San Diego Bay marinas: America's Cup (AC), Harbor Island West (HW) and Harbor Island East (HE). Coordinates for each site are listed in Appendix Table S1. To improve readability, figures are not to scale.

boats') and (ii) adjacent or opposite areas without boats that are open to the main channel (hereafter 'without boats') (Fig. 1). Samples were collected from a small vessel during low tide.

At each site, surface sediment (0–5 cm) was collected using a small custom-made gravity core containing a Plexiglas tube (surface area = 20. 4 cm^2). On board, the overlying water was gently removed and the sediment redox potential was measured in the top 1 cm using a portable Eh-meter (Mettler Toledo). In addition, a small syringe core (1.13 cm^2 × 1 cm depth) was taken for sediment Chl *a* and pheopigment concentrations in preweighed 15-ml polypropylene tubes and stored at −20 °C until analysis. Then the top 5-cm fraction of sediment was used for further analysis of sediment Cu using an ICP-OES Perkin Elmer Optima 3000 DV analyzer. Blanks and certified standard reference material PACS-2 (National Research Council of Canada) were used for accuracy and quality control (~88 ± 0.6% recovery; n = 6).

Dissolved Cu was measured on 200-ml filtered (polycarbonate membrane 0.45 μm) surface (50 cm below the surface) and bottom water (30 cm above the bottom) seawater and acidified (pH < 2 with HNO3 Optima, Fisher Scientific) using a Varian 880Z graphite furnace atomic absorption spectrometer (GFAAS). Accuracy and precision were assessed by analysis of Canadian Research Council coastal seawater certified standards (CASS-4) (91 ± 3.9% recovery; n = 6).

For determination of suspended particulate matter, pre-weighed polycarbonate filter membranes (0.45 μm) were air-dried to constant weight under laminar flow conditions and re-weighed. For determination of particulate Cu, filters with suspended particulate matter were transferred into acid-cleaned polypropylene vials, and digested in two steps with concentrated HNO_3 (Optima) and a mixture of HNO_3/HCl as described by Neira *et al.* (2009). Samples were analyzed in triplicate using an ICP-OES (Perkin Elmer Optima 3000DV).

The Orion 94-29 Cu-Ion Selective Electrode (Cu-ISE) was used to determine the concentration of Cu^{++} in unfiltered seawater samples collected from the surface, bottom and porewater. The ISE electrode measures hydrated divalent Cu ions in seawater in terms of pCu, where pCu = $-\log_{10}$ (Cu^{++}) when calibrated with ethylenediamine Cu buffer at three different pH values prepared in 0.45 μm filtered seawater (Belli & Zirino 1993;

DeMarco *et al.* 1997; Zirino *et al.* 1998b). The electrode is precise at 0.06 pCu units, and covers a range of pCu between 9.4 to 13 or more, as the response of the electrode to these buffers is linear (Belli & Zirino 1993). Cu titrations on unfiltered surface and bottom seawater collected at sites representing the head, the center, and the mouth portion of each basin were conducted for determination of Cu complexation capacity (CuCC) concentration. Further details of the cell used for measurements and procedure are described by Delgadillo-Hinojosa *et al.* (2008b).

Sediment properties

The total organic matter (TOM) content of sediment samples was determined on ~30 g freeze-dried, homogenized sediment by mass loss after firing at 500 °C for 4 h in a muffle furnace (Byers *et al.* 1978). Percent sand (>63 μm) and silt-clay (<63 μm) were determined as described by Neira *et al.* (2009). Sediment chlorophyll *a* (chl *a*) and pheopigments (0–1 cm) were determined spectrophotometrically (Thermo Spectronics, Genesis 20) from freeze-dried sediment (Hagerthey *et al.* 2006) after extraction with 90% acetone (Dalsgaard *et al.* 2000). The sum of Chl *a* and pheopigments is given in chloroplastic pigment equivalents (CPE) (Pfannkuche & Soltwedel 1998). Water content and porosity were determined by weight loss after freeze-drying the top 1-cm section of sediment used for pigment determination, assuming a sediment and water density of 2.65 g·cm^{-3} (quarz) and 1.025 g·cm^{-3}, respectively (Buchanan 1984).

Macrofauna

For analysis of macrofauna, three replicate cores (5.1 cm i.d; 20.4 cm^2) were collected at the same sites as for Cu analysis, using a mini-gravity corer at 15 stations in AC, 12 stations in HW, and 8 stations in HE (Fig. 2). Immediately after collection, the uppermost 5 cm were extruded and transported in a cooler to the laboratory. Samples were preserved in 8% buffered formaldehyde solution with rose Bengal stain. In the laboratory, sediment samples were sieved on a 0.3-mm-mesh sieve, and retained invertebrates were sorted in fresh water under a dissecting microscope. The use of a 0.3-mm-mesh sieve has been increasingly adopted as better representing the entire community (*e.g.* Talley & Levin 1999; Levin *et al.* 2002; Neira *et al.* 2009, 2011; Gooday *et al.* 2009; Ingole *et al.* 2010). This mesh size retains most juveniles and thin forms among macrofaunal taxa, providing an accurate estimate of macrofaunal abundance and biodiversity. Mostly, early life history stages of macrofauna are smaller than 1 mm and are often those that are the most vulnerable to physical disturbances or chemical contamination. Specimens were counted and identified to the lowest taxon possible, with putative species designated for diversity calculations. Cu in invertebrate tissue was determined on samples collected at selected sites in the three marinas. Details of faunal extraction and procedure for Cu in tissue analysis are described in Neira *et al.* (2011).

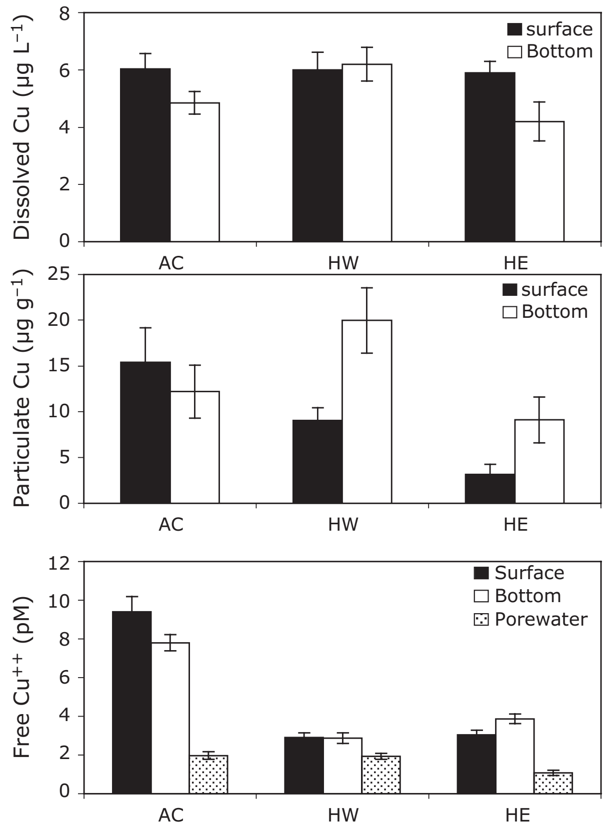

Fig. 2. Mean (±1 SE) copper concentrations. From top to bottom: dissolved Cu, particulate Cu, and free Cu^{++} measured in surface and bottom water. Free Cu^{++} was also measured in porewater. Note different units. AC = America's Cup; HW = Harbor Island West; HE = Harbor Island East.

Data analysis

A detailed description of the data analyses is found in Neira *et al.* (2011). Briefly, probability data were produced using kriging calculations performed in MATLAB 2008 (Trauth 2007) and then mapped using ARCGIS (ESRI, 2006). Data were tested for normality and when necessary, were square root-transformed. One standard error (SE) about the mean is presented with mean data unless otherwise indicated. Unless otherwise indicated, n-values are 15, 12, and 8 for America's Cup, Harbor Island West and Harbor Island East, respectively. Univariate tests, ANOVA and *Post-Hoc* tests were performed to detect the differences between the sites. Univariate analyses were performed using the software package JMP 6.0.3. Standard diversity metrics such as Pielou's evenness (J′), the Shannon–Wiener diversity index ($H' \log_{10}$) and Rank 1 dominance (the proportion of the most abundant taxa) were calculated to describe macrofaunal assemblage structure.

Community composition was analyzed based on a Bray –Curtis similarity matrix and visualized using non-metric multidimensional scaling analysis (nMDS). Differences in composition between sites and treatments were tested with analysis of similarity (ANOSIM). Composition proportion and dominance differences were tested with similarity percentage (SIMPER) (Clarke 1993). All community and diversity analyses were performed using PRIMER 6.

Ordination and gradient analysis routines such as redundancy analysis (RDA) and partial redundancy analysis (pRDA) were used to explore multivariate relationships between macrofaunal community structure and environmental variables (ter Braak & Šmilauer 1998, 2002) and to identify prospective indicator species of Cu contamination (Kremen 1992). A Two Way Indicator Species Analysis (TWINSPAN) was performed to complement the results from the pRDA triplot.

A forward stepwise selection procedure was applied to select a set of explanatory environmental variables, all measured independently, that account for the composition variation in the faunal data. A Monte Carlo permutation test was used to determine significant relationships between the species and environment, as well as the environmental variables that best explain changes in community. Ordination and gradient analysis were performed using CANOCO for Windows 4.5.

To determine how the explanatory variables influence the distribution of macrofaunal communities, we modeled regression trees of the species dataset using the environmental data as predictor variables. Regression trees function by recursively partitioning the dataset into binary subsets (also called nodes) in which the values of the response variable are successively more and more homogeneous (De'ath & Fabricius 2000; Sutton 2005). Regression trees were modeled using STATISTICA v.8 (StatSoft).

Results

Marina environmental properties

Within marinas, water temperature ranged from 19 °C to 21.1 °C at the surface and from 18.6 °C to 20.4 °C at the bottom. Salinities ranged from 33.1 to 34.1 at the surface and 34.1 to 35.2 at the bottom. In AC and HW, water temperatures were slightly higher at the head of the basin and surface water relative to the mouth of the basin and bottom water, whereas in HE, temperatures at the surface and bottom water were slightly lower at the head relative to the mouth of the basin. Salinity varied slightly between both surface and bottom water (from 33.7 to 34.3), and between the head and mouth of the basin (from 34.0 to 33.5 and from 34.6 to 34.1, for surface and bottom water, respectively). Overall, the Cu complexation capacity (CuCC; n = 3) averaged 259 ± 14 nM, 264 ± 53 nM and 325 ± 19 nM in surface water, and 275 ± 33 nM, 276 ± 58 nM and 321 ± 30 nM in bottom water, for AC, HW and HE, respectively. A summary of the main sediment environmental properties (other than Cu) measured in AC, HW and HE is presented in Appendix Table S1. In AC sediment organic matter ranged from 2.2% to 7.2%, whereas in HW organic matter ranged from 0.9% to 9.9%. In HE, organic matter content ranged between 2.3% and 5.3%. At all marinas, sediment organic matter was positively correlated with mud content (<63 μm) (R^2 = 0.56; n = 15, 0.73; n = 12, 0.94; n = 8) for AC, HW and HE, respectively; all $P < 0.05$).

Concentration and spatial distribution of Cu species

Dissolved Cu (DCu) concentrations in surface and bottom water of all three marinas exceeded the EPA water quality criteria of 3.1 $\mu g \cdot l^{-1}$. Surface water DCu concentration averaged 6 ± 0.3 $\mu g \cdot L^{-1}$ for all three marinas. Bottom water concentrations were slightly lower at AC (4.8 ± 0.4 $\mu g \cdot L^{-1}$) and HE (4.20 ± 0.7 $\mu g \cdot L^{-1}$), while at HW, the concentrations were similar (6.2 ± 0.6 $\mu g \cdot L^{-1}$) (Fig. 2). Peak concentrations were 10.7 $\mu g \cdot L^{-1}$ in AC and 11.2 $\mu g \cdot L^{-1}$ in HW. Particulate Cu concentration varied among marinas and in surface *versus* bottom water. In surface water and bottom water of AC, mean Cu concentrations were 15.4 ± 3.7 and 12 ± 2.9 $\mu g \cdot g^{-1}$ respectively, whereas at HE, the concentrations were the lowest (3.1 ± 1.1 and 9.1 ± 2.5 $\mu g \cdot g^{-1}$, respectively). Levels of particulate Cu at HW were intermediate (9.1 ± 1.4 $\mu g \cdot g^{-1}$) in surface water and greatest in bottom water (19.9 ± 3.5 $\mu g \cdot g^{-1}$) (Fig. 2). The contributions of particulate Cu to the total Cu pool in surface and bottom water were 15.3 ± 2.6 and 15.6 ± 3.2%, 11.4 ± 1.3 and 20.8 ± 3.0%, and 1.9 ± 0.6 and 6.9 ± 1.2% for AC, HW and HE, respectively. Measurements of Cu activity [pCu, where pCu = $-\log_{10}$ (Cu^{++})] at three vertical horizons (surface, bottom and sediment porewater) revealed that free Cu^{++} in the water column was greater in AC than in the two Harbor Island marinas, and much higher in surface water than in sediment porewater, especially in AC and HE ($P < 0.001$) (Fig. 2).

On average, sediment Cu concentrations for all the marinas exceeded the effects range low (ERL) of 34 $mg \cdot kg^{-1}$, *i.e.* where adverse effects to fauna may occur

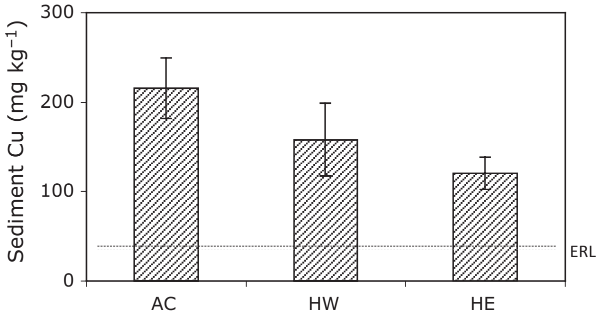

Fig. 3. Mean (±1 SE) sediment copper concentrations at America's Cup (AC), Harbor Island West (HW) and Harbor Island East (HE). Dashed line indicates the threshold effect range low (ERL) of 34 mg kg^{-1}, *i.e.* the range where adverse effects on fauna may occur (NOAA 1999).

(NOAA 1999) and was greatest in AC (215.6 ± 34 mg·kg^{-1}), followed by HW (157.9 ± 41 mg·kg^{-1}) and HE (120.5 ± 18 mg·kg^{-1}) (Fig. 3).

Probability maps of Cu concentration distribution were generated based on the actual Cu levels in the water column and sediment, over a range of exposure to boat hulls and distance from the mouth of each marina. The spatial distributions of dissolved Cu in surface and bottom water, free Cu^{++} in surface water and Cu in sediment (solid phase) are shown in Fig. 4A–C for AC, HW and HE, respectively. Cu distribution in sediments exhibits a clear gradient, with the highest concentrations (hotspots) found beneath the moored boats. Peak concentrations of over 460 and 400 mg·kg^{-1} were registered at AC and HW, respectively. These greatly exceed the effects range medium (ERM) of 270 mg·kg^{-1}, *i.e.* at which concentrations are frequently toxic (NOAA 1999). At HE, levels of Cu in sediments were relatively low and did not exceed 176 mg·kg^{-1}.

Macrofaunal density, composition and diversity

Comparison between marinas

Mean total macrofaunal densities (±1 SE), averaged for all sampled sites within each marina, were 39 ± 12 ind. 20.4 cm^{-2} core (19,117 ind. m^{-2}) at AC (AC), 42 ± 19 (20,588 ind. m^{-2}) at HW, and 65 ± 28 (31,765 ind. m^{-2}) at HE. Densities among marinas were not significantly different ($F_{2,34}$ = 2.4; P = 0.102). Faunal assemblage composition did not differ among marinas (ANOSIM P = 0.199 AC *versus* HW, P = 0.445 AC *versus* HE, P = 0.133 HW *versus* HE). In general, a total of 54 taxa were identified. Contribution of oligochaetes to the total abundance was much higher at AC (37 ± 5.5%) and HW (25.8 ± 3.7%) than at HE (11.6 ± 1.8%) ($F_{2,34}$ = 6.9; P = 0.003). At HE the percent contribution of amphipods (32.6 ± 5.6%) and other crustaceans (11.9 ± 1.8%, isopods tanaids, ostracods and cumaceans) was greater than in AC (4.7 ± 1.5%) and HW (6 ± 1.3%) ($F_{2,34}$ = 5.5; P = 0.008) (Appendix Table S2). Species richness, diversity indices (evenness J′, Shannon H′ and dominance) did not differ significantly among marinas (all P > 0.05) (Table 1).

The influence of boats

Total macrofaunal densities at sites with boats did not differ from sites without boats in each marina (Table 1). Sites with boats had mean total densities of 7–69, 8–37 and 47–69 ind. 20.4 cm^{-2} core, whereas at sites without boats, densities were 34–63, 35–149 and 19–131 in 20.4 cm^{-2} core, for AC, HW and HE, respectively. Mean macrofaunal densities for each taxon and its percent contribution to the total are presented in Appendix Tables S3–S5, for AC, HW and HE, respectively. Faunal assemblages at all sites with boats in all three marinas exhibited consistent composition differences (ANOSIM, P = 0.001) relative to sites without boats (Fig. 5). At sites without boats there were greater abundances of some amphipods such as the species *Desdimelita* sp., *Harpinia* sp., *Aoroides* sp., *Corophium* sp., *Podocerus* sp., bivalves such as *Lyonsia californica*, *Musculista senhousia*, *Macoma* sp., and polychaetes such as *Diplocirrus* sp. In contrast, at sites with boats, densities of *Pseudopolydora paucibranchiata*, *Polydora nuchalis*, *Euchone limnicola*, *Exogone lourei* and *Tubificoides* spp. were enhanced.

Average taxon richness, diversity H′ as well as rarefaction diversity (Fig. 6) were lower at sites with boats at AC and HW and higher at sites without boats. Evenness (J′) was also significantly lower at sites with boats at AC. Rank 1 dominance (R1D) (an inverse indicator of assemblage evenness) was highest at sites with boats at AC (Table 1). In contrast to AC and HW marinas, no significant differences in abundances and community diversity descriptors were observed between boat and no-boat sites within HE (Table 1).

Macrofauna in relation to Cu and environment

Initial examination of relationships between environmental variables and the univariate measures derived from macrofaunal data was conducted with a Spearman rank correlation analysis (Table 2). Some diversity descriptors were negatively correlated with sediment Cu and TOM, whereas dominance was positively correlated with sediment Cu concentration. Macrofaunal species richness (S) was lower at sites with high sediment Cu, where boats were present (Fig. 7). This applies primarily to AC and HW; no significant differences were found at HE.

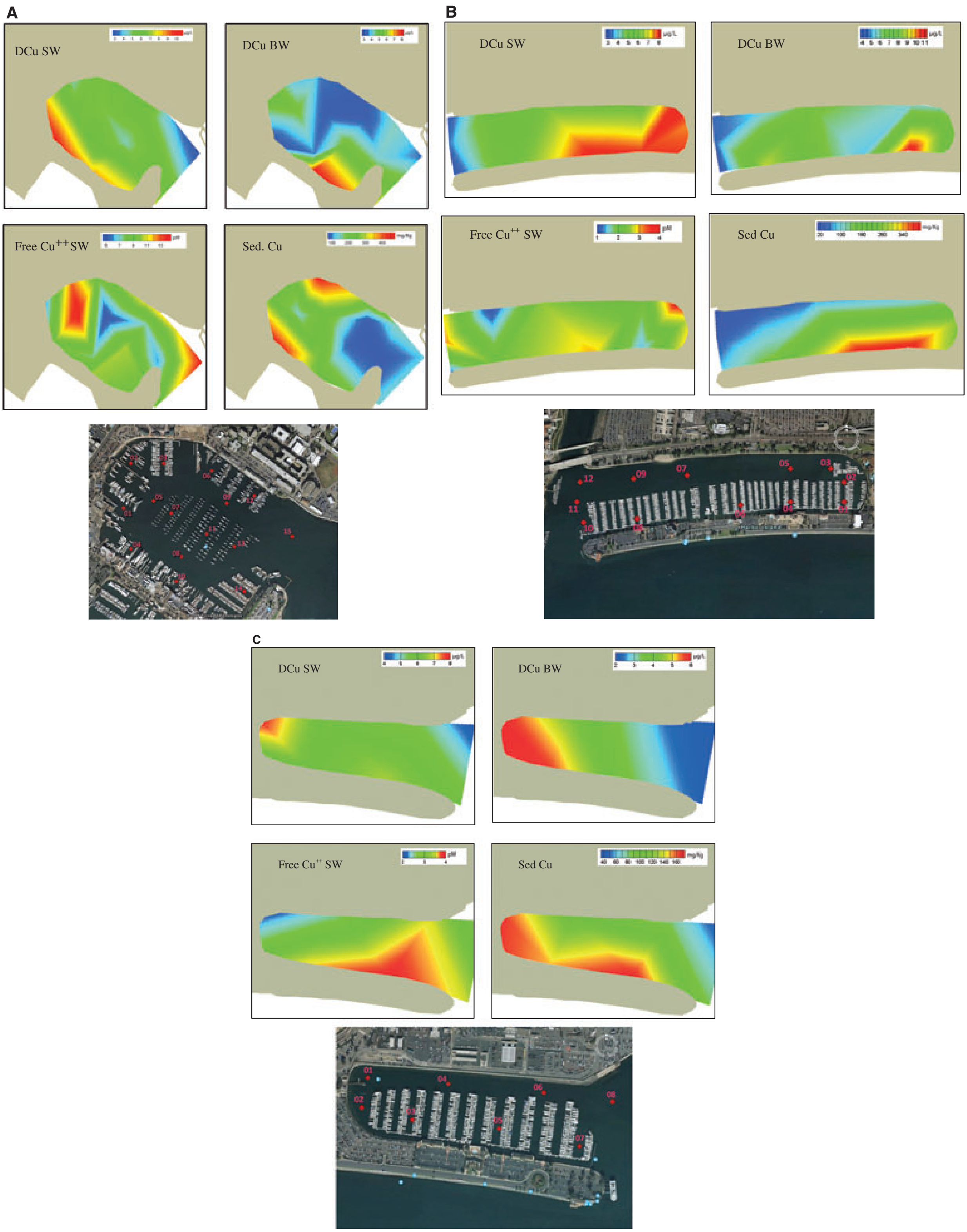

Fig. 4. Probability maps showing the spatial distribution of copper concentrations in San Diego Bay marinas. (A) America's Cup, (B) Harbor Island West, (C) Harbor Island East. DCu SW = dissolved Cu in surface water; DCu BW = dissolved Cu in bottom water; free Cu^{++} SW = free Cu^{++} in surface water; Sed Cu = sediment Cu. Colors are assigned different values in each panel. Cu species are presented in different units.

Table 1. Mean species richness (S), density (N; ind 20.4 cm^2 core), eveness (J′), Shannon–Wiener diversity index (H′ log_{10}), and Rank 1 Dominance (R1D) for all sites, and sites with boats and without boats, at America's Cup and Harbor Island West and East in San Diego Bay, California. P values reflect results of one-way ANOVA testing for differences between sites with and without boats.

Marinas	Sites	S	N	J′	H′(log_{10})	R1D%
America's Cup	all sites	18	39	0.76	0.94	38.00
	with boats	14	35	0.697	0.790	48.49
	without boats	25	45	0.863	1.170	22.23
		$F_{1,14} = 10.9$	$F_{1,14} = 1.4$	$F_{1.14} = 7.5$	$F_{1,14} = 16.3$	$F_{1,14} = 9.7$
		$P = 0.014$	$P = 0.259$	$P = 0.017$	$P = 0.001$	$P = 0.008$
Harbor Island West	all sites	18	42	0.820	1.000	29.70
	with boats	13	27	0.821	0.888	33.04
	without boats	25	62	0.821	1.146	25.02
		$F_{1,11} = 31.0$	$F_{1,11} = 4.1$	$F_{1,11} < 0.01$	$F_{1,11} = 7.1$	$F_{1,11} = 1.6$
		$P < 0.001$	$P = 0.069$	$P = 0.990$	$P = 0.023$	$P = 0.237$
Harbor Island East	all sites	19	65	0.790	1.010	27.40
	with boats	18	57	0.804	1.002	27.33
	without boats	21	73	0.777	1.022	27.41
		$F_{1,7} = 0.8$	$F_{1,7} = 0.4$	$F_{1,7} = 0.1$	$F_{1,7} < 0.1$	$F_{1,7} < 0.1$
		$P = 0.387$	$P = 0.540$	$P = 0.797$	$P = 0.914$	$P = 0.993$

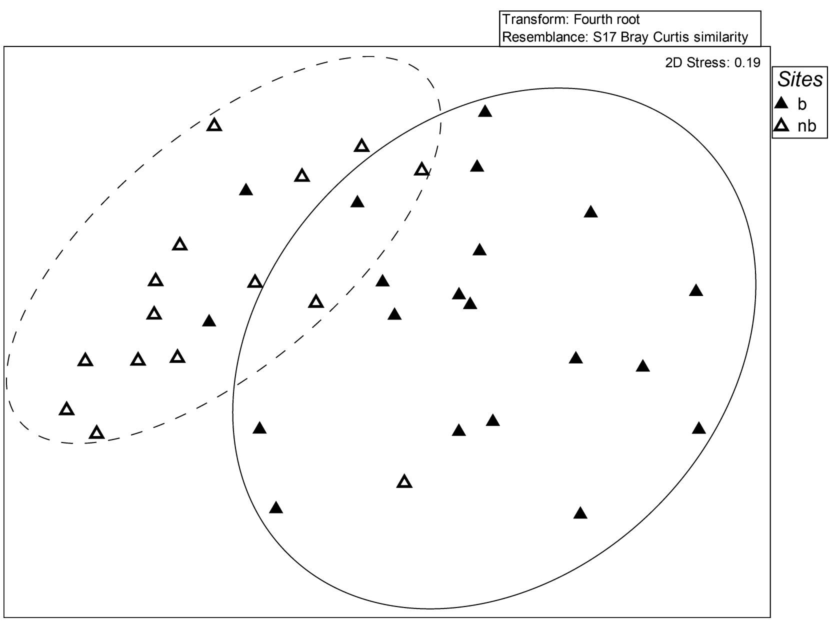

Fig. 5. MDS plot of macrofaunal composition from sites with boats (b; black triangles, solid line) and sites without boats (nb; open triangles, dashed line) at America's Cup (AC), Harbor Island West (HW) and Harbor Island East (HE). SIMPER dissimilarity between sites with and without boats was 56.5%. ANOSIM P = 0.001.

A further forward stepwise selection procedure identified a set of environmental variables associated with the benthic environment that best explained the variation in macrofaunal community composition (Table 3). These results point to sediment Cu as the main variable driving changes in macrofaunal composition. Furthermore, all these variables were used in the ordination models. Thus, the RDA yielded four axes that explained 94.2% of the variance in macrofaunal community structure with environmental variables, and the species–environmental relationships were highly significant ($P = 0.001$) for all canonical axes (Appendix Table S6). In the ordination triplot (Fig. 8), there were a few species (clustered together on the right side of the plot) that related primarily to sediment Cu and total organic matter and secondarily to other Cu species (*e.g.* free Cu^{++} in porewater and dissolved Cu in bottom water). Most macrofaunal species (at the left side of the plot) were associated with environmental vectors other than Cu species (Fig. 8).

The analysis of natural variables as covariables using partial RDA (pRDA) (Appendix Table S7) allowed us to decompose (variance partitioning) the total variability into a part that can be explained partially by the influence of each of the Cu chemical species on macrofaunal

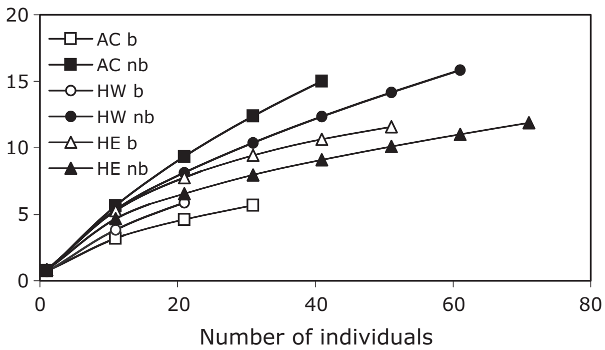

Fig. 6. Rarefaction curves showing macrofaunal species richness from sites with and without boats at America's Cup (AC), Harbor Island West (HW) and Harbor Island East (HE). b = with boats; nb = no boats.

community. Thus, we calculated that 38.6% of the variance of community composition was explained by Cu species (sediment Cu, DCu in bottom water, free Cu^{++} in porewater and free Cu^{++} in bottom water). Of this proportion, sediment Cu alone explained 25.6%. An additional 12.6% of variation was explained by the natural covariables (TOM, chl *a*, pheopigments, CPE, mud, sand, redox potential) and 48.8% of the variation remained unexplained.

Regression trees were used to provide an alternative description of the dependence of the community descriptors on the environmental variables of interest. We built a regression tree to depict the response of the community species richness in relation to Cu explanatory variables. Sediment Cu concentration was the key explanatory variable associated with species richness variation, and dissolved Cu in bottom water and free Cu^{++} in porewater were also explanatory in partitioning species richness variation. The first partition grouped 37.1%, containing the highest number of species, with the lowest sediment Cu concentration range (11–115 mg kg^{-1}) and 62.9% of the sites with greatest sediment Cu range (115–480 mg kg^{-1}), containing lower species richness. Further partitioning was dissolved Cu in bottom water and free Cu^{++} in porewater, respectively (Fig. 9).

Table 2. Spearman's rank correlation coefficients between environmental variables and macrofauna data in San Diego Bay marinas. Significant correlation and level of significance are indicated in bold and by asterisk, respectively.

	macrofauna				
variable	no species	density	H′(log_{10})	J′	R1D
America's Cup					
Cu^{++} PW	0.206	0.128	−0.0357	−0.021	0.078
Cu^{++} BW	0.233	−0.053	0.135	0.189	−0.2
DCu BW	0.229	0.51	−0.0214	−0.082	0.061
Cu Sed	−0.764***	−0.575*	−0.782***	−0.578*	0.671**
TOM	−0.741**	−0.548*	−0.573*	−0.211	0.391
Chl a	0.305	−0.087	−0.370	−0.334	0.37
Phaeo	−0.656**	−0.439	−0.600*	−0.382	0.45
CPE	−0.641*	−0.409	−0.602*	−0.396	0.455
Mud	−0.715**	−0.542*	−0.617*	−0.228	0.443
Sand	0.717**	0.542*	0.617*	0.228	−0.442
SRP	0.317	0.153	0.275	0.103	−0.189
Harbor Island West					
Cu^{++} PW	0.049	0.126	0.314	0.056	−0.307
Cu^{++} BW	−0.195	0.119	−0.427	−0.308	0.185
DCu BW	−0.202	−0.311	−0.189	−0.119	0.056
Cu Sed	−0.875***	−0.685*	−0.776**	−0.301	0.454
TOM	−0.836***	−0.629*	−0.790**	−0.363	0.447
Chl a	−0.590*	−0.573	−0.426	0.083	−0.069
Phaeo	−0.763**	−0.419	−0.685*	−0.363	0.342
CPE	−0.769**	−0.447	−0.678*	−0.307	0.286
Mud	−0.784**	−0.713**	−0.741**	−0.237	0.426
Sand	0.783**	0.713**	0.741**	0.237	−0.426
SRP	0.804**	0.739**	0.620*	0.199	−0.322
Harbor Island East					
Cu^{++} PW	−0.216	−0.333	−0.023	−0.024	0.047
Cu^{++} BW	0.289	0.595	−0.071	−0.071	0.047
DCu BW	−0.469	−0.381	−0.214	−0.214	0.166
Cu Sed	−0.783*	−0.214	−0.476	−0.476	0.428
TOM	−0.759*	−0.214	−0.405	−0.405	0.381
Chl a	−0.12	0.024	0.0234	0.024	−0.095
Phaeo	−0.301	0.143	−0.095	−0.095	0.071
CPE	−0.361	0.095	−0.095	−0.095	0.024
Mud	−0.711*	0.071	−0.405	−0.405	0.357
Sand	0.711*	−0.071	0.405	0.405	−0.357
SRP	0.301	−0.143	0.095	0.095	−0.0714

Cu^{++} PW = free Cu^{++} porewater; Cu^{++} BW = free Cu^{++} bottom water; DCu BW = dissolved Cu bottom water; Cu Sed = sediment Cu; TOM = total organic matter; Chl *a* = chlorophyll a; Phaeo = phaeopigments; CPE = chloroplastic pigment equivalent; SRP = sediment redox potential.

Indicator species

The RDA triplot (Fig. 8) served as a first approach to detect species that were associated with high sediment Cu concentration. These included *Polydora nuchalis* and *Pseudopolydora paucibranchiata*, *Euchone limnicola* and *Typosyllis aciculata*; all appeared to exhibit tolerance to Cu. In contrast, another more diverse group of species, primarily amphipods (*Podocerus* sp., *Harpinia* sp., *Corophium* spp., *Aoroides* sp.), appeared to be Cu-sensitive indicators. The TWINSPAN analysis provided similar results. Samples were separated in two groups at the highest level (Fig. 10), with the spionid polychaetes *P. nuchalis* and *P. paucibranchiata* as prospective Cu-tolerant indicators, and *Podocerus* sp., *Harpinia* sp. and the polychaete *Diplocirrus* sp. as prospective Cu-sensitive indicators. Furthermore, the high-Cu tolerant assemblages were subdivided into a group characterized by *Euchone limnicola* and a group characterized by *Leptochelia dubia*, *Typosyllis aciculata* and *Dedimelita* sp. The Cu-sensitive

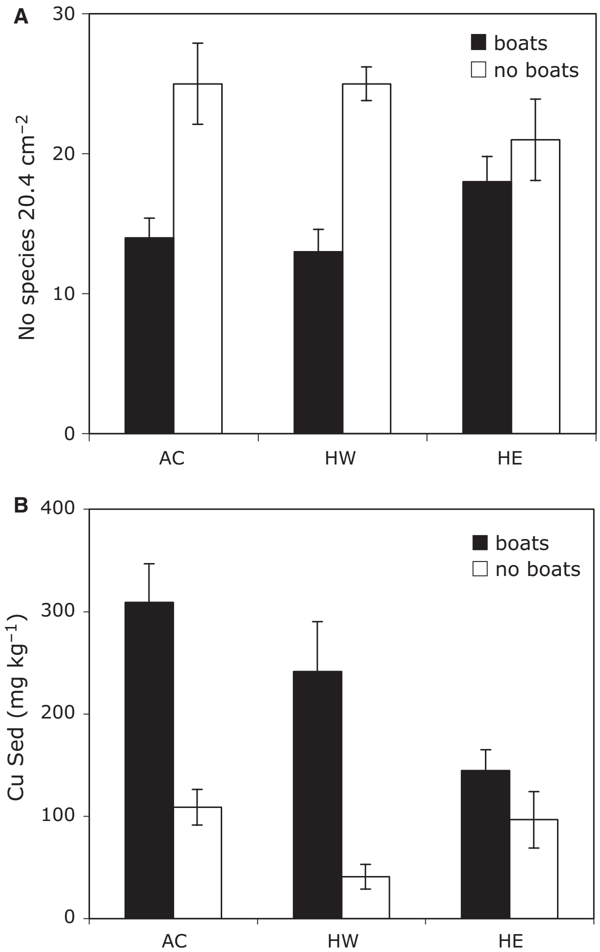

Fig. 7. Number of macrofaunal species (A) and sediment copper concentration (B) at sites with boats and without boats in America's Cup (AC), Harbor Island West (HW) and Harbor Island East (HE).

Table 3. Results of the forward stepwise selection procedure analyzing environmental variables of three San Diego Bay marinas. Significant variables that most likely influence macrofaunal community structure (P < 0.05) are shown in bold.

environmental variables	F	P
sediment Cu	**11.364**	**0.001**
dissolved Cu bottom water	**3.2053**	**0.001**
sediment redox potential	1.7296	0.063
free Cu^{++} bottom water	1.6215	0.078
free Cu^{++} porewater	1.3002	0.238
mud content (<63 μm)	1.1687	0.317
sand content (>63 μm)	1.6118	0.093
sediment phaeopigments	0.73005	0.693
sediment chl a	0.63487	0.798
total organic matter	0.49992	0.883

assemblage was subdivided into a group characterized by *Cossura* and a group characterized by *Macoma* sp. and *Lyonsia californica* (Fig. 10).

Cu in macrofaunal tissues

Cu concentrations in macrofauna collected from sites with and without boats at the three marinas ranged from 0.7 to 3258 μg g^{-1} (Appendix Table S8). Considering the species as 'replicates' within a site, average Cu concentration in tissue exceeded Cu concentrations of surrounding sediment, and was higher at sites with boats than sites without boats ($F_{1,12}$ = 4.6; P = 0.05) (Fig. 11A). However, when the Cu concentrations are examined at a lower taxonomic level, differences in responses are evident. Some species, such as the polychaetes *Lumbrineris* sp., the olichochaete *Tubificoides* spp. and the crustaceans *Leptochelia dubia* and *Desdimelita* sp., exhibited tissue concentrations approximately twice that observed for the sediment ($F_{1,36}$ = 12.2; P = 0.001). In contrast, species such as *Pseudopolydora paucibranchiata* and *Polydora nuchalis*, *Cossura* sp. had concentrations much lower than the surrounded sediment ($F_{1,13}$ = 30.6; P < 0.001), even at sites with sediment Cu exceeding 400 μg g^{-1} (Fig. 11B).

Discussion

Dissolved, particulate, free Cu, and complexation capacity

To date, Cu partitioning has been studied in four marinas in San Diego Bay (this study, Neira *et al.* 2009). Dissolved Cu concentration at the three marinas exceeded the threshold of water quality of 3.1 μg l^{-1} (SWRCB 2000; US EPA 2000), averaging 6.0 μg l^{-1} in surface water and 5.2 μg l^{-1} in bottom water, with a maximum of ~11 μg l^{-1} measured in HW and AC. These values fall within the range of concentrations reported in early studies for these marinas (McPherson & Peters 1995; Schiff *et al.* 2007). In AC, Cu concentrations of 3.7–8.8 μg l^{-1} were reported by Schiff *et al.* (2007), values slightly lower than those of <5–11 μg l^{-1} reported by McPherson & Peters (1995). In HW, concentrations of 9.1–10.4 and 5.0 –7.0 have been documented by Schiff *et al.* (2007) and McPherson & Peters (1995), respectively.

Gradients of dissolved Cu, especially in surface water, were evident from the basin head to the mouth at AC, HW and HE (Fig. 4A-C). As in SIYB (Neira *et al.* 2009), higher concentrations were measured in surface water than in bottom water (6.0 *versus* 4.9 μg·L^{-1}, P = 0.024 in AC; 5.9 *versus* 4.2 μg·L^{-1} in HE, P = 0.009). In HW, differences between surface and bottom water average concentrations were not evident (~ 6.0 μg·L^{-1}). The constant influx of Cu, along with poor or incomplete tidal flushing, may contribute to a strong Cu gradient from the basin mouth to the inner portion. A lower concentration of dissolved Cu in bottom water relative to surface water may be attributed to a net transfer of Cu from the surface

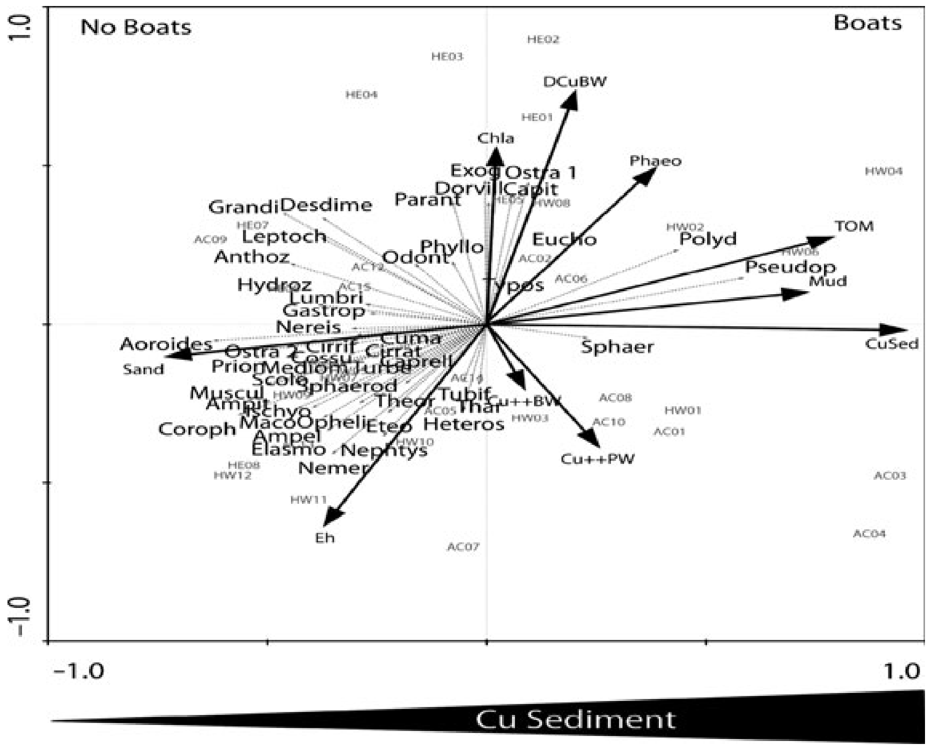

Fig. 8. Redundancy analysis (RDA) triplot displaying the position of macrofaunal species in relation to benthic environmental variables that best explain their distribution along sites with boats (*i.e.* higher Cu) and sites without boats (*i.e.* lower Cu) in America's Cup (AC), Harbor Island West (HW) and Harbor Island East (HE). Solid arrows are the explanatory variables; the dashed arrows are macrofaunal taxa. Arrows pointing in the same relative direction are correlated, and longer arrows indicate increasing values. The first two axes explained 81.6% of the species-environment variance. The relationships were highly significant (P = 0.001; 999 permutations in Monte Carlo permutation test).

to the sea bed via complexation and adsorption to suspended particulate matter, including clay and organic particles (Chadwick *et al.* 2004; Minaberry & Gordillo 2007). Suspended colloidal and particulate matter are important components in the process of trapping and transfer of trace elements to the sediments because of their strong sorption capacities (Bloundi *et al.* 2008; Zirino *et al.* 2013).

During this study the contribution of particulate Cu to the total Cu pool was about 15% in AC and HW and comparable to that of SIYB surface water (13.5%). In HE, the percent contribution of particulate Cu was much lower (~4%). As in SIYB, the percent contribution of particulate Cu to the total is slightly higher in bottom water than in surface water. Differences may reflect the spring–summer peak vessel activities (Neira *et al.* 2009), which could involve more intense hull-cleaning activities at AC and HW relative to HE, with the subsequent release of rapidly settling paint chips and debris (Valkirs *et al.* 1994). Resuspension of sediment has also been suggested as a mechanism to explain enhanced particulate Cu contribution in bottom water (Neira *et al.* 2009). The elevated free Cu^{++} concentrations, especially in AC (Fig. 2), could also be the result of hull-cleaning operations. Valkirs *et al.* (1994) found that free Cu^{++} levels increased 10-fold during cleaning operations. In SIYB, free Cu^{++} levels measured in surface, bottom and porewater (Neira *et al.* 2009) are even higher than those measured in the present study for AC, HW and HE. In SIYB there are enhanced contributions of particulate Cu in summer relative to winter, and in bottom water relative to surface waters (Neira *et al.* 2009).

Metal titrations provided important information about the total metal buffering capacity of the system. The free metal ion (Cu^{++}) is the key variable of biological uptake (Campbell 1995). All other forms of the metal present in the water are bound to dissolved organic substances and colloidal matter found in the water. The property of seawater that binds microconstituents has been termed the complexation capacity (CC) (Zirino *et al.* 2013). The CuCC include covalent bonding among dissolved ions and organic and inorganic molecules as well as adsorption on colloids and particles (Zirino *et al.* 2013). The CuCC is related to the level of productivity, turbulence, and rate of particle formation (Zirino *et al.* 2013). Our measurements of CuCC at key sections of each marina revealed a slight

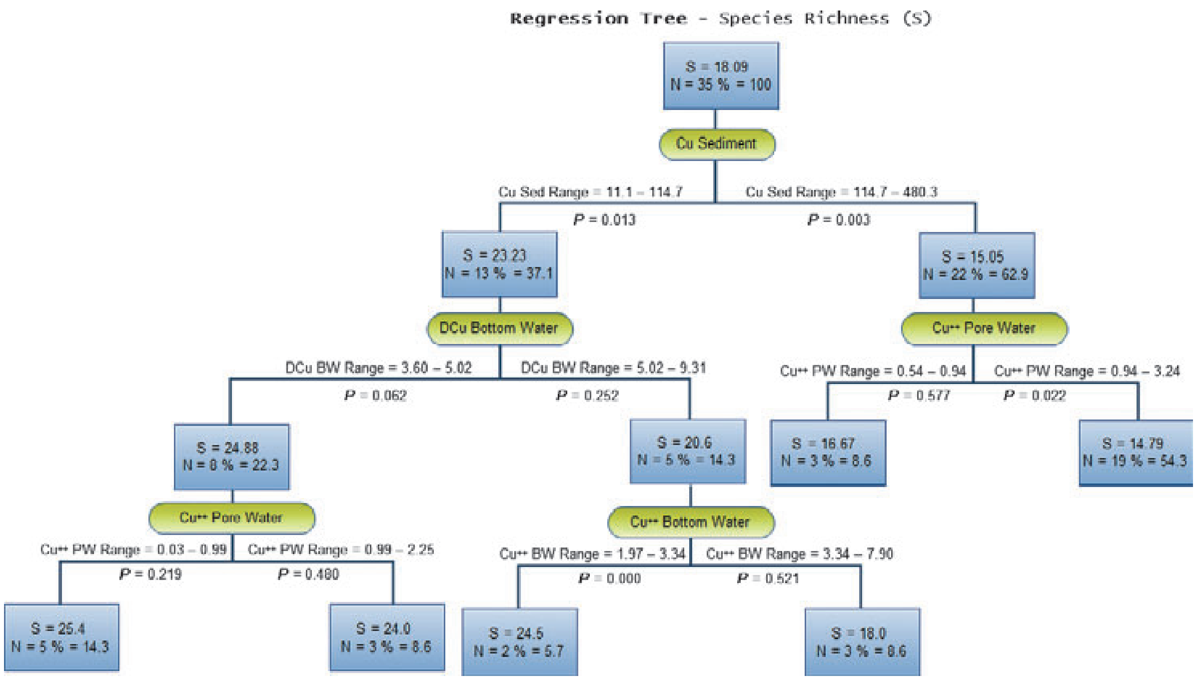

Fig. 9. Regression tree analysis for species richness of three marinas: America's Cup (AC), Harbor Island West (HW) and Harbor Island East (HE). Species richness is the response variable and the multiple explanatory variables (predictors) are sediment Cu (Cu Sed), dissolved Cu in bottom water (DCu BW), and free Cu^{++} in porewater (Cu^{++} PW). Variables important in explaining species richness variation appear at the terminal nodes along with the concentration range. Significance (P) of the split as well as the number and percent of observations in the group are shown.

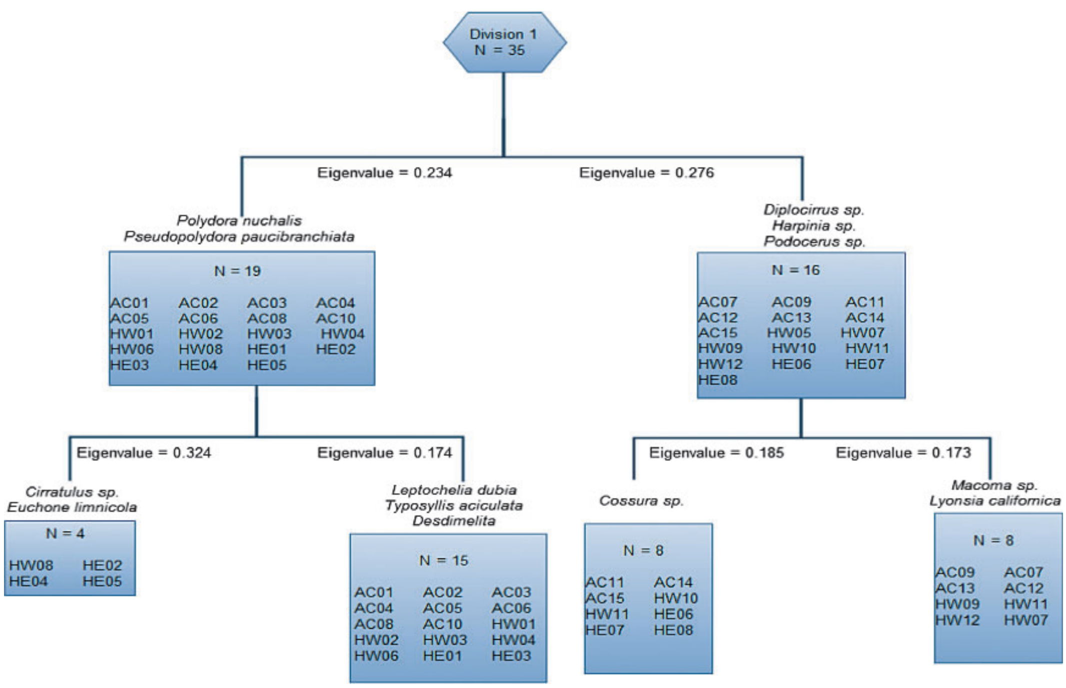

Fig. 10. TWINSPAN analysis of benthic macrofaunal communities in America's Cup, Harbor Island West and Harbor Island East. Analysis is based on occurrence weighted according to abundance. Prospective 'Cu-tolerant indicators' and 'Cu-sensitive indicators' are given on the left and on the right, respectively. The division is constructed on the basis of a correspondence analysis (CA) ordination. In the boxes are indicated the number of sites (N) and the site names included in the classification. The eigenvalue is a measure of the explanatory power of the axis.

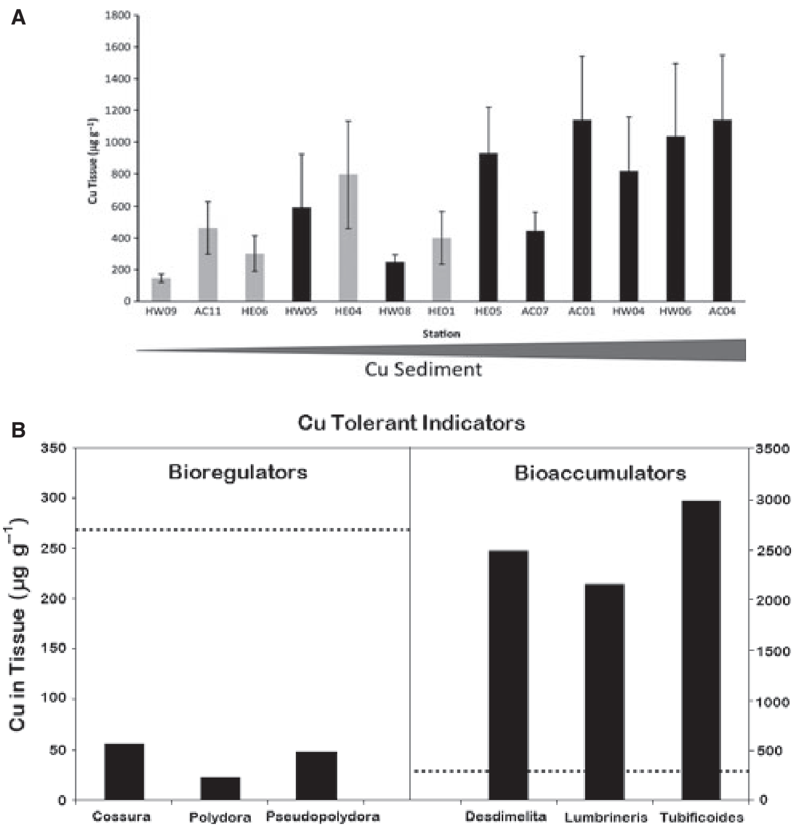

Fig. 11. (A) Average Cu concentration in tissues considering the species as 'replicates' within sites with and without boats along the gradient of Cu in sediment of three San Diego Bay marinas (light bars: sites without boats, black bars: sites with boats) and (B) Cu concentration in tissues of species associated with high background sediment Cu and proposed as Cu-tolerant indicators in three San Diego Bay marinas. Species exhibiting Cu concentration above levels of the surrounding environment (dashed line) are suggested to function as bioaccumulators. Species with much lower Cu concentrations than surrounding sediments are suggested to function as Cu bioregulators. Dashed line represents background sediment Cu concentrations above the effect range medium (ERM), *i.e.* concentrations frequently toxic (NOAA 1999).

decline in CuCC from the basin head to the mouth. CuCC values were comparable to those reported for SIYB (306–589 nM; Neira *et al.* 2009), Venice Lagoon, Italy (95–573 nM; Delgadillo-Hinojosa *et al.* 2008b), Humber Estuary, NE England (200–420 nM; Van Veen *et al.* 2001), and San Diego Bay (98 and 338 nM; Rivera-Duarte *et al.* 2005). In all cases, the CuCC exceeds the dissolved Cu in the water. In HE, CuCC exceeded the concentration of dissolved Cu in bottom water by about five times, and in AC and HW, CuCC by about three times. These results suggest that CuCC is more effective at HE than HW or AC. However, this merits further investigation.

Cu in sediments

In the USA, NOAA's National Status and Trends Program analyzed samples of surface sediment collected at almost 300 coastal and estuarine sites and listed San Diego (along with Boston, New York, Los Angeles and Seattle) as having the highest Cu concentration in sediment (NOAA 1991). Spatial distributions of Cu concentrations in marina sediments of San Diego Bay exhibit a clear gradient and hotspots associated with the presence of boats, as modeled in the probability maps (Fig. 4A-C). In some areas of AC and HW, Cu concentration exceeded the ERM of 270 mg kg^{-1} that is toxic to organisms (NOAA 1999). In contrast, at HE, Cu concentrations fell between the ERL and the ERM, where adverse effects occur only occasionally (NOAA 1999).

In general, very few sediment Cu data are available for the San Diego Bay marinas other than SIYB (*e.g.* Salazar & Salazar 1985; PTI Environmental Services 1990; California Department of Fish & Game 1995).

According to previous models proposed for San Diego Bay by Zirino *et al.* (1998a), Chadwick *et al.* (2004) and Blake *et al.* (2004), Cu from ships' hulls enters the water in dissolved form and agglomerates into and onto colloids before being adsorbed onto particulates that ultimately sink to the seabed. A net removal rate of Cu from the water to the sediment ranging from 6% to 9% day^{-1} (48% of the input) has been estimated for San Diego Bay (Chadwick *et al.* 2004). While sediment-bound Cu can be reintroduced into the water column by a variety of biogeochemical and physicochemical processes (*e.g.* bioturbation, bioirrigation, excretion, desorption from resuspended particles and diagenetic processes (Boudreau & Jørgensen 2001; Gee & Bruland 2002; Buck *et al.* 2007), net transport is always to the sediments. The Cu patterns presented here (Fig. 4A–C) support this model, with greater scavenging by particulates at the head of marinas.

Effects on macrofaunal communities

Cu at high concentrations is a known toxin and can have effects at the species and ecosystem levels (Rygg 1985; Perrett *et al.* 2006; Neira *et al.* 2011). Differing taxon composition, species richness and diversity as well as elevated dominance in communities associated with sediments underneath recreational boats relative to communities at sites without boats indicate a linkage to Cu toxicity, especially at AC and HW. In HE, none of the community attributes were affected. Although average macrofaunal abundance is lower at sites with boats in all marinas, differences were not significant; the same situation was observed in SIYB (Neira *et al.* 2011). At sites without boats, abundances of crustacean amphipods, bivalves and some polychaetes were greater, whereas at sites with boats polychetes and oligochaetes (*Tubificoides* spp.) were better represented.

The association of Cu concentration with community effects is difficult to establish in terms of cause–effect relationships because factors such as other pollutants, hydrodynamics, sediment stability, competitive interactions, bioturbation, recruitment patterns and natural variability (Oug 1998) could also cause differences between sites. However, our analyses support a causal relationship between elevated sediment Cu at sites with boats and reduced macrofaunal diversity and changes in community composition. In this regard, all univariate (Tables 1 and 2, Fig. 9) and multivariate analyses (Table 3, Fig. 8, Appendix Table S6, S7) pointed to sediment Cu as the main driver responsible for changes in community composition and reduction in macrofaunal diversity. Similar results were obtained recently for SIYB (Neira *et al.* 2011). In the present study the total faunal variance for all three marinas explained by sediment Cu (26.5%) was higher than that reported for SIYB (18.3%) Neira *et al.* (2011) or by Olsgard (1999) (17.4%), who examined effects of Cu on macrofaunal recolonization in the Oslofjord, Norway. The remaining 48.8% of the total variation corresponded to residual variability due to unmeasured or physical variables such as presence of other pollutants, hydrodynamics, fluxes from sediments or competitive interactions (*e.g.* predation).

Peracarid crustaceans (primarily amphipods) and bivalves were the most affected by elevated sediment Cu in terms of species composition and relative abundance (Appendix Tables S3–S5). Field experiments examining effects of Cu on soft-sediment fauna showed that in treatments where Cu was introduced, the abundance of amphipods and cumaceans was reduced compared with controls (Morrisey *et al.* 1996). A similar effect of reduced diversity was observed in faunal communities of Norwegian fjords by Rygg (1985) who found that Cu was more closely linked to reduction in macrofaunal diversity than were zinc and lead. In laboratory experiments using soft sediment collected in Grays Point, Australia, Stark (1998) found that crustaceans were very sensitive to Cu exposure; controls had the greatest abundances of total crustaceans, amphipods and copepods whereas the lowest abundances were observed in Cu-enriched treatments. Their mobility may reduce their need to evolve Cu tolerance.

Interestingly, measurements of Cu in tissue (Fig. 11A–B, Appendix Table S8) revealed not only a wide range of Cu concentrations in different species, but also within the same species. Several species exhibited Cu concentration above levels in the surrounding environment. We interpret this to reflect bioaccumulation. The annelids *Lumbrineris* sp. and *Tubificoides*. sp., and the amphipod *Desdimelita* sp. contained high levels of Cu, suggesting they function as Cu bioaacumulators. In contrast, the spionid polychaetes *Polydora nuchalis* and *Pseudopolydora paucibranchiata* had much lower Cu concentrations than surround sediments, suggesting they function as Cu bioregulators. These differences may reflect species variations in feeding strategies, degree of tolerance to the metal and tendency to bioaccumulate. Differences in digestive capacity, digestive tract biochemistry, throughput time, and assimilation efficiencies of Cu bound and sorbed to sediment and detritus may also affect Cu uptake (Chen & Mayer 1998; Wang *et al.* 1999; Chen *et al.* 2002).

Those macrobenthic invertebrates in San Diego Bay marinas that attain highest densities in the face of Cu pollution (*e.g. Polydora nuchalis*, *Pseudopolydora paucibranchiata*, *Euchone limnicola*, *Typosyllis aciculata*, *Tubificoides* spp.) (Figs 8 and 10) are here proposed as prospective indicators of high-Cu conditions. More Cu-sensitive taxa (*e.g. Podocerus* sp., *Aoroides* sp., *Harpinia* sp., *Macoma* sp., *Lyonsia californica*), are proposed as indicators of healthier conditions. Similar indicators were suggested for SIYB (Neira *et al.* 2011). These responses suggest multiple mechanisms are involved in the effects, and clearly some species are able to regulate their body Cu concentration. Lifestyles, feeding habits, behavioral avoidance, degrees of mobility, routes of exposure, and ability to accumulate and detoxify have been proposed (Reish 1993; Correia *et al.* 2002; Mardsen & Rainbow 2004; Wiklund *et al.* 2006). The sediment-dwelling fauna is in direct contact with sediment and their metal exposure does not appear to be controlled by porewater concentration but rather through ingestion of organic matter associated with sediment particles. Lee *et al.* (2000) reported a weak association between porewater and bioaccumulation of metals in several invertebrates. Direct ingestion of sediment (regardless of sulfide content) was the most probable explanation. Free Cu^{++} in porewater was strongly reduced in these sediments relative to bottom and surface water, especially at HE. Relatively high Cu complexation capacity in bottom water, suggests a lowering of Cu^{++} in porewater to below toxic levels (Di Toro *et al.* 2005; Neira *et al.* 2009). TOM may serve as a potential source of ligands to porewater and overlying water (Skrabal *et al.* 1997). Although sediments are a rich source of organic ligands, these can be dominated by weak ligands, causing Cu to become more bioavailable, and thus more toxic to animals (Gerringa *et al.* 1991). The patterns of excess CuCC across all four marinas in San Diego Bay indicate that sediment Cu appears to be the primary variable responsible for changes in macrofaunal communities in San Diego Bay marinas.

Potential ecosystem consequences of elevated Cu

In the benthic environment, the high sediment Cu levels but limited free Cu^{++} raises concerns about the role of Cu species in structuring faunal communities. A key aspect is to know the potential effects of Cu on benthic fauna once Cu enters the food web. Low free Cu^{++} and relatively high organic matter content may yield sediments that are non-toxic to infaunal invertebrates. However, the present results for AC and HW and HE, along with our recent studies in SIYB (Neira *et al.* 2009, 2011), suggest that sediment Cu associated with boat presence strongly influences faunal communities. The main effects of elevated sediment Cu include changes in faunal assemblage composition, reduction of species richness and diversity, with loss or scarcity of crustaceans, primarily amphipods and some polychaetes and bivalves. Diversity of benthic communities is of paramount importance in estuarine and coastal habitats and has been linked to ecosystem functions such as resource availability, elemental cycling, benthic–pelagic coupling and production of biomass (Snelgrove *et al.* 2000; Levin *et al.* 2001). Reduced biodiversity (species richness) and structural complexity may have a direct negative effect on functional diversity (*i.e.* number of functional roles

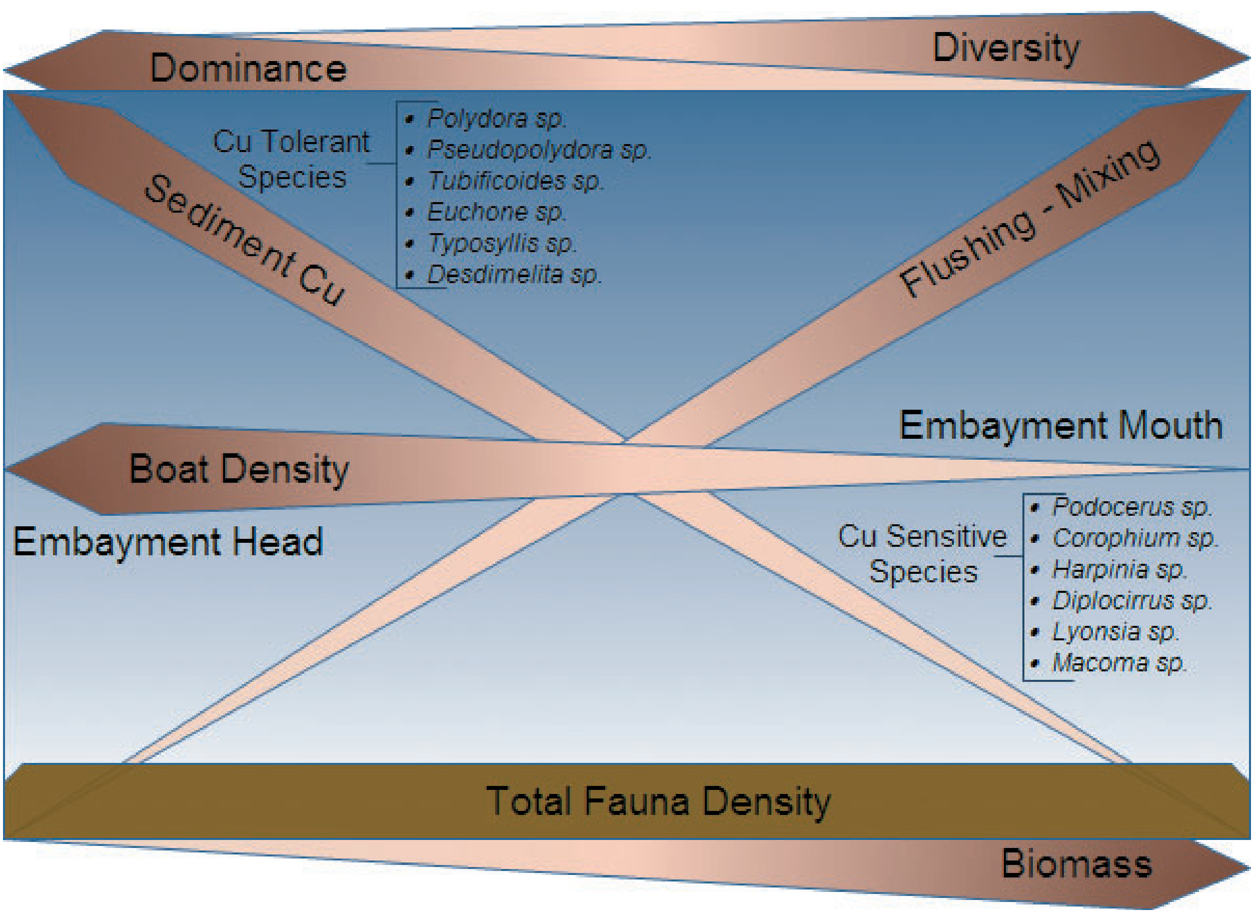

Fig. 12. Schematic diagram summarizing underlying mechanisms involved in the effects of Cu pollution on macrofaunal communities of San Diego Bay marinas.

held by species in the ecosystem) (Lohrer *et al.* 2004; Danovaro *et al.* 2008). The loss or reduction of highly active bioturbators and secondary producers will have considerable consequences for ecosystem function due to the lower contribution to the remineralization of organic matter, reduced bioirrigation and remobilization of toxic substances from the sediment. Other potential consequences of biodiversity reduction and community alteration are reduced contribution of prey for higher trophic levels, more time to search for food by predators, and disruption of predator–prey interactions (Pyle & Mirza 2007; Boyd 2010).

Based on the results presented here and those of Neira *et al.* (2011) in SIYB, we summarized general mechanisms underlying Cu pollution and its effects on macrofaunal communities of San Diego Bay marinas (Fig. 12). The information provided in this study suggests that using sediment quality guidelines based solely on contaminant concentrations (Long *et al.* 1995; MacDonald *et al.* 1996; NOAA 1999) and laboratory toxicity assays would be unlikely to reflect the complexity of the interactions and factors contributing to community level response to Cu pollution. It is necessary to apply holistic strategies and regulatory policies for assessing the effects of Cu pollution as well as to develop novel mitigation protocols for Cu in San Diego Bay and other estuarine environments. The identification of taxa that are sensitive or tolerant to Cu will allow the development of Bay-specific, multispecies models of macrofaunal status and health. Given the extensive use of Cu globally as an antifouling paint additive, we predict that the Cu–macrofauna interactions observed in San Diego Bay are likely to be relevant to urbanized marinas on an international scale.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Location of sites sampled in America's Cup, Harbor Island West and Harbor Island East, San Diego Bay, California, and their sediment properties.

Table S2. Mean density (No individuals 20.4 cm-2 core) ±1 SE of macrofaunal taxa at sites of America's Cup, Harbor Island West, and Harbor Island East, San Diego Bay, California.

Table S3. Mean density (No individual 20.4 cm-2 core) ±1 SE of macrofauna taxa at sites with and without boats in America's Cup, San Diego Bay, California.

Table S4. Mean density (No individual 20.4 cm-2 core) ±1 SE of macrofauna taxa at sites with and without boats in Harbor Island West, San Diego Bay, California.

Table S5. Mean density (No individual 20.4 cm-2 core) ±1 SE of macrofauna taxa at sites with and without boats in Harbor Island East, San Diego Bay, California.

Table S6. Redundancy analysis (RDA) describing relationships between community structureand the environmental variables (sediment Cu, dissolved Cu in bottom water, free Cu++ in bottom water, free Cu++ in porewater, mud, sand, chl *a*, phaeopigments, redox potential, total organic matter) identified by a forward stepwise selection.

Table S7. Partial redundancy analysis (pRDA) describing relationships between macrofaunal structure and variables of interest (sediment Cu, dissolved Cu in bottom water, free Cu++ in bottom water, free Cu++ in porewater, after removing effects of the non Cu variables.

Table S8. Tissue Cu concentrations in invertebrate species at sites with and without boats in America's Cup (AC), Harbor Island West (HW), and Harbor Island East (HE), San Diego Bay, California. Background Cu concentrations sediment are indicated at the last column.