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Authors
Neira, Carlos
Mendoza, Guillermo
Porrachia, Magali
et al.

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Macrofaunal Recolonization of Copper-Contaminated Sediments in San Diego Bay

Carlos Neira [a]*, Guillermo Mendoza [a], Magali Porrachia [b], Chris Stransky [c], and Lisa A. Levin [a]

[a] Integrative Oceanography Division and Center for Marine Biodiversity and Conservation, Scripps Institution of Oceanography, La Jolla, CA 92093-0218, USA

[b] Geosciences Research Division, Scripps Institution of Oceanography, La Jolla, CA 92093-0244, USA

[c] AMEC Environment & Infrastructure, San Diego, CA 92123, USA

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* Corresponding author. Tel.: +1 858 534 1864; fax: +1 858 822 0562.

E- mail address: cneira@ucsd.edu (C. Neira)

ABSTRACT

Effects of Cu-loading on macrofaunal recolonization were examined in Shelter Island Yacht Basin (San Diego Bay, California). Sediments with high and low Cu levels were defaunated and Cu-spiked, translocated, and then placed back into the environment. These demonstrated that the alteration observed in benthic communities associated with Cu contamination occurs during initial recolonization. After a 3-month exposure to sediments with varying Cu levels, two primary colonizing communities were identified: (1) a “mouth assemblage” resembling adjacent background fauna associated with low-Cu levels that was more diverse and predominantly dominated by surface- and subsurface-deposit feeders, burrowers, and tube builders, and (2) a “head assemblage” resembling adjacent background fauna associated with high-Cu concentrations, with few dominant species and an increasing importance of carnivores and mobile epifauna. Cu loading can cause reduced biodiversity and lower structural complexity that may last several months if high concentrations persist, with a direct effect on community functioning.

Keywords: recolonization; copper pollution; macrofauna diversity; sediment; San Diego Bay; California

1. Introduction

Copper (Cu) pollution has become a global environmental problem due to increasing demand for Cu for multiple uses (Nriagu 1979; Rauch and Pacyna 2009). Cu comes mostly from anthropogenic activities such as mining and smelting, fish farm activities, disposal of wastes and sewage sludge, leaching of antifouling paints and wood preservatives (Clearwater et al. 2002; Berkum 2005; Ramirez et al. 2005; Dean et al. 2007; Parks et al. 2010; Mayor and Solan 2011; Biggs and D'Anna 2012; Ramirez-Llodra et al. 2015). Approximately 1.8 million metric tons of Cu enter the global ecosystem annually of which 15.7% is deposited in freshwater and in the coastal ocean, 80.7% in terrestrial areas, and 3.6% to the atmosphere (Eiler 1998). Trace amounts of Cu are fundamental for the growth and metabolism of all living organisms due to its central role in a range of enzymes (Festa and Thiele 2011), but Cu can be toxic to marine organisms at slightly higher levels (Buck et al. 2007). The release of Cu into the marine environment can contribute to the degradation of the health of the aquatic ecosystems (Bylyard 1987; Burd; 2002; Pascal et al. 2010; Vogt 2013). This is of particular relevance because increasing Cu input is occurring synergistically with ocean warming-linked acidification which is predicted to increase the bioavailability of Cu and hence its toxicity (Millero et al. 2009; Richards et al. 2011; Roberts et al. 2013; Gomiero and Viarengo 2014).

Cu has been identified as a primary contaminant of concern in coastal benthic ecosystems such as bays, estuaries and fjords due to its increasingly extensive use in antifouling paints applied to marine vessels and other submerged structures such as fish farm cages (Terlizzi et al. 2001; Yebra et al. 2004; Carson et al. 2009; Dafforn et al.

2011; Mayor and Solan 2011). Cu entering the marine environment ultimately deposits and accumulates in sediments through binding and adsorption processes (Zirino et al. 2013). Elevated Cu concentrations have the potential of alter benthic community structure (Josefson et al. 2008); this is often reflected in enhanced dominance and reduced richness, diversity, and biomass (Rygg 1985; Schwinghamer 1988; Morrisey et al. 1996; Perrett, et al. 2006; Neira et al. 2011, 2014). Benthic communities play critical roles not only in the food web, as direct or indirect food source for higher trophic levels, but also in overall ecosystem functioning by promoting habitat heterogeneity that enhances recruitment for other organisms, by decomposing organic matter, and through digestion and bioturbation, with subsequent mixing and oxygenation of deeper sediments (Aller and Yingst 1985; Petersen et al.1998; Jones and Turner 2010; Kuwabara et al. 2012). Hence, changes in abundances, community composition and diversity of benthic communities can affect the functioning of the entire ecosystem (Levin et al. 2001; Danovaro et al. 2008). In this regard, our recent studies conducted in Shelter Island Yacht Basin (Neira et al. 2009, 2011) and in America's Cup and Harbor Island West and East (San Diego Bay, California) revealed that established macrofaunal communities differ in terms of composition, diversity, dominance and biomass, but not or little in total density between sites with high and low Cu (Neira et al. 2011). Cu bound to sediment particles appears to be the primary Cu form affecting macrofaunal communities (Neira et al. 2011, 2014). The results of these studies raise new ecological questions such as whether the effects observed are also reproduced at initial stages of recolonization and inhibition of larval settlement (Reichelt-Brushett and Harrison 2000).

Recolonization is considered a fundamental structuring process in soft-bottom benthic communities; however, little is known about recolonization of benthic communities in areas contaminated with trace metals (e.g. Olsgard et al. 1999; Lu and Wu 2003; Trannum et al. 2004; Chariton et al. 2011). Inhibition of larval settlement has been reported as one of the negative effects of Cu on soft-bottom communities (Reichelt-Brushett and Harrison 2000). Larval recruitment from the water column and macrofaunal immigration has been mentioned as a common route to recolonize defaunated sediments or replace fauna after mortality (Sibert 1981; Frid 1989; Watzin and Roscigno 1997).

Due to a gradient in sediment Cu concentrations from high at the head of the basin to low at the mouth of the basin, Shelter Island Yacht Basin provides a natural scenario for examining the potential effects of Cu on the initial colonization of soft-bottom macrofauna. The study examined the effects of differing sediment Cu levels on macrofaunal recolonization of defaunated soft-bottom sediments. Through a manipulative experiment involving defaunation, spiking, replacing and translocation, we tested the hypothesis that initial recolonization of defaunated sediments by macrofauna would differ in terms of abundances, assemblage composition, structural and trophic diversity between (1) Cu-levels that occur at the head and mouth of the basin, and (2) that these differences would intensify in corresponding Cu-spiked sediments.

2. Materials and methods

2.1. Study site

In this study, we took advantage of a naturally occurring Cu gradient in SIYB (Neira et al. 2009) to conduct a field experiment at two sites with contrasting Cu concentration: near the mouth (hereafter “mouth”) (32˚42.55’N; 117˚14.08’W), with low Cu concentration (~34 mg kg^{-1}) and at the head (hereafter “head”) (32˚43.09N; 117˚13.56W) about 3 km away, with relatively high Cu concentration (~200 mg kg^{-1}). SIYB has a surface area of about 740,000 m^2, with a volume of 31,000,000 m^3 (CARWQCB 2005). Both sites have similar depth (~ 4.5 m), with bottom water dissolved oxygen of ~8.5 mg L^{-1} (5.9 ml L^{-1}) at the mouth and ~7.3 mg L^{-1} (5.1 ml L^{-1}) at the head. No smell of sulfide was noted from the sediment collected for the experiment, in line with previous sampling in the area and oxygenated bottom water conditions. Further details of the study location are reported in Neira et al. (2009, 2011).

2.2. Experimental design

Sediment used in the experiment was collected from the high-Cu and low-C study sites by multiple deployments of a small custom-made gravity corer containing a removable Plexiglas tube (surface area = 20.4 cm^{-2}). Sediment from each site (mouth and head) was defaunated by successive freezing and thawing at -20˚C (Santos and Simon 1980; Bolam et al. 2004; Stocum and Plante 2006; Ryu et al. 2010). Sediment was further wet-sieved (500 μm) to remove large particles, shell remains and dead fauna. Sediment from each location was split in two fractions: (a) one fraction was used as a control representing sediment from the mouth with a low background Cu concentration ("mouth natural") and from the head, with a high background Cu concentration ("head natural"), and (b) another fraction was spiked with Cu. For Cu additions, sediment was transferred into pre acid-washed plastic containers and spiked following recommendations of Simpson et al. (2004). Sediment was spiked with Cu at room temperature by multiple doses of $CuCl_2$ (2.54 g per 500 ml SIO pier seawater [salinity ~33.63] each time). Every time after adding the doses of Cu, sediment was thoroughly homogenized using a plastic spatula, decanted and excess overlying water was removed. Furthermore, added Cu was allowed to equilibrate with the sediments for at least 24 h before pH was checked and adjusted to 8.3 using additions of 1 M NaOH prepared in seawater. After adding the last doses of Cu, sediment was allowed to equilibrate two weeks (with pH checked periodically). Depending on sediment type and its binding capacity, equilibrium of Cu-spiked sediments appears after 10-15 days (Simpson et al. 2004). After a total period of ~1 month, final concentrations of 1000 mg kg^{-1} (mouth sediment) - 1500 mg kg^{-1} (head sediment) were obtained. These spiked concentrations were chosen based on peak background sediment Cu concentrations reported for different marinas and shipyards in San Diego Bay (e.g. Valkirs et al. 1994; Exponent 2003; CARWQCB 2005; Neira et al. 2009, 2014), and to cover a wide range of Cu concentrations measured in macrofaunal tissue (Neira et al. 2011, 2014). Head sediments are finer and with higher organic matter content than mouth sediments (Neira et al. 2009) and hence have higher binding capacity and faster equilibration rates (Simpson et al. 2004). Concurrently, six trays (59.7 cm L x 47 cm W) made of three high-density polyethylene (HDPE) (0.6 cm thick) plates, two of them provided with 12 holes (8.5 cm diameter), were assembled to contain 250 ml polypropylene beakers (Fisher). Each beaker was perforated with two opposite holes (2 cm diameter) and covered with a 25-μm mesh Nitex mesh intended to allow porewater exchange with overlying water.

To compare Cu levels associated with the sediments from two locations within the same basin, seven treatments were designed using background natural Cu concentrations and artificially Cu-spiked sediment. Descriptions of the treatments are given in **Table 1**. Treatment names were abbreviated: the first letter represents the location where the trays were deployed (mouth or head), the second letter refers to the provenance of the sediment and the third letter means "spiked" or "natural" concentration. MMN = mouth sediment with naturally low Cu concentration redeployed at the mouth site, HHN = head sediment with naturally high Cu concentration redeployed at the head site, MMS = mouth sediment spiked with Cu redeployed at the mouth site, HHS = head sediment spiked with Cu and redeployed at the head site, MHS = spiked head sediment translocated to the mouth site, also HMS = spiked mouth sediment translocated to the head site, and HMN = mouth sediment with naturally low Cu concentration translocated to the head site. The design allowed us to contrast the effects of Cu on faunal recolonization at three levels:

(1) Location: (a) A site located near the mouth is exposed to better water flushing and mixing, with relatively coarser grain size and lower organic matter in sediments, fewer boats docked nearby, and hence has low background sediment Cu. (b) a site located at the head of the basin has poorer water flushing and mixing, relatively higher organic content in finer sediments, higher density of boats and hence

higher background Cu than at the mouth (Neira et al. 2009).

(2) Artificial Cu spiking: this treatment exposed recolonizing fauna to much more elevated Cu concentration than under more natural conditions.

(3) Community recovery: By comparing resemblance of 3-month recolonizing fauna with the background macrofauna.

In July 2011, the defaunated sediments representing each treatment were transferred into the pre acid-washed polypropylene beakers, separated according to the deployment location (mouth and head) in box coolers, and frozen at -20°C in a walk-in freezer room for about 20 h. In the field on July 25, 2011, frozen beakers assigned to be deployed at the mouth or head were placed randomly in triplicate trays. The use of frozen sediment avoided effectively any disturbance or resuspension by turbulence when trays were introduced into the water. Freezing (and sieving) of sediment prior to deployment has been used extensively in field experiments examining the effects on recolonization or community structure of a broad range of pollutants such as metals (e.g. Austen et al. 1994; Olsgard 1999; Trannum et al. 2004), octylphenol (Ryu et al. 2010), crude oil (Berge et al. 1987; Berge 1990); and tributyltin (Austen and McEvoy 1997) without apparent alteration of bioavailability. The tray design (**Appendix A Fig. A1**), with four rings on top near the corners, allowed us to lower them manually to the bottom from a boat in a few minutes, by extending polyester ropes. Weights, attached shortly before deployment, held the trays in place. This was tested prior to the experiment and confirmed by divers upon retrieval (see below). At each location, trays were deployed a few meters apart, and position was registered with a GPS. To facilitate further recovery, a small underwater floating marker was fixed to one of the rings of each tray with a line approximately 50 cm from the bottom.

On October 26, 2011, after 3 months exposure, the trays were gently retrieved and brought to the surface by divers. Beakers were transferred to coolers for further transport. At each location (mouth and head), four replicate cores were collected from the adjacent sediment, and were designed AM = "adjacent mouth", and AH = "adjacent head" (**Table 1**). They make comparisons with background macrofauna possible as the contained sediments are barely different from those background sediments surrounding them, and were under the same local environmental influences.

2.3. Sediment properties and faunal analysis

From all treatments, a sediment fraction was collected for determination of total organic matter, chlorophyll *a*, phaeopigments, grain size, and bioavailable sediment Cu. We also collected four sediment cores from the adjacent experimental area for comparisons with the ambient macrofaunal communities. An additional core was collected for sediment properties and sediment Cu after 3 months deployment. Sediment (top 5 cm) for macrofauna analysis was collected from the center of each beaker with a plexi-glass liner (20.4 cm^2), preserved with 8% buffered formalin and stained with Rose Bengal. Sediments were then sieved on a 300-μm sieve, and fauna were sorted and identified to lowest attainable taxonomic level under a binocular microscope.

Sediment total organic matter (TOM) content was determined from mass loss of freeze-dried, homogenized sediment after combustion at 500°C for 4 h in a muffle furnace. Percent sand (>63 μm) and silt-clay (<63 μm) were determined on sediment digested with hydrogen peroxide (30%) to remove organic matter as described by Neira et al. (2009).

Sediment chl *a* (chl *a*) and phaeopigments (phaeo) were determined spectrophotometrically from freeze-dried sediment after extraction with 90% acetone.

Sediment Cu was determined using an ICP-OES Perkin Elmer Optima 3000 DV analyzer, after digestion with 45% nitric acid (Optima). This ensures complete extraction of the leachable (i.e. bioavailable) Cu associated with organic matter adsorbed to sediment particles (Cook et al. 1997; Deheyn et al. 2005).

2.4. Data analysis

Spearman rank correlation analysis was performed to test for possible relationships among Cu, organic-matter descriptors, sand and mud content. Macrofaunal density (N), species richness (S) and diversity indices such as Pielou's evenness (J'), Shannon-Wiener diversity (H' $\log_{10}$) and Rank 1 dominance (R1D – the proportion of the most abundant taxon, i.e. an inverse indicator of assemblage evenness) were calculated to describe colonizing macrofaunal assemblage structure. Taxon richness was also examined through rarefaction curves (number of species per number of individuals sampled – Hurlbert, 1971). Feeding modes and lifestyle composition were used as a measure of functional diversity. Data (mean ±1 SE) were tested for normality using goodness-of-fit tests and square root transformed to achieve normality when possible. Comparisons across treatments were made with univariate analyses (ANOVA, with a posteriori Tukey HSD test) or when parametric assumptions could not be fulfilled, with the non-parametric Wilcoxon's test, using JMP 9.0.0. Community composition was analyzed based on a Bray-Curtis similarity matrix and visualized using non-metric multidimensional scaling ordination (nMDS), using Primer v6. Species abundance data were fourth-root transformed prior to analysis. Differences in composition between treatments were tested with analysis of similarity (ANOSIM). The Primer similarity percentage (SIMPER) procedure was applied to identify which taxa and in what proportion contributed to any difference among sediment treatments, including both the translocated and the adjacent background sediments.

To test the interaction and influence of the two different locations (head and mouth) with the experimental Cu treatments a two-way fixed factor permutational ANOVA was performed on biological variables (species richness, density and dominance. In order to overcome normality issues with the data, a Montecarlo permutation (9999) was performed. Univariate data were square root transformed. For the community data, interactions were tested using PERMANOVA+ routines for PRIMER with fourth-root transformed data and a Bray Curtis dissimilarity matrix (Anderson et al. 2008).

3. Results

3.1. Sediment properties

A summary of sediment properties of the different treatments and adjacent sediment measured after 90 days experimental period is shown in **Table 2**. Spiked sediments from the head that were placed back at the head still were quite high in Cu as were head spiked sediment translocated to the mouth. Cu concentrations differed significantly among treatments (Wilcoxon $X^2 = 45.2$, df = 8, $P < 0.0001$), with peak Cu concentration of 1256 mg kg^{-1} in spiked head sediments replaced at the head (HHS) (**Table 2**). Sediment TOM also showed significant differences among treatments ($F_{8,17} = 7.7$; $P = 0.003$), while no significant differences were detected in mud content and chloropigment-derived organic matter as a function of location (Wilcoxon $X^2 = 3.4$, df = 1; P = 0.062) or Cu treatment (Wilcoxon $X^2 = 13.3$, df = 8; $P = 0.102$) (**Table 2**). Cu concentrations were positively correlated with TOM ($P < 0.01$) and chl *a* ($P < 0.05$).

3.2. Recolonizing faunal density, composition and diversity

A total of 42 colonizing taxa were identified from all sediment samples, with annelids and crustaceans as the dominant groups followed by bivalves and "others" represented by cnidarians, nemerteans and holothurians (**Fig. 1**). Mean total densities were significantly different among treatments (ANOVA $F_{8,37} = 2.4$, $P = 0.040$), with the lowest abundance at the spiked mouth sediment translocated to the head site (HMS) with 4.7 ind. ±1.2 ind. 20.4 cm^{-2} and the highest at mouth sediment with naturally low Cu concentration redeployed at the mouth site (MMN) with 33.6 ±6.0 ind. 20.4 cm^{-2}, the latter showing recovered densities similar to these of the adjacent sediment (35 ±2.5 ind. 20.4 cm^{-2}) (**Fig. 2**). The same pattern was observed for taxon richness and H' diversity of recolonizing fauna, while Pielou evenness (J') did not show significant differences (**Table 3**). Rank 1 dominance was greatest primarily in treatments associated with the head site such as those spiked mouth sediments translocated to the head (HMS), head sediment spiked with Cu and redeployed at the head site (HHS), and adjacent head sediments (AH) (**Table 3**). Rarefaction diversity was greatest in mouth sediments with naturally low Cu concentration redeployed at the mouth (MMN), and lowest in head sediment spiked with Cu and redeployed at the head (HHS), spiked mouth sediments translocated to the head (HMS), and adjacent head sediments (AH) (**Fig. 3**).

Polychaetes, one of the most dominant taxa, were represented by 15 families. They showed differing composition in relation to location, with a higher family richness at the mouth sediment with naturally low Cu concentration redeployed at the mouth site (MMN), as was the adjacent mouth (AM) (**Fig. 4A**). Within the polychaetes, spionids and lumbrinerids were a numerically important component of the recolonizing assemblages. They appear to exhibit tolerance to Cu particularly in the "head" and Cu-spiked sediments, contributing > 40% of the total, while colonizing capitellids were well-represented in mouth-associated sediments (**Fig. 4A**). Among the peracarid crustaceans, the number of colonizing amphipods was reduced in those treatments associated with head and Cu-spiked sediments, with a dominance of *Desdimelita* sp., *Caprella californica*, Cumacea and Ostracoda of > 60% of the total (**Fig. 4B**).

Fauna colonizing sediments exhibited consistent assemblage composition differences as a function of location and Cu treatment (MDS and ANOSIM; **Fig. 5**). At the location level, the mouth site and head site exhibited different assemblage composition (ANOSIM, $P = 0.001$), with assemblage dissimilarity between locations reaching 77.26% (SIMPER) (**Table. 4**). Differences were primarily driven by the relatively high abundances of *Mediomastus* sp., *Prionospio* sp., Ischyroceridae, *Podocerus* sp., and Ostracoda sp. 2 in the mouth site sediments, and the high percent contribution of *Desdimelita* sp., Hydrozoa, *Lumbrineris* sp., and *C. californica* in the head site sediments. Within-location assemblage similarities were low in both groups (SIMPER 37.39% group "mouth, SIMPER 36.17% similarity group "head"). The assemblage similarity within the group "mouth" was driven by the high relative abundances of the capitellid *Mediomastus* sp. and the spionid *Prionospio* sp., while the within-head site similarity (**Table 4**) was driven by the high relative abundances of the amphipod *Desdimelita sp.*, Hydrozoa, *Prionospio* sp., *C. californica*, *Lumbrineris* sp., and Cumacea. Effects of Cu concentrations on colonizing fauna were also reflected in assemblage composition differences among Cu-treatments (MDS; **Fig. 5**), ANOSIM, SIMPER (**Table 4**). The main taxa contributing to dissimilarity between significantly treatments and adjacent sediment are shown by SIMPER in **Supplement Table S1**.

Regarding the biological response to Cu treatments and location, results of permutational two-way ANOVA indicate that taxon richness of the colonizing macrofauna was affected by a significant interaction between the two factors; this may primarily be driven by Cu (pseudo F = 12.35; $P = 0.0003$) compared to location (pseudo F = 7.22; $P = 0.013$) (**Supplement Table S2A**). In contrast, for macrofaunal density, the significance of the interaction was weak (pseudo F = 3.22; $P = 0.058$), with no significant effect from treatment or location ($P > 0.05$) (**Supplement Table S2B**). R1D did not show a significant interaction (pseudo F = 0.60; $P = 0.55$) but the effects of location and Cu treatment were significant when tested independently (pseudo F = 8.246; $P = 0.002$ and pseudo F = 4.90; $P = 0.038$, respectively) (**Supplement Table S2C**).

In addition, a series of pairwise tests were performed for the interaction terms. Since the interaction affecting taxon richness was mainly driven by Cu (**Supplement Table S3A**), the treatment and location were tested separately for each of the treatment series. In the mouth, the natural (MMN) treatment was significantly different to the spiked (MMS) treatment (t = 4.33, $P = 0.0037$). In contrast, at the head there was no significant difference between the same treatment levels, i.e. HHN and HHS (pseudo t = 0.53, $P = 0.609$). No significant difference was found for the MMS and the head Cu-spiked sediment translocated to the mouth (MHS) (pseudo t = 0.52, $P > 0.05$), while the head Cu-spiked sediment translocated to the mouth (MHS) and the MMN were significantly different (pseudo t = 3.21, $P = 0.017$) (**Supplement Table S3A**). For density (**Supplement Table S3B**), significant differences were observed between spiked mouth sediment translocated to the head (HMS) and spiked head sediments (HHS) (**Fig. 1**) while for R1D (**Supplement Table S3C**) significant differences were observed for mouth natural sediments (MMN) and mouth-spiked sediments (MMS), and between spiked-head sediments translocated to the mouth (MHS) and replaced natural mouth sediments (MMN).

3.3. Cu effect on functional diversity and community recovery

After 90 days, the colonizing fauna of low-Cu sediments seem to recover more readily resembling those of adjacent sediments, either located at the head (AH) or located at the mouth (AM), while in spiked sediment poor recovery was observed. The recovery is reflected in terms of structural diversity, e.g. richness of polychaetes (**Fig. 4A**) and crustacean (**Fig. 4B**), and in terms of functional diversity, e.g. feeding modes (**Fig. 6A**) and lifestyles (**Fig. 6B**). This also is supported by the analysis of macrofaunal assemblage dissimilarities between treatments (SIMPER). In both cases, recolonizing assemblages of spiked sediments (in location and translocated) were more similar to each other (e.g. MHS vs MMS 58.1%) but much dissimilar relative to adjacent mouth sediment (AM), with i.e. 78.8% and 77.9%, respectively (**Table 4**). Similarly, assemblages of spiked sediments (in place and translocated to the head) were more similar to each other (i.e. 67.2% HHS vs HMS), while assemblage dissimilarity was greater relative to adjacent head sediment (AH), with 69.3% and 83.9% for HHS and HMS, respectively (**Table 4**).

In general surface-deposit feeding (SDF) was the most common feeding mode after the 3-month recolonization period. However, the proportional representation of each feeding mode varied among treatments. For instance, contrasting trends in percent contribution during recolonization were observed in relation to the other feeding modes, and in terms of recovery status when contrasted with either adjacent mouth (AM) or adjacent head (AH) sediment (**Fig. 6A**). Cu spiked mouth sediments translocated to the head (HMS) showed the lowest number of feeding modes, with only SDF (93%) and omnivores (3%). Carnivores gained importance in both Cu-spiked and natural collected sediments and those replaced at the head (HHS 19.5% and HHN 27%, respectively). The latter showing a recovery contribution approaching that observed at adjacent sediment

(AH 40%). At the mouth, subsurface-deposit feeding (SSDF) was well represented (40%) in head Cu-spiked sediments translocated to the mouth (MHS) and Cu-spiked sediments collected and replaced at the mouth (MMS). In terms of recovery, mouth natural sediment replaced at the mouth (MMN) had assemblages exhibiting similar feeding modes as found in the adjacent mouth (AM) sediments (**Fig. 6A**). Regarding lifestyles, mobile fauna were over 50% colonizers, except in Cu-spiked sediments collected and replaced at the mouth (MMS 26%) and head Cu-spiked sediments translocated to the mouth (MHS 21%) where tube builders and burrowers were the main recolonizers (~70%) (**Fig. 6B**). Epifaunal recolonizers were well represented in Cu-rich sediments associated with the head. Thus, in sediment that were Cu-spiked (HHS) or natural collections replaced at the head (HHN), the macrofauna seemed to have achieved similarity to those in adjacent head (AH) sediments, with ~39% epifaunal lifestyles. In contrast, at the mouth the proportional contribution of lifestyles was divergent (**Fig. 6B**). Mobile colonizer contribution was relatively low in Cu-spiked sediments (MMS 26% and MHS 21.6%) and increased in mouth natural sediments (MMN 58%), while burrowers showed an inverse trend. In terms of recovery, natural sediments collected and replaced at the mouth (MMN) exhibited nearly the same contribution of lifestyles as those of the adjacent mouth sediment (**Fig. 6B**).

4. Discussion

4.1. Sediment properties

Cu concentrations in spiked treatments were still very high compared to non-spiked ones after 3 months, although, on average, reduction by ~27% in Cu concentration was observed (**Table 2**). This is most likely attributable to leaching of Cu into the water (Hall and Frid 1995) by bioturbation that enhances oxidizing solutes and solubility (Lu and Chen 1977; Jones and Turner 2010), and dilution of Cu-spiked fines with less contaminated sediments deposited from surrounded environment (Hill et al. 2011). Indeed, fine sediment deposited on top of the jars was observed upon retrieving the trays. In spite of the fact that trays near the mouth are subjected to stronger flushing, tidal mixing, and shorter residence time than at the head of the basin, VanderWeele 1996; Seligman and Zirino 1998), no significant differences in mud content were observed after the 3 month experiment. We consider that these findings highly supportive of previous results showing that mud content was not significantly influencing faunal community structure (Neira et al. 2011) and rather strongly suggest a causal relationships between Cu and the effects observed on fauna, i.e., chemical impairment given by toxic Cu was more important in determining faunal recolonizing response than the physical substrate. Early studies have shown less sensitivity of macrofauna and meiofauna to physical disturbance and destabilization of sediment than to changes in the chemical environment (Warwick et al. 1990; Hall et al. 1991; Höpner and Michaelis 1994; Neira and Rackemann 1996).

4.2. Recolonizing assemblage response

In general, recruitment and metamorphosis of planktonic larvae are considered the most sensitive phases in the development of macrobenthic invertebrates (Woodin 1976; Watzin and Roscigno 1997), and thereby highly vulnerable to contaminants (Bonsdorff et al. 1990). However, benthic communities can also become established by juvenile and adult migration (Dauer and Simon 1976, Palmer 1988; Neira and Rackemann 1996), either through active migration (swimming and selective re-entry into the sediment (Butman 1989) or passive transport and deposition (with sediment particles resuspension) (Savidge and Taghon 1988; Levin and DiBacco 1995). Pearson and Rosenberg (1978) suggested that during the initial colonization of areas that have experienced total defaunation, adult migration might play a greater role than larval settlement. Adult macrofauna are capable of recolonizing relatively small sediment patches over a short period of time (Bell and Devlin, 1983). Although recolonization is expected to be mainly through recruitment driven by water column sources (i.e. larval settlement of planktonic stages of macrofauna), we do not discard the possibility that initial recolonization also occurred by active or passive migrating juvenile and adults resulting from horizontal advection and or dispersal after resuspension (Pearson and Rosenberg 1978; Santos and Simon 1980, Neira et al. 2006). Because their shallow nature, sediments of yacht embayments can easily be resuspended through ship movements and propellers' stirring. Therefore, in this study, we considered "initial recolonization" in a *sensu latu* context, i.e. through larval settlement and juvenile and adults transportation (sampling was on a 300 μm sieve).

The manipulative approach used with trays containing jars with defaunated sediment treatments over 3 months seemed to be sufficiently powerful to examine initial recolonization by macrofauna and thus obtain additional ecologically relevant information to determine the effects of Cu pollution. The colonizing assemblages described quantitatively for each location and treatments mirrored the biological response of recolonizing organisms to the environmental conditions present. The lower species richness and diversity and low dominance at Cu-spiked mouth and head sediments relative to corresponding non Cu-spiked settings (**Fig. 3**, **Table 3**) suggests a major role for Cu in early succession by inhibiting settlement, early colonization and adult migration. This also reflects the fact that the location near the mouth is a cleaner environment with regards to Cu pollution levels (Neira et al. 2009), and hence a wider variety of species can recolonize and become established there (Neira et al. 2011).

4.3. Location and Cu effects on recolonization

Two main recolonizing benthic assemblages emerged from this experiment (**Fig. 5**): (1) one associated with location “mouth” clustering those assemblages from the mouth or translocated from the head and placed at the mouth, converging to adjacent mouth (AM) of lower Cu-loading sediments, and 2) one associated with location “head” clustering those treatments converging toward adjacent head (AH) conditions of higher Cu-loading sediments (**Fig. 5**). This supports findings of previous studies on effects of Cu on community composition and diversity measured from established communities (Neira et al. 2011). In sites with high sediment Cu, shifts in macrofaunal community composition occurred, with scarcity of crustaceans, primarily amphipods, and reduced polychaete family richness (**Fig. 4**). In general, crustaceans have been considered more sensitive to Cu contamination than, for instance, mollusks, oligochaetes, or fish (Jin et al.

2015).

Furthermore, there were significant differences among treatments (**Fig. 5**, **Table 4**), which support the efficacy of the tray design suggests in revealing selective recolonization as well as species-specific tolerances and sensitivities to Cu exposure. Differences in assemblage composition also suggest different larval settlement, different mortality after settlement and different migration strategies of juveniles and adults (Olsgard 1999). Treatments with much higher Cu (spiked) represent an environment more stressful for initial recolonizing fauna, as evidenced by reduced species richness and diversity (**Fig. 3**), and elevated dominance (**Table 3**) relative to those subject to lower Cu levels (i.e. natural). There is a strong interaction between locations and Cu treatments due to differing composition of recolonizing communities, as shown by the polychaete (**Fig. 4A**) and crustacean (**Fig. 4B**) assemblages as well as feeding modes (**Fig. 6A**) and lifestyles (**Fig. 6B**) PERMANOVA.

4.4. Cu effects on recolonization and community recovery

The linkage between metal stress and recolonization is difficult to establish in terms of cause-effect due to other environmental factors and stressors such as hydrodynamics, substrate, competitive interactions, other pollutants and natural variability (Oug 1998; Olsgard 1999; Chariton et al. 2011). However, results suggest that the differences in recolonizing assemblage composition and biodiversity (structural and functional) in San Diego marina ecosystems are driven by Cu, and take effect at early stages of colonization. Our earlier studies on established marina communities indicated that among Cu species, sediment Cu was the primary driver responsible for changes in macrofaunal composition and reduction in macrofaunal biomass and diversity (Neira et al. 2011, 2014). From previous studies in San Diego Bay marinas, we know that sediment Cu is the main driver explaining the variance of established species composition; however, a relatively large percent (~47%) of the variance in composition remained unexplained. Thus, presumably other physical and biological factors (Neira et al. 2011) are influencing faunal recolonization as well. The relatively short experimental period experiment (90 days) and the pre-treatment of experimental sediment were intended to minimize or eliminate potential confounding factors and thus test for Cu effects. Studies testing multiple stressors (Folt et al. 1999; Schiedek et al. 2007) can further elucidate the extent of the effects of Cu and other factors on recolonization and community structure.

Early studies indicate that coastal invertebrates exposed to high levels of Cu (and other heavy metals) are under selective pressure to evolve metal tolerance (Klerks and Weis 1987; Krantzberg and Stokes 1989; Hummel and Paternello 1994), and that this may be a heritable trait (Grant et al. 1989; Dauvin 2008). However, discrepancy in tolerance response has been reported, most likely due to differences in feeding strategies, digestive tract biochemistry, digestive capacity, tendency to accumulate, throughput time, and assimilation efficiency (Ward and Hutchings 1996; Chen and Mayer 1998; Wang et al. 1999; Chen et al. 2002). A field study in Norwegian fjords showed that *Capitella* and *Tubificoides* sp. were tolerant of high sediment Cu concentration (500 mg kg^{-1}) (Rygg 1985), while in a microcosm study where Cu concentration reached up to 411 mg kg^{-1}, abundances of *Tubificoides* and *Capitella* were reduced (Hall and Frid 1995). In our study the capitellid *Mediomastus* sp. had relatively high abundances in mouth-spiked sediments (**Fig. 4A**). In a moderately polluted location in the Oslofjord, Norway, Olsgard (1999) examined faunal recolonization at different concentrations of Cu in sediment and found that several species were negatively correlated with increased sediment Cu concentration, among them some spionid species such as *Pseudopolydora paucibranchiata*. In a follow-up experiment on benthic recolonization in the same Oslofjord, Trannum et al. (2004) observed significant changes in the composition of recolonized assemblages, with sediment Cu concentrations of 400-1500 mg kg^{-1}, however, *P. paucibranchiata* was not affected, as has occurred for San Diego Bay marinas (Neira et al. 2011, 2014). In the present study, spionids, especially *Prionospio* sp., seem to thrive as recolonizers in natural and Cu-enriched sediments (**Fig. 4A**).

Recolonization of Cu-enriched sediments suggests that marina macrofauna have evolved tolerance to environmental Cu stress, as observed for natural benthic communities (Neira et al. 2011). Species have evolved mechanisms for detoxifying and storing Cu within their bodies, thus minimizing stress when exposed to elevated Cu levels (Betzer and Yevich 1975; Correia et al. 2002). The differing composition of recolonizing assemblages reflects the diversity of response at the species level, and even within a species. Differing recolonization patterns of translocated Cu-spiked sediment strongly suggest that fauna coming from the chronically more polluted area (the head), are more tolerant and most likely recover faster than fauna from the more pristine area (the mouth). This is supported by observation of the lowest community diversity values (**Table 3**) and mean densities (**Fig. 2**) at mouth-spiked sediments translocated to the head (HMS), and the opposite in head-spiked sediments translocated to the mouth (MHS). Level of background stress caused by contaminants (e.g. Cu) may play an important role in the sensitivity response of the recolonizing assemblages (Luoma and Carter 1991; Austen and Sommerfield 1997). More favorable hydrodynamic conditions, e.g. tidal flushing and lower residence time, and resource availability at the mouth, may have facilitated recolonization. This seems to be supported by the assemblage of mouth natural sediments translocated to the head (HMN), which began to resemble the head faunal community (**Fig. 5**) in having a diversity and dominance intermediate relative to AM and AH sediments (**Fig. 3, Table 3**).

Results of this study suggest that Cu can cause impairment in faunal recolonization that may last several months if high concentrations persist. That may be exacerbated at the head where tidal flushing is poor (VanderWeele 1996). A 2-year microcosm experiment in which sediment concentration reached 411 mg kg^{-1}, demonstrated that sediment Cu concentrations decreased rapidly when exposed to uncontaminated water, however faunal recovery took over 1 year depending on taxon (Hall and Frid 1995). The incipient macrofaunal recolonization of Cu-spiked sediments suggest that some species-specific tolerance to Cu stress is occurring, reflecting a broad diversity in sensitivities at the species level, and even within species. Common community-level outcomes to environmental stressors are reduced species diversity and elevated dominance by stress tolerant taxa (Luoma and Carter 1991; Austen et al. 1994). In the present study, polychaete species such as *Prionospio* sp., *P. paucibranchiata*, *Mediomastus* sp., the oligochaete *Tubificoides* sp., hydrozoans, and the amphipod *Desdimelita* sp. were well represented in recolonized Cu-spiked sediments. These are the same species proposed as prospective indicators of high-Cu conditions (Neira et al.

2014). *Desdimelita* sp. and *Tubificoides* sp. have the capacity to bioaccumulate Cu (Neira et al. 2014), which might contribute to detoxification of the surrounding environment (Icely and Nott 1980; Bat et al. 1998).

5. Conclusions

This study provides ecologically relevant information on the community-level response to Cu-contaminated sediment during the critical initial recolonization phase and contributes to a better understanding of species tolerance and sensitivity to Cu. It also gives insights into the complexity of Cu effects on recolonizing macrobenthos and community resilience.

While it is difficult to establish a cause-effect relationship between Cu concentration and community structure due to differing hydrodynamics, other pollutants, and natural variability (Oug 1998), the results suggest that the differences in recolonizing assemblage composition and biodiversity (structural and functional) in a Cu-impacted marina ecosystem, can take effect at early stages of colonization. Still further studies are necessary to elucidate succession patterns as well as recolonization under multiple stressors. The experimental design applied in the present study, using a manipulative approach involving *in situ* experimentation using defaunated sediments, with contrasting natural Cu loading, spiked sediments, translocation, and replacement into the environment, appears to be a powerful tool for examining recolonization. It not only provides a unique look at the response of initial stages of recolonization by macrofauna in response to Cu contamination, but also broadens the understanding of how Cu may impact established benthic communities. The recolonization process has been considered as being "open ended" because the outcome can be highly variable, as it may be affected by seasonality, disturbances, and the substrate being colonized (Osman 1977). The different response of the recolonizing fauna at the marina mouth and head reflects the spatial complexity of interactions and factors contributing to community level-response to Cu (Neira et al. 2011, 2014).

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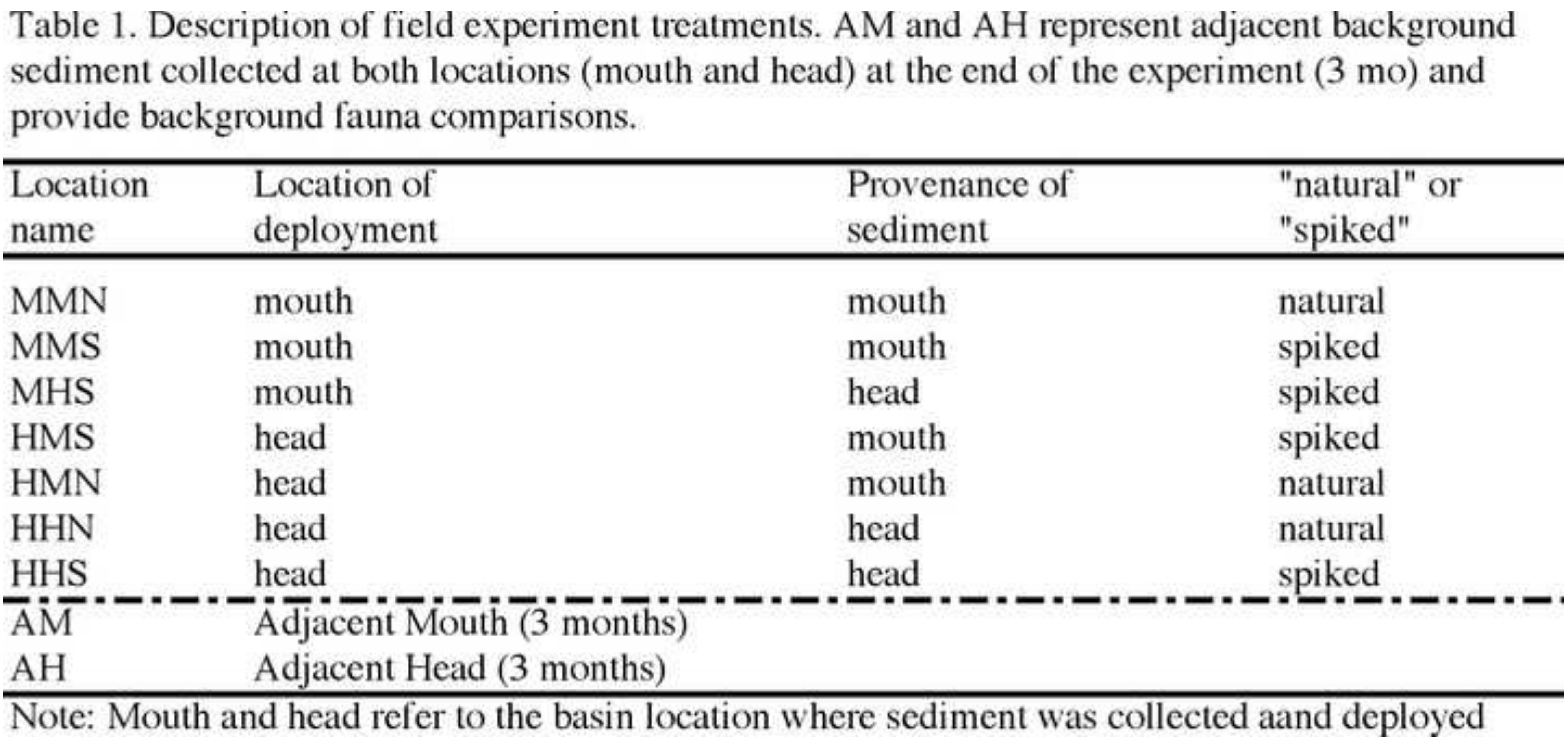


Table 1. Description of field experiment treatments. AM and AH represent adjacent background sediment collected at both locations (mouth and head) at the end of the experiment (3 mo) and provide background fauna comparisons.

Location name	Location of deployment	Provenance of sediment	"natural" or "spiked"
MMN	mouth	mouth	natural
MMS	mouth	mouth	spiked
MHS	mouth	head	spiked
HMS	head	mouth	spiked
HMN	head	mouth	natural
HHN	head	head	natural
HHS	head	head	spiked
AM	Adjacent Mouth (3 months)		
AH	Adjacent Head (3 months)		

Note: Mouth and head refer to the basin location where sediment was collected aand deployed

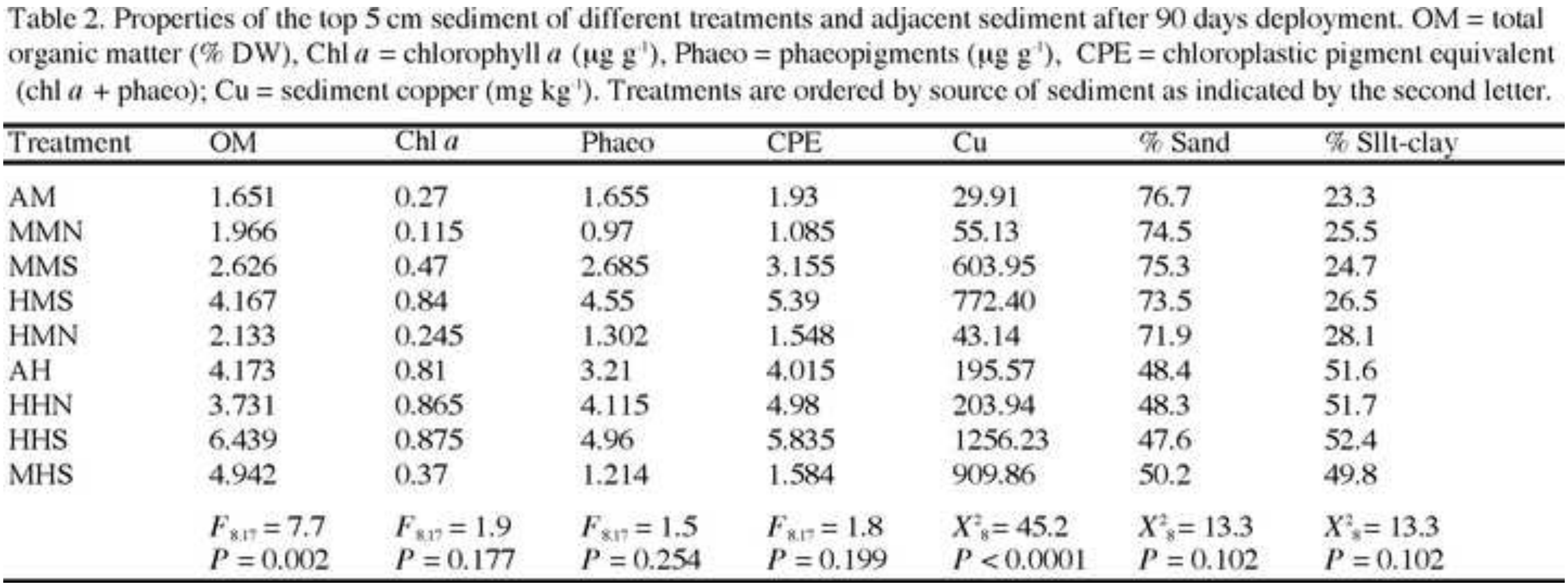


Table 2. Properties of the top 5 cm sediment of different treatments and adjacent sediment after 90 days deployment. OM = total organic matter (% DW), Chl *a* = chlorophyll *a* ($\mu g\ g^{-1}$), Phaeo = phaeopigments ($\mu g\ g^{-1}$), CPE = chloroplastic pigment equivalent (chl *a* + phaeo); Cu = sediment copper ($mg\ kg^{-1}$). Treatments are ordered by source of sediment as indicated by the second letter.

Treatment	OM	Chl *a*	Phaeo	CPE	Cu	% Sand	% Silt-clay
AM	1.651	0.27	1.655	1.93	29.91	76.7	23.3
MMN	1.966	0.115	0.97	1.085	55.13	74.5	25.5
MMS	2.626	0.47	2.685	3.155	603.95	75.3	24.7
HMS	4.167	0.84	4.55	5.39	772.40	73.5	26.5
HMN	2.133	0.245	1.302	1.548	43.14	71.9	28.1
AH	4.173	0.81	3.21	4.015	195.57	48.4	51.6
HHN	3.731	0.865	4.115	4.98	203.94	48.3	51.7
HHS	6.439	0.875	4.96	5.835	1256.23	47.6	52.4
MHS	4.942	0.37	1.214	1.584	909.86	50.2	49.8
	$F_{8,17} = 7.7$ $P = 0.002$	$F_{8,17} = 1.9$ $P = 0.177$	$F_{8,17} = 1.5$ $P = 0.254$	$F_{8,17} = 1.8$ $P = 0.199$	$X^2_8 = 45.2$ $P < 0.0001$	$X^2_8 = 13.3$ $P = 0.102$	$X^2_8 = 13.3$ $P = 0.102$

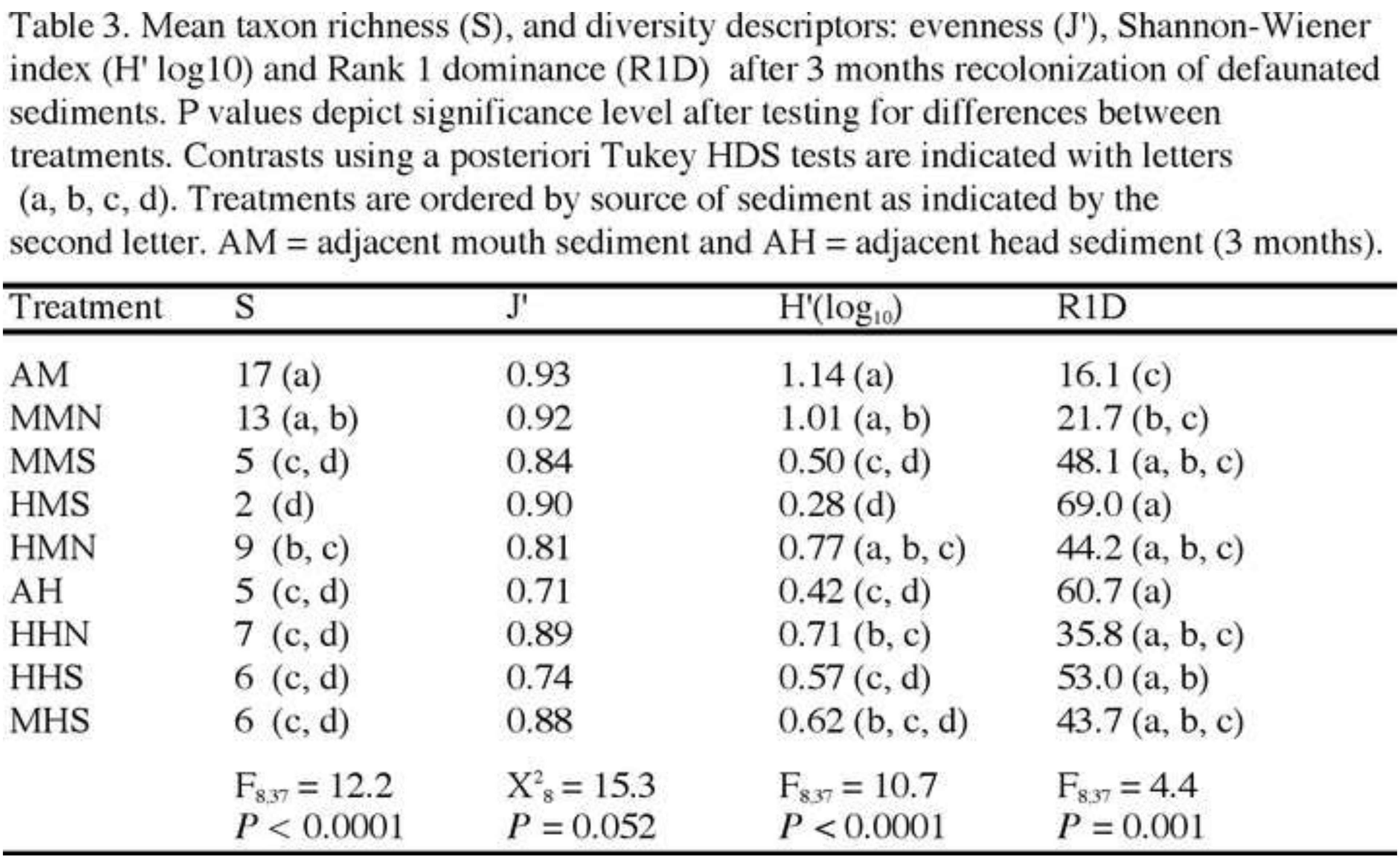


Table 3. Mean taxon richness (S), and diversity descriptors: evenness (J'), Shannon-Wiener index (H' log10) and Rank 1 dominance (R1D) after 3 months recolonization of defaunated sediments. P values depict significance level after testing for differences between treatments. Contrasts using a posteriori Tukey HDS tests are indicated with letters (a, b, c, d). Treatments are ordered by source of sediment as indicated by the second letter. AM = adjacent mouth sediment and AH = adjacent head sediment (3 months).

Treatment	S	J'	H'($\log_{10}$)	R1D
AM	17 (a)	0.93	1.14 (a)	16.1 (c)
MMN	13 (a, b)	0.92	1.01 (a, b)	21.7 (b, c)
MMS	5 (c, d)	0.84	0.50 (c, d)	48.1 (a, b, c)
HMS	2 (d)	0.90	0.28 (d)	69.0 (a)
HMN	9 (b, c)	0.81	0.77 (a, b, c)	44.2 (a, b, c)
AH	5 (c, d)	0.71	0.42 (c, d)	60.7 (a)
HHN	7 (c, d)	0.89	0.71 (b, c)	35.8 (a, b, c)
HHS	6 (c, d)	0.74	0.57 (c, d)	53.0 (a, b)
MHS	6 (c, d)	0.88	0.62 (b, c, d)	43.7 (a, b, c)
	$F_{8,37} = 12.2$ $P < 0.0001$	$X^2_8 = 15.3$ $P = 0.052$	$F_{8,37} = 10.7$ $P < 0.0001$	$F_{8,37} = 4.4$ $P = 0.001$

Table 4. Pairwise one-way Analysis of Similarity (ANOSIM) tests for recolonizing faunal similarities between treatment assemblages are given above the diagonal. SIMPER within-treatment similarities are given on the diagonal (gray boxes), and SIMPER percent dissimilarities are given below the diagonal. Significant values are indicated in bold.

	AM	AH	MMN	HHN	MMS	HHS	MHS	HMS	HMN
AM	64.6	**0.029**	0.270	**0.005**	**0.029**	**0.005**	**0.029**	**0.029**	**0.029**
AH	79.8	35.7	**0.008**	**0.005**	**0.029**	**0.005**	**0.029**	**0.029**	**0.029**
MMN	44.6	88.2	53.0	**0.002**	**0.008**	**0.002**	**0.018**	**0.018**	**0.036**
HHN	68.7	72.3	77.1	51.7	**0.005**	**0.002**	**0.012**	**0.012**	0.071
MMS	77.9	93.8	70.5	75.5	38.6	**0.005**	0.714	**0.029**	**0.029**
HHS	72.3	69.3	74.2	57.5	81.2	52.6	**0.012**	**0.012**	0.131
MHS	78.8	93.2	71.9	68.2	58.1	76.2	35.5	0.100	0.100
HMS	82.8	83.9	82.4	72.8	89.3	67.2	84.1	42.5	0.100
HMN	50.8	74.5	57.9	58.9	85.5	54.2	79.6	74.5	49.1

Figures caption

Fig. 1. Percent composition of main macrofaunal taxa represented in treatments with differing Cu levels. Treatment description are given in Table 1. Others = Cnidaria, Nemertea, Holothurida. AM = Adjacent Mouth sediment (3 months); AH = Adjacent Head sediment (3 months).

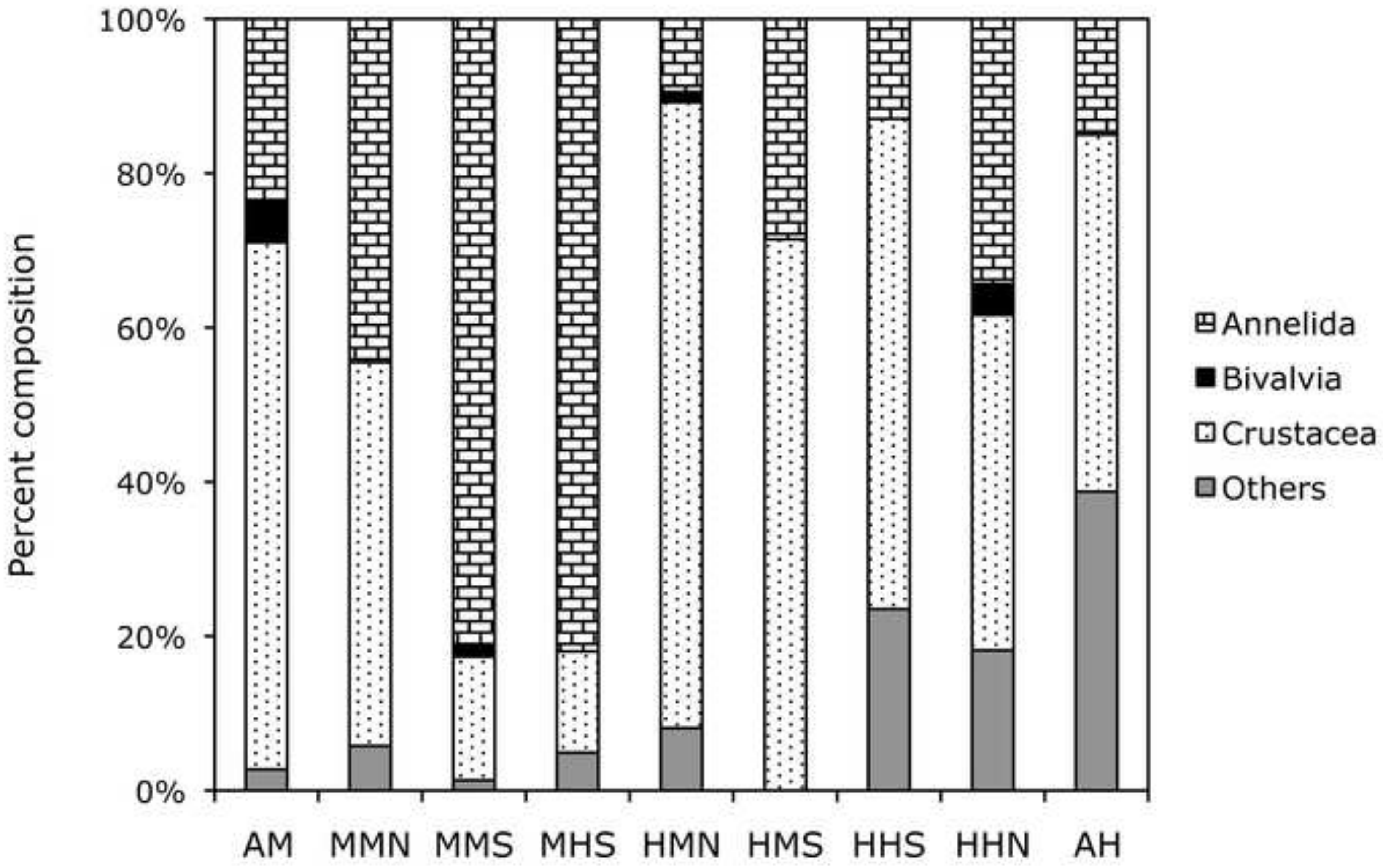
Fig. 2. Mean total densities of recolonizing macrofauna after the 3-month field experiment. Treatment description are given in Table 1.

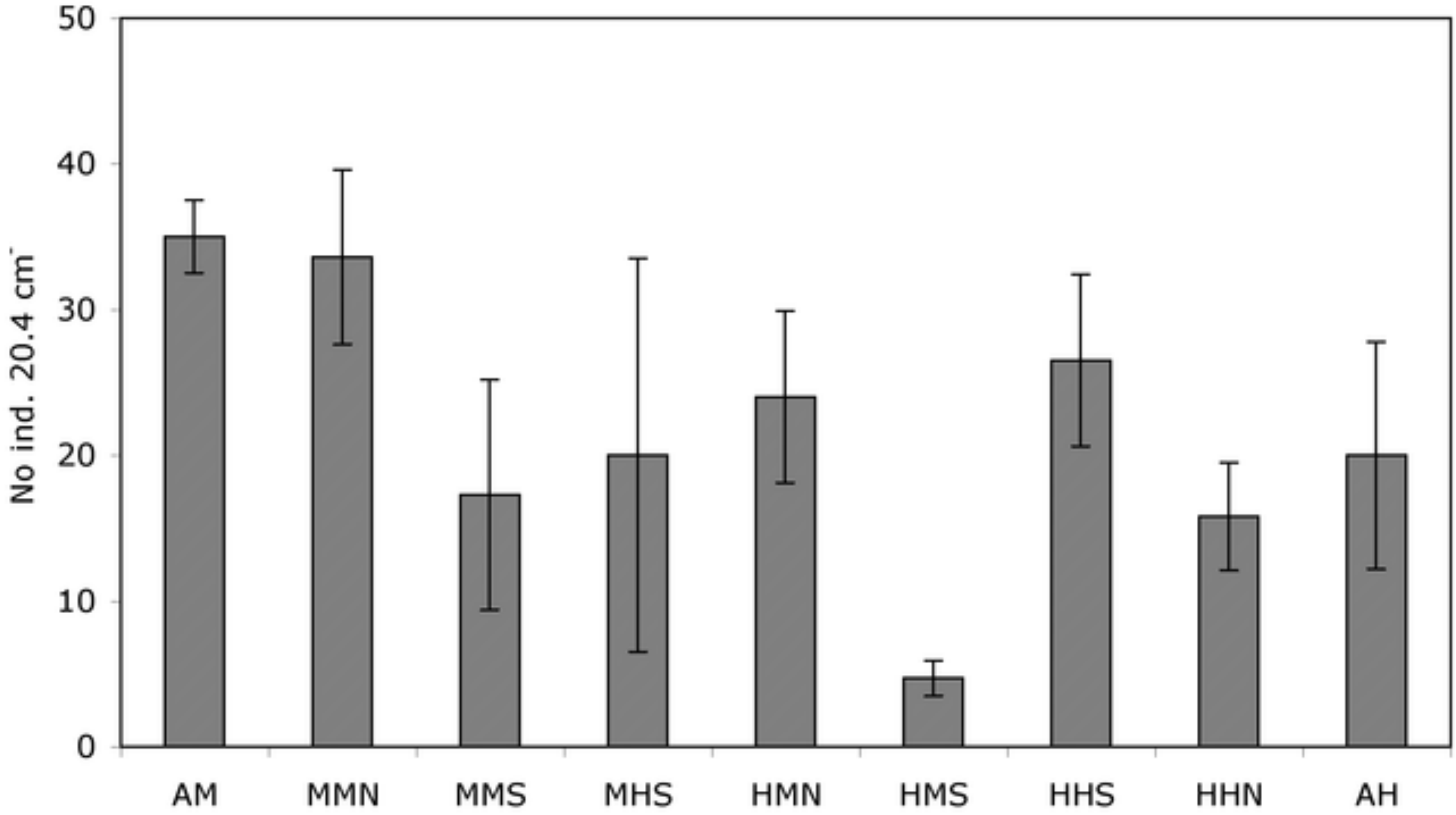
Fig. 3. Comparative rarefaction curves illustrating the diversity of recolonizing assemblages on naturally Cu-loaded sediments and Cu-spiked sediments. Treatment description are given in Table 1.

Fig. 4. Percent composition of recolonizing Polychaeta families (A) and peracarid Crustacea (B) in the different treatments. Treatment descriptions are given in Table 1.

Fig. 5. Multidimensional scaling (MDS) plot displaying recolonizing macrofaunal assemblages of differing treatments. Circles indicate two major assemblages (ANOSIM, P = 0.001; Dissimilarity = 77.3%): one associated with the mouth of the basin, with naturally low Cu concentration (solid line) and one associated with the head of the basin, with naturally high Cu concentration (dash line). Detailed results of Analysis of Similarity (ANOSIM) for recolonizing macrofauna between Cu treatments are given in Table 4.

Fig. 6. Feeding mode (A) and lifestyle (B) representation of recolonizing macrofauna in the differing Cu treatments. Treatment description are given in Table 1. SDF = surface deposit feeders; SSDF = subsurface deposit feeders.


Percent composition
100%
80%
60%
40%
20%
0%
AM
MMN
MMS
MHS
HMN
HMS
HHS
HHN
AH
Annelida
Bivalvia
Crustacea
Others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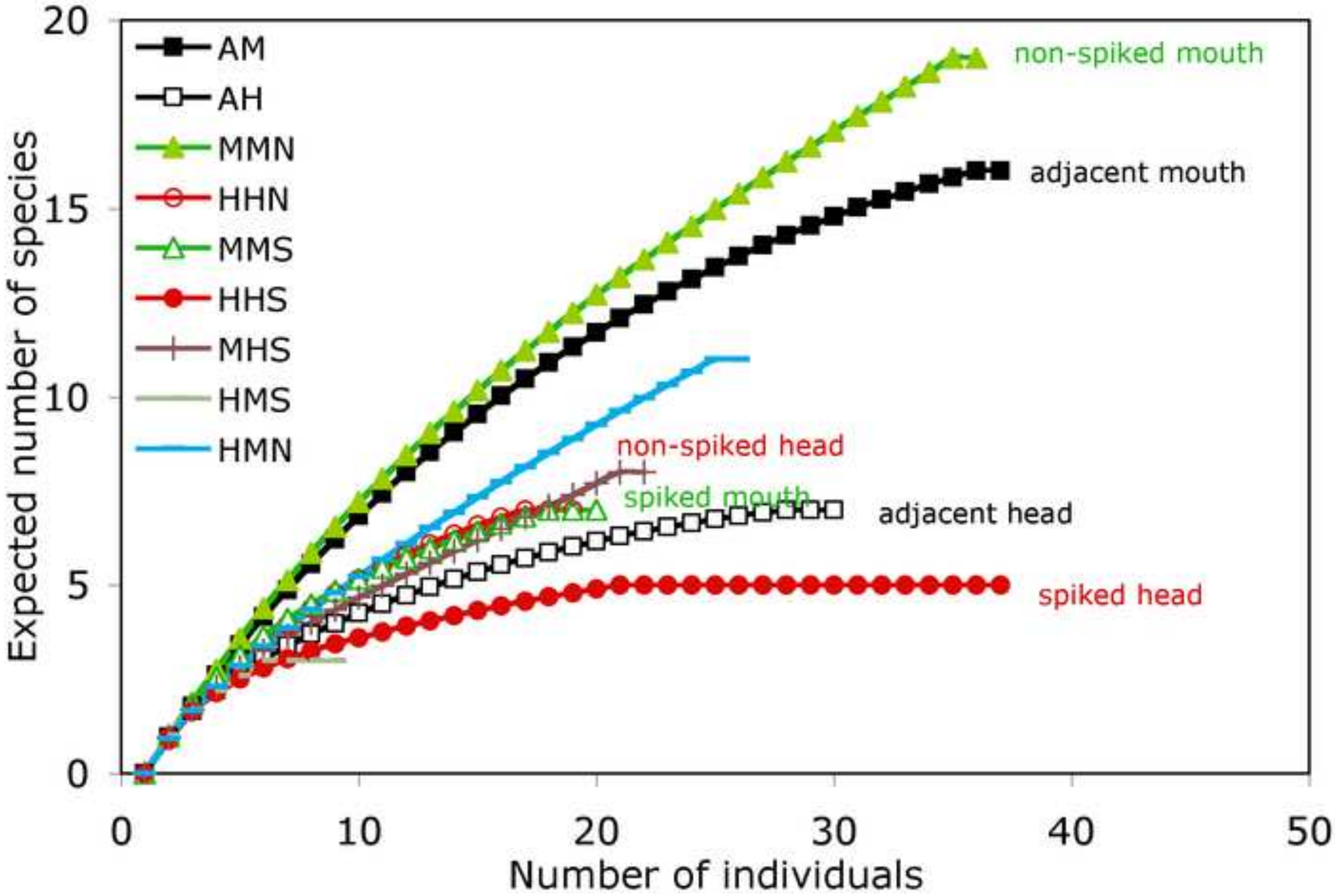
20
15
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5
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Expected number of species
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40
50
Number of individuals
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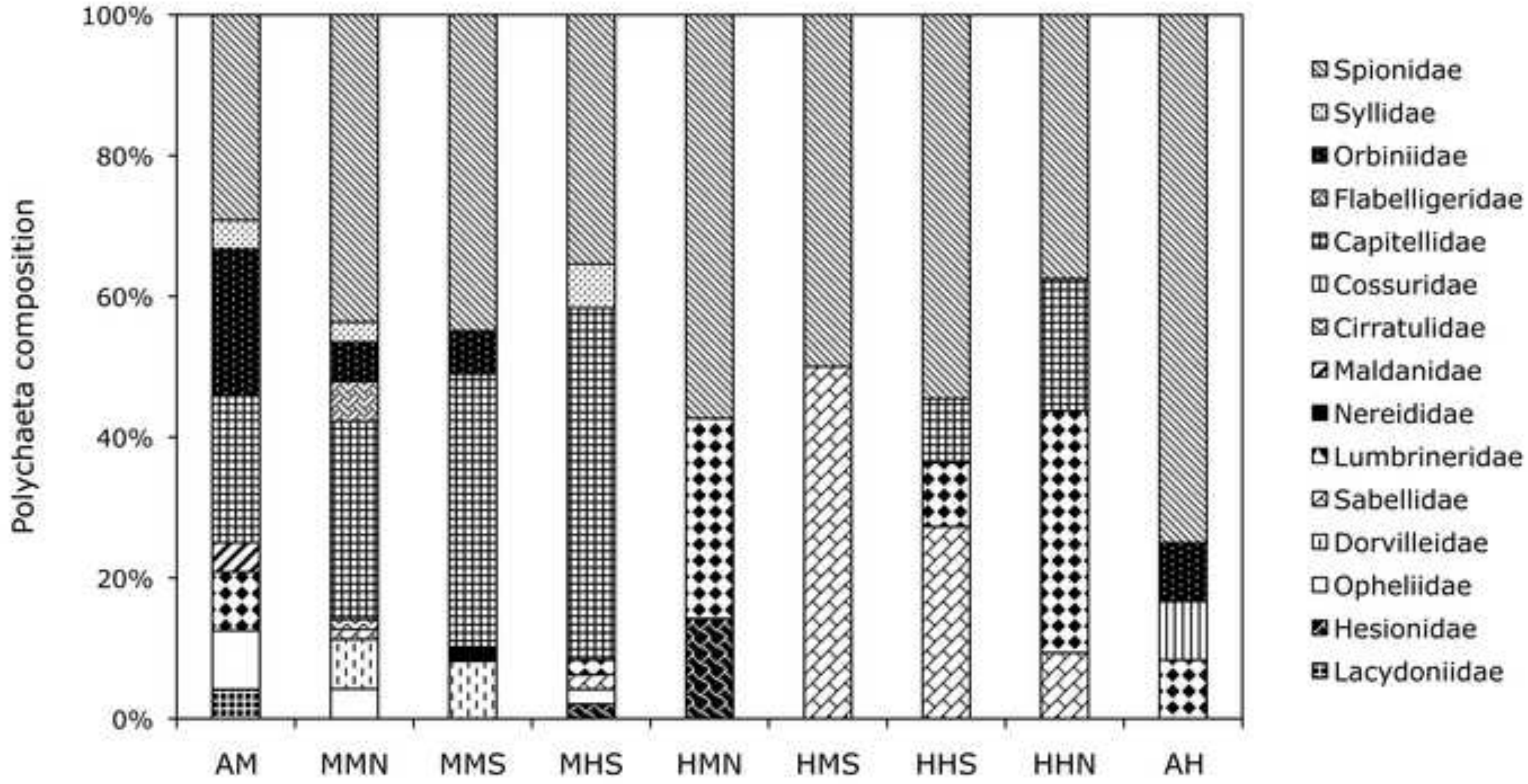
non-spiked head
spiked mouth
adjacent head
spiked head


100%
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20%
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Polychaeta composition
AM
MMN
MMS
MHS
HMN
HMS
HHS
HHN
AH
Spionidae
Syllidae
Orbiniidae
Flabelligeridae
Capitellidae
Cossuridae
Cirratulidae
Maldanidae
Nereididae
Lumbrineridae
Sabellidae
Dorvilleidae
Opheliidae
Hesionidae
Lacydoniidae

Figure(s)

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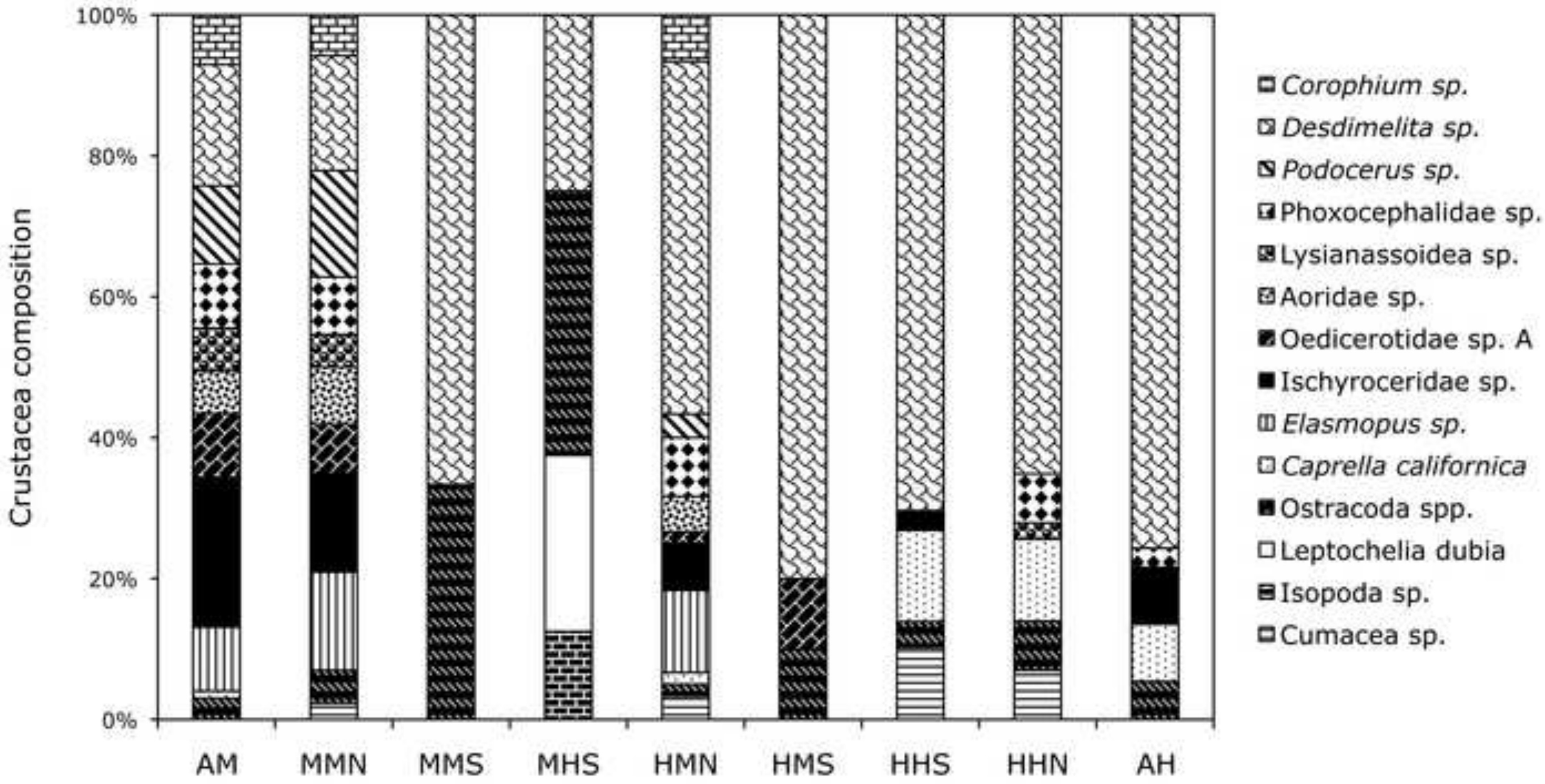

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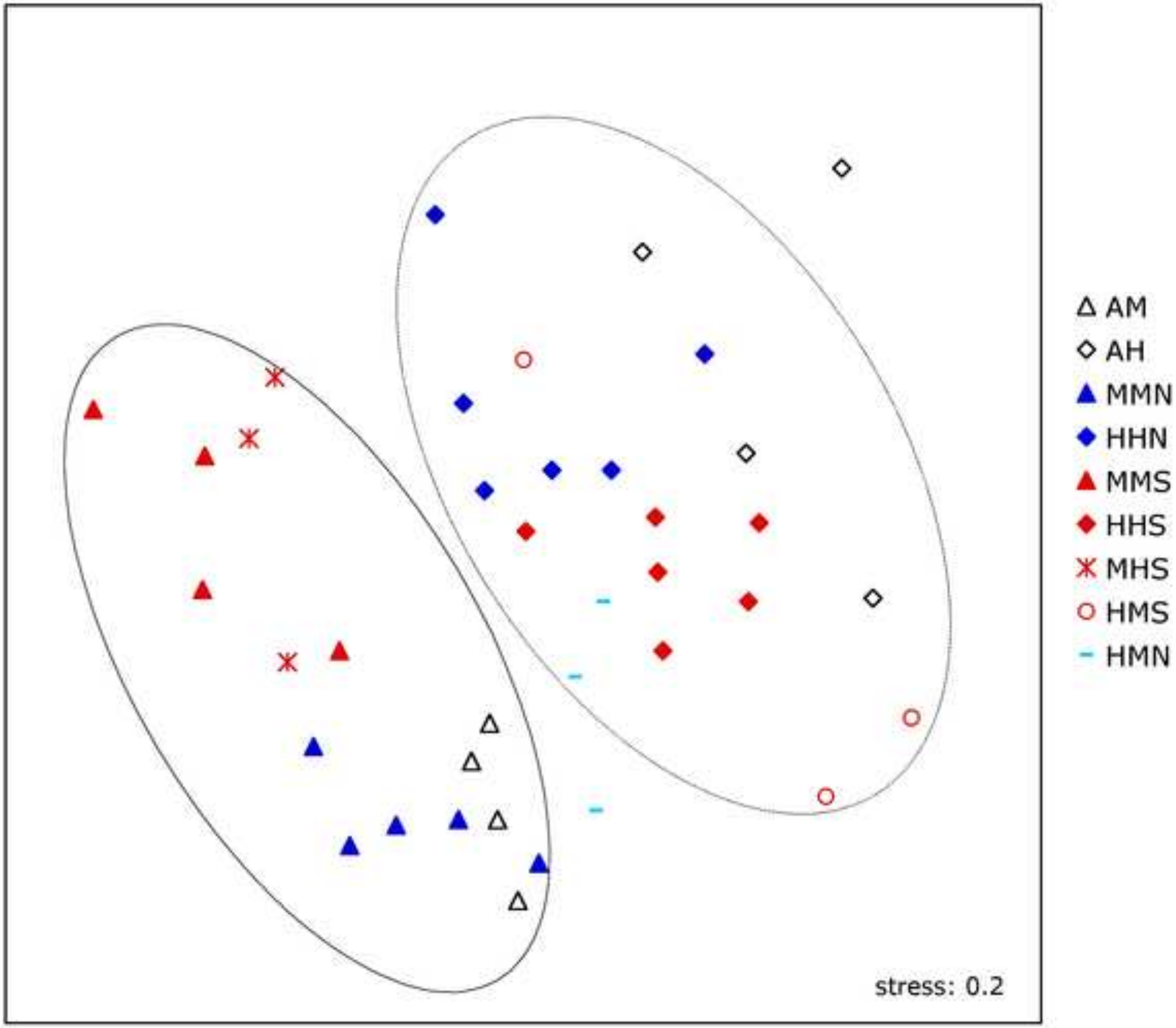

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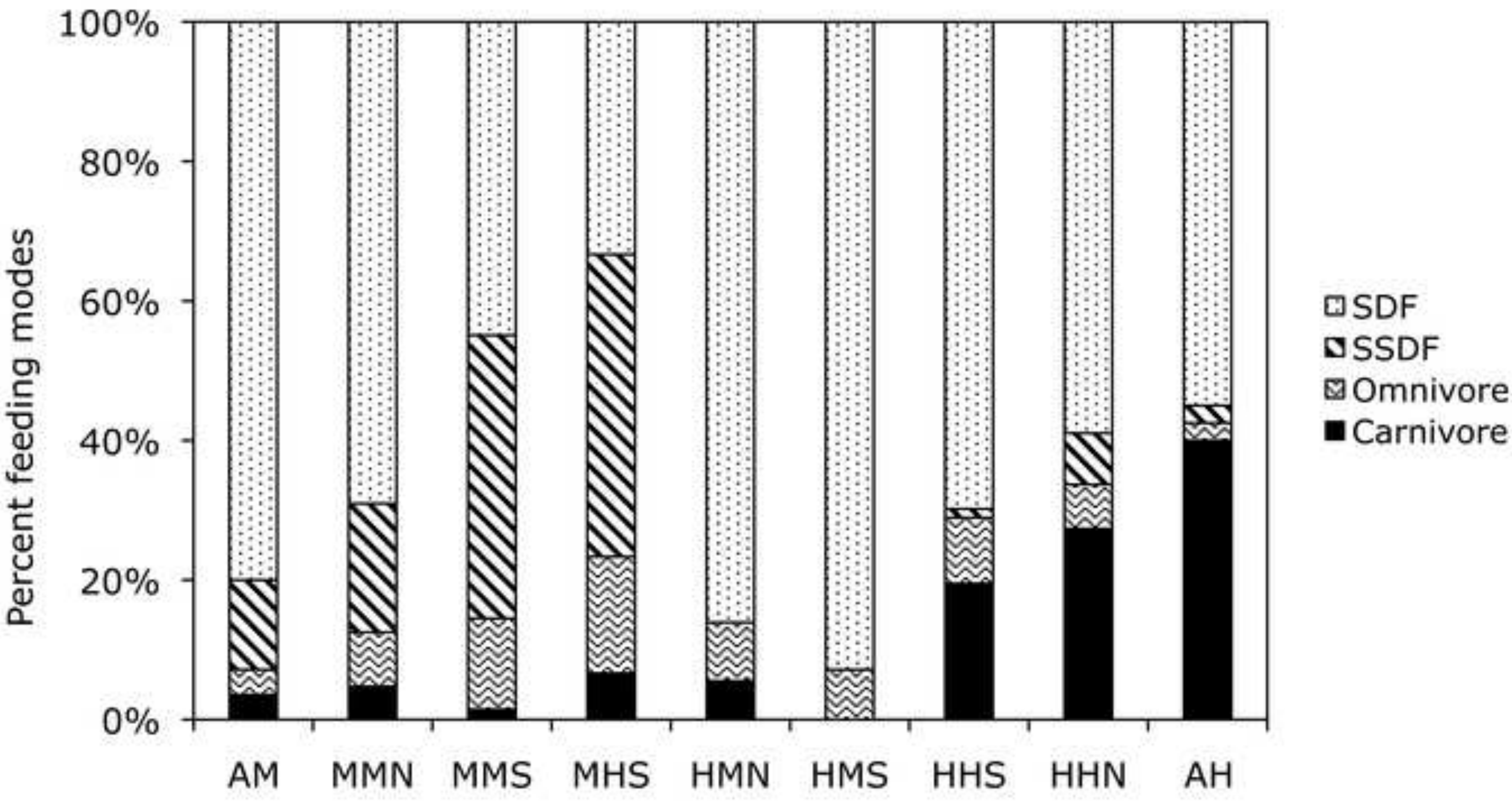

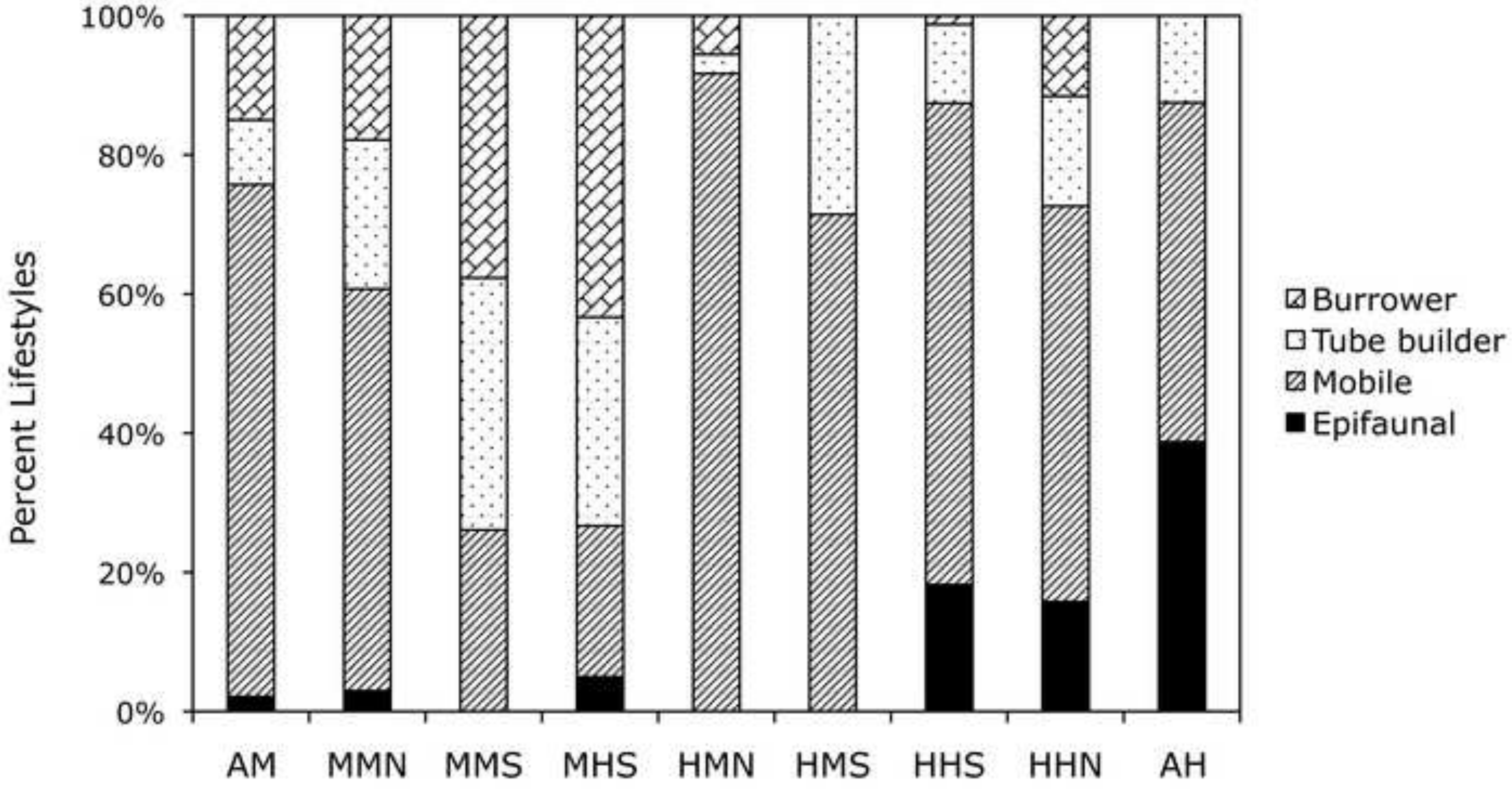
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☐ Tube builder
▨ Mobile
■ Epifaunal