



Macrobenthic community response to copper in Shelter Island Yacht Basin, San Diego Bay, California

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ABSTRACT

We examined Cu contamination effects on macrobenthic communities and Cu concentration in invertebrates within Shelter Island Yacht Basin, San Diego Bay, California. Results indicate that at some sites, Cu in sediment has exceeded a threshold for “self defense” mechanisms and highlight the potential negative impacts on benthic faunal communities where Cu accumulates and persists in sediments. At sites with elevated Cu levels in sediment, macrobenthic communities were not only less diverse but also their total biomass and body size (individual biomass) were reduced compared to sites with lower Cu. Cu concentration in tissue varied between species and within the same species, reflecting differing abilities to “regulate” their body load. The spatial complexity of Cu effects in a small marina such as SIYB emphasizes that sediment-quality criteria based solely on laboratory experiments should be used with caution, as they do not necessarily reflect the condition at the community and ecosystem levels.

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1. Introduction

Benthic communities (assemblages of coexisting species) are considered effective indicators of habitat condition because of their importance to overall ecosystem structure and function. Many infaunal species are sedentary and trophically diverse (Lenihan and Micheli, 2001) and their communities integrate the effects of water and sediment changes over time. In addition, benthic fauna play an important ecological role within food webs. They are a direct and indirect food source for many animals, including large crustaceans, fishes, marine birds and marine mammals (Feder and Jewett, 1981). Benthic animals also can alter physical and chemical conditions at the sediment–water interface, promote decomposition of sediment organic matter (OM), and are important mediators in nutrient recycling from the sediments to the water column through bioturbation and suspension feeding activities (Rhoads, 1974; Aller and Yingst, 1985). Hence, changes in community composition, abundances and diversity of benthic fauna can affect the functioning of the entire ecosystem (Bylyard, 1987).

Benthic fauna are most directly exposed to metal contaminated sediments and hence vulnerable to potential toxic effects. The

extent of the effect will depend on the species-specific tolerances to the metals present (Luoma and Carter, 1991) and the influence of metals on species interactions. The interaction of animals with the geochemical characteristics of the sediment are mediated by the animal physiology, life habit, and feeding mode. The uptake of trace metals in benthic invertebrates can affect their reproductive processes, feeding rates, respiration, protein utilization, and cause morphological abnormalities and histological problems in adult invertebrates (Luoma and Carter, 1991).

Copper (Cu hereafter) is a common contaminant in coastal waters, particularly in industrialized harbors, bays, and estuaries, due to its extensive use in antifouling paints (Salomons and Förstner, 1984; Terlizzi et al., 2001; Carson et al., 2009). Trace concentrations of Cu are essential for the proper function of many life processes, but Cu can be toxic at high levels (Buck et al., 2007). There is increasing concern regarding the ecological risk that Cu pollution poses in bays and marinas because Cu is toxic not only to the targeted fouling organisms but also to other organisms in the surrounding environment.

In San Diego Bay (32°40'N; 117°14'W) elevated concentrations of dissolved Cu have raised concerns about potential effects on benthic faunal communities, because in some areas dissolved Cu levels exceed state and national water quality criteria of 3.1 µg L⁻¹ (49 nM) (US EPA, 1995; Neira et al., 2009). One of these areas is Shelter Island Yacht Basin (SIYB) (32°43'N; 117°13'W), a small,

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man-built enclosure whose “mouth” (0.2 km) faces the main (and only) entrance channel to San Diego Bay. Because SIYB has no through flow, it is among the most Cu contaminated basins in the southern California area (Schiff et al., 2007).

Cu speciation in SIYB is believed to be similar to that in San Diego Bay where Cu is principally associated with OM (99%, Zirino et al., 1998), with the remainder, perhaps one percent, being associated with inorganic complexes (Zirino and Yamamoto, 1972). Cu species in San Diego Bay include the so-called “free ion” (Cu^{2+} , and Cu inorganic complexes), Cu associated with dissolved organic ligands ($\text{CuL} \approx 50\%$), Cu in colloidal forms, principally organic in nature ($\text{CuL}_{\text{coll}} \approx 30\%$) and Cu in particulates, again, principally organic in nature ($\text{CuL}_{\text{part}} \approx 10\text{--}20\%$) (Chadwick et al., 2004; Blake et al., 2004).

Recently, Neira et al. (2009) examined the overall effect of number of boats and distance on Cu species levels in the water column and sediments along with their spatial variability within the basin. Spatial models of Cu distribution in water and sediments revealed the presence of gradients and “hotspots” of Cu concentration. They found high concentrations of dissolved Cu in water and total Cu in sediments but limited amounts of free Cu^{2+} . This raised questions about the impact of Cu on benthic faunal communities living underneath and immediately adjacent to recreational boat basins.

The implementation by California regional Water Quality Control Board of the Total Mean Daily Load (TMDL) program in December 2005 (SDRWQCB, 2005), was intended to gradually reduce Cu levels in San Diego waters. This and the eventual phase-out of Cu-based hull paints on recreational boats in San Diego Bay (Carson et al., 2009) has created the need to understand the present status of benthic faunal communities in SIYB. In general, the impact of pollution on marine organisms has usually been assessed and predicted from studies with single species under controlled conditions (Widdows, 1985; Clark et al., 2001; Stark, 1998). Although this approach is valid, it does not lead to predictions about the effects of toxicants on entire communities (Kimball and Levin, 1985; Luoma, 1996). It has been well established that the main metal contaminant in SIYB is Cu (Zirino et al., 1998; SDRWQCB, 2005) and that a strong gradient of Cu occurs both in water and sediments linked to boat distribution (Neira et al., 2009). SIYB offers a unique “natural” mensurative experiment to contrast changes in macrobenthic communities between sites of high Cu (many boats) and sites of low Cu (no/few boats) levels.

Stress is a ubiquitous feature of coastal environments and is increasing under anthropogenic influence (Parker et al., 1999). Grime (1989) has defined stress as “external constraints limiting the rates of resource acquisition, growth or reproduction of organisms”. Excess Cu can have these effects on marine invertebrates (e.g. Luoma and Carter, 1991; Krång and Ekerholm, 2006; Roberts et al., 2006; Hollows et al., 2007). However, it is difficult to extrapolate effects on single functions of single species to consequences for higher levels of organization such as communities and ecosystems (Parker et al., 1999). Comparative analyses of community responses and species responses to stressors suggest that community effects cannot be extrapolated from single species responses alone (e.g. Van den Brink et al., 1996). Community level feedbacks are not well understood but could involve trophic cascades, facilitation, or animal–sediment interactions. Current paradigms suggest that reduced species diversity, elevated dominance by stress tolerant taxa, and increased homogeneity are common ecological, community-level outcomes of environmental stressors (Luoma and Carter, 1991; Austen et al., 1994). Stress may however promote evolutionary diversity over longer time scales (Klerks and Levinton, 1989; Korol, 1999; Nevo, 2001). Below we generate a series of predictions about the potential for Cu stress in protected embayments to modify elements of macrofaunal community structure based on stress theoretical considerations.

We hypothesize that (a) reductions in macrobenthic density, biomass, body size and diversity are associated with elevated Cu concentration gradients, or with specific Cu chemical species, (b) other identifiable factors such as sediment OM, grain size, and chlorophyll *a* (chl *a*) play a secondary role in structuring communities, (c) different feeding modes occur at different Cu concentrations, with deposit feeders proportionally decreased at highest Cu concentrations, and that (d) the community present under highest Cu stress should be comprised of a mixture of species with evolved tolerance and those with lifestyles that limit exposure to Cu (i.e. mobile and tube builders).

Information on metal concentrations in invertebrate tissues is an important component of any coastal and estuarine assessment because metal concentrations are site-specific and related to local environmental condition. A major factor determining the metal content in an organism is the concentration of the metal in the surrounding environment. Benthic animals receive metals directly from overlying waters, or via porewater, or when contacting and ingesting sediment particles. Because the benthic animals are permanently associated with their sediments, the question arises whether invertebrates inhabiting sediments with higher Cu will have enhanced Cu in their tissues. In general, little information is available on Cu concentration in the macrofauna of bottom sediments (Davydkova et al., 2005), but given the diversity of microhabitat, developmental biology, feeding modes and lifestyles among the macrobenthic taxa, we can expect a large range of exposures and sensitivities to Cu contamination. Thus we hypothesize that invertebrate tissue Cu concentrations will not directly reflect Cu in sediment, and that macrofaunal species' tolerance and sensitivity to Cu concentrations, assessed by tissue concentrations and distribution patterns, will vary across and within taxa.

2. Materials and methods

2.1. Study site

Shelter Island Yacht Basin is located in the north end of San Diego Bay near the bay mouth. It is a semi-enclosed, man-made basin of approximately 3.2 km long, with its opening facing the main entrance channel of San Diego Bay (Fig. 1). The mean width of the basin is 475 m with a mouth width of 200 m and a greatest width of 750 m in the center, and an average volume estimated at $5.9 \times 10^6 \text{ m}^3$ (Johnston, 1989). The mean depth is about 5 m (MLLW). SIYB is flushed by the largely semidiurnal tidally-driven current that enters San Diego Bay via the main channel. The average residence time is about 5 days. SIYB is heavily used for boat berthing as well as repair and repainting. About 13.5% (~2300) of the recreational boats of the San Diego Bay region are moored there permanently (Lenihan et al., 1990; Neira et al., 2009). A total Cu load of approximately 2200 kg y^{-1} has been estimated to enter the water column in SIYB, of which >92% is derived from passive leaching from hull paints (SDRWQCB, 2005).

2.2. Sampling design

In 2006 an exploratory sampling based on a grid of 32 stations projected onto an orthophoto of SIYB using ArcGIS (ESRI, 2006) was conducted (Neira et al., 2009). The design ensured that the entire basin was equally represented, including both open areas and areas with moored boats. This detailed determination of Cu levels in sediments and water, and their spatial distribution (vertical and horizontal) in relation to the recreational boat distribution, detected a Cu gradient and hotspots with different degrees of Cu concentration (Neira et al., 2009). This provided the basis for sampling in the present study. Therefore, in spring 2007 we sampled a total

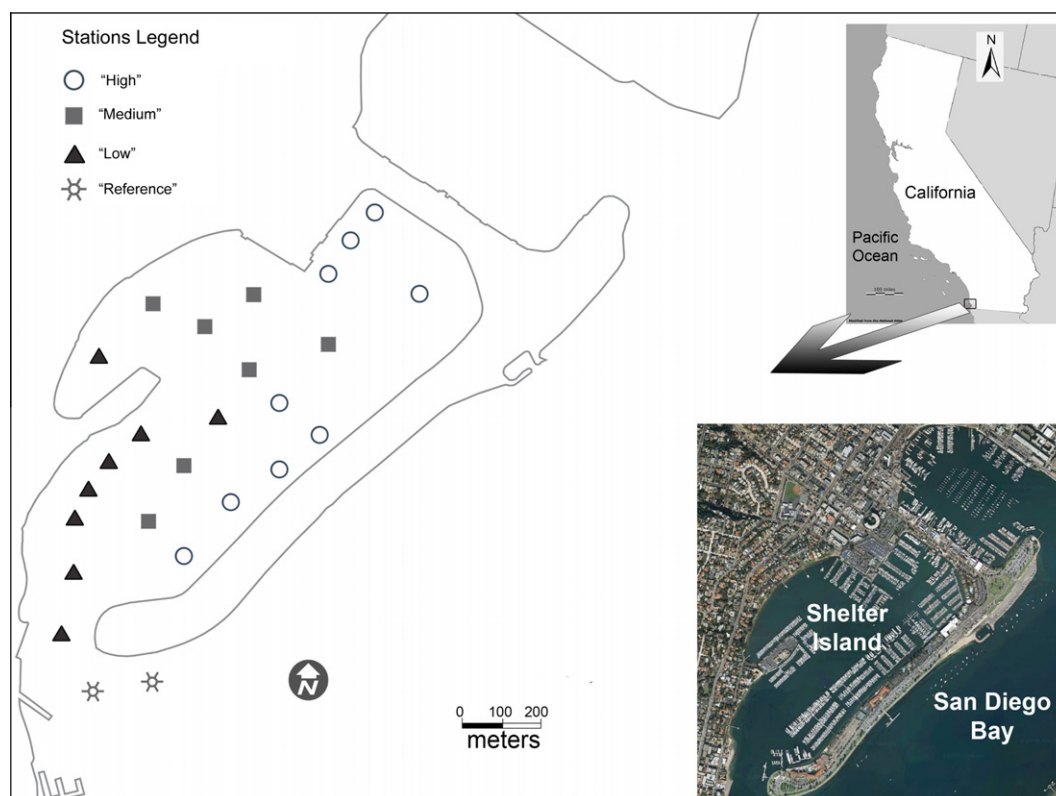


Fig. 1. Location of Shelter Island Yacht Basin in San Diego bay, California. Study sites, operationally designated as “high”, “medium”, and “low” Cu levels (based on a previous exploratory study by Neira et al. (2009), are shown with symbols. Two sites outside the basin served as “reference”.

of 26 stations that we categorized in terms of sediment Cu as “high” ($236.1 \pm 40.9 \text{ mg kg}^{-1}$; 9 stations), “medium” ($183.2 \pm 19.6 \text{ mg kg}^{-1}$; 7 stations), and “low” ($111.5 \pm 25.0 \text{ mg kg}^{-1}$; 8 stations). Two stations outside the basin served as reference sites ($18.9 \pm 2.7 \text{ mg kg}^{-1}$) (Fig. 1). More details of the study site and sampling design are given in Neira et al. (2009).

2.3. Sediment sampling, processing and analyses

At each station, samples of surface sediment (0–5 cm) were collected by SCUBA divers using plastic tube corers (8.1 cm i.d., 51.5 cm²). Onboard, the overlying water was removed and the redox potential was measured in the top 1 cm using a portable redox-meter (Mettler Toledo). In addition, a small syringe core (1.13 cm² × 1 cm depth) was taken from the same core for later sediment chlorophyll *a* (a proxy of microalgae biomass) and phaeopigments analysis. The subsample was placed in pre-weighed 15 mL polypropylene tubes and stored at -20°C . Then, the top 5 cm fraction of sediment was transferred into an acid-cleaned Nalgene® jar and transported on ice to the laboratory for further analysis of sediment Cu using atomic absorption spectrometry. Certified reference material (MESS-3) (National Research Council, Canada) was used for quality control (recovery ~94%) (Table 1).

“Surface” (50 cm below the surface) water was collected at each site with acid-washed 250 mL Nalgene® bottles. For the determination of dissolved Cu, 200 ml of seawater was filtered through a pre-weighed polycarbonate membrane (0.45 μm pore size, 47 mm diameter) using an acid-cleaned Nalgene® analytical filter unit. All filtered water samples were acidified to pH <2 with Optima grade HNO₃ (Fisher) and stored until analysis. Each filter containing suspended particulate matter was stored in a pre-cleaned Petri dish and kept frozen inside a Ziploc® bag. Porewater was extracted by centrifuging ~30 cc of homogenized sediment in polypropylene

Table 1

Analysis of the certified standards for seawater (CASS-4), sediment (MESS-3), and tissue (DOLT-2). The 95% confidence interval is given in parenthesis.

Certified standard	Certified value	Measured value	% Recovery	Detection limit	N
For seawater: CASS-4	0.59 (±0.06) μg L ⁻¹	0.54 (±0.02) μg L ⁻¹	90.7 (±3.9)	0.05 μg L ⁻¹	6
For sediment: MESS-3	33.90 (±1.60) mg kg ⁻¹	31.88 (±1.17) mg kg ⁻¹	94.0 (±3.4)	0.03 mg kg ⁻¹	6
For tissue: DOLT-2	25.8 (±1.1) mg kg ⁻¹	22.75 (±2.51) mg kg ⁻¹	88.2 (±5.20)	0.002 mg kg ⁻¹	3

tubes at 4000 rpm for 10 min. The supernatant was filtered through a 13 mm diameter, 0.45 μm pore size PTFE filter. The filtered pore water (~5 mL) was acidified to pH <2 with Optima grade HNO₃ and stored until analysis of dissolved Cu. Cu was measured using a Varian 880Z graphite furnace atomic absorption spectrometer (GFAAS). Accuracy and precision were assessed by analysis of certified coastal seawater standards (CASS-4) (National Research Council, Canada) (recovery ~91%) (Table 1). OM was determined on ~30 g dried, homogenized sediment by mass loss after combustion at 500 °C for 4 h (Byers et al., 1978). For grain size analysis, ~100 g of sediment were mixed with 25 mL of 30% hydrogen peroxide to remove the OM. The slurry was allowed to stand for 3–4 days until no trace of OM was left. After adding distilled water, the mixture was sieved wet through a 63-μm mesh sieve. Both fractions of the sample (>63 and <63 μm) were dried at 60 °C and weighed to determine percent sand and silt-clay, respectively. Sediment chlorophyll *a* (chl *a*) and phaeopigments, were determined spectrophotometrically (Thermo Spectronics, Genesis 20) from freeze-dried sediment (Hagerthey et al., 2006) after extraction with 90% acetone. The sum of chl *a* and phaeopigments is referred as chloroplastic pigment equivalent (CPE) (Pfannkuche and Soltwedel, 1998).

2.4. Macrofauna collection and processing

At each site, sediment cores were collected by SCUBA divers with plexiglass tubes (5.1 cm i.d; 20.4 cm²) for analysis of macrofauna. Immediately after collection, the uppermost 5 cm were extruded and transported in plastic jars to the laboratory. Samples were fixed in 8% buffered formaldehyde solution with rose Bengal stain. In the laboratory, sediment samples were sieved on a 0.3 mm mesh sieve, and the retained invertebrates were sorted in fresh water under a dissecting microscope. Specimens were counted and identified to the lowest taxon possible, with putative species designated for diversity calculations. Wet weighed biomass of each species was determined on a Sartorius[®] analytical balance.

2.5. Cu concentration in invertebrates

Sediments were collected in Ziploc[®] bags by SCUBA divers, and transported in an insulated container to SIO. Sediment was sieved on 300 µm mesh while washed with filtered seawater. Animals were picked from the sediment under a dissecting microscope and placed into Petri dishes with filtered seawater for 24–36 h to allow the gut to empty. Animals were identified to the lowest taxonomic level (mostly species). When necessary, smaller, same-species individuals from the same Cu-category sediment, though from different stations, were combined to obtain the critical mass for analysis. Because most of the animals were of small size, the whole animal was used for analysis. Soft parts of larger organisms (e.g. bivalves, gastropods) were dissected from the shell. The animals and tissue were carefully rinsed with MilliQ water and placed into acid-washed, pre-weighed polypropylene vials, dried at 60 °C, and reweighed prior to digestion and metal analysis. At each sample (1–15 mg DW), 500 µL of 45% ultra pure nitric acid (Optima, Fisher) was added. The digestion was made in a microwave oven (Ethos One, Milestone Inc.) at 80 °C for 4 h (15 min to reach 80 °C, 3 h at 80 °C, 20 min ventilation). The resulting residue was diluted with 5 mL MilliQ water, gently shaken, and allowing to rest for 48 h. Clear supernatant was transferred into ICPMS polystyrene tubes and measured with a ICP-OES Perkin Elmer Optima 3000 DV analyzer. For accuracy and precision additional blanks were prepared, and an identical exercise was performed using DOLT-2 standard (National Research Council of Canada) (~90% recovery) (Table 1).

2.6. Data analysis

Correlations among the investigated environmental variables and community parameters (abundance, biomass, diversity) were evaluated with the non-parametric Spearman's rank correlation coefficient (Zar, 1996). Total abundance (*N*), biomass, as well as diversity indices such as Pielou's evenness (*J'*), Shannon-Wiener diversity index ($H' \log_{10}$) and Rank1 dominance (the proportion of the most abundant species), were calculated to describe macrofaunal assemblage structure. Species richness was examined as a function of area (species per core) (*S*), and as a function of the number of individuals via rarefaction curves (Hurlbert, 1971). The differences between sites were tested applying one-way ANOVA. The post hoc HSD-Tukey (Honest Significant Difference) test was applied when differences were significant ($p < 0.05$; Sokal and Rohlf, 1997). Data were tested for normality, and when necessary, square root transformed. Univariate analyses were performed using the software package JMP 6.0.3. Given the fact that only one basin was studied, we acknowledged that all of our within-basin samples, grouped into zones are effectively pseudoreplicates (*sensu* Hurlbert, 1984). This may limit the inferences that can be made to SIYB.

Feeding modes were evaluated by assigning each species to one of the following categories: as surface-deposit feeders (SDF),

subsurface-deposit feeders (SSDF), omnivores and carnivores according to information in the literature for higher taxa, families, or genera (e.g. Fauchald and Jumars, 1979; Lastra et al., 1991; Rakocinski et al., 1997; Levin et al., 2006; Shields and Hughes, 2009).

Differences in the assemblages of taxa among sites of "high", "medium" and "low" Cu were examined using non-metric multidimensional scaling analysis (nMDS), based on Bray-Curtis similarity indices. Stress values indicate how well the solution (2-dimensional MDS plot) reflects high-dimensional relationships among samples. Values <1 are good and <0.2 are useful (Clarke, 1993). Data were double square root transformed to reduce the influence of disproportionately abundant taxa. Pairwise comparisons for significant differences in macrofaunal composition between sites were made using analysis of similarity (ANOSIM) (Clarke, 1993). Analysis of macrofaunal dissimilarities between sites and similarities within-groups was carried out using similarity percentage (SIMPER). A Principal Component Analysis (PCA) was used for the ordination of species based on Cu concentrations in tissue. These multivariate analyses were performed using PRIMER 5.2.2 (Plymouth marine Laboratory, Clarke, 1993; Clarke and Warwick, 1994).

A suite of ordination and gradient analysis routines were used to assess variation in assemblage structure, explore multivariate relationships between macrofaunal community structure and environmental variables (ter Braak and Šmilauer, 1998), and identify prospective indicator species of Cu contamination. As a classification method, Detrended Correspondence Analysis (DCA) was performed to determine the appropriate response model for the invertebrate data. DCA searches for major gradients in the species data irrespective of any environmental variables (ter Braak and Prentice, 1988). The length of the gradient derived from DCA can help to determine which method of direct gradient analysis is most appropriate to use on the dataset (Lepš and Šmilauer, 2003). The DCA performed on the invertebrate data indicated that the longest gradient was less (shorter) than 3.0 standard deviations; therefore we used Redundancy Analysis (RDA), the linear method of direct gradient analysis (Van Wijngaarden et al., 1995; ter Braak and Šmilauer, 2002). When the longest gradient is larger than 4.0, unimodal methods such as CCA are more appropriate (ter Braak and Šmilauer, 2002).

Partial RDA (pRDA) was used to separate the effects of explanatory variables from those of covariables (Lepš and Šmilauer, 2003). In our pRDA analysis, we included natural variables with a highly significant effect on community variation as covariables. In the case of pRDA, the sum of all canonical eigenvalues accounts only for the effects of the Cu species but the inclusion of the other covariables allows for the calculation of variance partitioning (Lepš and Šmilauer, 2003).

Ordination and gradient techniques, in their graphical form, can also aid in the identification of any potential invertebrate indicator species (Kremen, 1992). A TWINSpan (Two Way Indicator Species Analysis) was performed to complement and facilitate the interpretation of the RDA triplot.

A forward stepwise selection procedure was applied to select a set of explanatory variables (acceptance level: $p < 0.05$), which could account for the maximum variation in the faunal data, to be used in the ordination model (ter Braak, 1988; ter Braak and Šmilauer, 1998). A Monte Carlo permutation tests (999 permutations) was used to determine statistical significance of the species–environmental relationships (ordination axes). Species were square root transformed, while environmental variables were automatically centered and standardized by the CANOCO software (Jongman et al., 1995). The Monte Carlo permutation model allowed the selection of the most important environmental variables to perform the final RDA analysis. 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 data set using the environmental data as predictor variables. Regression trees handle a single response variable and multiple explanatory predictors (Merler et al., 1996) without data transformation and produce decision trees to display class memberships by recursively binary partitioning of the data set into subsets (also called nodes) that are successively more and more homogeneous in the values of the response variable (De'ath and Fabricius, 2000; Sutton, 2005). How each node is split into two sub-nodes is analogous to variable selection in regression. The split that maximizes the homogeneity and the difference between the resulting two subgroups is then selected. Regression and classification trees have been used previously in Southern California bays to model halibut fish nursery habitats and the influence of environmental variables (Fodrie and Mendoza, 2006). Regression trees were modeled using Statistica v.8 (Statsoft, 2008).

3. Results

3.1. Basin environmental properties

Patterns of Cu distribution and environmental parameters measured in this study were similar to those measured in spring 2006 and confirmed the longitudinal gradient of increasing Cu both in water and sediment. Dissolved Cu in surface water averaged $8.0 \pm 0.4 \mu\text{g L}^{-1}$, exceeding the EPA water quality criteria of $3.1 \mu\text{g L}^{-1}$. Sediment Cu showed a clear gradient from outside to the head of the basin, ranging on average from 18.9 mg kg^{-1} at the reference sites to 236.1 mg kg^{-1} at the “high” Cu sites, with an absolute peak concentration of 442 mg kg^{-1} . Pigment content in sediment, as CPE (sum of chl *a* and phaeopigments) ranged from 7.1 to $31.4 \mu\text{g g}^{-1}$, being it highest at “medium” and “high” Cu sites. Same picture was observed for mud content and OM, while the inverse occurred for sand content. Sediment redox potential was lower at sites with higher Cu, and inversely correlated with OM and pigments (Neira et al., 2009). A summary of the environmental properties measured in spring 2007, representing the different designated sites from outside to the head of the basin, is presented in Table 2. Details of the spatial distribution of Cu species and their relationships with sediment properties and number of boats are given in Neira et al. (2009).

3.2. Macrofaunal density, biomass and body size

Mean total macrofaunal densities did not vary significantly (Wilcoxon $\chi^2 = 3.3$, $df = 3$, $P = 0.340$) among designated sites, ranging from $94.4 (\pm 1 \text{ SE } 27.9)$ to $212 (\pm 1 \text{ SE } 57.2)$ ind 20.4 cm^{-2} core ($46,274\text{--}103,921$ ind m^{-2}) (Table 3). The lowest absolute densities (37 ind 20.4 cm^{-2}) core were found at the head of the basin. In contrast, mean total biomass was an order of magnitude lower (biomass: $42.8 \pm 1 \text{ SE } 7.9 \text{ mg } 20.4 \text{ cm}^{-2}$ core) at sites with “high” Cu

than those of “low” Cu and reference sites (biomass: $495.7 \pm 1 \text{ SE } 88.6 \text{ mg } 20.4 \text{ cm}^{-2}$ core) (Wilcoxon $\chi^2 = 18.74$, $df = 3$, $P = 0.0003$) (Table 4). The same occurred for the average body size (individual biomass), which was 8.5 mg at reference sites versus 1.1 mg at the “high” Cu sites (Wilcoxon $\chi^2 = 21.53$, $df = 3$, $P < 0.0001$).

3.3. Composition and diversity

A total of 48 taxa were identified (Table 3), with annelid polychaetes (16 families) (Fig. 2A) and peracarid crustacean (amphipods, isopods, ostracods, cumaceans) (Fig. 2B) as the dominant groups (overall >77% of total). Other minor taxa present included Mollusca, Cnidaria, Turbellaria, Nemertea, Phoronida, and Holothuria (overall <9.8%). Tubificid oligochaetes were numerically important outside and at the head of the basin (Table 3). Among the polychaete families, there was a higher family richness at “low” Cu and reference sites than in sites with higher Cu (Fig. 2A). Syllids were the dominant group overall, with enhanced percent contribution in the “high” Cu sites. In contrast, the percent contribution of spionid, orbinid and capitellid polychaetes was lower at “high” Cu sites (Fig. 2A). Peracarid crustaceans were the most affected when sediment Cu was high (Fig. 2B). The number of amphipods was reduced to only 4 species at “high” Cu sites, with *Caprella californica* and *Grandidirella japonica* being the dominant (~93% of the total crustaceans) while at “low” Cu and reference sites, 10 species were present, in relatively equitable proportion (Fig. 2B).

Samples from sites with different Cu levels exhibited consistent composition differences with respect to both density (MDS, Fig. 3A) and biomass (MDS, Fig. 3B). Communities of the “high” sediment Cu sites differed significantly from those of the “medium” sediment Cu sites (ANOSIM, $P = 0.004$), “low” Cu sites (ANOSIM, $P = 0.002$), and reference area (ANOSIM $P = 0.036$). Within-site assemblage similarity was highest outside the basin (SIMPER, 76.1% similarity) and the lowest at the “high” Cu sites (SIMPER 47.6% similarity). Assemblage dissimilarities were highest between “high” and “low” Cu sites (SIMPER 69.1% dissimilarity) (Fig. 3A). Differences were driven by enhanced abundances of *Exogone lourei*, *Tubificoides*, *C. californica*, and *Leptochelia dubia* at the “high” Cu sites. The changes in assemblage composition based on biomass were even more noticeable, with communities of the “high” Cu sites differing from those of the “medium” ANOSIM $P = 0.001$, “low” (ANOSIM $P = 0.001$), and reference sites (ANOSIM $P = 0.018$) (MDS, Fig. 3B). Dissimilarities between “high” Cu assemblages and those of “medium”, “low”, and reference, were 67.4%, 80.2% and 80.9% (SIMPER), respectively. Reduced biomass of several species of polychaetes, molluscs, and peracarid crustacean at the “high” Cu sites (Table 4) contributed to the high dissimilarities. Within-site assemblage homogeneity was greatest at reference sites outside (SIMPER, 77.7% similarity) and least at the “high” Cu sites (SIMPER, 42.2% similarity).

Table 2

Average (± 1 SE) of concentrations/measurements of environmental variables for designated sites in Shelter Island Yacht Basin. OM = sediment organic matter, Eh = redox potential (mV), chl *a* = chlorophyll *a* ($\mu\text{g g}^{-1}$), Phaeo = phaeopigments ($\mu\text{g g}^{-1}$), CPE = sum of chl *a* and Phaeo ($\mu\text{g g}^{-1}$), DCuSW = dissolved Cu in surface water ($\mu\text{g L}^{-1}$), CuSed = sediment Cu (mg kg^{-1}), DCuPW = dissolved Cu in porewater ($\mu\text{g L}^{-1}$), Cu⁺⁺ SW = free Cu⁺⁺ in surface water (picomolar), Cu⁺⁺ PW = free Cu in porewater (picomolar).

Sites	% OM	% Silt-clay	% Sand	Eh	Chl <i>a</i>	Phaeo
“High” Cu	5.1 (0.9)	57.1 (10.5)	42.9 (10.5)	-77.8 (26.5)	5.8 (1.5)	23.8 (5.8)
“Medium” Cu	6.3 (0.5)	50.8 (14.0)	49.2 (14.0)	-101.6 (18.5)	6.4 (3.5)	24.9 (4.5)
“Low” Cu	3.9 (0.5)	55.3 (6.9)	44.7 (6.9)	-32.9 (28.3)	3.8 (0.7)	13.5 (2.5)
Reference	1.3 (0.2)	8.8 (0.3)	91.2 (0.3)	-63.0 (11.0)	3.3 (0.2)	3.8 (0.9)
Sites	CPE	DCuSW	CuSed	DCuPW	Cu ⁺⁺ SW	Cu ⁺⁺ PW
“High” Cu	29.7 (7.3)	8.8 (0.5)	236.1 (40.9)	7.8 (0.7)	9.35 (0.5)	0.27 (0.1)
“Medium” Cu	31.4 (5.8)	7.9 (0.5)	183.2 (19.6)	7.3 (0.3)	9.30 (1.0)	0.34 (0.2)
“Low” Cu	17.3 (3.1)	8.5 (0.2)	111.5 (25.0)	6.8 (0.6)	9.15 (0.3)	0.89 (0.3)
Reference	7.1 (1.1)	3.8 (0.5)	18.9 (2.7)	7.2 (0.1)	5.40 (0.2)	1.56 (0.7)

Table 3
Mean density (No individuals 20.4 cm⁻² core) ±1 SE of macrofaunal taxa at sites along Shelter Island Yacht Basin representing designed sites of “high”, “medium”, and “low” Cu levels. Two sites outside the basin served as reference.

Taxa	“High” Cu			“Medium” Cu			“Low” Cu”			Reference		
	Average	S.E.	%	Average	S.E.	%	Average	S.E.	%	Average	S.E.	%
<i>Tubificoides</i> spp.	28.1	12.1	13.3	1.9	0.6	1.5	2.8	1.4	2.9	23.0	5.0	18.9
<i>Pseudopolydora paucibranchiata</i>	7.9	2.0	3.7	5.4	2.6	4.5	5.6	3.6	6.0	0.5	0.5	0.4
<i>Polydora nuchalis</i>	5.0	1.5	2.4	2.0	1.2	1.6	0.6	0.7	0.7	0.0	0.0	0.0
<i>Prionospio</i> sp.	0.7	0.3	0.3	1.0	0.4	0.8	1.9	0.9	2.0	2.0	1.0	1.6
<i>Exogone lourei</i>	76.1	31.0	35.9	12.1	6.2	10.0	6.4	5.5	6.8	23.0	13.0	18.9
<i>Odontosyllis cf. phosphorea</i>	1.4	0.8	0.7	5.9	2.9	4.8	2.9	2.1	3.0	1.0	0.0	0.8
<i>Sphaerosyllis cf. californiensis</i>	4.8	2.7	2.3	1.0	0.6	0.8	1.1	1.0	1.2	3.5	0.5	2.9
<i>Scoloplos</i> sp.	3.8	1.0	1.8	3.7	1.5	3.1	4.3	1.6	4.5	8.5	2.5	7.0
<i>Diplocirrus</i> sp.	0.0	0.0	0.0	0.3	0.2	0.2	0.9	0.6	0.9	1.5	0.5	1.2
<i>Mediomastus</i> sp.	1.3	0.5	0.6	0.6	0.2	0.5	3.0	1.2	3.2	9.5	1.5	7.8
<i>Capitella</i> spp.	0.8	0.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.4
<i>Anaitides</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0
<i>Eteone californica</i>	0.3	0.2	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cossura</i> sp.	3.7	2.3	1.7	0.4	0.3	0.4	0.5	0.3	0.5	0.0	0.0	0.0
<i>Tharyx</i> sp.	0.1	0.1	0.1	0.0	0.0	0.0	0.5	0.2	0.5	0.0	0.0	0.0
<i>Cirratulus</i> sp.	6.4	4.2	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cirriformia spirobrachia</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.5	0.5	3.7
<i>Clymenella californica</i>	0.7	0.7	0.3	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0
<i>Nereis procera</i>	0.4	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Lumbrineris</i> sp.	1.6	0.5	0.7	1.3	0.7	1.1	2.5	0.8	2.6	0.5	0.5	0.4
<i>Euchone limnicola</i>	6.2	2.1	2.9	10.9	4.4	8.9	3.3	1.5	3.4	1.5	1.5	1.2
<i>Nephtys cornuta</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.5	2.0
<i>Dorvillea rudolphi</i>	4.1	1.0	1.9	0.4	0.2	0.4	0.6	0.6	0.7	0.0	0.0	0.0
<i>Ophelia limacina</i>	0.1	0.1	0.1	0.0	0.0	0.0	0.5	0.3	0.5	1.5	0.5	1.2
Sphaerodoridae	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0
<i>Musculista senhousia</i>	0.0	0.0	0.0	2.0	1.7	1.6	1.1	1.3	1.2	0.0	0.0	0.0
<i>Macoma</i> sp.	0.2	0.1	0.1	0.0	0.0	0.0	0.5	0.3	0.5	1.5	0.5	1.2
<i>Lyonsia californica</i>	0.0	0.0	0.0	0.3	0.2	0.2	0.6	0.4	0.7	0.0	0.0	0.0
Gastropoda	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.0	0.0	0.0
<i>Corophium</i> spp.	0.0	0.0	0.0	0.1	0.1	0.1	5.5	1.6	5.8	2.5	0.5	2.0
<i>Grandidierella japonica</i>	16.8	5.8	7.9	40.1	13.8	33.1	9.8	3.0	10.3	8.0	5.0	6.6
<i>Podocerus</i> sp.	0.0	0.0	0.0	10.1	2.7	8.4	4.6	1.2	4.9	3.5	0.5	2.9
<i>Harpinia</i> sp.	1.0	0.8	0.5	1.3	0.6	1.1	3.3	1.1	3.4	3.0	1.0	2.5
<i>Aoroides</i> sp.	0.0	0.0	0.0	0.9	0.9	0.7	4.8	1.2	5.0	2.0	1.0	1.6
<i>Amphithoe</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	3.4	0.9	3.6	2.0	1.0	1.6
<i>Elasmopus</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.4	1.9	2.5	0.5	2.0
<i>Ampelisca</i> sp.	0.0	0.0	0.0	0.1	0.1	0.1	2.8	1.0	2.9	1.5	0.5	1.2
<i>Caprella californica</i>	14.3	3.6	6.8	7.7	3.1	6.4	12.9	12.9	13.6	1.0	1.0	0.8
<i>Leptochelia dubia</i>	15.6	8.3	7.3	1.3	0.8	1.1	0.3	0.3	0.3	2.5	1.5	2.0
<i>Heteroserolis</i> sp.	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.4
<i>Paranthurus elegans</i>	1.3	0.8	0.6	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0
Ostracoda	0.8	0.3	0.4	0.7	0.4	0.6	0.4	0.3	0.4	2.5	0.5	2.0
Cumacea	0.6	0.2	0.3	0.1	0.1	0.1	0.4	0.3	0.4	2.0	1.0	1.6
Cnidaria	5.1	2.0	2.4	9.0	2.6	7.4	3.9	1.6	4.1	3.0	1.0	2.5
Tubellaria	0.6	0.3	0.3	0.6	0.3	0.5	0.1	0.1	0.1	0.0	0.0	0.0
Nemertea	0.2	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.5	0.5	0.4
<i>Phoronis</i> sp.	1.8	1.8	0.8	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0
Holothurida	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.0	0.0	0.0
Total	212.0	57.2	100.0	121.4	20.0	100.0	94.4	27.9	100.0	122.0	25.0	100.0
No m ⁻²	103921.6	28031.4		59523.8	9790.3		46262.3	13693.1		59803.9	12256.8	

Average species richness ($S = 27.5$) per 20.4 cm⁻² core as well as diversity ($H' = 1.25$) was greatest at sites with lowest Cu ($S = 27.5$, $H' = 1.25$, respectively), and lowest at sites with “medium” and “high” Cu ($S = 14.9$ and 16.6, respectively) (Table 5), while evenness (J') was not significantly different. Rank 1 dominance (the proportion of the most abundant species) was greatest in sites with “medium” and high Cu levels (Table 5). Rarefaction diversity was greatest at reference and “low” Cu sites, and lowest at the “medium” and “high” Cu sites (Fig. 4).

3.4. Macrofaunal feeding modes and lifestyles

The dominant macrofaunal organisms in the SIYB were surface-deposit feeders (SDF), with a presence of more than 50% at “medium” and “low” sediment Cu sites, followed by subsurface-deposit feeders and omnivores. Carnivores contributed only a small percentage of the total (Fig. 5A). However, the proportional represen-

tation of each feeding mode varied between the designated sites. For instance, omnivores were dominant ($X^2_4 = 15.6$; $P = 0.0036$) at the sites of “high” Cu, with about 50% contribution; in contrast, subsurface-deposit feeders (SSDF) were dominant at the reference sites ($F_{3,25} = 3.5$, $P = 0.032$) (Fig. 5A). Mobile fauna were well represented (>50%) at all sediment Cu sites. Burrowers contributed over 30% at the reference sites. Tube builders were fairly equally represented at all sites within the basin (~21%), while their contribution was reduced (~12%) at the reference sites outside the basin (Fig. 5B). Species' feeding modes and lifestyles are given in Appendix A, Table A1.

3.5. Macrofauna in relation to the environment

Spearman rank correlation analysis was used as a first approach to explore possible relationships between the environmental variables and the univariate measures derived from macrofaunal data.

Table 4

Mean biomass (mg 20.4 cm⁻² core) ± 1 SE of macrofaunal taxa at sites along Shelter Island Yacht Basin representing designated sites of “high”, “medium”, and “low” Cu levels. Two sites outside the basin served as reference.

Taxa	“High” Cu			“Medium” Cu			“Low” Cu			Reference		
	Average	S.E.	%	Average	S.E.	%	Average	S.E.	%	Average	S.E.	%
<i>Tubificoides</i> spp.	2.04	2.31	4.8	0.10	0.03	0.1	0.16	0.07	0.0	1.44	0.31	0.3
<i>Pseudopolydora paucibranchiata</i>	5.15	6.21	12.0	1.78	0.68	2.3	3.12	1.67	0.9	1.29	1.29	0.3
<i>Polydora nuchalis</i>	2.84	3.10	6.6	0.54	0.36	0.7	0.38	0.38	0.1	0.00	0.00	0.0
<i>Prionospio</i> sp.	0.48	0.59	1.1	2.80	1.05	3.6	3.96	2.54	1.2	3.79	1.90	0.8
<i>Exogone lourei</i>	2.27	2.72	5.3	2.33	0.56	3.0	0.86	0.68	0.3	2.76	1.56	0.6
<i>Odontosyllis cf. phosphorea</i>	2.23	2.75	5.2	13.35	8.63	17.0	3.89	2.38	1.2	29.74	0.00	6.0
<i>Sphaerosyllis cf. californiensis</i>	0.10	0.12	0.2	0.02	0.01	0.0	0.03	0.02	0.0	0.43	0.33	0.1
<i>Scoloplos</i> sp.	2.86	3.20	6.7	2.08	0.78	2.6	36.19	8.33	11.0	39.27	7.93	7.9
<i>Diplocirrus</i> sp.	0.00	0.00	0.0	0.81	0.55	1.0	9.30	5.38	2.8	16.52	5.51	3.3
<i>Mediomastus</i> sp.	1.41	1.74	3.3	2.29	1.14	2.9	2.80	1.00	0.8	14.31	2.26	2.9
<i>Capitella</i> spp.	0.48	0.59	1.1	0.00	0.00	0.0	0.00	0.00	0.0	1.18	1.18	0.2
<i>Anaitides</i> sp.	0.00	0.00	0.0	0.00	0.00	0.0	0.31	0.31	0.1	0.00	0.00	0.0
<i>Eteone californica</i>	0.38	0.22	0.9	0.65	0.65	0.8	0.00	0.00	0.0	0.00	0.00	0.0
<i>Cossura</i> sp.	0.09	0.08	0.2	0.01	0.01	0.0	0.02	0.01	0.0	0.00	0.00	0.0
<i>Tharyx</i> sp.	0.00	0.00	0.0	0.00	0.00	0.0	0.88	0.51	0.3	0.00	0.00	0.0
<i>Cirratulus</i> sp.	1.57	1.94	3.7	0.00	0.00	0.0	0.00	0.00	0.0	0.00	0.00	0.0
<i>Cirriformia spirobrachia</i>	0.00	0.00	0.0	0.00	0.00	0.0	0.00	0.00	0.0	147.47	16.39	29.7
<i>Clymenella californica</i>	0.04	0.05	0.1	0.00	0.00	0.0	0.51	0.51	0.2	0.00	0.00	0.0
<i>Nereis procera</i>	2.56	3.16	6.0	0.00	0.00	0.0	0.00	0.00	0.0	0.00	0.00	0.0
<i>Lumbrineris</i> sp.	0.69	0.55	1.6	3.07	1.59	3.9	6.40	3.19	1.9	0.59	0.59	0.1
<i>Euchone limnicola</i>	0.25	0.17	0.6	4.87	1.73	6.2	4.57	1.52	1.4	0.54	0.54	0.1
<i>Nephtys cornuta</i>	0.00	0.00	0.0	0.00	0.00	0.0	0.00	0.00	0.0	28.08	0.33	5.7
<i>Dorvillea rudolphi</i>	2.16	2.39	5.0	0.37	0.27	0.5	0.72	0.64	0.2	0.00	0.00	0.0
<i>Ophelia limacina</i>	0.00	0.00	0.0	0.00	0.00	0.0	0.06	0.03	0.0	2.87	0.96	0.6
Sphaerodoridae	0.00	0.00	0.0	0.00	0.00	0.0	0.04	0.04	0.0	0.00	0.00	0.0
<i>Musculista senhousia</i>	0.00	0.00	0.0	23.83	11.39	30.3	93.19	93.20	28.2	0.00	0.00	0.0
<i>Macoma</i> sp.	1.12	1.39	2.6	0.00	0.00	0.0	58.30	40.51	17.7	14.63	4.88	3.0
<i>Lyonsia californica</i>	0.00	0.00	0.0	1.33	0.88	1.7	8.99	5.09	2.7	0.00	0.00	0.0
Gastropoda	0.00	0.00	0.0	0.00	0.00	0.0	0.50	0.50	0.1	0.00	0.00	0.0
<i>Corophium</i> spp.	0.00	0.00	0.0	0.02	0.02	0.0	23.88	6.78	7.2	1.84	0.37	0.4
<i>Grandidierella japonica</i>	1.90	2.34	4.4	5.50	1.69	7.0	3.43	0.88	1.0	35.37	13.98	7.1
<i>Podocerus</i> sp.	0.18	0.22	0.4	6.24	2.64	7.9	3.09	0.80	0.9	22.40	3.20	4.5
<i>Harpinia</i> sp.	0.00	0.00	0.0	1.44	0.80	1.8	5.33	0.54	1.6	5.88	1.96	1.2
<i>Aoroides</i> sp.	0.00	0.00	0.0	1.47	1.47	1.9	37.20	9.76	11.3	30.20	15.10	6.1
<i>Ampithoe</i> sp.	0.00	0.00	0.0	0.00	0.00	0.0	6.00	0.70	2.0	6.10	2.95	1.2
<i>Elasmopus</i> sp.	0.00	0.00	0.0	0.00	0.00	0.0	4.04	0.93	1.2	4.26	3.15	0.9
<i>Ampelisca</i> sp.	0.00	0.00	0.0	0.04	0.04	0.0	2.60	0.99	0.8	16.49	5.50	3.3
<i>Caprella californica</i>	3.16	3.74	7.4	1.97	0.80	2.5	4.89	3.71	1.5	0.34	0.34	0.1
<i>Leptochelia dubia</i>	0.70	0.86	1.6	0.14	0.09	0.2	0.03	0.03	0.0	2.47	1.06	0.5
<i>Heteroserolis</i> sp.	2.22	2.75	5.2	0.00	0.00	0.0	0.00	0.00	0.0	50.05	50.06	10.1
<i>Paranthurus elegans</i>	1.56	1.93	3.6	0.00	0.00	0.0	0.06	0.06	0.0	0.00	0.00	0.0
Ostracoda	0.54	0.67	1.3	0.74	0.43	0.9	0.39	0.27	0.1	0.36	0.07	0.1
Cumacea	0.05	0.06	0.1	0.02	0.02	0.0	0.17	0.11	0.1	5.81	2.91	1.2
Cnidaria	0.79	0.98	1.8	0.69	0.17	0.9	1.89	0.82	0.6	8.88	2.96	1.8
Tubellaria	0.70	0.87	1.6	0.16	0.08	0.2	0.03	0.03	0.0	0.00	0.00	0.0
Nemertea	0.19	0.04	0.5	0.00	0.00	0.0	0.11	0.11	0.0	0.45	0.45	0.1
<i>Phoronis</i> sp.	2.08	2.57	4.9	0.00	0.00	0.0	0.14	0.14	0.0	0.00	0.00	0.0
Holothurida	0.00	0.00	0.0	0.00	0.00	0.0	1.05	1.05	0.3	0.00	0.00	0.0
Total	42.78	7.89	100.0	78.63	16.90	100.0	330.09	88.99	100.0	495.73	88.56	100.0
g m ⁻²	20.97	3.87		38.55	8.28		161.81	43.62		243.01	43.41	

Noticeably, number of species and biomass were negatively correlated with sediment Cu and TOM while dominance (R_1) was positively correlated with sediment Cu (Table 6). There was a consistent decrease of macrofaunal biomass with increasing Cu in sediment ($y = -71.02\ln(x) + 432.07$, $r^2 = 0.30$; $P = 0.015$) (Fig. 6).

The forward stepwise selection procedure identified a set of five environmental variables (of the ten measured) that best explained the variation in the species data (free Cu⁺⁺, dissolved Cu in pore water, sediment Cu, TOM, and chl *a*) (Table 7). These variables, which can significantly influence macrofaunal community structure, were used in the ordination models. The RDA yielded four axes that explained 97.5% of the variance in the relationships between macrofaunal community structure and environmental variables, and the species–environmental relationships were highly significant ($P = 0.001$) for all canonical axes (Table 8A). In

the ordination triplot (Fig. 7), there were a few species that clustered together, influenced by sediment Cu and TOM, while the highest species representation was related to descending environmental vectors. Community structure and the RDA axes synthesizing environmental variation were strongly correlated ($R > 0.73$, Table 8A). The removal of the two natural variables (TOM and chl *a*) as covariables using partial RDA, allowed us to decompose (variance partitioning) the total variability into a part that can be explained solely by the influence of the Cu chemical species (variables of interest) on macrofaunal assemblage structure. By using the results of the redundancy analyses (Table 8B), we calculated that 18.3% of the variance of biological species composition was explained by the Cu species (sediment Cu, Cu⁺⁺ in porewater and dissolved Cu in porewater) and 34.4% is explained by the covariables TOM and chl *a* (total of 52.7%); a 47.3% of the variation remained unexplained. All the significant variables accounted for

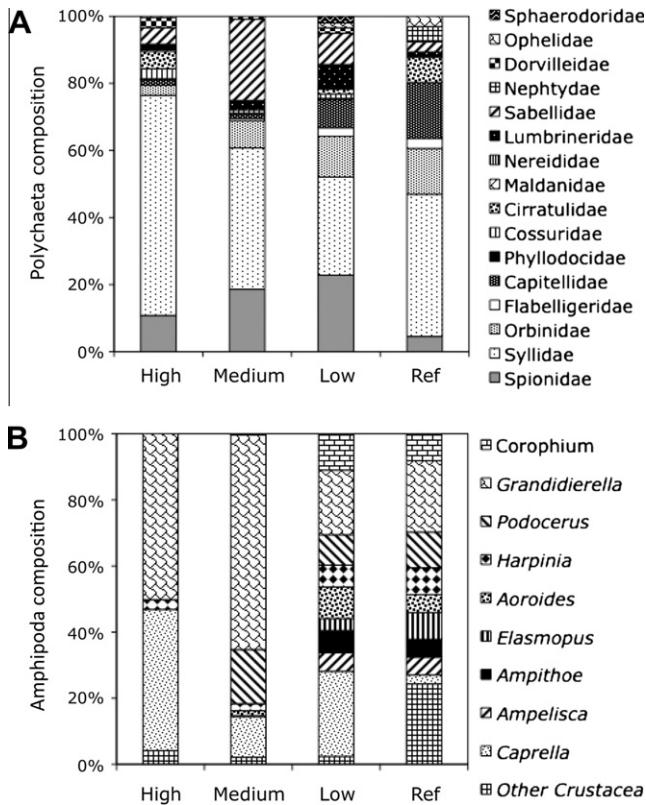


Fig. 2. Percent composition of Polychaeta families (A) and peracarid Crustacea (B), at the different study sites.

52.7% of the variance. The remaining unexplained variance is presumably due to other physical and biological factors such as presence of other pollutants, hydrodynamics, sediment stability, competitive interactions (e.g. predation), bioturbation, recruitment patterns, or natural variability (Oug, 1998).

3.6. Indicator species and regression trees

Species such as *Polydora*, *Pseudopolydora*, *Euchone*, *Lumbrineris* and *Caprella* are associated with high sediment Cu and TOM (clustering together many “high” Cu sites) (see RDA plot, Fig. 7). This suggests they are more tolerant to Cu and hence are prospective “Cu tolerant indicators”. In contrast, we find a group of species that can be considered as “Cu sensitive indicators”, such as the amphipods *Podocerus*, *Corophium*, *Elasmopus*, *Ampithoe* and *Ampelisca*, and the polychaetes *Ophelia*, *Diplocirrus*, and the bivalves *Musculista* and *Macoma*. TWINSpan analysis provides comparable results. Samples were classified into 2 groups at the highest level (Fig. 8), with the spionid *Polydora* as a prospective “Cu tolerant indicator”, while *Podocerus* and *Corophium* represent those sites with “low” Cu and hence we suggest are prospective “Cu sensitive indicators”. Furthermore, the high Cu assemblages were subdivided into a group characterized by *Euchone* and a group characterized by *Odontosyllis* and *Mediomastus* as indicator species (Fig. 8). The lower Cu assemblages, with *Podocerus* and *Corophium* as indicator species was subdivided into a group dominated by *Grandidierella*, *Scoloplos* and *Lumbrineris*, and a group characterized by *Sphaerosyllis*. The results of the RDA confirm the groupings obtained with TWINSpan.

By using regression trees (a non-parametric regression model), we described the dependence of the response of community descriptors (such as macrofaunal biomass) on the environmental variables. Given the strong influence of the pRDA natural variables and to see the role of the Cu explanatory variables in the variance partitioning model, we built regression trees using the variables sediment Cu, dissolved Cu in porewater and free Cu⁺⁺ in porewater. Sediment Cu concentration appears to be the key explanatory variable associated with macrofaunal biomass variation (Fig. 9) while dissolved Cu and free Cu⁺⁺ in porewater were additionally important variables in partitioning biomass and species richness variation. The first partition grouped 65% of the sites with the lowest sediment Cu range (16.1–191.2 mg kg⁻¹) that contained the highest biomass, and 35% of the sites with greatest sediment Cu range (191.2–442.3 mg kg⁻¹) containing the lowest biomass.

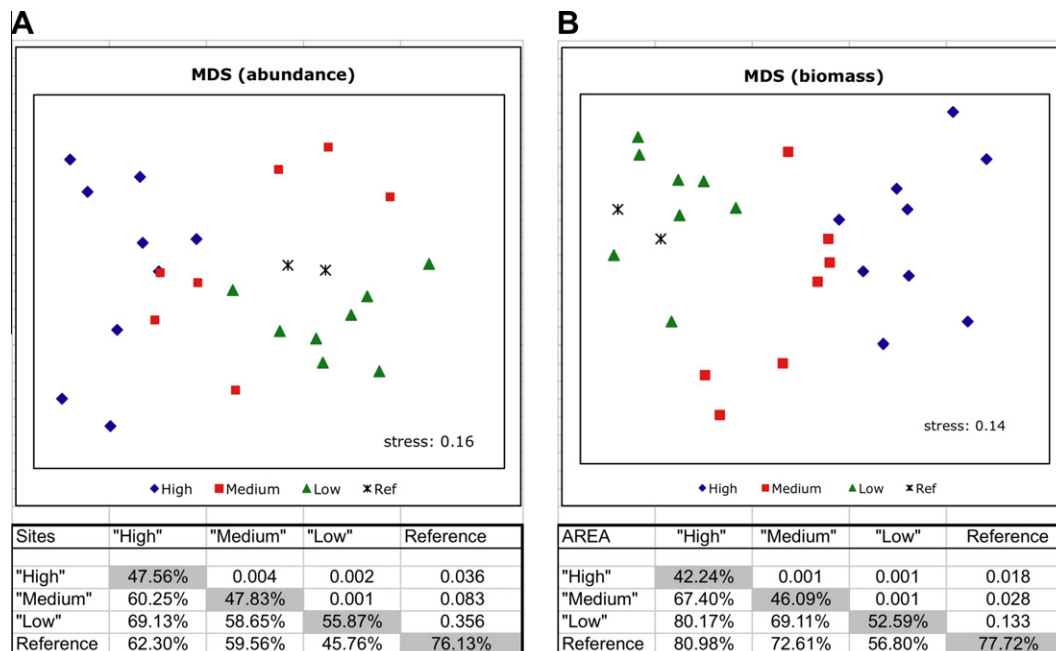


Fig. 3. MDS plots of macrofaunal community, (A) based on abundance composition, (B) biomass composition. The bottom shows comparisons of macrofaunal assemblages in the different study sites. Pairwise one-way Analysis of Similarity (ANOSIM) tests for macrofaunal similarities between sites are given above the diagonal. SIMPER within group similarities are given on the diagonal (dark), and SIMPER percent dissimilarities are given below the diagonal. Significance was set at $\alpha = 0.05$.

Table 5

Mean number of taxa (*S*), abundance (*N*), biomass (*B*), evenness (*J'*), Shannon–Wiener diversity index (*H'*), and rank 1 dominance (R1D) for sites operationally designated as “high”, “medium”, and “low” Cu. Two sites outside the basin served as reference. Significance levels are shown in the bottom of the Table. Contrasts made using a posteriori Tukey HDS tests are indicated with letters (a, b, c).

Sites	<i>S</i>	<i>N</i>	<i>B</i>	<i>J'</i>	<i>H'</i> (log ₁₀)	R1D
“High” Cu	16.6	212.0	42.8	0.75	0.90	32.99
“Medium” Cu	14.9	121.4	78.6	0.72	0.85	37.68
“Low” Cu	20.6	94.4	330.1	0.80	0.98	17.32
Reference	27.5	122.0	495.7	0.83	1.25	21.52
	$F_{3,25} = 8.23$ $P = 0.0007$ $R^a; L^{a,b}; H^{b,c}; M^c$	$\chi^2_3 = 3.3$ $P = 0.340$	$\chi^2_3 = 3.3$ $P = 0.0003$ $R^a; L^a; H^b; M^b$	$\chi^2_3 = 3.85$ $P = 0.278$	$F_{3,25} = 5.56$ $P = 0.0054$ $R^a; L^{a,b}; H^b; M^b$	$F_{3,25} = 4.90$ $P = 0.0093$ $M^a; H^a; R^{a,b}; L^b$

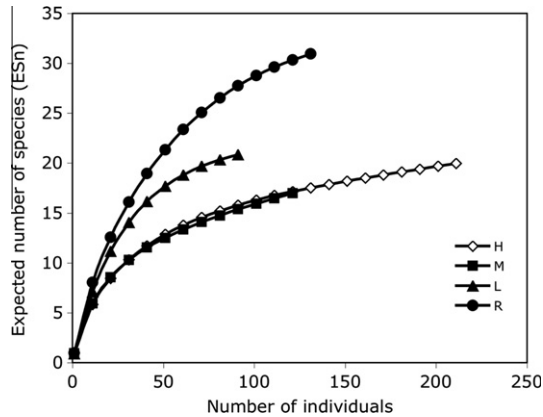


Fig. 4. Comparative rarefaction curves illustrating macrofaunal diversity in SIYB sites of different sediment Cu pollution levels (“high”, “medium”, “low”, and reference).

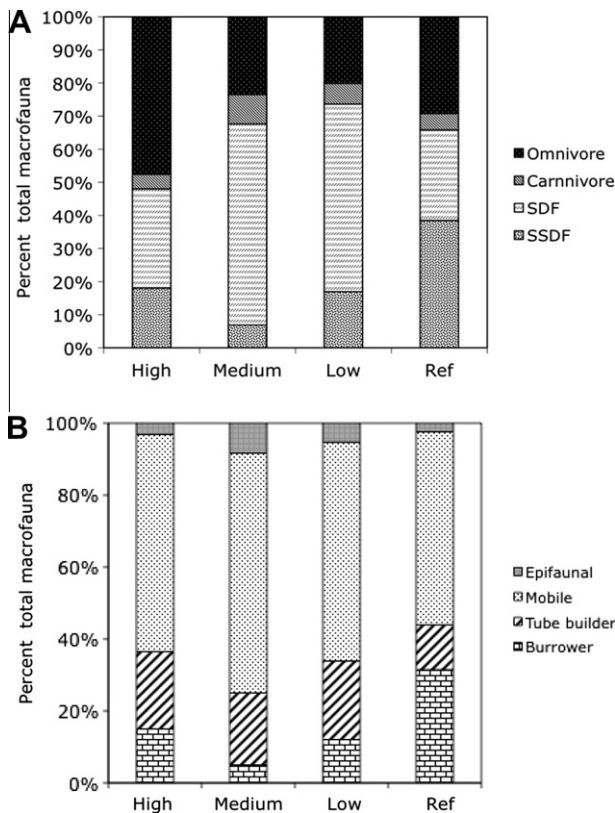


Fig. 5. Macrofaunal feeding modes (A) and lifestyles (B) as a function of Cu in sediment. SDF = surface-deposit feeders; SSDF = subsurface-deposit feeders.

3.7. Cu concentrations in macrofauna

Cu concentrations in the fauna collected at sites of different sediment Cu levels are shown in Table 9. Concentrations ranged from 3.5 to 1712 $\mu\text{g g}^{-1}$. Some species such as the polychaetes *Lumbrineris*, *Scoloplos*, *Euchone*, the oligochaete *Tubificoides*, and the crustaceans *Leptochelia*, *Caprella* and *Paranthurus* showed enhanced Cu content (>4 times the sediment Cu concentration) coinciding with the greatest Cu concentrations in surrounding sediments. In contrast, species such as *Polydora* and *Pseudopolydora*, *Harmothoe*, *Ophelia*, *Exogone*, and *Musculista* had concentrations 2.8–40 times lower than in the “high” Cu sediments. A PCA ordination identified patterns in the data highlighting similarities and differences of the Cu concentration in invertebrates, grouping them in relation to the different sediment Cu levels (“high”, “medium”, “low”, and reference) (Fig. 10). The first two axis of the PCA ordination explained 62.5% of the total variance of the Cu in tissue. The ordination grouped those species with high Cu (e.g. *Lumbrineris*, *Tubificoides*, *Paranthurus*, *Caprella*) associated with the “high” Cu sediments. Some species such as *Ostracoda* sp.1 and *Dorvillea* grouped separately from the other species, as they had the greatest Cu concentrations in their tissues at sites of “medium” and “low” Cu in sediment, respectively.

4. Discussion

4.1. Sediment Cu concentrations in SIYB

Sediment Cu concentrations measured in spring 2007 ranged from 16.1 outside the mouth to 442.3 mg kg^{-1} at the head of the basin (on average $166.8 \pm 1 \text{ SE } 20.9 \text{ mg kg}^{-1}$). These results were consistent with our previous exploratory study conducted in spring 2006, which indicated a strong gradient associated with number and distance of boats in SIYB (Neira et al., 2009). There is evidence that sediment in SIYB acts as a sink for Cu from the water column. In San Diego Bay, about 48% of the Cu input is deposited into the sediment (Chadwick et al., 2004). Prior to the detailed study conducted by Neira et al. (2009) that addressed spatial distribution of sediment Cu and other Cu species, there were few reports on sediment Cu concentration in SIYB. The limited Cu data available suggest that sediment Cu in SIYB may have increased since 1975. In 1975, concentrations of 60–70 mg kg^{-1} were reported (Peeling, 1975). More recently, Valkirs et al. (1994) determined Cu concentrations in 1991 and 1993 ranging from 133 to 212 mg kg^{-1} . Furthermore, three stations examined from 1993 to 1994 showed Cu concentrations ranging from 86 to 150 mg kg^{-1} (SWRCB et al., 1996). Van der Weele (1996) reported Cu concentrations from five stations that ranged from 86.4 to 188.4 mg kg^{-1} . Also, in sediment collected at four stations, Gieskes et al. (2002) found Cu concentrations ranging from just over 34 mg kg^{-1} to >270 mg kg^{-1} . Although it was known that Cu can build up in

Table 6

Spearman's rank correlation coefficients between studied environmental variables and macrofauna data. Significant correlations and level of significance indicated in bold and asterisks, respectively. Cu⁺⁺ PW = free Cu⁺⁺ porewater; DCu PW = total dissolved Cu porewater; CuSed = sediment Cu; TOM = total organic matter; chl *a* = chlorophyll *a*; Phaeo = phaeopigments; CPE = chloroplastic pigment equivalent; SRP = sediment redox potential; DCu SW = total dissolved Cu surface water; Cu⁺⁺ SW = free Cu⁺⁺ in surface water.

Variable	Macrofauna					
	No species	Density	Biomass	H'	J'	Dominance R1
Cu ⁺⁺ PW	0.216	-0.28	0.432*	0.039	0.06	-0.317
DCu PW	-0.057	-0.077	-0.265	-0.253	0.049	0.006
CuSed	-0.389*	0.302	-0.571**	-0.354	-0.299	0.538**
TOM	-0.486*	0.015	-0.446*	-0.283	-0.139	0.339
Chl <i>a</i>	-0.066	0.279	-0.104	-0.247	-0.366	0.447*
Phaeo	-0.209	0.269	-0.261	-0.312	-0.376	0.448*
CPE	-0.187	0.284	-0.245	-0.302	-0.377	0.461*
Mud	-0.139	0.103	-0.227	0.019	0.086	-0.067
Sand	0.139	-0.103	0.227	-0.019	-0.086	0.067
SRP	-0.082	-0.405*	0.078	0.057	0.244	-0.418*
DCu SW	-0.109	-0.047	-0.273	0.146	0.238	-0.279
Cu ⁺⁺ SW	-0.044	-0.003	-0.208	-0.015	0.059	-0.075

* Significance level: $0.01 \leq p < 0.05$.

** Significance level: $0.001 \leq p < 0.01$.

*** Significance level: $p < 0.001$.

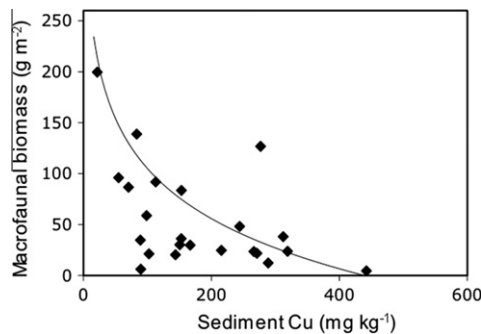


Fig. 6. Relationship between macrofaunal biomass and sediment Cu concentration ($y = -71.02\ln(x) + 432.07$, $r^2 = 0.30$; $P = 0.015$).

Table 7

Results of the forward stepwise selection procedure. Significant variables influencing faunal community structure ($p < 0.05$) are shown in bold.

Environmental variables	F	P
Free Cu ⁺⁺ porewater	2.76	0.001
Dissolved Cu porewater	2.43	0.001
Sediment Cu (solid phase)	2.27	0.004
Total organic matter	2.13	0.006
Sediment chlorophyll <i>a</i>	1.80	0.015
Free Cu ⁺⁺ surface water	1.05	0.432
Dissolved Cu surface water	0.95	0.542
Mud content (<63 μm)	0.76	0.729
sediment phaeopigments	0.72	0.752
Sediment redox potential	0.58	0.903

Table 8A

Redundancy analysis (RDA) describing relationships between community structure and the five environmental variables identified by a forward stepwise selection. The eigenvalue is equivalent to the percent variation explained by each axis and is a measure of the relative importance of the ordination axis. All *P*-values were obtained from Monte Carlo permutation tests.

RDA axis	1	2	3	4
Eigenvalues	0.357	0.085	0.055	0.016
Species–environment correlations	0.902	0.891	0.736	0.782
Cumulative percentage variance of species–environment relation	67.8	83.9	94.4	97.5
Sum of all eigenvalues	1.000			
Sum of all canonical eigenvalues	0.527			
Significance <i>P</i> first canonical axis:	0.001			
Significance <i>P</i> all canonical axes:	0.001			

sediment and persist over time, and that concentrations in some areas of SIYB exceeded the Effect Range Medium of 270 mg kg^{-1} (NOAA, 1999), none of these studies gathered data on benthic community structure. This was recognized by the San Diego Regional Water Quality Control Board (SDRWQCB, 2005), and is addressed in the present study.

4.2. Effects on macrofaunal composition, biomass and diversity

The lower diversity, total biomass and individual biomass (body size) found in assemblages associated with sediments of “high” background Cu contamination, relative to “low” Cu and reference sites assemblages, suggest a linkage to Cu toxicity. Another element that supports the argument for Cu toxicity is the lower total biomass and individual biomass at “medium” and “high” Cu sites. At these sites OM content of sediments is greater than at reference and “low” Cu sites (Table 2). There is a strong positive correlation of OM with sediment Cu (Neira et al., 2009). At sites where low diversity and biomass occur, oxygen concentrations measured in bottom waters are above 6.2 mg L^{-1} ($\sim 4.3 \text{ ml L}^{-1}$) and thus hypoxia is not a confounding factor. Similar oxygen concentrations have been reported previously in SIYB (e.g. Van der Weele, 1996).

Gray (1982) also found trends of reduced average body size within macrofauna in polluted sediments (including Cu). Benthic communities sampled at several sites in Frierfjord/Langesundfjord (Norway) exposed to a gradient of Cu and diesel oil contamination resulted in reduced biomass–size spectra (Schwinghamer, 1988). A correlative study between benthic macrofauna diversity and heavy metal content in sediment, indicated that Cu was more closely

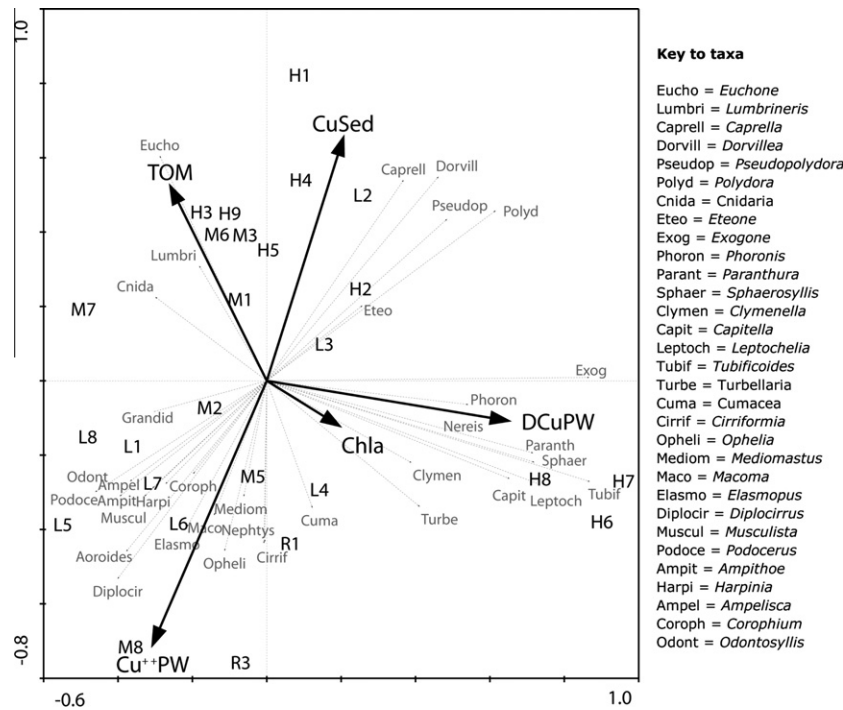


Fig. 7. Redundancy analysis (RDA) triplot displaying the position of macrofaunal species in relation to sediment environmental variables that best explain their distribution among sites. Solid arrows are the environmental vectors representing sediment Cu (CuSed), free Cu⁺⁺ in porewater (Cu⁺⁺ PW), total dissolved Cu in porewater (DCuPW), total organic matter (TOM), and chlorophyll a (chl a); the dashed arrows are the invertebrate taxa. Arrows pointing in the same relative direction are correlated, and longer arrows indicate increasing values. The first two axes explained 83.9% of the variance of species–environment relation and the species–environment relationships were highly significant ($P = 0.001$; 999 permutations in Monte Carlo test).

Table 8B

Partial redundancy analysis (pRDA) describing relationships between macrofaunal community structure and variables of interest after removing effects of the natural covariables.

RDA axis	1	2	3	4
Eigenvalues	0.284	0.035	0.025	0.133
Correlations between community structure and environmental axis	0.887	0.769	0.648	0.000
Cumulative percentage variance of species–environment relation	82.6	92.7	100	0.0
Sum of all eigenvalues	0.817			
Sum of all canonical eigenvalues	0.344			
Significance P all canonical axes:	0.001			

linked to reduction in diversity than were zinc and lead (Rygg, 1985). The lesser macrofaunal biomass and individual biomass (body size), as well as lower diversity occurring in SIYB “high” Cu sites, appear to support Gray’s hypothesis that smaller animals will dominate the macrofauna in stressed communities (Gray, 1982).

Impairment of chemoreception in aquatic animals has been indicated as an important effect of Cu contamination (Carreau and Pyle, 2005; Krång and Ekerholm, 2006; Pyle and Mirza, 2007). Among the potential ecological consequences of impaired chemosensory abilities in invertebrates, is reduced ability to search for and acquire food. For example, reduced response to food odors as well as reduced feeding was detected in crustacean postlarvae after acute exposure to Cu (Santos et al., 2000). Invertebrates have a chemosensory epithelium lining the gut to facilitate post-ingestive feeding which can be blocked by the contaminant, inhibiting the reception of chemical stimuli (Blaxter and Ten Hallers-Tjabbes, 1992; Pyle and Mirza, 2007). Reduction of biomass may also be magnified by indirect toxic effects on food sources such as microphytobenthos, which is intimately linked to sediment OM, the main food source for benthic organisms. In recent microcosm studies, sediments polluted by Cu (10 mM) caused a significant reduc-

tion of total biomass of cyanobacterial microalgae (from 3.8 to 0.4 mg C cm⁻³ of sediment) after 7 days (Burnat et al., 2009).

4.3. Crustacean sensitivity to Cu

Within the benthic community of SIYB, peracarid crustaceans, primarily amphipods, were the most negatively affected by elevated Cu concentrations. A reduced number of crustacean species (Fig. 2B), density (Table 3) and biomass (Table 4) were observed at “high” Cu sites relative to “low” and reference sites. Based on laboratory and field experiments, Stark (1998) reported that crustaceans appeared to be very sensitive to Cu exposure. He found that the controls (untreated with Cu) had greatest abundances of total crustaceans, amphipods and copepods while the least abundances occurred in the Cu treatments. Field experiments to examine effects of Cu on soft sediment fauna indicated that in treatments where Cu was introduced, amphipods and cumaceans had reduced abundances, compared to controls untreated with Cu (Morrisey et al., 1996). Similar effects were observed by Rygg (1985). Amphipods are mobile animals which search for food primarily in uppermost sediment layers, although some of them (e.g. *Corophium*) are also capable of burrowing to search for food items bound to the sediment, such as microalgae and detritus. Interestingly, while we found that there is a substantial reduction in number of amphipod species and their biomass at sites of “high” Cu, at the same time there are other crustaceans such as the amphipods *C. californica* and *G. japonica*, and the tanaid *L. dubia* that apparently cope well at those sites (Fig. 2B).

In contrast, Annelida, and in particular Oligochaeta seem not to be affected by Cu contamination. This pattern has been observed for oligochaetes and nemerteans in laboratory and field experiments (Stark, 1998). In SIYB, polychaetes showed a broad spectrum of responses, as reflected by the polychaete composition at the different sites (Fig. 2A).

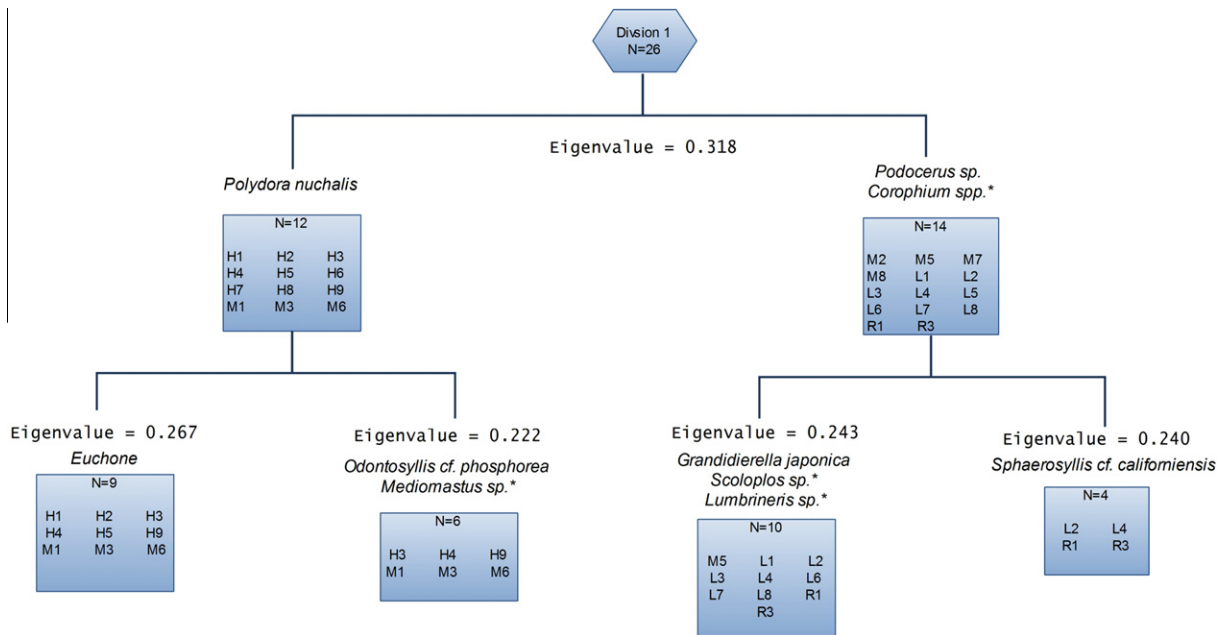


Fig. 8. TWINSpan analysis of benthic macrofaunal assemblages in SIYB based on species occurrences weighted according to abundance. Prospective “Cu tolerant indicators” and “Cu sensitive indicators” are given on the left and right, respectively.

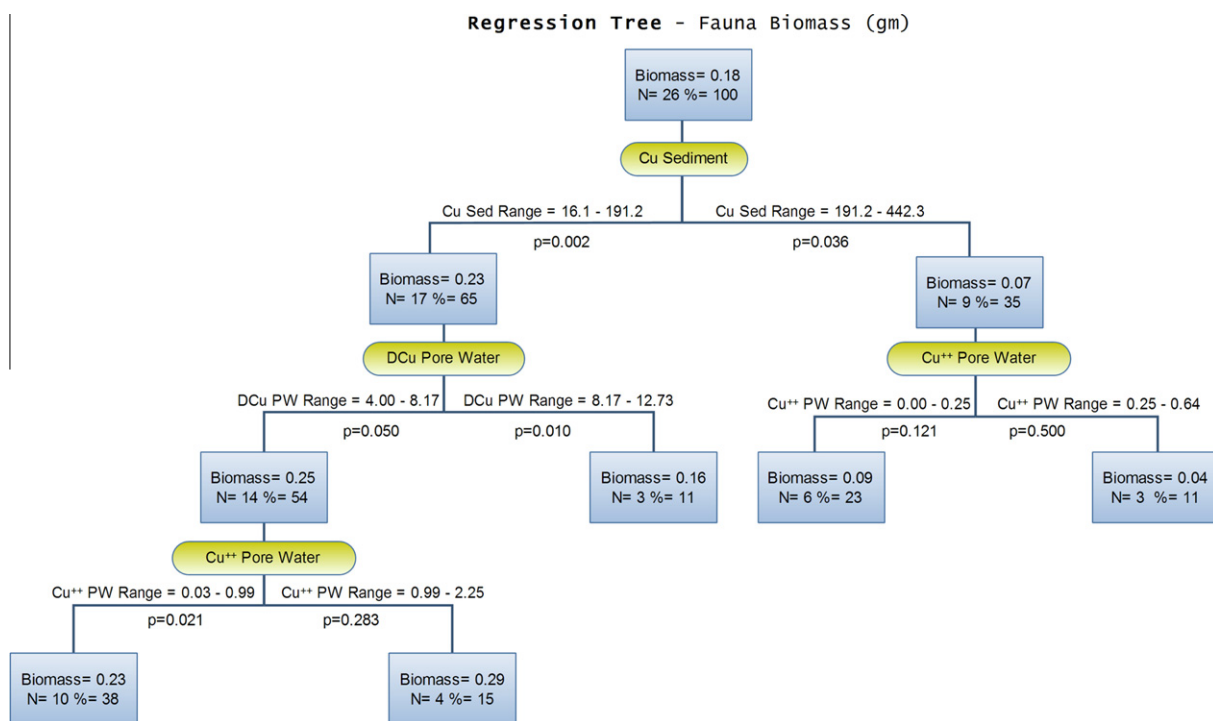


Fig. 9. Regression tree analysis for macrofaunal biomass. Biomass is the single response variables while the multiple explanatory variables (predictors) used were sediment Cu (Cu sediment), free Cu⁺⁺ in porewater (Cu⁺⁺ PW) and total dissolved Cu in porewater (DCuPW). Variables important in explaining variation appear at terminal nodes along with the concentration range, significance P of the split as well as the number and percent of observations in the group.

It has been suggested that coastal invertebrates exposed to high levels of Cu (or heavy metals, in general) are under selective pressure to evolve metal tolerance (Klerks and Weis, 1987; Luoma and Carter, 1991; Hummel and Paternello, 1994). Clearly, SIYB results (Figs. 7, 8, and 10) indicated that some macrobenthic invertebrates were disadvantaged by Cu pollution (e.g. *Podocerus*, *Corophium*, *Aoroides*, *Ampithoe*, *Musculista*) while others were “favored” by it (e.g. *Caprella*, *Grandidierella*, *Polydora*, *Dorvillea*, *Polydora*, *Pseudopolydora*, *Euchone*). This, along with the fact that many species accumulate Cu

differently in their tissues at differing background sediment Cu concentrations (Table 9), suggests that the effects cannot be explained by a single mechanism. There are clearly differing responses to Cu involving toxicity and diverse habitat, lifestyles, feeding habits, behavioral avoidance, degrees of mobility, routes of exposure, and ability to accumulate and detoxify (Rees, 1983; Reish, 1993; Correia et al., 2002; Mardsen and Rainbow, 2004; Wiklund et al., 2006).

Indirect effects of Cu on mobile species such as *Caprella* can occur through changes in species associations and changes in habitat

Table 9

Mean (\pm SE) tissue copper concentrations in Shelter Island Yacht Basin benthic invertebrates species ($\mu\text{g g}^{-1}$ DW) collected in spring 2007 at sites designed according to their copper concentrations in sediments. Ref = reference; (-) = single sample.

Taxon	Sites			Reference
	"High" Cu Average Cu in tissue ($\mu\text{g/g}$)	"Medium" Cu	"Low" Cu	
Polychaeta				
<i>Lumbrineris</i>	1166.5 (330.7)	533.6 (151.9)	504.9 (159.1)	82.6 (29.8)
<i>Exogone</i>	83.4 (-)	20.1 (-)		1.8 (-)
<i>Odontosyllis</i>	21.5 (6.3)	13.3	38.7 (10.5)	
<i>Typosyllis</i>	780.8 (-)			
<i>Harmothoe</i>	19.6 (4.7)	13.3 (-)		
<i>Diplocirrus</i>	216.4 (71.2)	237.9 (-)	278.6 (70.3)	34.2 (10.5)
<i>Pseudopolydora</i>	18.9 (4.3)	15.9 (-)	53.5 (-)	
<i>Polydora</i>	72.8 (19.4)			
<i>Prionospio</i>		101.5 (-)	88.5 (25.6)	199.8 (-)
<i>Dorvillea</i>	217.7 (52.5)	27.9 (-)	614.9 (-)	
<i>Mediomastus</i>	430.6 (124.8)		49.9 (16.7)	89.3 (-)
<i>Scoloplos</i>	310.5 (75.2)	13.1 (-)	76.9 (20.7)	6.5 (1.6)
Cirratulidae	406.4 (96.6)			179.9 (-)
<i>Clymenella</i>	185.1 (53.9)		104.8 (-)	
<i>Euchone</i>	484.7 (-)	122.2 (-)	15.4 (3.5)	
<i>Nereis procera</i>	190.3 (70.2)			
<i>Eteone</i>		88.0 (-)		174.5 (-)
<i>Ophelia</i>	56.7 (-)			
<i>Nephtys</i>				29.5 (6.9)
Oligochaeta				
<i>Tubificoides</i>	1712.2 (-)	202.1 (-)	568.5 (-)	126.0 (-)
Isopoda				
<i>Paranthura</i>	1048.7(-)	268.3 (67.1)		
Amphipoda				
<i>Elasmopus</i>		302.2 (-)	179.8 (-)	272.9 (-)
<i>Harpinia</i>		266 (-)	98.3 (22.7)	147.1 (-)
<i>Grandidierella</i>			35.6 (-)	36.0 (10.9)
<i>Corophium</i>			49.8 (-)	
Caprellidae				
<i>Caprella</i>	448.6 (99.3)	137.0 (31.7)	14.3 (-)	
Tanaidacea				
<i>Leptocheilia</i>	1264.3 (455.2)			584.9 (-)
Cumacea	245.8 (-)		89.9 (-)	
Ostracoda sp.1	582.6 (-)	1655.3 (-)		
Ostracoda sp.2	185.7 (46.1)	9.8		
Decapoda				
Paguridae sp.	586.1 (168.7)			
Gastropoda				202.7 (-)
Bivalvia				
<i>Musculista</i>	5.9 (-)		3.5 (0.8)	
<i>Macoma</i>		1230.2 (-)		91.5 (-)
<i>Lyonsia californica</i>		190.7 (-)		11.5 (-)
Cnidaria				
Anthozoa sp.1	108.9 (-)		98.1 (-)	65.1 (-)
Anthozoa sp.2		378.1 (-)	104.1 (25.8)	24.8 (-)

(Perrett et al., 2006). Reduced feeding ability and reduced colonization was observed in gammarid amphipods fed with Cu-spiked diets (Weeks and Rainbow, 1993; Roberts et al., 2006). In SIYB, the presence of some amphipod genera such as *Caprella* and *Grandidierella* and several polychaete genera (e.g. *Exogone*, *Polydora*, *Pseudopolydora*, *Dorvillea*, *Euchone*) at high Cu sites, suggests some tolerance to Cu through detoxification and excretory processes (Correia et al., 2002; Mardsen and Rainbow, 2004).

Despite the association of Cu concentration with community effects, we acknowledge that it may be difficult to establish categorically cause-effect relationships to Cu contamination because other factors such as presence of other pollutants, and differing patterns of circulation could also cause differences among stations. Furthermore, no other contaminant has ever been thoroughly studied in SIYB. Nevertheless, our results appear to support a causal relationship between elevated sediment Cu concentrations, reduced macrofaunal biomass and diversity, and changes in macrobenthic community composition. This result is supported by our pRDA analysis in which we included natural variables with a highly significant effect on community variation as covariables. The total

faunal variance (18.3%) was explained by Cu; this is similar to the 17.4% of variance explained by Cu in Olsgard (1999), who examined effects of Cu on macrofaunal recolonization.

4.4. Cu in benthic invertebrate tissue

SIYB sediments not only appear to be an important sink for Cu (SDWQRCB, 2005; Neira et al., 2009), but also seem to be a Cu-enriched food source for benthic invertebrates. Cu concentration in SIYB invertebrate tissues varied between species and within the same species (Table 9). Some species followed a pattern of increasing Cu concentrations in tissue with increasing Cu in sediment, while others did not. Several species exhibited Cu magnification above concentrations in the surrounding environment, whereas other species had lower concentrations. This may reflect species variations in feeding strategies, digestive capacity, digestive tract biochemistry, throughput time, and assimilation efficiencies of Cu sorbed to sediment and detritus (Chen and Mayer, 1998; Wang et al., 1999).

Three major factors have been suggested to influence metal concentration in the tissues of marine invertebrates, namely the

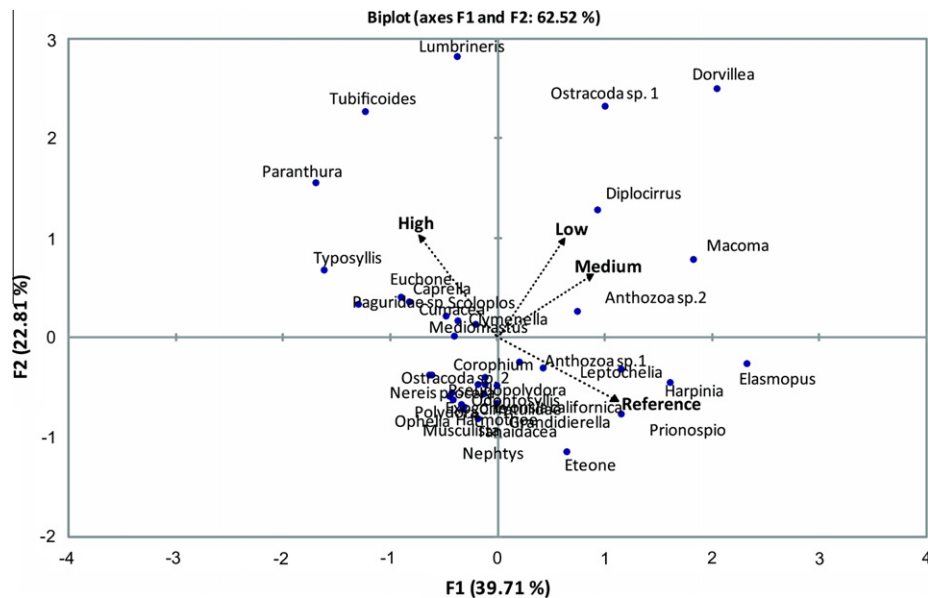


Fig. 10. Biplot showing the first two principal axes from a PCA explaining the total variance of Cu concentration in macroinvertebrates tissue at sites of different sediment Cu levels.

environmental bioavailability of the metal, the rate of diffusion across tissue boundaries (including the gut), and the extent to which metals are retained within the animal (Depledge and Rainbow, 1990). Several authors have noted that concentrations of metals in tissues of marine invertebrates vary with species, specific tissue, individual age, size, physiological state, reproductive condition (Eisler, 1981; Depledge and Bjerregaard, 1990). Metal exposure of benthic animals is not necessarily controlled by porewater concentration, but primarily through ingestion of particles. Studies have shown that a weak association between porewater and bioaccumulation of metals exist in several invertebrates, and the most probable explanation is that these invertebrates accumulate metals mainly through direct ingestion of sediment, regardless of sulfide content (Lee et al., 2000). Our results (Figs. 7 and 9) suggest that total dissolved Cu and free Cu^{++} in porewater may have a minor effect on benthic faunal composition and biomass. Dissolved Cu averaged $7.3 \mu\text{g L}^{-1}$ and did not exhibit large variations among sites, while free Cu^{++} was very low (pCu 14.6–11.7) (Neira et al., 2009); pCu values of 11 or lower can become toxic to phytoplankton (Sunda and Guillard, 1976; Brand et al., 1986), copepods (Sunda et al., 1987) and mussel larvae (Rivera-Duarte et al., 2005).

In addition, in SIYB we found a relatively high and uniform Cu complexation capacity (i.e. occurrence of Cu-binding ligands) in waters overlying sediments, lowering Cu^{++} to below toxic levels (Neira et al., 2009). In marine invertebrates, conditions within the digestive system differ substantially from those in surrounding sediments. Invertebrate gut fluids contain high concentrations of organic ligands. Cu concentrations in tissue suggest that there are some species able to “regulate” their body load through mechanisms that need further investigation, most likely associated with detoxification, storage and excretion abilities. However, because we determined Cu concentrations from the whole body (due to the small size of the animals), variation in Cu levels in different tissues may have been masked.

4.5. Potential ecosystem consequences

It is well documented that pollution stress leads to structural changes in benthic communities (Pearson and Rosenberg, 1978). Diversity is a key aspect of benthic community structure in estuarine and coastal areas and may be linked to a number of ecosystem

functions (e.g. resource availability, elemental cycling, nutrient exchange, production of biomass) (Danovaro et al., 2008). The loss or scarcity of key bioturbators that are secondary producers, primarily amphipod crustaceans and polychaetes such as *Capitella* and *Mediomastus*, as observed in “high” Cu sites of SIYB, may have substantial consequences for ecosystem function since there is reduced contribution to the remineralization of OM, as well as reduced contribution as prey for higher trophic levels. In addition, the greater the loss of bioturbators, the more settling and concentration of contaminants in sediments occurs. Lower macrobenthic diversity may support lower rates of ecosystem processes such as production and remineralization, and a decreased efficiency with which they are performed. A lower structural biodiversity (species richness) has a direct negative effect on functional diversity (i.e. the number of functional roles species represent in the ecosystem) (Lohrer et al., 2004; Danovaro et al., 2008). In addition, altered faunal community composition and impaired biomass at high Cu sites may affect potential predators, requiring more time to search for and more food to meet nutritional requirements. Heavy metals, including Cu, can also influence predator/prey interactions by degrading the ability of prey to respond to predators (McPherson et al., 2004).

5. Conclusions

Sediment Cu is associated with alteration of the SIYB benthic community. Previous measurements of Cu complexation capacity (CuCC) revealed relatively high CuCC in bottom and surface waters but low free Cu^{++} concentrations in porewater. Neira et al. (2009) suggested that SIYB is “self detoxifying”, modulated by ligands produced *in situ* and those released from the sediment (Neira et al., 2009). The macrofaunal results presented here indicate that at some sites, primarily at the head of SIYB, Cu in sediment has exceeded a threshold for “self defense” mechanisms and highlight the potential negative impacts on benthic macrofaunal communities where Cu accumulates and persists in sediments. Chronic Cu stress allows little opportunity for benthic assemblages to recover, causing permanent reduction in biomass, body size and diversity, but not in density.

Although SIYB is small in size (relative to San Diego Bay as a whole), high-spatial resolution sampling revealed significant gradients in Cu species and faunal community structure. Previous sam-

pling efforts have focused on bay-wide analyses, and have failed to identify the small spatial scale (tens of m) on which Cu contamination can act. Identification of fine-scale community responses to environmental Cu will be useful to managers and decision makers, enhancing the design of monitoring programs by allowing prioritization of the areas and taxa that should be targeted.

The combined effects of reduced biomass, body size, and diversity resulting from elevated Cu levels in sediments lead to dramatic alteration of the system at the community level. However, the picture is complex, reflecting a diversity of responses at the species level, and even within a species. More information regarding the life habits of organisms under Cu “stress” will help to distinguish the reasons for such differences in their responses. This study is unique among other studies of Cu contamination, in that our results are wholly based on field data, thereby reflect actual environmental conditions. The information obtained from this study highlights the spatial complexity of Cu effects in a small marina such as SIYB and emphasizes that sediment-quality criteria based solely on laboratory experiments should be used with caution as they do not

necessarily reflect the situation at the community and ecosystem levels.

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Appendix A

Table A1

Feeding modes and lifestyles for Shelter Island Yacht Basin macrofauna SDF = surface deposit feeder, SSDF = subsurface deposit feeder.

Taxa	Class	Family	Feeding mode	Lifestyle
<i>Tubificoides</i> spp.	Oligochaeta	Tubificidae	SSDF	Burrower
<i>Pseudopolydora paucibranchiata</i>	Polychaeta	Spionidae	SDF	Tube builder
<i>Polydora nuchalis</i>	Polychaeta	Spionidae	SDF	Tube builder
<i>Prionospio</i> sp.	Polychaeta	Spionidae	SDF	Tube builder
<i>Exogone lourei</i>	Polychaeta	Syllidae	Omnivore	Mobile
<i>Odontosyllis cf. phosphorea</i>	Polychaeta	Syllidae	Omnivore	Mobile
<i>Sphaerosyllis cf. californiensis</i>	Polychaeta	Syllidae	Omnivore	Mobile
<i>Scoloplos</i> sp.	Polychaeta	Orbiniidae	SSDF	Tube builder
<i>Diplocirrus</i> sp.	Polychaeta	Flabelligeridae	SDF	Burrower
<i>Mediomastus</i> sp.	Polychaeta	Capitellidae	SSDF	Burrower
<i>Capitella</i> spp.	Polychaeta	Capitellidae	SSDF	Burrower
<i>Phyllodoce mucosa</i>	Polychaeta	Phyllodocidae	Carnivore	Mobile
<i>Eteone californica</i>	Polychaeta	Phyllodocidae	Carnivore	Mobile
<i>Cossura</i> sp.	Polychaeta	Cossuridae	SSDF	Mobile
<i>Tharyx</i> sp.	Polychaeta	Cirratulidae	SDF	Mobile
<i>Cirratulus</i> sp.	Polychaeta	Cirratulidae	SDF	Mobile
<i>Cirriformia spirobrachia</i>	Polychaeta	Cirratulidae	SDF	Mobile
<i>Clymenella californica</i>	Polychaeta	Maldanidae	SSDF	Tube builder
<i>Nereis procera</i>	Polychaeta	Nereididae	Omnivore	Mobile
<i>Lumbrineris</i> sp.	Polychaeta	Lumbrineridae	Carnivore	Mobile
<i>Euchone limnicola</i>	Polychaeta	Sabellidae	SDF	Tube builder
<i>Nephtys cornuta</i>	Polychaeta	Nephtyidae	Carnivore	Mobile
<i>Dorvillea rudolphi</i>	Polychaeta	Dorvilleidae	Omnivore	Mobile
<i>Ophelia limacina</i>	Polychaeta	Opheliidae	SSDF	Burrower
<i>Sphaerodoridae</i> sp.	Polychaeta	Sphaerodoridae	Carnivore	Mobile
<i>Musculista senhousia</i>	Bivalvia	Mytilidae	SDF	Burrower
<i>Macoma</i> sp.	Bivalvia	Tellinidae	SDF	Burrower
<i>Lyonsia californica</i>	Bivalvia	Lyonsiidae	SDF	Burrower
<i>Gastropod</i> sp.	Gastropoda	–	SDF	Mobile
<i>Corophium</i> spp.	Malacostraca	Corophiidae	SDF	Mobile
<i>Grandidierella japonica</i>	Malacostraca	Aoridae	SDF	Mobile
<i>Podocerus</i> sp.	Malacostraca	Podoceridae	SDF	Mobile
<i>Harpinia</i> sp.	Malacostraca	Phoxocephalidae	SDF	Mobile
<i>Aoroides</i> sp.	Malacostraca	Aoridae	SDF	Mobile
<i>Ampithoe</i> sp.	Malacostraca	Ampithoidae	SDF	Mobile
<i>Elasmopus</i> sp.	Malacostraca	Gammaridae	SDF	Mobile
<i>Ampelisca</i> sp.	Malacostraca	Ampeliscidae	SDF	Mobile
<i>Caprella californica</i>	Malacostraca	Caprellidae	SDF	Mobile
<i>Leptocheilia dubia</i>	Malacostraca	Leptocheiliidae	Omnivore	Mobile
<i>Heteroserolis</i> sp.	Malacostraca	Serolidae	Omnivore	Mobile
<i>Paranthura elegans</i>	Malacostraca	Paranthuridae	Omnivore	Mobile
Cumacea	Malacostraca	–	Omnivore	Mobile
Ostracoda	Ostracoda	–	Omnivore	Mobile
Cnidaria	Anthozoa	–	Carnivore	Epifaunal
Tubellaria	Tubellaria	–	Carnivore	Mobile
Nemertea	–	–	Carnivore	Mobile
<i>Phoronis</i> sp.	Phoronida	–	SDF	Tube builder
<i>Holothurid</i> sp.	Holothuriidea	–	SDF	Burrower

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