

In Situ Approach to the Examination of the Impact of Copper Pollution on Marine Meiobenthic Copepods

Graham R. Saunders* and Colin G. Moore**

School of Life Sciences, Heriot-Watt University, Edinburgh, Scotland EH14 4AS, UK

(Accepted February 10, 2004)

Graham R. Saunders and Colin G. Moore (2004) In situ approach to the examination of the impact of copper pollution on marine meiobenthic copepods. *Zoological Studies* 43(2): 350-365. The aim of this study was to identify the influence of copper pollution on benthic copepod populations by means of dosing seabed sediments with copper. Superficial muddy sediments were removed from a shallow subtidal experimental site on the west coast of Scotland and dosed with copper powder to produce nominal dry weight concentrations of 0, 50, 500, and 5000 µg/g sediment. Four replicate 100 cm³ polycarbonate bottles were filled with spiked sediment for each concentration, and the bottles were implanted into the seabed at the experimental site. After 1 mo, the bottles, together with replicate core samples from the untreated background sediment, were collected, the meiofauna was extracted using colloidal silica, and the copper concentration was measured. In relation to the controls, copepod abundance was significantly depressed in the high-copper treatment. Composite diversity indices (Shannon-Wiener and Simpson's) and evenness failed to exhibit any copper-induced changes, although the number of species and genera were depressed at the highest concentration. Differences in the responses of copepods to copper were found between copepodites and adults and among different species. In general, the dominance of *Tachidiella minuta* and species of *Halectinosoma* and *Longipedia* in the control and low copper concentrations was replaced by dominance of species of *Cletodes*, *Laophonte*, and *Stenhelia* at the higher concentrations. The abundance of *Cletodes longicaudatus* was significantly enhanced in the high-copper treatment. The only statistical evidence for an impact of copper on the copepod assemblage at levels lower than the high treatment was revealed by applying the multivariate test ANOSIM (analysis of similarities) to the copepodite abundance data at the generic level, with the copepodite assemblage in the medium treatment being of a significantly different composition to that in the controls. Results are discussed in relation to other studies on the impact of pollution on the meiofaunal taxa, the likely mechanisms bringing about the observed changes, and the validity of this in situ approach. <http://www.sinica.edu.tw/zool/zoolstud/43.2/350.pdf>

Key words: Harpacticoids, Diversity, Metals, Disturbance.

Any study designed to evaluate the effect of perturbations on the marine environment is likely to encounter a formidable array of problems. Difficulties may arise from the wide range of physical parameters that have to be considered, or in the interpretation of the sometimes subtle effects on the structure of the ecological community. Separation of natural fluctuations from changes initiated by the introduction of a chemical disturbance presents a considerable additional challenge.

Confounding factors can take many forms

such as depth, oxygen levels, salinity and temperature fluctuations, patchiness of food sources, current regimes, and granulometric variations. Coull and Chandler (1992) noted that field studies evaluating the effect of pollution on meiofauna are invariably one of 2 types: (1) monitoring of an area or site that has undergone either continuous chronic contamination (e.g., sewage effluent) or a more-sudden, acute event such as an oil spill, and (2) a more-manipulative approach where contaminants are experimentally introduced to sites that are usually thought to be previously uncontaminat-

* To whom correspondence and reprint requests should be addressed. Tel: 44-131-4462456. Fax: 44-131-4462405.

E-mail: Graham.Saunders@snh.gov.uk

Current address: Scottish Natural Heritage, 2 Anderson Place, Edinburgh, Scotland EH6 5NP, UK

**E-mail: C.G.Moore@hw.ac.uk

ed. In the 1st approach, the selection of control or reference sites may amount to a “best guess” as to what may constitute an environmentally equivalent area. The 2nd strategy, by its very nature, provides for a greater degree of control with pre-contamination sampling, but in situ manipulation and controlled contamination of subtidal sediments, particularly those with a high silt-clay content, are notoriously difficult. In addition, the contaminated plot may need to cover a relatively large area to accommodate replicate sampling, and even then, repeated sampling of the same location within the plot may accidentally occur. Any polluted area will require the control plot to be located at a distance that will minimize the possibility of cross-contamination, and this then increases the chances that one or more of the factors mentioned above may, at some stage, come into play. For the most part, this is essentially a problem of scale. Large, well-separated plots are clearly more likely to incur greater differences in environmental variables than smaller, more-localized sites. But a reduced separation distance presents the very real possibility that the experiment will be invalidated due to control contamination.

The small size and often high densities of meiofaunal taxa means that, in practice, a test plot need not be particularly large if it is to be infrequently sampled or recovered in its entirety, and this presents the possibility of an entirely different approach. A number of studies have explored the use of an experimental strategy that incorporates enclosures, trays, or containers arranged in arrays at, or near, the seabed. Here, sediments are collected from a particular site, treated or modified for the parameter being examined, and then returned to the site for sampling at a later date. The contained sediments are therefore theoretically no different from those surrounding them, and should also be under the same local environmental influences.

However, application of this approach has been largely orientated towards investigations into the dynamics of settlement and recolonization, and so the test sediment is usually rendered azoic by drying or repeated freezing and thawing before placing it on the seabed. Both Hall and Frid (1997) and Olsgard (1999) investigated the effect of copper on recolonization patterns of subtidal macrobenthic species with some degree of success. Hall and Frid (1997) found rapid but sporadic recolonization over a 24 h period by relatively few species, many of which appeared to be attracted to the remains of the individuals killed in the freez-

ing process, but there was little by way of an impact that could be attributed to a copper contamination effect. Olsgard (1999), however, had the benefit of a background community with a much higher abundance, and was able to demonstrate a significant negative effect of a copper concentration of 300 $\mu\text{g/g}$ on selected polychaete species; the majority of taxa, though, exhibited no response even at the highest concentration of approximately 2000 $\mu\text{g/g}$.

Similar studies using meiofauna are surprisingly rare and have almost exclusively concentrated on the effect of hydrocarbon contamination (Alongi et al. 1983, Decker and Fleeger, 1984, Palmer et al. 1988, Spies et al. 1988, Christie and Berge 1995). Alongi et al. (1983), for example, examined the colonization process within a shallow subtidal meiobenthic sand community subjected to oil contamination at a range of concentrations. After 16 d, all of the treatment communities in the surface sediment layer had achieved parity with the background communities, but the return to natural nematode abundance in the subsurface anoxic zone occurred more slowly.

The use of azoic sediments can provide answers to specific issues relating to the rate and nature of faunal immigration into sediments. However, the methodology is intrinsically orientated towards a somewhat unnatural occurrence, and the possibility of artifactual interference must always be present. Reports similar to that of Hall and Frid (1997), for which the attraction of organic remains is suspected of being implicated in the abundance increases, are common. In more extreme treatments where all organisms are removed, the converse situation can occur with the colonization process retarded due to loss of sedimentary organic binding properties or an absence of microflora (Fegley 1988).

For a realistic assessment of pollution impacts, it is clearly more desirable to deal with natural sediments complete with a fully intact, non-stressed community. Any form of manipulation may introduce concerns as to the effect of the experimental process on the ability of a study to accurately reflect the true situation.

Previous experience by the authors suggests that most meiofaunal taxa can withstand the rigors of displacement and some degree of physical manipulation. In this experiment, the feasibility of live-community manipulation under field conditions was investigated, while being simultaneously applied to evaluation of the effects of a range of copper concentrations on a muddy subtidal

meiobenthic community. Individually enclosed replicate community units were treated by mixing with copper powder and subsequently inserted into the sediment on the seabed. To ensure accurate identification of potential cross-contamination events, background, control, and copper-amended samples were randomly allocated a position within a grid array.

Manipulative field experiments on the impacts of pollution on the meiofauna are rare, and therefore this study probably represents a new approach to the evaluation of the effects of metals on meiobenthic communities.

MATERIALS AND METHODS

Experimental Preparation and Deployment

The study site was located on the southern side of Loch Creran, a fiordic sea-loch on the west coast of Scotland. An exploratory dive was initially performed to determine conditions of the site, and a preparatory sediment sample was collected. This fresh sample was weighed while wet, then dried to a constant weight to determine the water content in order to allow calculation of the nominal sediment concentration in $\mu\text{g/g}$ dry weight of sediment. A 3 x 3 m grid with thirty-six 0.25 m² sectors was constructed in situ by a diver with garden stakes and polypropylene string at a depth of 9.5 m below the chart datum at the location 56°31.39'N 5°20.05'W (WGS84 datum). The orientation of the grid was roughly in a north-south direction.

Sediment from close to the grid site was collected by a diver who gently scooped the surface layer into 5 L buckets. On returning to land, the sediment was homogenized in a single 20 L bucket by gentle manual stirring. Aliquots were taken and made up to a predetermined weight in 1 L acid-washed plastic beakers. Copper powder (-200 mesh, 99%, Sigma-Aldrich, Poole, UK) was added to achieve nominal dry weight concentrations of 50, 500, and 5000 $\mu\text{g/g}$. Each preparation was again gently but thoroughly homogenized before dispensing to 100 cm³ wide-mouthed, screw-cap polycarbonate bottles (75 mm tall with a 42 mm internal mouth diameter). The bottles were filled to very close to the brim, but care was taken to leave the lip thread clean so that the lid could be securely sealed for transportation without leakage. Four replicate bottles for each concentration were prepared, with a further 4 control replicates, treated identically, but without the addition of copper. Both

the bottles themselves and the lids were labeled with an indelible marker. This procedure took approximately 1.5 h. Once capped, the bottles were placed in buckets containing cold seawater and transported with a minimum of agitation to the site of deployment.

Each bottle was placed within the center of a grid square. The location within the grid was determined by random allocation. Bottles were gently pressed into the sediment until the capped lip was just protruding from the surrounding sediment surface. After all of the bottles had been positioned, the lids were very gently unscrewed but not completely removed. They were then left to equilibrate and settle overnight.

The next day, 4 background core samples for meiofauna and 1 each for metal content and particle size analyses were taken from randomly allocated squares within the grid. The cores were identical in bore diameter and depth to the experimental bottles so that the recovered surface area and volume would be comparable. After this had been completed, the lids of the experimental bottles were completely unscrewed and removed, with a great deal of care taken not to disturb the surface of the sediment within.

Thirty days later, the bottles were gently but securely recapped and returned to the surface, where they were immediately preserved in formalin prior to meiofaunal extraction, or were frozen before metal content analysis. After this was completed, repeat background samples for meiofauna, metal content, and particle size analyses were taken from randomly allocated squares not previously sampled.

Meiofaunal extraction

The meiofauna passing a mesh of 1 mm but retained by a mesh of 45 μm was extracted from the sediment by 4 decantation stages, followed by 2 density separation stages using Ludox-TM at a specific gravity of 1.115 (Moore and Stevenson 1997).

Univariate analyses

Total copepod abundances and effects on individual taxa were examined by one-way analysis of variance (ANOVA) on treatment, control, and background samples, followed by Tukey's a posteriori test in the case of significant ANOVAs. Prior to performing the ANOVA, all abundance data were log-transformed, and Bartlett's and Levene's

tests were applied to confirm the homogeneity of the variance.

Differences in community structure were determined by performing one-way ANOVA on a variety of diversity indices. Where logarithms were used in the calculations, \log_2 was selected.

Multivariate analyses

Non-parametric multidimensional scaling (MDS) was applied to copepod species abundance data, together with one-way analysis of similarities (ANOSIM) tests performed on groups of community samples. Both MDS and ANOSIM results were obtained using the PRIMER software package (Primer-E, Plymouth, UK).

A range of transformations of varying levels of severity was applied to the data prior to analysis.

Metal content analyses

Frozen samples were slowly thawed and homogenized. Three replicate 25 g samples of wet sediment were removed from each bottle and oven-dried at 60°C for 4 d. The dried sediment was ground with a mortar and pestle, and 2 g was accurately weighed and placed in a 50 ml plastic sample tube. Five milliliters of deionized distilled water was added to the sediment followed by 5 ml of concentrated nitric acid (trace metal analysis grade: Fisher, Loughborough, UK). After the initial reaction had subsided, the sample tubes were capped and placed in a water bath at 70°C for 4 h, with agitation by inversion every 30 min. After acid digestion, the tubes were removed and allowed to cool before adding a further 40 ml of deionized distilled water. Samples were then left to settle before instrumental analyses. Replicate reagent blanks were simultaneously taken throughout the entire process.

Table 1. Nominal and measured mean sediment copper levels

Copper level	Sediment Copper Concentration ($\mu\text{g/g}$)	
	Nominal	Actual
Background (1 day)	0	16.2
Background (30 day)	0	13.5
Control (30 day)	0	14.7
Low (30 day)	50	91.9
Medium (30 day)	500	893.4
High (30 day)	5000	8662.3

Copper concentrations were determined on a dry weight basis by atomic absorption spectroscopy (Instrumental Laboratories S11 oxyacetylene, Thermo Unicam, Cambridge, UK). Copper standards were made up in deionized distilled water to cover the range of 0–50 $\mu\text{g/ml}$ (985 $\mu\text{g Cu/ml}$ in 1% HNO_3 ; Sigma - Aldrich, Poole, UK). However, it was subsequently established that a linear response lay within the range of 0–10 $\mu\text{g/ml}$, and so all samples were diluted, when necessary, to give an absorbance value within this range.

RESULTS

The nominal and actual sediment copper concentrations in the core and bottle-confined samples are shown in table 1. Background and control concentrations did not vary between the start and the end of the experiment despite the proximity of the high-concentration samples. The introduced metal remained largely bound in the sediment matrix, with minimal loss via solubilization. The measured concentrations were substantially higher than intended, but remained within an experimentally relevant range.

Particle size analyses indicated that there was very little change in sediment character over the course of the experiment. The silt-clay composition did fluctuate slightly, but was not considered to be sufficient to affect the meiobenthic community structure.

Copepod community effects

Combined age group abundance

There appeared to be little or no effect of copper on total copepod abundance at the low and medium concentrations in comparison to the control abundance (Fig. 1). The high-concentration exposure did, however, exhibit a marked reduction in numbers, and its mean abundance significantly differed from those of the control, and low- and medium-level communities, but not from that of the background samples.

Examination of the effects at the level of individual taxa revealed a more-complex pattern of response, with an elevated abundance in either the low or medium treatments common. Figure 1 shows a range of typical responses by three of the most abundant species in the combined treatments. The highest copper concentration produced a clear abundance reduction for most

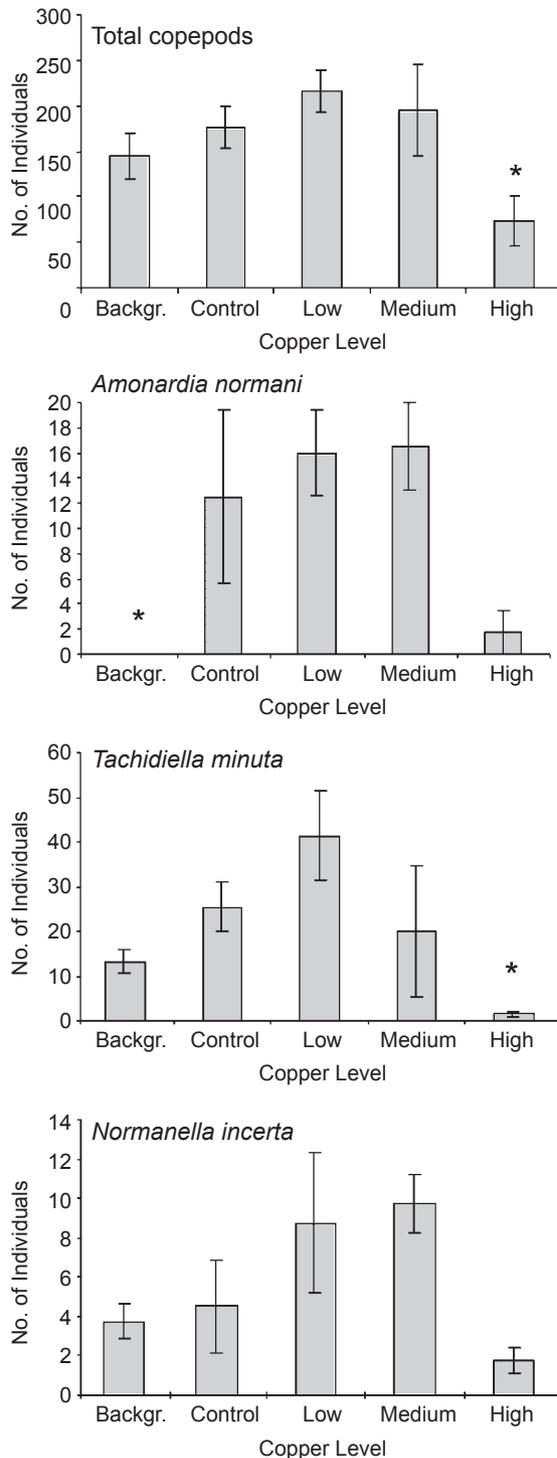


Fig. 1. Bar chart showing mean abundance of total copepods of *Amonardia normani*, *Tachidiella minuta*, and *Normanella incerta* (all age groups) in copper-treatment bottle enclosures and background samples. Error bars denote the standard error, and asterisks (*) indicate statistically significant differences compared to the control ($p < 0.05$).

species, although these reductions significantly differed from the controls in only two of the 11 species which had sufficient numerical abundance for statistical analysis. A higher frequency of significant differences occurred between the elevated abundance in the low- and medium-concentration treatments and the depleted high concentrations. One species, *Cletodes longicaudatus*, was notable in achieving a significant abundance increase in the high-copper treatment.

The overall pattern of effect was broadly maintained at the higher taxonomic levels of genera (Fig. 2) and family, with the majority of taxa undergoing a significant abundance drop corresponding to the high copper contamination. However, the Cletodidae (predominantly species of *Cletodes*) continued to collectively maintain an elevated abundance at the greatest contamination level, although it was not statistically significant ($p > 0.05$).

When the most abundant individual genera were ranked and grouped by the treatment in which they reached their highest abundance (Table 2), there was a degree of consistency in the relationship between some taxa and copper level tolerance. In general, the dominance of *Tachidiella minuta* and species of *Halectinosoma* and *Longipedia* in the control and low-copper samples was replaced by *Cletodes*, *Laophonte*, and *Stenhelia* in the higher-concentration treatments. This trend was largely maintained at the family level with the highest mean abundance of the Ectinosomatidae and Tisbidae found in the control or low treatments, while the higher copper concentrations promoted a peak abundance of the Cletodidae. The status of the Diosaccidae was less well defined, but tended towards a tolerance of higher copper levels.

Adult and copepodite abundances

In order to investigate the copepod community response in greater detail, the abundances of adults and copepodites were independently examined. It is clear that the overall patterns of effect differed (Fig. 3), with the adult component exhibiting an abundance reduction corresponding to increasing copper, while the copepodite numbers did not conform to a similar pattern. The ANOVA results for adult abundance confirmed a significant difference between the communities subjected to the highest copper level and those in the control, low, and background sediments, respectively.

Further examination of adult sensitivity was difficult at the species level because of low individ-

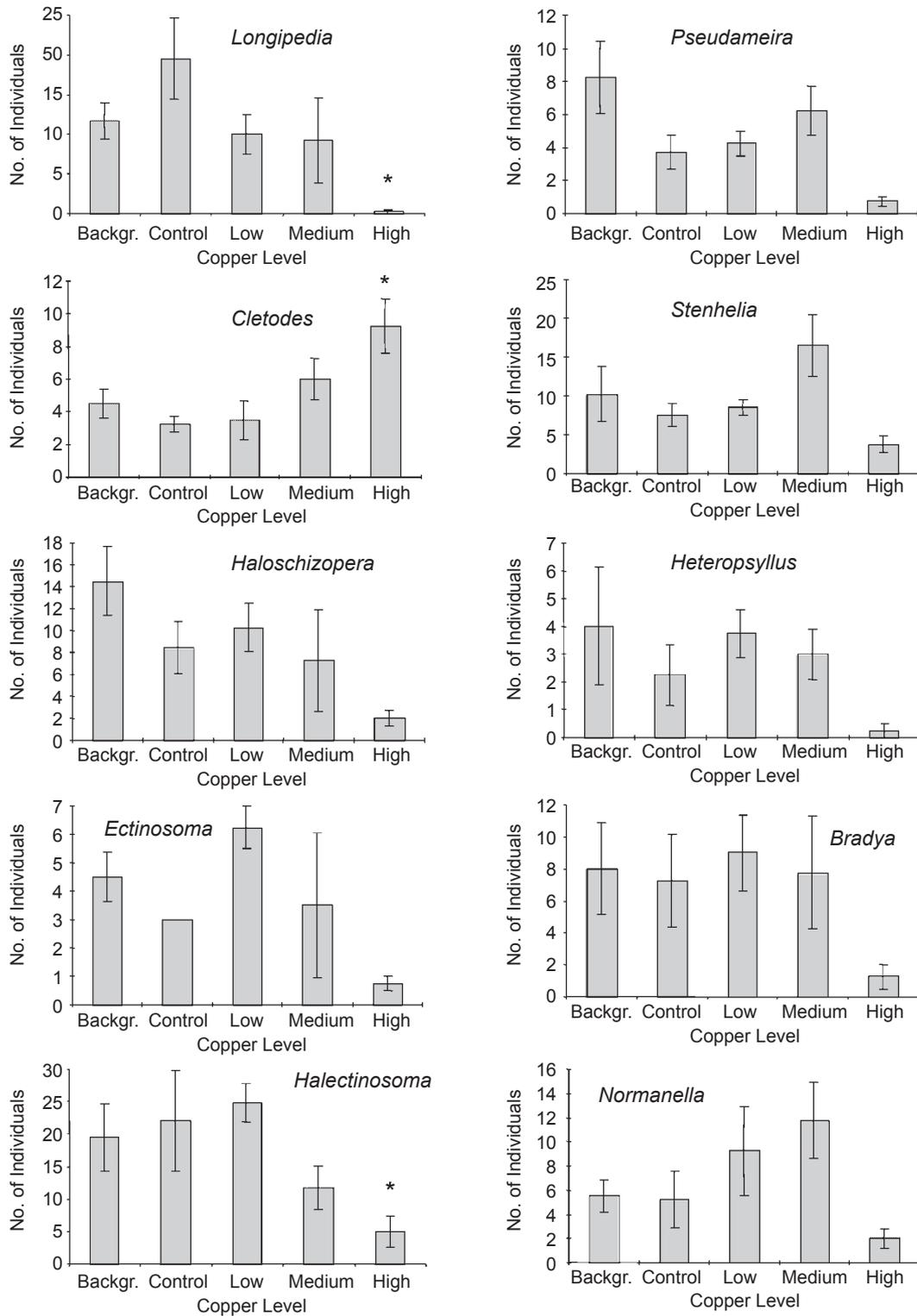


Fig. 2. Mean abundance of copepods by genera (all age groups) in the enclosure treatments and background cores. Error bars denote the standard error, and asterisks (*) indicate statistically significant differences compared to the control ($p < 0.05$).

ual species abundances. Only *Tachidiella minuta* adults were present in sufficient numbers to warrant statistical attention, and fig. 3 reveals a clear effect at the high copper concentration, which was supported by the ANOVA results. This differed from the results incorporating all age groups in the ability to detect a significant difference between the medium- and high-concentration exposures.

The copepodite component of the harpacticoid community was collectively the most abundant in all of the samples, giving rise to mean values far in excess of adult numbers (Fig. 3). In addition, the overall copepodite response to metal contamination appeared to follow a slightly different pattern to the adult response. While the mean combined adult abundance declined with increasing copper concentration, copepodite counts reached greatly elevated numbers with low and medium copper concentrations, giving rise to significant differences between those treatments and the high-copper exposure.

The proliferation of copepodites in these contaminated sediments did not seem to be confined to particular species. Figure 4 shows the abundance charts for all of the numerically important genera (species were not used because of the difficulties associated with juvenile identification). It is clear that the majority of copepodite taxa were able to thrive in the low and medium concentrations, and that it was this population increase that largely gave rise to the observed statistically significant effects. Only copepodites of *Amonardia* and *Longipedia* displayed a response that could be said to approach a negatively correlated relation-

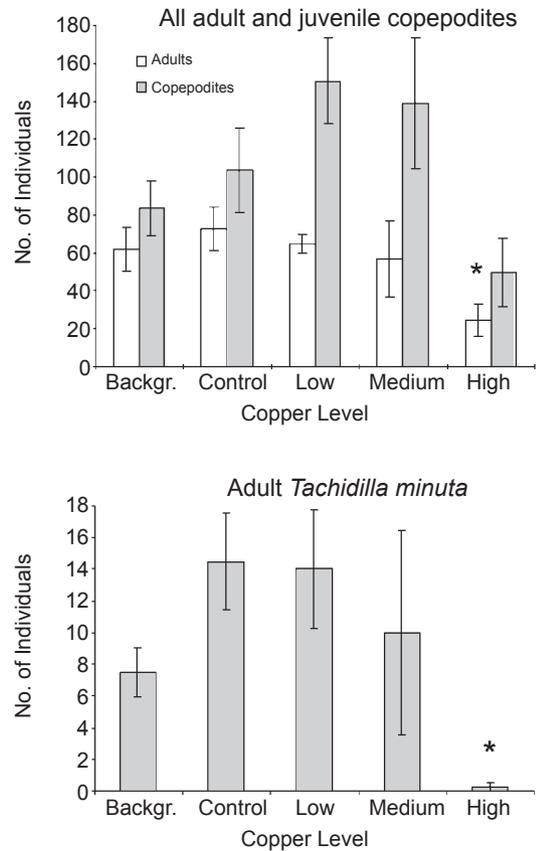


Fig. 3. Mean abundance of total adults and copepodites and mean abundance of adult *Tachidiella minuta* in background cores and copper-treatment enclosures. Error bars denote the standard error, and asterisks (*) indicate statistically significant differences compared to the control ($p < 0.05$).

Table 2. Ten most-abundant genera ranked by percentage mean abundance for each treatment: all age groups

Rank	Background		Control		Low		Medium		High	
	Genus	Percent mean abundance	Genus	Percent mean abundance						
1	<i>Stenhelia</i>	12.89	<i>Tachidiella</i>	14.63	<i>Tachidiella</i>	18.64	<i>Stenhelia</i>	13.88	<i>Cletodes</i>	12.29
2	<i>Halectinosoma</i>	12.89	<i>Halectinosoma</i>	12.63	<i>Halectinosoma</i>	11.19	<i>Tachidiella</i>	9.91	<i>Stenhelia</i>	9.97
3	<i>Haloschizopera</i>	9.59	<i>Longipedia</i>	11.19	<i>Stenhelia</i>	9.49	<i>Amonardia</i>	8.18	<i>Rhizothrix</i>	7.97
4	<i>Rhizothrix</i>	9.42	<i>Stenhelia</i>	8.75	<i>Amonardia</i>	7.23	<i>Rhizothrix</i>	7.31	<i>Laophonte</i>	7.31
5	<i>Tachidiella</i>	8.76	<i>Amonardia</i>	8.61	<i>Haloschizopera</i>	4.63	<i>Halectinosoma</i>	5.82	<i>Halectinosoma</i>	6.64
6	<i>Longipedia</i>	7.77	<i>Haloschizopera</i>	4.88	<i>Longipedia</i>	4.52	<i>Normanella</i>	5.82	<i>Amonardia</i>	2.66
7	<i>Pseudameira</i>	5.45	<i>Bradya</i>	4.16	<i>Normanella</i>	4.18	<i>Pseudobradya</i>	5.08	<i>Normanella</i>	2.66
8	<i>Bradya</i>	5.29	<i>Rhizothrix</i>	3.73	<i>Laophonte</i>	4.18	<i>Longipedia</i>	4.58	<i>Haloschizopera</i>	2.66
9	<i>Normanella</i>	3.64	<i>Normanella</i>	3.01	<i>Bradya</i>	4.07	<i>Bradya</i>	3.84	<i>Paradactylopodia</i>	2.33
10	<i>Cletodes</i>	2.98	<i>Pseudameira</i>	2.15	<i>Rhizothrix</i>	4.07	<i>Haloschizopera</i>	3.59	<i>Tachidiella</i>	1.99

ship with copper concentration (Fig. 4), and thus showed a significant population reduction between the control and high metal concentration.

Examination of differences in the copepodite community composition between treatments revealed a pattern of modification similar to that exhibited by adult species with the dominance of *Tachidiella* in the low-concentration sediments replaced by cyclopoids, *Cletodes* and *Rhizothrix*, at higher concentrations.

Of incidental interest was the absence of *Amonardia* species from the background samples (Fig. 4), while maintaining a proportionately high presence in treated samples. This appeared to be the only taxon that exhibited a clear attraction for the treatment enclosures.

Diversity

Of the suite of diversity indices that were

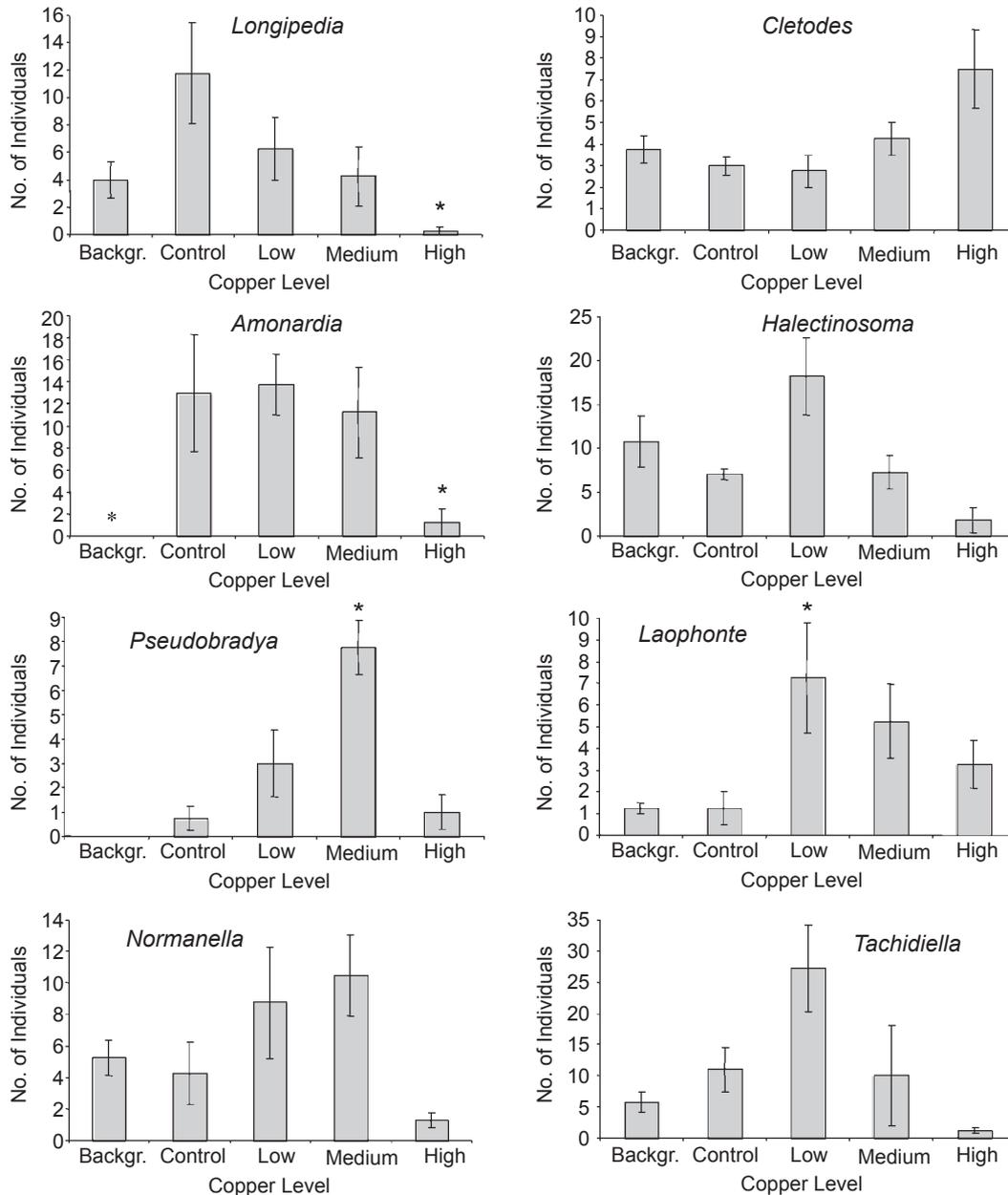


Fig. 4. Mean copepodite treatment abundance within dominant genera. Error bars denote the standard error, and asterisks (*) indicate statistically significant differences compared to the control ($p < 0.05$).

applied to the copepod community, only those dependent on the number of taxa present rather than individual abundances were able to adequately display a visible effect (Fig. 5). The communities subjected to the low copper concentration appeared enhanced in terms of numbers of species and genera and species richness, but they did not markedly differ from the control communities. The communities exposed to the high concentration did, however, undergo a clear decline when compared to the controls.

The ANOVA results provided confirmation of these effects, although the level of sensitivity varied for each index. The Shannon-Wiener, Simpson, and evenness indices showed no statistically significant variation between the contaminated communities. Species richness was able to

detect a difference between the elevated low concentration value and the obviously impacted high concentration. It was, however, the simple enumeration of species and genera that provided the clearest indication of copper's impact.

MDS ordinations

Figure 6 displays the results of MDS applied to all age group data. It is clear that the communities exposed to the high concentration remain spatially segregated, despite a comprehensive range of data transformations. This was confirmed by ANOSIM. The maintenance of the integrity of this segregation in the binary (presence/absence) transformation strongly implied that the nature of the community difference was taxon-dependent,

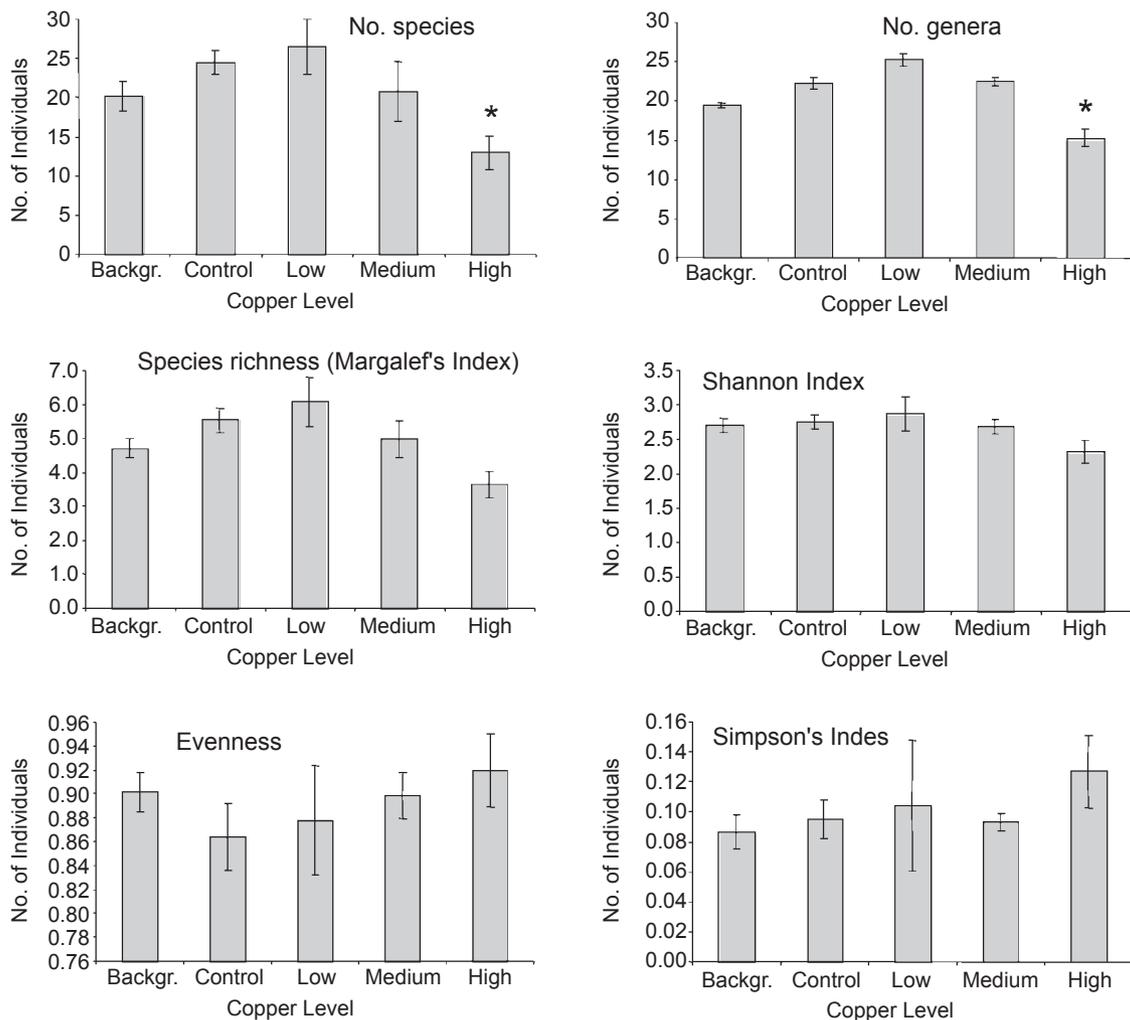


Fig. 5. Mean copepod community diversity in background and treatment samples. Error bars denote the standard error, and asterisks (*) indicate statistically significant differences compared to the control ($p < 0.05$).

thus confirming the conclusions drawn from the univariate diversity analyses.

When the same analysis was performed with adult-only abundance data, the overall response was largely unchanged.

The copepodite results (Fig. 7) also indicated divergence of the high-concentration communities, but here there was also evidence from ANOSIM, after transformation, that the medium-concentration exposure was also modifying the species composition of the juvenile component, with significant differences when compared against both the control and low exposures (Table 3). This was the only firm statistical indication of an effect for any of the concentration levels below that of the high concentration. Significant differences could also be seen between the control and background communities (Table 3) indicating the presence of an “enclosure effect”.

DISCUSSION

An important basic requirement for the success of this experiment was that the integrity of the sediment-copper concentration be maintained throughout the experimental period. Other studies have reported substantial losses by diffusion. Hall and Frid (1995) in a contaminated microcosm experiment with 30% of the water replaced at 2-wk intervals (with a silt-clay content comparable to the present experiment at about 59%) found that copper was exported from the sediment at the rate of $19.4 \mu\text{g}/\text{m}^2/\text{d}$ over a 1-yr period. This rate however did not fully reflect the exponential nature of the transfer with a loss of $161 \mu\text{g}/\text{g}$ of copper in the 1st week of the experiment.

Similarly Watzin and Roscigno (1997) found that the introduction of zinc-contaminated sediments to field conditions resulted in a concentra-

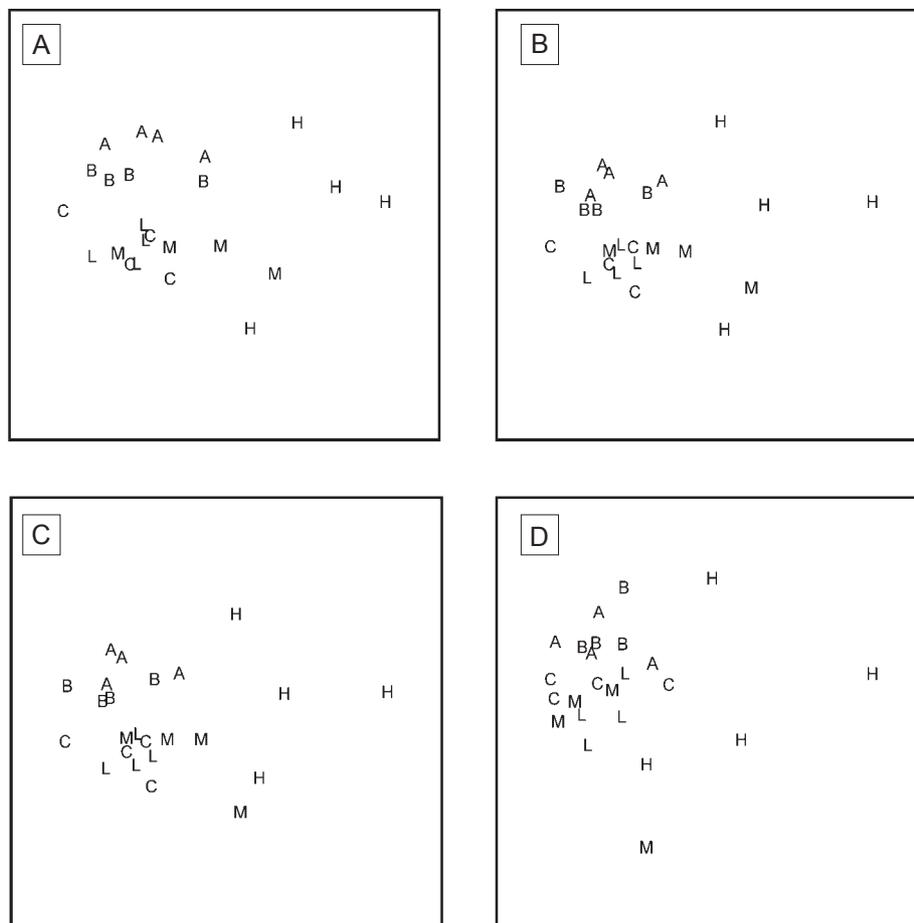


Fig. 6. MDS ordinations of copepod species (all age groups) from treatment enclosures and background cores. A, non-transformed; B, square root-transformed; C, Log_e -transformed; D, presence/absence. Stress values were 0.15, 0.16, 0.15, and 0.17, respectively. Treatment groups are C, control; L, low; M, medium; and H, high. Background cores are A, 1 d and B, 30 d.

tion reduction in the surface 2 cm of sediment greater than 1/2 the original value over a 1-wk period. In contrast, Olsgard (1999), in an experiment similar in nature to the present study, reported only low-level transfer of copper from the upper 1 cm into the water column.

By implication, if the high diffusion rates of some studies occurred in the present study, then the initial sediment concentration of copper must have been substantially higher than the calculated value. The freshly collected mud may well have had a higher water content than the preliminary test sediment, and this would have caused a higher copper loading than intended. This indeed was the case since the measured concentration was almost exactly double the intended initial concentration after a month in the field. However, if the diffusion rates indicated by the above studies were applied here, the initial concentrations would have had to have been unrealistically high. It seems

likely that the constraints imposed by the small containers, low local water movement, and the use of copper powder may have served to restrict copper movement to the overlying water column. It is unfortunate that an aliquot of the freshly mixed sediment was not taken before field deployment.

Given a relatively stable copper sediment loading, the relationship between concentration and copepod community composition was not one of a simple toxicity-mediated reduction in abundance. The copper contamination at intermediate levels (91.9~893.4 $\mu\text{g/g}$ dry weight of sediment) clearly gave rise to conditions which not only were many taxa able to comfortably tolerate but that actively stimulated an increase in abundance. It appears that the plotting of a generalized taxon response, such as the total number of copepods, does not wholly reflect the complexities of the underlying patterns of effects on component species. Within the community, there were a few

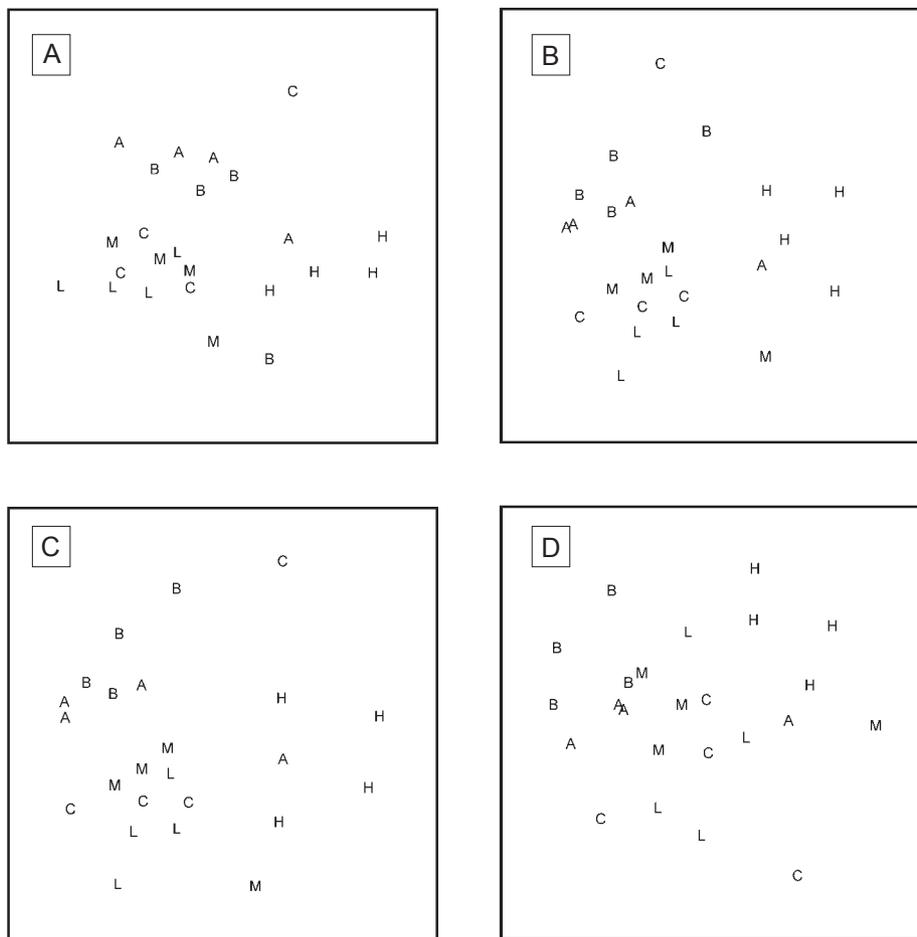


Fig. 7. MDS ordinations of copepodite genera from treatment enclosures and background cores. A, non-transformed; B, square root-transformed; C, Log_e -transformed; D, presence/absence. Stress values were 0.18, 0.19, 0.20, and 0.21, respectively.

species that exhibited a high degree of sensitivity to relatively low copper concentrations (*Longipedia* spp. and *Tachidiella minuta*) and those that appeared highly copper tolerant (*Cletodes* spp. *Stenhelia* spp., and species of Cyclopoida).

This study provides clear evidence that the majority of copepod taxa normally present within uncontaminated sediments were able to survive and even flourish in what might be considered moderately contaminated conditions. Detailed analyses of the copepod species data clearly show contamination-related increases in both the number of species and the number of individuals within some of these species, but it is the diversity measures which emphasize changes in the number of taxa that most comprehensively define shifts in the

community structure.

This phenomenon of species number increases at intermediate levels of community stress has been previously reported for a different form of environmental perturbation. Connell (1978) proposed the “intermediate disturbance hypothesis” to explain observed diversity increases after moderate levels of physical disturbance were applied to established communities such as those found in rain forests or on coral reefs. In those systems, it was suggested that physical disturbance plays an important role in maintaining diversity by preventing competitively dominant species from excluding others, with diversity maximized at an optimum level of disturbance frequency and intensity. At greater and lesser extremes, if the disturbance is weak or infrequent then the process of competitive exclusion is not prevented, while under an intense or frequent disturbance regime sensitive or trophically specialized species suffer high mortality or are excluded. In both cases, diversity, specifically the number of species, would be expected to decline.

A number of studies have succeeded in both experimentally re-creating conditions in which such diversity increases have been seen to occur, and identifying incidences where a natural disturbance has been observed to promote such an effect. The response of meiofaunal microcosm communities to a range of simulated disturbance regimes was investigated by Schratzberger and Warwick (1998), while Widdicombe and Austen (1998) and Austen et al. (1998) examined the effects of burrowing and feeding activities of experimentally controlled densities of specific macrobenthic species in a series of mesocosm experiments. In all of those studies, the predictions of the intermediate disturbance hypothesis were considered to have been upheld.

Conceptually, the effects of chemical contamination and physical disturbance have a great deal in common, with similarities in the modification mechanism and outcome of community change. Indeed, Alongi et al. (1983) made no distinction between the different sources of community impacts and defined disturbance as “a perturbation causing significant mortality of individuals”. In this respect, it is, perhaps, entirely valid to expect similar processes to occur in both chemical and physical disturbance events, and thus the underlying concepts of the intermediate disturbance hypothesis should be equally applicable to the present study. Here, a stable sandy mud community underwent contamination that may have removed,

Table 3. ANOSIM results for copepodite abundance. √ denotes a significant difference ($p < 0.05$)

Transformation		Treatment				
		Control	Low	Medium	High	Background at 1 d
None	Low					
	Medium					
	High	√	√	√		
	Background at 1 d	√	√	√		
	Background at 30 d	√	√	√	√	√
Square root	Low					
	Medium	√	√			
	High	√	√	√		
	Background at 1 d	√	√		√	
	Background at 30 d	√	√	√	√	√
Log _e	Low					
	Medium	√	√			
	High	√	√	√		
	Background at 1 d	√	√			
	Background at 30 d	√	√	√	√	√
Presence/absence	Low					
	Medium					
	High		√			
	Background at 1 d		√			
	Background at 30 d	√	√		√	

or reduced the abundance of, copper-sensitive competitively dominant species at low and medium copper levels. At high contamination levels, it is likely that meiobenthic mortality due to simple toxicity played the greatest role in reducing the number of species.

While some studies on the effects of metallic pollutants on the meiobenthos have reported overall abundance increases at intermediate contamination levels (Alongi et al. 1983, Austen and McEvoy 1997), few have demonstrated increases in the number of taxa or other diversity measures. This is perhaps largely due to the low number of studies performed in field conditions with natural communities. Another important factor may be that the range selection and number of concentration points have simply not been suitable for detecting such an effect. However, Somerfield et al. (1994), when sampling adjacent areas of long-term metal contamination, found that the site with the low to medium levels of contamination supported a copepod community with both the greatest mean number of genera and the highest Shannon-Wiener index. The nematode community, however, tended towards a reduction in diversity (number of species and Shannon-Wiener index) with increasing metal content, although there was a rise in abundance corresponding to median contamination levels.

The dynamics of the community restructuring process involved in sediment contamination may be revealed by examining the species compositions of sample communities in greater detail. However, in doing so, we must also consider the possible modifying effects of the experimental methodology, since the initial manipulative process necessarily involves a degree of physical disturbance. In addition, one must also take into account the consequences of community confinement within enclosure structures.

To some extent, such issues have been addressed by way of a number of essentially similar approaches. In the present study, an attempt was made to preserve the natural community structure, but other studies have documented the recovery of an extreme situation, that of recolonization of defaunated sediment. Evidence from those experiments tends to suggest that since the test sediments are replaced in the same location from which they were taken, the community is likely to return to its original composition over a fairly short period despite the barrier to within-sediment recruitment. Recruitment from the water column is probably a common route of mortality replacement

and taxon composition, and the abundance of suspended communities has been shown to be equivalent (Sibert 1981) or in excess (Fegley 1988) of that of the sediment. Chandler and Fleeger (1983) demonstrated that recolonization by suspension and resettlement was at least as important as the through-sediment route in muddy sediments, while Scheibel (1974) found that both nematodes and copepods were rapid colonizers of azoic sediments even when suspended over 2 m above the seabed. Similarly Watzin and Rosigno (1997), in a field experiment examining macro- and meiofaunal recruitment into zinc-contaminated sediment found large numbers of adult copepods entering the sediment via the water column.

Recolonization experiments within Loch Creran were performed by Olafsson and Moore (1992). In those experiments, they noted a disproportionate abundance of *Tachidiella minuta*, *Laophonte longicaudata*, and *Pseudobryda similis*, in the early stages of colonization. *Tachidiella minuta* clearly appeared to be a successful early colonizer in azoic sediments, but Harries (pers. comm.) observed no such dominance in studies on dredging disturbance, again in Loch Creran. In the present study *T. minuta* did achieve dominance, both as copepodites and adults, in the exposure treatments, but it was also present in relatively high abundance in the background communities, such that there was no significant difference between these and the control communities. The reduction in abundance of this species in the medium concentration and its almost complete loss in the high-concentration sediments formed the greatest contribution to treatment differences and was thus one of the major copper-mediated effects.

Both *Laophonte longicaudata* and *Pseudobryda similis* were found in the treatment samples, but unlike *T. minuta*, they appeared to be able to tolerate and probably exploit conditions in the contaminated sediments. *Laophonte longicaudata*, although not high in absolute abundance, achieved its greatest proportional abundance in the highest copper concentration with both adults and copepodites being well-established.

Many of the species that contributed to the overall community are epibenthic foragers, and it is possible that these species, particularly at the intermediate concentrations, were able to gain advantages of the food resource within the contaminated sediments while avoiding direct and prolonged contact with the contaminant. At the highest concentration, a small number of cyclopid

species achieved overall dominance. These taxa, together with *Amonardia normani*, were rarely found in the background samples and appeared to have a positive association with the enclosure treatments. However unlike the Cyclopoida, *A. normani*, mostly represented as copepodites, was clearly sensitive to copper at the high concentration.

Most of these taxa probably constitute opportunist species. Warwick et al. (1988) found that the substantial reduction in diversity in response to increasing levels of a mixture of oil and copper was almost entirely due to elevated abundances of *Tisbe*, *Danielssenia typica*, and an unidentified diosaccid. They concluded that since opportunistic copepods such as *Tisbe* had higher colonizing potential than nematodes, the response was general in nature due to organic enrichment brought about by addition of hydrocarbons and to the mortalities of large macrobenthic species.

Some epibenthic copepod species have been shown to exhibit an active affinity for sediment pits and depressions, and will settle within them after transport through the water column (Sun and Fleeger 1994). In addition, Fegley (1988) suggested that drifting meiofauna may have the facility to select the sediments in which they will eventually settle. Collectively these mechanisms may provide both an explanation for the “enclosure effect” (by which a small depression forms at the circular lip of the enclosure) and the apparent insensitivity of some species, as it is conceivable that they may spend little time exposed to the copper before being displaced by the next tidal cycle. In addition, the high abundance of *Amonardia normani*, a phytal species (Hicks and Coull 1983), may indicate the periodic deposition of plant material within the enclosure depression. Elevated abundance of *A. normani* copepodites was also noted by Bunker (pers. comm.) during an experiment in which trays of sediment were placed at a site close to the location of the present experiment.

The high proportional abundances of *Cletodes*, *Stenhelicia*, and *Rhizothrix*, all endobenthic species, in the high-concentration samples, at 1st sight tend to rather contradict much of what has previously been proposed. However, *Stenhelicia* is known to have the ability to build small closed-ended tubes near the sediment surface (Chandler and Fleeger 1984) which may provide protection against the more-severe toxic effects of copper. In support of this, Somerfield et al. (1994) found that endobenthic species were largely absent from the most-polluted parts of the Fal

Estuary, UK, while epibenthic species such as *Tachidius*, *Pseudobradya*, and *Microarthridon* were common. Some endobenthic tube-building species, including *Stenhelicia*, however, were still present.

The sand-dwelling *Rhizothrix minuta* was found to be comparatively copper-tolerant in microcosm experiments carried out by the authors (unpubl. data), and in this experiment, the persistence of *R. curvata* tends to suggest that this is a general characteristic of the genus.

The presence of species of *Cletodes*, a genus reported to be slow to colonize (Olafsson and Moore 1992), is perhaps a good indicator of the maturity of the enclosure communities. This group is thought to disperse only by movement within the sediment and is known to be robust, with a thick, heavy cuticle, and this may resist contaminant diffusion into body tissues.

One of the major goals of this experiment was to evaluate the ability of a number of commonly used statistical techniques to detect any effects of metals on the meiobenthos. The nominal copper levels were originally selected to span a wide concentration range, such that there should be clearly defined differences between at least two of the treatments. The actual range proved to be somewhat wider than intended at 13.5 to 8662.3 µg/g dry weight of sediment. The highest concentration was very much greater than would be found in even the most polluted of waters, but the experimental low and medium levels (91.9 and 893.4 µg/g) are representative of what might be considered as low to moderately high levels in the context of global anthropogenic sediment contamination.

Univariate measures of diversity and abundance gave widely varying indications of the nature and severity of the effects of these levels of contamination, which highlights the need for a pragmatic approach to these types of field data. Elevation of species numbers and abundances at the intermediate concentrations was unexpected, and clearly may not have been observed at all if the copper range had been more refined, or indeed of a substantially greater range. Perhaps of more concern, particularly in relation to the previous field experiment, is the interaction between details of community attributes and the number of treatments. If this experiment had been performed with only the control and high treatment, not only would a great deal of detail have been lost, but total copepod abundance and species richness together with all of the other major taxa abun-

dances would have indicated that there was no significant copper effect. It is clear from this that the graphing of treatment points is essential for the determination of subtle non-linear effects.

Similarly, the selection of a range of diversity indices was of crucial importance. Because of the sparse and relatively even distribution of individuals among species present in the adult community (from which the indices were calculated), it was the fluctuation in the number of taxa which largely defined the treatment differences. Commonly used indices such as Shannon-Wiener provided insufficient discrimination to determine diversity changes even in communities exposed to the highest concentration.

Multivariate ordination of copepod data, in general terms, provided no greater detail than species abundance charts, except to indicate a subtle divergence between the treatment and background assemblages (as discussed above).

ANOSIM tests on adult and all-age-group data did not greatly improve the discrimination obtained with univariate ANOVA results, but mainly reiterated differences in the greatly impacted communities exposed to the highest copper concentration. The copepodite component did, however, provide the only solid statistical basis for an effect in the medium-concentration treatments. This appears to largely have been due to the abundance peaks of copepodites of *Rhizothrix*, *Pseudobradya*, and *Stenhelia* at this concentration.

The application of both univariate and multivariate analyses to species data aggregated to higher taxonomic levels has been advocated by a number of authors. The benefits of reduced identification effort are particularly attractive for meiofauna because of the major expenditure of extra time often required to refine identification from family to species. In addition, abundance data may be discarded for some juveniles because of an inability to identify them to species. Warwick (1988) and Warwick et al. (1990) analyzed meiofaunal and macrofaunal data gathered along pollution gradients to a range of taxonomic levels. They suggested that soft-sediment benthic macrofaunal communities could be identified to phylum without appreciable loss of discrimination, while meiofauna could safely be identified to family without a significant reduction in information. Somerfield and Clarke (1995) remarked that the majority of studies were rather subjective in nature, relying on visual examination of ordinations or dendrograms often derived from unreplicated data sets. They under-

took a more-objective approach with data obtained from previous studies where clear responses to anthropogenic disturbance had been demonstrated. They concluded that aggregation to family incurred a small and usually inconsequential loss of discrimination for macrofauna, but a nematode community could not be aggregated beyond genus without major losses in discriminatory powers.

In this study when univariate analysis was performed on data aggregated to genus and family, the significant difference between the high-concentration and other treatments was largely maintained throughout. Indeed, aggregation was beneficial or even necessary in some instances since many individual taxa contributed such low abundances that analysis at lower taxonomic levels was inappropriate. Multivariate analyses applied to the same data gave similar results, with ANOSIM discrimination virtually identical among species, genus, and family levels.

As a means of addressing the problem of inadequate or absent controls in field pollution studies, this type of experimental approach has much to recommend it. The use of 100-cm³ bottles as sediment enclosures and final sampling units proved to be a sufficient size for most of the major meiobenthic groups, although sediments of different granulometric characteristics may require some modification in order to maintain the statistical relevance. In this experiment, a single sampling time of 1 mo was used, but there is considerable scope for developing strategies to examine the dynamics of community adjustment after contamination with the addition of a temporal dimension. However, before this type of study is attempted, further assessment of the potential effects of the initial physical disturbance component is required. In addition, the extent and persistence of a possible "enclosure effect" should be established so that it can be characterized and eliminated from any subsequent studies.

Acknowledgments: We are grateful to the Natural Environment Research Council UK for financial support for this work.

REFERENCES

- Alongi DM, DF Boesch, RJ Diaz. 1983. Colonisation of meiobenthos in oil-contaminated subtidal sands in the lower Chesapeake Bay. *Mar. Biol.* **72**: 325-335.
- Austen MC, AJ McEvoy. 1997. Experimental effects of tributyl tin (TBT) contaminated sediments on a range of meiobenthic communities. *Environ. Pollut.* **96**: 435-444.

- Austen MC, S Widdicombe, N Villano-Pitacco. 1998. Effects of biological disturbance on diversity and structure of meiobenthic nematode communities. *Mar. Ecol.-Prog. Ser.* **174**: 233-246.
- Chandler GT, JW Fleeger. 1983. Meiofaunal colonization of azoic sediment in Louisiana: mechanisms of dispersal. *J. Exp. Mar. Biol. Ecol.* **69**: 175-188.
- Chandler GT, JW Fleeger. 1984. Tube-building by a marine meiobenthic harpacticoid copepod. *Mar. Biol.* **82**: 15-19.
- Christie H, JA Berge. 1995. *In situ* experiments on recolonization of intertidal mudflat fauna to sediment contaminated with different levels of oil. *Sarsia* **80**: 175-185.
- Connell JH. 1978. Diversity in tropical rain forests and coral reefs. *Science* **199**: 1302-1310.
- Coull BC, GT Chandler. 1992. Pollution and meiofauna: field, laboratory and mesocosm studies. *Oceanogr. Mar. Biol. Annu. Rev.* **30**: 191-271.
- Decker CJ, JW Fleeger. 1984. The effect of crude oil on the colonization of meiofauna into salt marsh sediments. *Hydrobiology* **118**: 49-58.
- Fegley SR. 1988. A comparison of meiofaunal settlement onto the sediment surface and recolonization of defaunated sandy sediment. *J. Exp. Mar. Biol. Ecol.* **123**: 97-113.
- Hall JA, CLJ Frid. 1995. Responses of estuarine benthic macrofauna in copper contaminated sediments to remediation of sediment water quality. *Mar. Pollut. Bull.* **30**: 694-700.
- Hall JA, CLJ Frid. 1997. Estuarine sediment remediation: effects on benthic biodiversity. *Estuar. Coast. Shelf Sci.* **44(Supplement A)**: 55-61.
- Hicks GRF, BC Coull. 1983. The ecology of marine meiobenthic harpacticoid copepods. *Oceanogr. Mar. Biol. Annu. Rev.* **21**: 67-175.
- Moore CG, JM Stevenson. 1997. A possible new meiofaunal tool for rapid assessment of the environmental impact of marine oil pollution. *Cah. Biol. Mar.* **38**: 277-282.
- Olafsson E, CG Moore. 1992. Effects of macroepifauna on developing nematode and harpacticoid assemblages in a subtidal muddy habitat. *Mar. Ecol.-Prog. Ser.* **84**: 161-171.
- Olsgard F. 1999. Effects of copper contamination on recolonization of subtidal marine soft sediments - an experimental field study. *Mar. Pollut. Bull.* **38**: 448-462.
- Palmer MA, PA Montagna, RB Spies, D Hardin. 1988. Meiofauna dispersal near natural petroleum seeps in the Santa Barbara Channel: a recolonization experiment. *Oil Chem. Pollut.* **4**: 179-189.
- Scheibel W. 1974. Submarine experiments on benthic colonization of sediments in the Western Baltic Sea. II. Meiofauna. *Mar. Biol.* **28**: 165-168.
- Schratzberger M, RM Warwick. 1998. Effects of physical disturbance on nematode communities in sand and mud: a microcosm experiment. *Mar. Biol.* **130**: 643-650.
- Sibert JR. 1981. Intertidal hyperbenthic populations in the Nanaimo Estuary. *Mar. Biol.* **64**: 259-265.
- Somerfield PJ, JM Gee, RM Warwick. 1994. Soft sediment meiofaunal community structure in relation to a long-term heavy metal gradient in the Fal estuary system. *Mar. Ecol.-Prog. Ser.* **105**: 79-88.
- Somerfield PJ, KR Clarke. 1995. Taxonomic levels, in marine community studies, revisited. *Mar. Ecol.-Prog. Ser.* **127**: 113-119.
- Spies RB, DD Hardin, J Toal. 1988. Organic enrichment or toxicity? A comparison of the effects of kelp and crude oil in sediments on the colonization and growth of fauna. *J. Exp. Mar. Biol. Ecol.* **124**: 261-282.
- Sun B, JW Fleeger. 1994. Field experiments on the colonization of meiofauna into sediment depressions. *Mar. Ecol.-Prog. Ser.* **110**: 167-175.
- Warwick RM. 1988. The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. *Mar. Pollut. Bull.* **19**: 259-268.
- Warwick RM, MR Carr, KR Clarke, JM Gee, RH Green. 1988. A mesocosm experiment on the effects of hydrocarbon and copper pollution on a sublittoral soft-sediment meiobenthic community. *Mar. Ecol.-Prog. Ser.* **46**: 181-191.
- Warwick RM, HM Platt, KR Clarke, J Agard, J Gobin. 1990. Analysis of macrobenthic and meiobenthic community structure in relation to pollution and disturbance in Hamilton Harbour, Bermuda. *J. Exp. Mar. Biol. Ecol.* **138**: 119-142.
- Watzin MC, PR Roscigno. 1997. The effects of zinc contamination on the recruitment and early survival of benthic invertebrates in an estuary. *Mar. Pollut. Bull.* **14**: 443-455.
- Widdicombe S, MC Austen. 1998. Experimental evidence for the role of *Brissopsis lyrifera* (Forbes, 1841) as a critical species in the maintenance of benthic diversity and the modification of sediment chemistry. *J. Exp. Mar. Biol. Ecol.* **228**: 241-255.