THE EFFECTS OF COPPER ON MARINE MEIOBENTHIC COMMUNITIES: FIELD AND LABORATORY STUDIES

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Thesis submitted for the degree of
Doctor of Philosophy

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June 2000

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ACKNOWLEDGMENTS

I would like to extend my gratitude to all of those people who have contributed in any way to the work that has resulted in this Thesis.

Thanks are due to Dan Harries, Colin Moore, Jonathan Hunt, and Adrian Bunker for their diving expertise, without which the field sampling and/or microcosm construction would not have been possible. I am grateful to Ron Wylie and Dave Ashcroft for their much-needed boat support. I am further indebted to Ron Wylie and Jonathan Hunt for their almost unbelievable efforts during the microcosm experiments and to Dan Harries for his help with taxonomic identification. The Loch Creran fieldwork was often made more comfortable (and entertaining) by Jonquil and Jock Slorance at Creran Moorings. I gratefully acknowledge the receipt of a Natural Environment Research Council Studentship. Finally, I would like to express my sincere gratitude to Dr. Colin Moore for his support, constructive supervision, critical eye and, above all, sense of humour.

ABSTRACT

Copper is a major constituent of global metallic discharges and often comprises the greatest concentration in heavily contaminated areas. In addition, as a direct result of the restrictions on the use of tributyltin (TBT), copper has also been increasingly used as a marine antifoulant, where commercial preparations are designed to continually release the metal into the water column. In a series of field and laboratory experiments the effect of sediment copper contamination on meiobenthic communities was investigated.

Field-collected meiofaunal sand communities were maintained in microcosms for 18-20 days and subjected to copper concentrations of 57, 133, 188, 215, 1104 and 1977 $\mu g.g^{-1}$. Nematodes were found to be extremely sensitive, with large and rapid abundance decreases even at the lowest concentration. Copepods, particularly *Rhizothrix minuta*, were less sensitive but the effect was highly dependent on both the copper concentration and the length of time of exposure. During counting, distinction was made between live and dead animals. It was found that a very high proportion of dead, but well-preserved animals, particularly nematodes, remained in the contaminated microcosms, suggesting that the often-held assumption that dead or dying specimens are rapidly degraded is in error and may lead to a misinterpretation of results.

An experimental contamination of a muddy sediment field site by means of a static structure painted with a commercial antifoul preparation did not produce any measurable effect attributable to copper over a 90 day period, despite achieving a sediment metal concentration of 432 $\mu g.g^{-1}$.

In a second field experiment, the problem of replicated controls in field studies was addressed by randomly placing semi-enclosed muddy sediment units in a grid array, together with treatments contaminated with 91, 893 and 8662 $\mu g.g^{-1}$. Community effects were examined after 30 days using both univariate and multivariate statistical techniques. Although there was evidence of community modifications with increasing copper concentration only the highest concentration produced significant meiobenthic abundance reductions. In contrast, the lower concentrations tended to promote abundance increases, both at copepod species and major taxa level when compared to

control communities. It is proposed that analogous processes to those postulated for the 'intermediate disturbance hypothesis' may occur in metal-contaminated sediments. Some copepod species or groups exhibited reduced sensitivity to contamination, notably *Rhizothrix curvata*, *Stenhelia gibba* and Cletodidae spp. In addition, differences in copepod response patterns were observed between copepodite and adult stages with a general tendency towards greater copepodite tolerance. In contrast to the microcosm sand community, muddy sediment nematodes were found to be less sensitive than copepods.

This study demonstrates that a meiobenthic community response to metal contamination is complex and dependent on a number of factors such as sediment structure, life-stage composition and other community attributes. It is clear that the further development of both field and laboratory techniques are required to adequately define the nature of contamination effects.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Meiofauna: General Definition And History

The term ‘meiofauna’ (derived from the Greek *meio* meaning smaller) was first used by Mare (1942) to define an assemblage of mobile or hapto-sessile benthic invertebrates that were markedly smaller than the macrobenthos, yet larger than the microfauna. The current definitions hinge on mesh widths of sieves, with meiofauna constrained by a range of less than 1 mm to a lower limit of 42 μm. This does not preclude some meiofaunal organisms having at least one dimension greater than 1 mm, and also does not take into account whether a specimen is live or preserved.

Organisms of meiofaunal dimensions have been known since the early days of microscopy, but have only relatively recently been considered worthy of study in their own right. Much of the emphasis has necessarily focused on the taxonomy, and new meiofaunal groups continue to be discovered as ever greater areas of the benthos are explored. In addition, as ecological principles and techniques have developed, so too has the ability to characterise marine (and freshwater) meiobenthic communities, leading to fresh insights as to their importance in aquatic ecosystems.

For the past twenty years macrobenthic communities have been routinely used as indicators of pollution stress. However, meiofauna may offer a number of advantages over macrofauna, and a considerable amount of effort is being directed towards understanding the factors which modify meiobenthic assemblages under toxic or enriched conditions. Much of the data are conflicting, but it remains to be seen whether this is just a reflection of our lack of knowledge concerning the underlying mechanisms governing the physical and biological interactions.

The following is an attempt to ‘sketch’ a picture of the current status of meiofaunal ecology and outline its relevance in the determination of pollution effects. Particular emphasis is placed on the meiobenthos and the effect of trace metal pollution.

1.2. The Ecology Of Meiofaunal Communities

1.2.1. The Meiofaunal Groups

It is important to emphasise that the term ‘meiofauna’ is wholly artificial, in that it attempts to categorise organisms only in terms of body size, without any reference to taxonomic, trophic or habitat considerations. Many macrofaunal species can be considered as temporary meiofauna, as only part of their life history may be spent within the stated size range. A further complication has arisen with the relatively recent studies of deep sea communities, where there has been a tendency towards ‘miniaturisation’ (Gage and Tyler, 1991), to the point where taxa normally considered as macrofauna are only obtained in sieves of the meiofaunal mesh size. Faced with a paucity of animals from the 1 mm sieve, Hessler and Jumars (1974) suggested the terms ‘meiofauna taxa’ and ‘macrofauna taxa’ be used to allow the deep sea use of the traditional categories.

Of the 33 metazoan phyla, 22 are known to have at least some meiobenthic members, with the Gastrotricha, Gnathostomulida, Kinorhyncha, and Loricifera generally considered to be exclusively meiobenthic (Coull, 1988). Other notable members include the Turbellaria, Archiannelida, Coelenterata, Annelida, Nematoda and Copepoda. It is the nematodes and harpacticoid copepods that usually dominate the meiobenthos and hence are frequently the organisms of choice for studying meiobenthic community attributes.

1.2.2. Meiofaunal Habitats and Morphologies

There are a wide variety of recognised meiofaunal habitats, with communities in both marine and freshwater conditions. Habitat is a commonly used method of sub-grouping these meiofaunal communities, with representatives existing in the plankton; as commensals with shellfish, corals or algae; or in association with both inter- and subtidal sediments. The epibenthic and phytal forms have a tendency to be larger and often have the ability to swim for short distances.

Further differentiation is also possible within the different types of sediment, with often clearly defined sediment-type dependent morphologies. In general, sediment-dwellers

fall into one of two categories; those found in sand, or those preferring a muddy habitat, where the median particle diameter is less than 125μm. For taxa with representatives in both habitats (e.g. Copepoda, Nematoda, Turbellaria), the sand-dwellers are likely to be slender to allow movement through the interstices between the sand grains, and often have adhesive glands for attachment purposes. The mud fauna, although having no specific morphology, are usually larger with a predominantly burrowing tendency. The success of nematodes as the frequently dominant taxon may be partly ascribed to their shape, which is particularly well adapted to movement within interstitial spaces (Bouwman, 1987).

Warwick (1984) when investigating the size distribution of marine benthic organisms from a variety of locations, found a trough between the numbers of meio- and macrobenthic species at a body weight of 45 μg (dry weight). Bouwman (1987) observed that as the meiofaunal mode occurs around 0.6 μg and the macrofaunal mode at around 3 mg, the gap may be ecologically significant, separating an interstitial and burrowing mode of life.

1.2.3. Abundance and Diversity

Early marine and estuarine benthic studies tended to regard the meiofauna as energetically rather insignificant, with the secondary level mainly composed of herbivorous macrobenthos with access to an excessive food supply. However, more recent work, such as that of Kuipers *et al* (1981) in the Western Wadden Sea, suggests that the micro- and meiofauna attain high productivity (high P/B ratio) despite their small individual size and total biomass, ultimately contributing more in terms of primary consumption (and production of food for small carnivores) than was originally thought.

It is clear that abundance within the meiofauna is highly variable, with water temperature, seasonality, latitude, tidal exposure, water depth, habitat and grain size all conspiring against attempts at generalised density values. However, Coull (1988) arrives at an average figure for shallow (less than 100 m) waters of approximately 10^6 meiofaunal organisms per m^2 of sediment surface (or within the range of 200-3000

individuals/10 cm^2), producing a standing stock dry weight biomass of 1-2 $g.m^{-2}$. The highest values are frequently from intertidal muddy estuarine habitats while the lowest, by an order of magnitude, are from the deep sea (Theil, 1983). It has been suggested that sediment grain size plays the most important role in defining abundance and species composition, but that other factors such as those given above, with the additions of salinity and oxygen content, may also produce critical population modifications (Coull, 1988).

Within sediments nematodes typically dominate, often comprising over 50% of the total taxa, with harpacticoids usually the second most numerous. However, reviews suggest that overall meiobenthic composition can vary widely, and generalisations made on either a depth or geographical basis are difficult to make (McIntyre, 1969; Hicks, 1977; Hicks and Coull, 1983; Heip *et al.*, 1985). Harpacticoids are commonly present in higher proportions in coarse sediments, and indeed may sometimes dominate (Hicks and Coull, 1983), but as the sediment particle size decreases nematodes tend to prevail. Even in the deep sea nematodes predominate with values of 85-90% (after removal of agglutinating foraminiferans), followed by the harpacticoids as the next numerous at 2-3% (Gage and Tyler, 1991).

There are, however, reports of other taxa ranking at either first or second at particular locations. Coull (1985) found gastrotrichs to be the second most abundant at a sandy North Carolina (USA) site, while Hummon *et al* (1976) stated that ostracods were ranked second on a Delaware (USA) sandy beach. Alongi (1987), found turbellarians at 58-67% to be the dominant group in an Australian tropical mangrove estuary, and noted the low abundance of hard-bodied meiofauna when compared with temperate communities, suggesting that this may be due to comparatively greater physical stress in tropical intertidal communities.

Diversity is known to vary considerably throughout meiofaunal habitats, but in general, diversity values are greater in the deep sea than in equivalent shallow-water sediments (Coull, 1972; Gage and Tyler, 1991). An indication of the elevated deep sea diversity can be seen from the samples obtained by Wilson and Hessler (1987) from the equatorial East Pacific region, where 148 species were obtained from amongst 216

individuals. A number of speculative reasons have been proposed for this. Gage and Tyler (1991) suggest that abyssal conditions are physically more stable, allowing the establishment of a more heterogeneous community, while Coull (1988) offers the near-opposing view that a combination of factors such as predation and disturbance act to suppress dominance by reduction of competitors. Coull (1988) also suggests that the perception of a 'homogeneous' deep sea may be erroneous, and that microbiogenic structures present a greater number of habitats for species coexistence, citing the observations of Aller and Aller (1986) where meiofaunal abundance was 2-3 times higher proximal to detritus-filled macrofaunal burrows.

As a generalisation, it can be said that for shallow waters the highest densities and lowest species numbers are found in sheltered estuarine habitats, while highest diversities occur in marine subtidal habitats. Nevertheless, within this frame, Coull (1988) observes that in shallow water, regardless of sediment type, diversity tends to be similar with a characteristic 1-10 dominant species, implying a possible optimal diversity value for some, or most, shallow water assemblages. However, there is some evidence for modifying influences such as that of biogenic structure effects in shallow waters. For example, the highest diversity values recorded were for copepods obtained from *Laminaria* holdfast communities (Moore, 1973).

1.2.4. Vertical Zonation

The highest proportion of meiobenthic organisms are consistently reported to be concentrated in the surface layers of the sediment. Tietjen (1969) stated that 80% of all meiofauna (1184 - 5163/10 cm^2) obtained from a New England estuary was found in the top 3 cm, and similarly Feder and Bryson-Schwafel (1988) obtained greater than 90% from the upper 3 cm of an Alaskan intertidal zone. Hardy and Barnet (1986) found most of the harpacticoid population from the Firth of Clyde restricted to the top 1 cm.

Meiofaunal organisms have been found to occur at sediment depths of 30 cm and greater, many of which appear to be able to migrate vertically in response to a number of stimuli. The harpacticoid *Leptastacus rostratus* found in Florida (USA), tends to maintain a population maximum at a sediment depth of 2 cm and is rarely found in the

0-1 cm region. Flume experiments have shown that this species will migrate in response to near-bottom current flow, burrowing deeper when exposed to increased flow (Foy and Thistle, 1991), presumably to avoid suspension in the water column and thus perhaps exposure to predators.

Seasonal temperature may also be a controlling factor, particularly on exposed sandy beaches, where the meiofauna have been observed to move from the surface layers in summer to recorded depths of 50 cm in winter (Harris, 1972c). This is unlikely to induce vertical migration in the subtidal however, as temperature fluctuations here are not usually as great.

Dye (1983) studied depth distribution profiles of South African mangrove sediments and concluded that factors such as temperature and pH were not as important as the depth of the redox potential discontinuity layer (RPD). The RPD layer is effectively a transition zone between areas of aerobic and anaerobic sediments, and its depth is dependent on the amount of organic material available for decomposition, and the rate at which oxygen can diffuse into the sediment from the overlaying water. The limits of oxic sediments are to a great degree controlled by sediment particle size. In organic muds, oxygen diffusion rates are slow, allowing an aerobic layer only at the sediment surface. In sand, aerobic conditions can prevail to much greater depths, particularly on high energy beaches where turbulence may increase oxygen penetration.

Clearly, if the RPD layer is a significant factor determining vertical distribution, one would expect meiofaunal sediment depth profiles to reflect the aerobic/anaerobic status of the sediment. This does indeed seem to be the case. In muds and high detrital sediments, the majority of the meiobenthos is restricted to the surface layer often only a few centimetres or millimetres (Coull and Bell, 1979), while in sands much greater depths are reported; in excess of 50 cm on high energy beaches (Coull, 1988).

Some meiofauna, termed ‘thiobios’ (Boaden and Platt, 1971) are capable of penetrating the anoxic, high sulphide layer, but because of the difficulties in studying such organisms *in situ* there is still much debate over how this is possible, and indeed, whether such habitats are truly anoxic (Fenchel and Riedl, 1970; Powell *et al.*, 1980;

Meyers *et al.*, 1987; Giere, 1993). Reise and Ax (1979) reported the existence of 'oxygen islands' within the anaerobic zone. These appear to be oxic areas formed around macrofaunal burrows, created by large polychaetes, nemertines or amphipods, and many of the suspected anaerobic-tolerant meiofauna are consistently found in greater numbers in close proximity.

With the above reservations, it seems likely that the RPD layer is the primary determinant of vertical zonation. Coull (1988) suggested that oxygen could be thought of as a 'super parameter', controlling the redox potential and the oxidation states of sulphur and various sediment-bound nutrients. McLachlan (1978) observed that when the redox potentials dropped below +200 mV, meiofaunal densities were greatly reduced. It seems that the harpacticoid copepods are typically the most sensitive taxon to reduced oxygen tension (Hicks and Coull, 1983), with only two reported species *Apodopsyllus africanus* (=*bermudensis*) and *Paraleptastacus espinulatus* thought to be able to survive under anoxic conditions (Wieser *et al.*, 1974).

Many more nematode species, however, seem to be tolerant of low oxygen levels. Heip *et al* (1985) (following Ott, 1972) maintain that on beaches in North Carolina, USA, the deeper levels of sediment are not supporting lower numbers of surface fauna, but a fauna adapted to inhabiting these levels, with the dominant *Metrachromadora obesa* often found at the greatest depth. It should be noted that this investigation, and almost all others, concentrate on faunal distributions in coarse sandy sediments purely because the depth distribution has a sufficient spread to allow artificial segregation of layers. Consequently, in muddy sediments where the majority of the fauna is at, or near, the water-surface interface, there has been correspondingly less research.

Within the sandy sediments of beaches, meiofauna have to cope with the problem of desiccation brought about by the ebbing tide. Many meiofaunal organisms are able to respond to low pore water content (Jansson, 1968). McLachlan *et al* (1977) showed a migration linked to tidal periods, upward on the flood and downward on the ebb tide. The level of migration seems to be attenuated by seasonality, with vertical migration less in the winter months. This is thought to be at least partly due to the lower winter

temperatures and the associated reduction in desiccation at low tide. In addition, reduced migration is also observed at night, again possibly because of lower temperatures.

Seasonal temperatures also affect the depth of the RPD zone within the sediment, and with the increasing summer temperatures the RPD layer will move upwards forcing a displacement of sensitive organisms. Harris (1972c), working on a Cornwall intertidal sandy beach, was able to show a clear seasonal migration of the harpacticoid *Psammotopa phyllosetosa* over two years. During the months of June and July the greater density could be found in the top 4 cm of sediment. By December and January they had relocated to below 16 cm.

Although oxygen tension and temperature/seasonality are now almost universally accepted to be the major forces driving vertical meiofaunal migration, when viewed as a community event, it should be recognised that there may be other factors (perhaps still unknown) that may influence the movement of the constituent species. It is certainly true that there are some species of nematode that exhibit a form of differential distribution. Warwick and Gee (1984) studied the vertical distribution of the five dominant nematode species of a sandy mudflat, concluding that there was evidence for vertical segregation, possibly due to avoidance of interspecific competition. Blome (1983) discovered in a defined area of beach on a North Sea island, that the nematode species migrated deeper into the sediment at lower temperatures. Differences have also been noted amongst the life stages of a population (e.g. males, ovigerous females and copepodites) (Harris, 1972b).

1.2.5. Horizontal Zonation

Coull (1988) lists a number of studies where patterns of horizontal distributions have been detected. These include salinity gradients in estuaries, across the combined physical modifiers of intertidal sandy and muddy habitats, and with increasing water depth in subtidal environments.

Perhaps the most prominent among these is the effect of salinity, notably conspicuous in the estuarine environment. Many investigations have demonstrated a strong relationship between estuarine salinity gradients and meiofaunal community structure. Austen and Warwick (1989) using multivariate analysis implicated salinity as a major modifier of both nematode and copepod assemblages in the Tamar estuary (U. K.), noting that the nematode species distribution was relatively even at the mouth and head of the estuary, but not in the middle reaches. Copepods, however, exhibited more variation in both abundance and species richness, but overall populations became more dominated and less diverse with reduced salinities.

In a comparative study of two estuaries with very different levels of fresh water inflow, Montagna and Kalke (1992) showed a differential response between macro- and meiofauna. In general, macrofaunal density (and biomass) increased with decreasing salinity, while for meiofaunal densities the converse was true. In addition, in the estuary with the lower fresh water inflow the meiofaunal densities were significantly higher, suggesting either a lack of low salinity tolerance and/or possible macrofaunal interactions by means of competition or predation. However, the concept of a general meiofaunal susceptibility to low salinities conflicts with other studies where considerable densities of marine species in the higher limnic reaches have been reported (Alongi, 1990), although it does seem that certain taxonomic groups are more successful than others in these conditions (Giere, 1993). It is possible that some distinction should be made for the severity of salinity stress within the estuary. Over a semidiurnal tidal period, the interstitial salinity may remain relatively constant allowing nematodes to escape major effects by burrowing, whereas irregular and more intense events may require the development of a broad physiological tolerance (Heip *et al.*, 1985).

Salinity has been correlated with the success of particular nematode feeding types (Austen, 1989). At high salinities, selective and epigrowth feeders dominate, while the lowest salinities are characterised by omnivores and opportunistic non-selective deposit feeders, perhaps reflecting the greater availability of organic material at the higher reaches of the estuary.

It seems that taken as a single factor, diminished salinity can be broadly responsible for reductions in species richness, abundance per unit area and diversity (Capstick, 1959; Heip *et al.*, 1985; Coull, 1988). In most cases there is a seaward increase in abundance, suggesting that the changes are mainly due to migrational limits for 'euryhaline' species. There certainly appears to be a transition zone where diversity (Warwick, 1971) and species richness (Gerlach, 1954) may be suppressed due to the decline of marine species in favour of freshwater taxa.

Many authors have reported zonation across intertidal sandy beaches. Moore (1979), and subsequently Coull (1988), defined three zones constraining meiofaunal groups:

1. A sublittoral fringe guild.

2. Species of a wide biotopic range, but with their distribution usually centred on the lower shore.

3. Species limited to the upper shore.

It is likely that the zonation is controlled by a combination of factors, but primarily interstitial space at the lower limit and salinity/desiccation tolerance at the upper extreme.

McLachlan (1980) proposed that both vertical and horizontal distribution of density and species on sandy beaches were governed by two major factors; desiccation and dissolved oxygen content in the interstitial water. These factors were incorporated in a four-component stratification scheme for an average South African beach:

1. An upper dry stratum with low water saturation and supporting adapted nematodes and oligochaetes with few copepods and turbellarians.

2. A moist sand stratum beneath dry sand with essentially stable temperature and salinity. The well-oxygenated conditions promote high meiofaunal diversity and is particularly rich in harpacticoids.

3. A water table stratum; permanently moist with low oxygen tension and brackish salinities. Nematodes and crustaceans dominate but with reduced diversity and abundance.

4. A low oxygen stratum, which may be deep in high energy beaches. Chemically reduced conditions may prevail on beaches receiving high organic matter. Meiofaunal abundance is low, with nematodes dominating.

Modifications of this scheme abound, frequently incorporating other controlling factors from specific beaches, but the principles remain intact throughout. Coull (1988) comments that although zonation on sandy beaches is well documented, there appears to be 'no unequivocal and universal causative factors', but Giere (1993) maintains that, particularly in littoral areas, the correlation between sediment structure and meiofaunal distribution is such that it dominates all other factors.

Muddy shores generally contain high meiofaunal densities at or near the sediment surface. Indeed, mudflats have been reported to contain twice as many meiofaunal individuals in the upper 1 cm as can be found in a 10 cm column of sandy sediment (Smith and Coull, 1987). Giere (1993) observed that in muddy sediments of the North Sea the greater meiofaunal abundance is located near the low-tide level, in direct contrast to sandy shores where the maxima are closer to the mid- and high-tide line. Coull *et al* (1979), in a study of a muddy South Carolina (USA) salt marsh, showed that most copepod species were restricted to sub-habitats along a gradient extending from high intertidal marsh to the subtidal, with a single species *Microarthridion littorale*

present across the entire range. They concluded that the zonation patterns were the result of a combination of physical (water depth, tidal exposure) and biological (presence/absence of vegetation; macrofaunal disturbance/predation) factors determining sub-habitat allocation. Clearly, some of these factors, particularly those of a physical nature, may be directly related to the near-surface location of the mud-dwelling fauna.

Bouwman (1983) in a comparative study of the Ems estuary and Wadden Sea (Netherlands) noted a significant difference in nematode assemblages between the subtidal estuarine sediments and the tidal flat. The tidal flat supports a dense stock of microphytobenthos at the surface and is therefore dominated by diatom feeders. Most of these species are not found in the sublittoral sediments, suggesting that food preferences and tolerance to reduced salinities may force a differentiation between sublittoral and intertidal estuarine communities.

From the continental shelf to the deep sea the faunal composition is largely influenced by sediment and depth changes (Hicks and Coull, 1983). Sandy continental shelves exhibit the expected sandy substrate assemblages of shallower water, and changes at the taxon level tend to correlate with physical changes in the substratum such as a shift from sand to silty sand (Tietjen, 1971). In addition, depth related changes at the family level are evident for both copepods (Coull *et al.*, 1982; Hicks and Coull, 1983) and nematodes (Tietjen, 1976). The taxonomic associations at depth appear to be global with families and/or genera consistently found at similar depths. An exception to this is found in the Mediterranean and Arctic, where ‘deep-sea’ taxa are found in substantially shallower waters (<500 m), and this is thought to be related to sediment-type (Mediterranean) and temperature (Arctic) similarities with bathyal conditions.

1.2.6. Patchiness

Aggregative distribution of meiofauna on the micro-scale is a well documented phenomenon, but its causes are still a subject of much debate (Hicks and Coull, 1983). On the metre- to kilometre-scale physical factors such as salinity, sediment characteristics, oxygen concentration and tidal exposure can be evoked, but at smaller scales biological interactions may become more prominent. Biogenic structures such as animal burrows and tubes are thought to promote localised aggregation perhaps by increasing oxygen availability and access to current-borne nutrients (Sun and Fleeger, 1991). Reise and Ax (1979) demonstrated a meiofaunal abundance (predominantly nematodes) ten times that of the surrounding sediments around *Arenicola* burrows on an intertidal sand flat.

In the case of harpacticoids, associations have been shown between aggregation behaviour and life cycle stage. Heip and Engels (1977) found males tended to aggregate with non-gravid females but not with gravid females. Findlay (1981) using a range of core sizes to discriminate between spatial scales was able to show seasonally-related life stage differences in patchiness scales. Nauplii and adults exhibited similar distributional patterns in September, but in February nauplii were aggregated at a 5 cm^2 scale while adults at 3 and 31 cm^2. Findlay (1981) speculates that the smaller adult area may consist of reproductively active individuals necessarily in close association, while the larger area may be made up of non-reproductive adults. He goes on to suggest that the segregation is largely defined by ontogenic differences in food preferences with nauplii utilising a different 'resource patch' to that of the adults, perhaps ultimately determined by food particle size.

It does seem clear, regardless of life stage, that patterns of meiofaunal distribution can be spatially correlated with food sources, and this has been demonstrated on tidal flats where diatom patches are closely linked to meiofaunal patch area (Montagna, 1983; Blanchard, 1990). More recently it has been proposed that mucus and exopolymers produced by microorganisms and covering detritus may be of considerable nutritional importance for meiofauna (Biddanda and Riemann, 1992). These particulate substances, high in amino acid and labile nitrogen compound content, may form an extensive matrix

of amorphous organic material and may be the major carbon source for many benthic organisms (Decho, 1990). The distribution is known to be patchy and therefore may have a role to play in determining small-scale spatial distribution.

Clearly, there are a wide number of causative factors for patchiness and it is not inconceivable that some or all of them are relevant at any given location, requiring a multifactorial approach to field surveys. Also, one cannot disregard other factors that may directly or indirectly modify population distribution, in particular, irregular or temporary occurrences such as storm disturbance or increased food input from larval settling. It seem that the issue of patchiness may become increasingly complex as more information becomes available.

1.2.7. Dispersal

The greater proportion of meiofaunal organisms do not produce planktonic larvae, and so the common method of dispersal and colonisation in the marine environment is unavailable to the meiobenthos. Indeed, meiobenthic development, morphology and behaviour seems to be expressly aimed towards maintaining a sediment-bound existence (Hicks and Coull, 1983). Dispersal does occur, however, over both large and small distances; along contiguous beaches and across oceanic barriers.

Many species such as the turbellarian *Gyratrix hermaphroditus* (Sterrer, 1973) appear to be globally ubiquitous, presenting meiobenthologists with the problem of how such widespread distribution may arise. Moreover, it seems that certain groups have been more predisposed to a cosmopolitan distribution, with ‘soft-bodied’ meiobenthos such as interstitial polychaetes, ciliates, turbellarians, gnathostomulids and gastrotrichs particularly successful. On the other hand the ‘hard bodied’ taxa, notably the harpacticoids tend to be rather confined in their distribution often exhibiting a high degree of endemism (Wells, 1986).

A popular explanation for wide faunal distributions is transport by continental drift *via* plate tectonics (Sterrer, 1973). In recent years however, studies of geographically isolated populations have undermined the principles on which this theory rests, namely

the requirement for slow speciation. The Galapagos Islands, for example, have a rich and radiating meiofaunal population, but are geologically extremely young at 3 million years (Westheide, 1991).

Gerlach (1977) offered other possible means of dispersal:

1. *Via* airborne animals.

2. Rafting on drifting materials.

3. Transport in ballast of sailing ships.

4. Suspension in the water column.

There is little direct evidence for transport by attachment to airborne animals, although it would be unwise to dismiss the possibility out of hand. Clearly the potential is there for birds, particularly intertidally-feeding migratory species, to entrain meiofauna from the sediment or water column and carry them some distance. The extent of transport is debatable, but for attached organisms to be relocated alive it seems unlikely that pan-oceanic distances can be crossed.

Meiofaunal relocation by means of phytal rafts has been observed by a number of authors. Diatoms and cyanobacteria can excrete mucous, which, by means of its aggregative properties, may promote the formation of dense mats over the sediment surface. These mats have been observed to become suspended in the water column together with some of the surface sediment and associated epibenthic meiofauna (commonly copepods) and can subsequently be carried over a limited distance (Hicks, 1988).

Harpacticoids have been found on drifting *Sargassum* offering the possibility of transatlantic dispersal, although Hicks (1977) argued that the species concerned were of a wide biotopical range and may be expected in such a situation. He went on to say that phytal species were particularly specialised and were therefore unlikely to survive

transoceanic transport. Armonies (1989) suggested ‘sea foam’, a post-bloom algal excretion, as a possible meiofaunal carrier. The foam, which is deposited on beaches in considerable quantities, can be relatively long lasting and has been found to contain numerous meiofaunal organisms.

There have been many reports in recent times of colonisation by invading algae and macrofaunal species relocated by human activities, and this mechanism seems equally suitable for meiofaunal translocation. Gerlach (1977) pointed to the practices of the sailing vessels of the last century as a mode of dispersal. Stones and wet sand were often taken from local shores as ballast and then taken considerable distances before dumping, prior to cargo loading. This may well have resulted in transcontinental exchange of both macro- and meiofauna.

Suspension and entrainment within the water column is likely to take place in conditions of hydrodynamic turbulence, where breaking waves or strong currents will scour the sediment, resulting in a suspension containing meiofauna. Seabed structures such as boulders and plant culms may increase the turbulence and thus promote additional suspension (Palmer, 1986). A surprisingly high proportion of the interstitial meiofauna - around 10 - 30% of total drifting meiofauna - may find itself in the water column (Hagermann and Rieger, 1981), and with moderate currents, dispersal rates may attain 10 $km.day^{-1}$. In the extreme conditions of gales, scouring may occur at 25 m water depth and carry particles over distances of 50 km. Even under calm conditions, regular tidal currents are capable of displacing meiofauna (particularly epibenthic copepods) by suspension and resettlement (Armonies, 1990; Hicks, 1992; Giere, 1993).

Current and disturbance represent a passive means of dispersal, but colonisation experiments and analysis of the groups commonly found in suspension suggests that some taxa are able to actively enter the water column (Fegley, 1988). Harpacticoids, ostracods and turbellarians are most often found drifting in the water column, but oligochaetes and nematodes rarely so, suggesting that the former may elect to leave the sediment for a particular purpose.

Nematodes have been observed to swim in culture (Heip *et al.*, 1985), and the presence of female *Tripyla* in the gut of some pelagic plankton-feeding fish have led some authors to postulate that this nematode swims upward into the water column for spawning purposes (Gerlach, 1977). In general though, it seems that nematodes enter the water column relatively infrequently. In intertidal sediments Armonies (1988) was able to show that during a nocturnal high tide, 87% of copepods, 67% of ostracods, and 42% of platyhelminthes left the sediment but no nematodes or oligochaetes were observed to do so. Copepods have also been observed to leave the sediment in seagrass meadows where Arlt (1988) found greater than 50% ascending into the water column nightly. It appears that meiofaunal emergence is predominantly nocturnal and may constitute an effort to avoid visual predators.

Resettling after transport within the water column may involve an element of selectivity with active sampling for structural complexity and substrate roughness (Palmer, 1988; Eckman, 1990) possibly combined with biological and chemical signals (Giere, 1993).

Verification of emergence-settling activities have come from colonisation experiments where denuded or disturbed sediments have undergone surprisingly rapid recolonisation. Sherman and Coull (1980) reported recolonisation by copepods and nematodes of a 9 m^2 area of hand-turned intertidal mud flat over a single tidal cycle. Differential repopulation rates for particular species suggested that colonisation was taking place from the water column and through migration within the sediment. Other reports have detailed recolonisation taking weeks, months or even years. A determining factor may be habitat type, but Hicks and Coull (1983) advised caution when contrasting studies of this type, as differences in sampling and experimental design make objective comparisons difficult.

1.2.8. Seasonal Abundance

Seasonal variations in the meiobenthos of the intertidal and shallow sublittoral are well documented, with maximum abundance usually observed in the warmer summer months. Hicks and Coull (1983) listed a number of studies dealing with temporal changes of harpacticoid populations over not less than a year and noted that of the 84

species covered, only five exhibited abundance maxima in both warmer and cooler months of the year. Eskin and Coull (1987), sampling over three years on estuarine sand and mud sites found low nematode year to year variability at both sites but the mud species showed seasonal cycles while the sand species lacked any such fluctuations.

Palmer (1980) reported greater intertidal population densities of the copepod *Microarthridon littorale* during summer and autumn, with the maximum density occurring in July. Maximum subtidal densities occurred in October, with little numerical difference between zones throughout the rest of the year. Additionally, a far greater number of gravid females were observed subtidally at all times. This species is reported to breed throughout the year subtidally (Coull and Vernberg, 1975), but intertidal winter reproduction is suppressed (Fleeger, 1979). Heip *et al* (1985) noted that nematodes also exhibited a greater seasonal population stability in shallow subtidal areas when compared to intertidal sites. A number of factors were proposed by Palmer (1980) to account for the temporal differences; (1) variation in food quality and quantity, (2) increased subtidal exposure to epibenthic predators, and (3) different competitive pressures.

Alongi (1987) noted seasonally-linked increases in meiofaunal densities in tropical mangrove estuaries during the summer despite the excessive scouring and freshwater inflow resulting from summer monsoonal conditions. He concluded that the major stimulatory factor was temperature, but a high resilience to sediment disturbance in this habitat was clearly also important.

Hicks and Coull (1983) expressed some reservations however on the ability to generalise from these data, as it is not unusual for subsequent or parallel investigations at similar locations to yield abundance maxima for substantially different times of the year. Clearly, with significant intertidal and subtidal differences operating in the same location the site of sampling becomes of critical importance.

It seems logical to nominate reproductive peaks as the initial driving force behind most temporal abundance maxima, and reproductive and development rates have been consistently correlated (usually positively) with seasonal temperature (Hicks and Coull,

1983). In a study in the Firth of Clyde two subtidal harpacticoid species, *Asellopsis hispida* and *Harpacticus flexus*, each exhibited a clear reproductive period during the year. Gravid females were observed during January and February, followed by the appearance of copepodites from late April to June, with large numbers of adults present in June (Hardy and Barnet, 1986). Fleeger (1980) determined the combined adult and copepodite temporal density patterns for the nine most abundant species present in a low marsh intertidal site (South Carolina, USA), and found two different abundance patterns. Four species displayed an autumnal peak, centred around September, while another four species were at their lowest densities in June-September. The remaining species was present in high numbers all year except January and February.

Nematodes appear similarly disparate in their seasonal abundance. Bouwman (1983) described a number of species from the Ems-Dollard estuary (on the Dutch-German border), some of which reproduced all year round and thus maintained a relatively constant abundance (e.g. *Eudiplogaster pararmatus*), while others had notable peaks as with *Dichromadora geophila*, having juveniles present only from April to August, with a population maximum in May.

Some meiofaunal species are able to achieve a dormant or resistant condition during adverse circumstances or seasons and therefore may appear to be absent from the sediment (Warwick, 1980). Ostracods are known to overwinter as resting eggs, as may some nematode species, while some harpacticoids (e. g. female adult *Halicyclops magniceps*) hibernate in deeper layers of sediment (Heip, 1975), probably to avoid predators.

Sudden increases in predation pressure are thought to be of some importance in seasonally regulating meiofaunal abundance. The peak abundance of the harpacticoid *Harpacticus uniremis* was shown to sharply decline with the appearance of migrating juvenile salmon (Sibert, 1979) and Heip *et al* (1985) suggested predation by juvenile polychaetes may account for a rapid autumn decline in nematode populations in shallow New England estuaries.

In general, meiofaunal species tend to be narrowly specialised in terms of food preferences (Warwick, 1980), and population growth may be linked to periodic influxes of organic matter or food organisms. Heip *et al* (1985) noted that deposit-feeding estuarine nematodes tended to reach peak densities in autumn, winter or early spring, coincident with a high sediment content of dead *Zostera* leaves and other vegetation.

Given that some species may be in direct competition for food items perhaps 'seasonal staggering' may take place, where seasonal peaks are displaced to allow two or more species to share the same resource. Coull and Vernberg (1975) argued that an example of resource partitioning occurred in the case of the two closely related copepod species, *Halectinosoma* sp. and *Pseudobradya pulchella*, the former having an abundance peak in autumn, the latter in spring.

Although the occurrence of seasonally-determined abundance is well documented, the underlying reasons are not clear, perhaps because interactions between many or all of the factors discussed are taking place. It is clear that correlations and field observations exist for temperature, reproductive activity and predation, with some evidence for resource partitioning. However, there are no common patterns, and changes in temporal abundance remain essentially localised events.

1.2.9. Meiofaunal Food

Field data suggests that meiofauna are detrital, bacterial or diatom feeders, with varying levels of selectivity. In general, within shallow water environments of low to moderate water movement nematode feeding tends to be bacterial and/or detrital-based, while harpacticoids obtain nutrients from mainly microalgal sources (Montagna *et al.*, 1989). In laboratory-based experiments the benefits of meiofaunal selectivity have become very evident. Vranken *et al* (1984) tested the preference of a *Monohystera* (nematode) species for eleven bacterial species of which only one appeared to allow optimal growth. Similarly, Tietjen (1973) found only three out of the sixteen bacterial species offered were taken up by the nematode *Chromadora macrolaimoides*. When *C. macrolaimoides* was presented with 20 species of algae, only eleven were ingested in any quantity, with

five species able to sustain growth over a number of generations. Only two species, however, were able to sustain growth indefinitely.

Although these studies show specific preferences and to some extent the effect of diet on growth capability, other ontogenic factors may be affected. Lee *et al* (1976) tested the food preference and growth capability of the copepod *Nitocra typica* at various times from May to August, selectively controlling ingestion of particular algal species with rather spectacular effects. Some algae were able to sustain entire life cycles while others promoted rapid maturation or increased fecundity. Considering the advantages for particular life stages, it is perhaps not a surprise that *N. typica* was observed to modify its food preferences over time and between juvenile and adult stages. Similarly, Heinle *et al* (1977), in a study with *Scottolana canadensis* discovered that detritus supplied most of the copepod's energy needs, but the addition of algal cells was needed for egg production.

It is now generally accepted that most 'detrital feeders' do not obtain energy from the detritus particles themselves, but from the associated microbial colonists (Hicks and Coull, 1983). Indeed, it seems that most meiofaunal organisms lack the enzymic capability to utilise the detrital matter directly, and must rely on bacterial processing for the conversion to available nutrients. As the laboratory experiments have shown, there does appear to be an element of choice associated with many meiofaunal taxa and some reports suggest that meiofaunal density cannot be correlated with total bacterial density, but rather the composition in terms of specific strains or species of bacterium (Ravenel and Thistle, 1981).

Different methods of food uptake have been observed for various meiofaunal groups. Micro-organisms can be grazed directly from the substrate, or particles may be ingested whole and the microbial cells enzymically stripped within the gut lumen (Frithsen, 1984). Nematodes and copepods are also known to produce mucus traps which are able to capture bacteria. The bacterial/mucus mixture is then directly ingested.

Preferences for particular bacterial species (mainly by nematodes) may lead to niche partitioning, preventing competition for the same food resources. In some cases the

structure of nematode (Jensen, 1987) or copepod (Marcotte, 1984; Marcotte, 1986) mouthparts or buccal armatures can be correlated with the shape of the bacterial prey. Diatom feeders may be similarly specialised since mouth structures need to be able to deal with the hard siliceous test.

There is some evidence to suggest that the microorganism-meiofauna link goes beyond the simple predator-prey relationship, with meiofaunal organisms able to regulate the growth rates and distribution of microbiota. It has been shown that the growth of detrital microbiota is considerably enhanced by continuous meiofaunal grazing, which may serve to remove senescent cells and maintain microbial populations at an exponential level of growth (Montagna, 1984 - see below). In addition, mechanical fragmentation of the particles will enlarge the surface to volume ratio, allowing increased access for bacterial decomposition (Fenchel and Riedl, 1970; Cullen, 1973). Meiofaunal digging and burrowing activity may increase nutrient and oxygen advection rates, enlarging the habitats available to aerobic bacteria, while metabolic and mucal excretions by meiofauna provide sources of utilisable nitrogen and phosphorus, as does decaying meiofauna itself. In addition, the faecal pellets produced by meio- and macrobenthos attract dense colonies of bacteria, and cell numbers can be several orders of magnitude above the surrounding area (Meadows and Tait, 1985).

Meiofaunal utilisation of bacteria and algae has been calculated at approximately 0.1-10 μg dry wt. animal^{-1}. day^{-1} (Tietjen, 1980). In a further attempt to quantify intake, Admiraal *et al*, (1983) radioactively labelled diatoms and presented them to the nematode *Eudiplogaster paramatus*. Between six and seven were were taken per hour, representing a total daily carbon intake of 0.17 mg, or about double the average nematode body carbon.

Meiofaunal grazing of microflora is probably not food limited. Admiraal *et al* (1983) calculated that benthic diatom production was almost 2 g C. m^{-2}. day^{-1}, but nematode intake (as subsequently corrected by Heip *et al*, 1985) is unlikely to exceed 170 mg C. m^{-2}. day^{-1}. Montagna (1984), in a radiolabelling experiment obtained utilisation rates of 3% of bacterial and 1% of diatom standing stock per hour, suggesting that meiofaunal grazing may advantageously influence microbial population growth by maintaining

them in log phase. More recently, Epstein and Shiaris (1992) attempted to quantify bacterial consumption in the top 1 cm of sediment using fluorescence-labelled bacteria. This elegant study allowed visual identification of grazers and a numerical estimate of bacteria within the meiofaunal gut. They found that the majority of benthic organisms appeared to ingest the labelled bacteria, but nematodes were the major meiofaunal consumers, with considerably lower amounts consumed by harpacticoids. Foraminiferans and gastrotrichs were not observed to take up bacteria. They went on to estimate that only 0.03 - 0.2% of bacterial standing stock was consumed daily.

Clearly, much of the information concerning meiofaunal food selectivity and consumption rates has emerged from laboratory-based experiments, although the recent advances in radio- and fluorescence labelling technology has allowed a greater confidence in field- or mesocosm-derived results. In the past the meiofauna have often been considered in isolation as a 'small food web', unimportant and removed from higher trophic levels. But as information has emerged revealing the complex microbial-meiofaunal interactions, arguments have been advanced for an important ecological role as a significant link between bacteria and detritus, with repercussions at all trophic levels (Chardy and Dauvin, 1992).

1.2.10. Macrofaunal - Meiofaunal Interactions

The possible interactions and effects of macrofauna with respect to meiofaunal assemblages is still a subject of much debate and one that has been addressed with a number of field studies. The effects that have been reported overwhelmingly fall into one of three categories: (1) associations with macrofaunal structures; (2) disturbance; (3) predation. Direct competition may represent a fourth, but the difficulties in quantifying such a relationship have proved to be all but insuperable, with little or no data available.

With regard to the effect of macrofaunal structures, the possible advection effects of macrofaunal tube burrows is discussed elsewhere, as is the indirect nutritional potential of macrofaunal faecal pellets. Subsurface sediment and surface-protruding macrofaunal tubes whether inhabited or not have been reported to positively affect meiofaunal communities (Bell and Coen, 1982). Moore and O'Reilly (1993) noted a possible

commensal association between the harpacticoid *Bulbamphiascus imus* and the polychaete *Capitella capitata* in strongly organically polluted sediments. The macrofaunal structures may have a twofold beneficial effect for meiofauna: they provide a refuge from predatory macrofauna, and a food source in the membranous tube, or mucal tube lining.

Faecal pellets when present in sufficient quantities may impart a negative effect on meiofaunal populations and can thus be considered under the heading of disturbance. As an organic source, the faecal material may contribute to events leading to sediment oxygen deficiency. Similarly, bivalve biodeposits (e.g. in oyster and mussel beds) may accumulate as a thick layer, modifying the sediment properties and inducing the build-up of high interstitial ammonium levels. Dinet *et al* (1990) noted the decline of both nematode and copepod populations in such an environment, but Castel *et al* (1989) reported enhanced meiofaunal abundance in an oyster bed, concluding that the production of biodeposits represented a rich meiofaunal trophic resource. The contradiction is difficult to reconcile, but may be a function of differences in the quantity of accumulated material, or dissimilarity between sediment types.

Mechanical disturbance by the digging and reworking activities of fish, crabs and echinoids has been widely implicated in meiofaunal abundance reductions. Studies on the activities of the fiddler crab *Uca vocans* and *U. polita*, produced no evidence for direct predation on the meiobenthos, but when the crabs were excluded meiofaunal abundance increased two to five fold (Dye and Lasiak, 1986). A similar study with *U. pugnax* found a decrease in copepod abundance around crab burrows but increased nematodes (Bell *et al.*, 1978), perhaps pointing to a differential response with the passive more sediment-bound nematodes more resistant (or less able to move), while the generally more mobile copepods may enter the water column or migrate deeper into the sediment. Competition between meiofauna and crabs for food resources has also been suggested (Dye and Lasiak, 1986).

Meiofauna provide a food source for many other organisms at widely differing taxonomic levels. Common predators include shrimps, crabs, polychaetes, gastropods and a large number of juvenile fish species. It is still a matter of debate whether

macrofaunal predation pressures are able to modify or control meiofaunal community structure. Experiments involving the exclusion of macrofauna in intertidal habitats have been inconclusive, with some showing no measurable effects (Alheit and Scheibel, 1982; Hicks, 1984), and others producing meiofaunal population increases (Reise, 1979; Bell, 1980). Other reports claim reductions in meiofaunal densities in the presence of predators (Bell and Coull, 1978; Reise, 1979; Smith and Coull, 1987). The inherent dynamic characteristics of the intertidal environment may have made it difficult to separate true macrobenthic effects from physical influences (Olafsson and Moore, 1990). Subtidal investigations (Olafsson and Moore, 1990; Olafsson and Moore, 1992), also less than clear-cut, do however seem to suggest that macrofaunal predation, taken in isolation, is only a minor modifier of meiofaunal communities, and the overall mechanism is perhaps a complex interaction of many factors.

1.3. Meiofauna as a General Pollution Indicator

1.3.1. Why Meiofauna?

Any study designed to evaluate the effect of perturbations on the marine environment is liable to encounter a formidable array of problems, due to the multitude of physical parameters, and the complex ecological community structures. Much of the difficulty lies in the separation of natural fluctuations from changes initiated by the introduction of a chemical disturbance. Pelagic communities are unsuitable for this purpose because their movement within the water column makes it difficult to assess the length of time exposed to a particular pollution source. Also, repeated sampling for temporal studies would not be possible under these conditions (Warwick, 1988a). Benthic communities therefore present the obvious choice for the assessment of pollution effects in the field, and also to some extent in laboratory investigations.

Although the macrobenthos have been - and still are - used in pollution studies, meiofaunal organisms have particular attributes which make them of considerably greater value:

- Intimate association and dependence on sedimentary environments together with relatively sessile life styles means that exposure to a pollutant is consistent throughout.

- High abundance of individuals, even in habitats generally considered to be already under physical stresses, such as estuaries and exposed beaches. Numbers obtained are usually suitable for statistical analysis.

- High diversity, allows evaluation of changes in species composition with increased confidence.

- Some taxa exhibit extreme sensitivity to environmental stress (e.g. copepods), and their widespread distribution may allow direct site comparisons (Giere, 1993).

- Insensitivity of some meiofaunal taxa to mechanical disturbance gives the ability to distinguish between this and chemical stress (Warwick *et al.*, 1990).

- Meiofaunal species usually have benthic larvae, and so entire life cycles will be exposed to consistent levels of pollutant.

- Multiple generations within a year and rapid growth quickly produces useful information on sublethal effects on reproduction, growth rates, genetic expression, longevity and behaviour, sometimes in days or weeks. As many meiofaunal species complete their life cycles in 15 - 25 days, quick F1 generations for toxicity tests may quickly be produced.

- Rich assemblages may be found in areas of eutrophication, where hypoxia may exclude macrofauna (Austen and Wibdom, 1991).

- Meiofauna may react more rapidly than macrofauna to trace metal toxicity (van Damme *et al.*, 1984).

- Small, easily managed samples can be taken, with reduced costs in equipment and handling. The unprocessed samples may be conveniently transported back to the laboratory reducing the amount of time spent in the field.

There are, of course, disadvantages, some of which may prove to be quite substantial hurdles for some studies:

- The cost of processing increases as mesh size decreases, thus meiofauna processing may prove more expensive than for macrofauna.

- The identification of meiofaunal organisms often requires a greater dependence on high quality optical equipment and a specialist, as well as a less fully documented taxonomic literature.

- Studies cannot easily be transferred to regions of the world where fauna is poorly described and taxonomic expertise is lacking.

1.3.2. Practical Approaches

Moore & Bett (1989) proposed four major approaches to the use of meiofauna in pollution monitoring: (1) density of major taxa; (2) diversity; (3) species composition; (4) indicator species. Because of the obvious dominance of nematodes and copepods within the meiobenthos, these are consistently the organisms of choice for studying meiobenthic community attributes. Consequently, the majority of studies cited below consider one or both of these groups, almost to the exclusion of all others.

1.3.2.1. Density (Abundance) of Major Taxa

Most biomonitoring studies include, as an essential element, a measure of density or biomass of major taxa. The value of this type of data appears to be dependent on a number of factors, and is still the subject of some controversy.

Nematode density is thought to be relatively insensitive to pollution impact (Heip, 1980; Moore and Bett, 1989), and may fluctuate greatly temporally (Coull, 1985) and spatially (Findlay, 1981). Sediment characteristics may dictate abundance values, as finer sediments tend to have more nematodes than coarser sediments. This suggests that the usefulness of nematode abundance, taken in isolation, may be somewhat suspect, although major reductions have been observed in the case of oil pollution (Moore *et al.*, 1987).

Copepods are considered to be more sensitive to chemical stress, particularly pollutants that reduce the vertical distribution of sedimentary oxygen, as they are one of the most sensitive meiobenthic taxa to decreased oxygen and are generally only found in oxic habitats. However, they too exhibit both temporal and spatial variability with the addition of life-style preferences within the sediment. The combining of exposure results incorporating epibenthic, burrowing and interstitial forms may result in a 'generalised' copepod response, which is not a true representation of the effects on copepod abundance (Coull and Chandler, 1992).

Parker (1975) and Raffaelli & Mason (1981) proposed using both of the major taxa to compile an index based on their divergent reactions to pollution. The Nematode-Copepod ratio (N/C) effectively attempts to use the differential between the niche preferences of the two groups; a detrital/bacterial based food chain for nematodes, and an oxygen-sensitive microalgal bias for the copepods (Montagna *et al.*, 1989). Theoretically, if pollution in the form of organic enrichment is present, nematode numbers should increase, while impoverished copepod densities should become evident, thus the numerical value for N/C increases. Raffaelli and Mason (1981) showed that the overall N/C ratio increased with increasing organic load and inferred that ratios over a critical value of 100 indicated a polluted habitat.

The use of the Nematode-Copepod ratio provoked furious controversy (Coull *et al.*, 1981; Raffaelli, 1981), much of it centred around the modifying effects of sediment granularity, a problem recognised at the onset by Raffaelli and Mason (1981). Warwick (1981) suggested a refinement which required the identification of nematode feeding morphologies to equate the nematode-copepod trophic affinities (epigrowth, deposit or predatory feeders), producing a greater precision, but diminishing the appealing simplicity of the original method. Raffaelli (1987) subsequently corrected and restated his own stance, maintaining that differing habitat requirements created complications, which therefore restricted this method to the evaluation of organic pollution of sandy beaches, using only the interstitial group of copepods.

1.3.2.2. Diversity

Warwick and Clark (1991) critically assessed the merits and practical applications of several methods for measuring changes in benthic communities, broadly divided into three groups:

(1) Univariate methods: the relative abundance of different species are reduced to a single index (eg diversity indices).

(2) Graphical or distributional methods: relative abundance or biomass is plotted as a graph or curve e.g. dominance curves.

(3) Multivariate methods: the communities are compared on the basis species composition of the fauna as well as their relative importance in terms of abundance or biomass (e. g. ordination).

Pollution disturbance is known to be associated with changes in diversity, and diversity indices are commonly produced with any meiofaunal investigation. The manner in which diversity values change in response to pollution disturbance is somewhat variable and in many instances may be masked by other factors such as sediment granularity. However, some studies have reported a high level of success in using diversity indices to delineate a pollution gradient. Moore and Bett (1989) demonstrated a strong

correlation between the Shannon-Wiener index, derived from copepod taxa, and the distance from a sludge dump site in the Firth of Clyde, U. K. Copepod diversity was close to zero at the origin, and steadily increased both North and South of the site to a distance of 2 km. Other diversity reductions have been reported for organic enrichment (Moore and Pearson, 1986), oils (Moore *et al.*, 1987) and industrial pollutants (Heip *et al.*, 1984; van Damme *et al.*, 1984) but diversity enhancement has also been noted in the case of moderate pollution levels (Moore and Pearson, 1986).

One of the major problems with the application of diversity measures is the need to identify taxa to species level. However, the study of Moore and Bett (1989) referred to above, showed that diversity values calculated from both species and genera level paralleled each other, suggesting that a saving in taxonomic effort could be made with little loss of information, a conclusion supported by the reports of Heip *et al* (1988), Herman and Heip (1988), Somerfield and Clarke (1995) and Warwick (1988; 1988).

The use of *k*-dominance curves as a graphical representation of dominance for nematode assemblages has been proposed by Lambshead *et al* (1983), Shaw *et al* (1983), and Platt *et al* (1984). With this method the species are ranked in order of importance on the x-axis using a logarithmic scale, and the cumulative percentage dominance is plotted on the y-axis. A reduction in diversity is then indicated by a curve representing a high percentage dominance by a small number of species. Lambshead *et al* claimed that sufficient information is produced by this method to demonstrate a pollution effect, but Coull and Chandler (1992) point out that the calculation of a standard diversity index may be necessary. Platt *et al* (1984) recommend the use of the Cairns linked-estimator, a sequential comparison method developed to circumvent the need for species identification. However, Moore *et al* (1987) stated that some knowledge of taxonomy was necessary, with the error involved greatly reduced above 200 specimens.

Warwick (1986) proposed the application of a modified form of *k*-dominance curves to the detection of pollution effects on macrofaunal communities, with a graphical comparison of species diversities represented as abundance and biomass, referred to as the ‘ABC’ method. In undisturbed communities the relatively long-lived K-selected species, although low in numerical abundance are thought to maintain large body sizes

and therefore may dominate in terms of biomass diversity. After a disturbance event, a rapid colonising r-selected population may dominate, with a high abundance of a few species, but with low individual body size/biomass. Graphically, this is observed as the biomass curve elevated above the abundance curve in non-disturbed conditions, a convergence in moderate disturbance, and abundance in the elevated position in grossly disturbed conditions. A considerable attraction of this method is that it does not require samples from a "reference" site, a common problem in field studies. Warwick (1986) did however, point out that this method was probably not applicable to the meiobenthos, as there was no evidence of a similar relationship between pollution and species size, although it was noted subsequently that meiofauna may have a tendency towards larger species under organic pollution stress (Warwick, 1987).

Moore and Bett (1989) applied the same method to meiofauna subjected to organic pollution at the Clyde sewage sludge dumping ground. The results did indeed appear to demonstrate a converse effect to that shown for macrofauna, where the dominant meiofaunal species increased in physical size as the pollution density increased. To support this, Moore and Bett (1989) demonstrated a strong correlation between pollutant level and copepod body size along a transect through the dumping ground, concluding that this may offer a relatively simple monitoring method if further work can explain the conditions required for the occurrence of some diminutive dominating species.

Coull and Chandler (1992) pointed out the possible importance of the nature of the pollutant in terms of its effect on other aspects of meiofaunal morphology. Organic enrichment clearly holds the possibility of allowing a competitive advantage to those species that can utilise it as a food source. For some species of meiofauna this may in turn provide food organisms, or they may be able to feed directly on the polluting matter, stimulating a change in the community structure driven by feeding type.

The polluting material may alter the characteristics of the sediment, either by chemical adjustment or by the introduction of a different sediment granularity. Jensen (1986) stated that the length-width ratio of nematodes increased with increasing sedimentary sulphide, while Heip *et al* (1985) maintained that nematodes with deposit feeding morphologies were more common in muds than in sands. A note of caution is sounded

by Heip *et al* (1984) however, where their correlation of nematode feeding type with pollution was not as strong as the correlation of the former with sediment type, a clear indication of the importance of considering factors other than pollution during these investigations.

A different approach, originally developed for soil nematodes, has been advocated by Bongers (1990) and Bongers *et al* (1991). The most abundant nematode taxa (species, genera or families) were assigned a 'c-p value' between 1 and 5 relating to their tendency for K- or r-selected strategies, referred to as persisters and colonisers respectively (1 = extreme coloniser; 5 = extreme persister). With these values a 'maturity index' is calculated:

$$MI = \sum_{i=1}^{n} v(i).f(i)$$

where v(i) is the c-p value of taxon i, and f(i) its frequency. This index was applied to sites subjected to oil, organic and trace metal contamination with good discrimination between disturbed and non-disturbed sites. This method, as with others, does however require a certain amount of taxonomic skill and is thus limited to situations and locations where relevant keys and specialist personnel are available (Giere, 1993).

1.3.2.3. Species Composition

Univariate and graphical methods are not species specific and two or more widely varying communities (in terms of taxonomic composition) may have the same diversity or dominance, hence disturbance-induced changes in a community species composition may go undetected. Multivariate methods allow an examination of meiofaunal communities by simultaneously considering both species identity and their relative quantitative importance.

In the past, the most commonly used of the multivariate methods employed a form of hierarchical agglomerative clustering based on group averaging or nearest neighbour matrix sorting of sample similarities (frequently using the Bray-Curtis similarity measure). The results are usually presented as a dendrogram where species abundance or biomass may first be transformed to attenuate the influences of grossly dominant values, or to give a more appropriate scale. Frequently applied transformation functions are square root, double square root, log(x+1) or presence/absence.

The widespread availability of faster and more powerful computers has seen the rapid rise in popularity of ordination as a presentational tool, where similarities are represented by physical distance arranged in a two- or three-dimensional array. The currently prevailing methods in meiofaunal studies include Multidimensional Scaling (MDS), Detrended Correspondence Analysis (DECORANA), Principle Components Analysis (PCA) and Reciprocal Averaging (RA) derived from a similarity or correlation-based data matrix.

Moore and Bett (1989) applied both cluster analysis and DECORANA to data from a study of nematode populations in the Firth of Clyde (Lambshead, 1986), with replicate samples obtained from presumed clean and known polluted sites. With both these methods clear differentiation could be seen between samples from polluted and non-polluted environments.

Although Moore and Bett's study showed that multivariate methods were able to discriminate between clean and polluted sites, the process of site selection ensured that

two widely differing environmental conditions were under examination, thus giving no indication of the limits of detection. Extensive comparative tests of multivariate methods were carried out at the GEEP (Group of Experts on the Effect of Pollution) Oslo Workshop, using field and mesocosm samples with varying degrees of pollution difference between them (Gray *et al.*, 1988; Heip *et al.*, 1988; Warwick, 1988; Warwick *et al.*, 1988). Both meio- and macrofauna were sampled. The results indicated that all of the multivariate methods tested were able to display disturbance effects when the sample differences were great, but when the level of dissimilarity was reduced, none of the methods gave markedly clear indications of community contrasts. They were forced to conclude that the selection of a particular method was a matter of personal preference based on availability of suitable computing facilities and programs.

The field data did however show that the level of taxonomic discrimination could be reduced with little loss of ordinal spatial separation (Heip *et al.*, 1988). The nematodes appeared to be the most robust group, with spatial integrity breaking down at the level of sub-order and above, while for copepods it was evident at the family level (subsequently confirmed by Moore and Bett, 1989). This perhaps serves to diminish some of the criticism levelled at these techniques, namely that they represent a substantial investment in time and expertise if the required amount of raw data is to be obtained. With the pressure to identify taxa to species level relieved, the time spent processing samples may be reduced by as much as 90% (Heip *et al.*, 1988).

Although multivariate methods are relatively sensitive when compared to univariate and graphical methods, they are unfortunately only able to indicate community change, and not the cause of such a change (Warwick and Clark, 1991). This means that careful interpretation of results must be made to decide what factors are exerting an influence, as the possibility exists for natural variables such as sediment variability or salinity to subvert a pollution effect at the species composition level.

1.3.2.4. Indicator Species

The persistence of one or more species in disturbed environments has commonly been used to classify polluted sites. The use of such indicator species has stimulated much

criticism (Gray, 1979), but some meiofaunal species are known to proliferate in particular conditions.

For copepods, representatives of the genus *Tisbe* and large epibenthic diosaccid copepods have been reported to achieve high densities in polluted conditions. Marcotte and Coull (1974) showed that *Tisbe* sp. consistently dominated in samples obtained during the summer from their most polluted site in the Bay of Piran. In the winter months another species *Bulbamphiascus imus* succeeded *Tisbe*. *B. imus*, considered by some to be the only true meiofaunal indicator species (Coull and Chandler, 1992), is known to flourish in organically polluted sediments and has recently been reported as a possible commensal on the highly pollution tolerant macrofaunal species *Capitella capitata* (Moore and O'Reilly, 1993). Two different heavily polluted areas of the Baltic have been demonstrated to support dominant populations of *Nitocra* spp. and *Tachidius discipes* (Arlt, 1975; Anger and Scheibel, 1976).

Of the nematodes, the genus *Pontonema* is commonly reported in high densities in organically enriched sediments (Lorenzen *et al.*, 1987; Bett and Moore, 1988), while freshwater rhabditid nematodes have been found to dominate around domestic sewage outfalls where the effluent is diluted with water of low-salinity (Keller, 1986).

Meiobenthic oligochaetes can proliferate in low salinity areas, presenting the possibility of some species, particularly *Limnodrilus* spp., being useful indicators of industrial pollution in oligohaline areas (Coull and Chandler, 1992).

Because there are a number of other factors (some of which are still unknown) other than pollution that may lead to numerical species dominance within a community, a degree of caution is essential. There are as yet no established rules which set the parameters, within which a site may be designated as polluted, and much of the responsibility for a definitive assessment falls on the individual researcher's observational skills and experience. Typically, other methods will convey evidence of pollution stress before indicator species predominance is discernible (Warwick, 1987), and so this type of information is probably best used as a confirmatory tool.

1.3.3. Sediment - Pollutant interactions

Coastal and estuarine environments in particular are characterised by high levels of suspended clays, organic colloids and other particulates. As contaminants enter the water column a process of rapid binding and co-sedimentation will take place with the pollutant-sediment complex settling and integrating into the benthos. If the pollution input was a single event, the overlying waters will, over a period of days or weeks return to the pre-contamination condition. In contrast, the sediment-bound pollutant may persist for months or years presenting a possible long term toxic hazard to macro- and meiobenthic organisms.

The level of severity of the toxic effect depends on the inherent toxicity of the pollutant itself, its propensity for natural degradation or chemical conversion and, importantly for persistent chemicals, the way in which it is partitioned within the sedimentary matrix. Coull and Chandler (1992) refer to four benthic compartments; the sediment particles, the pore water, the overlying bottom water and the biota. Contaminant partitioning will depend on the affinity of the compound for a given compartment. For many trace metal ions and non-polar pollutants such as PCBs and PAHs, the concentrations on the sediment particles are higher than in the pore water. However, the pore water, in turn, is typically higher than the overlying water. Meiofauna that do not graze by sediment ingestion are exposed only to the pollution levels retained in the pore water. For sediment-ingesting species the potential toxicant load is greater because of the additional route of ingestion.

Therefore for ‘worst case’ representation of contamination effects, studies should endeavour to include species with a constant and intimate association with a sedimentary life style.

1.4. Effect Of Trace Metals On Meiofauna

1.4.1. Trace Metals and Marine Benthos

It has long been recognised that many metals are essential in trace amounts for the growth and development of aquatic organisms. Iron, for instance, is required in haemoglobin, the respiratory pigment found in all vertebrates and many invertebrates. Haemocyanin, performs an analogous function in many molluscs and higher crustaceans, but contains copper, while vanadium is accumulated for an, as yet, unknown purpose in the Ascidiacea (Kustlin and McLeod, 1983). Other transitional metals, such as zinc and cobalt are incorporated into enzymes or their prosthetic groups (e.g. zinc in vitamin B_{12}). Zinc and copper in high concentrations are also known to perform structural functions in polychaete worms, with nereids accumulating copper, and *Glycera* likewise zinc as a strengthening material for the tips of the jaws. Copper is accumulated for quite different reasons in the gills of ampharetid *Melinna palmata*, where it renders the animal unpalatable to most potential predators. Reports of trace metal requirements in meiofaunal organisms are few, but it is not unreasonable to assume that many of the known biochemical mechanisms utilised for accumulation or regulation in other marine organisms, may also function in the meiobenthos.

Clearly, with the potential for susceptible or tolerant taxa in meiobenthic assemblages, the monitoring of the effect of trace metal pollution as an indicator of environmental stress seems an attractive option. However, the majority of published studies have been conducted with a purely toxicological objective, often with a single, laboratory-reared species subjected to graduated levels of toxicant. Coull and Chandler (1992) noted the paucity of field experiments, commenting that of the 47 published metal toxicity investigations included in their review, one was conducted in a mesocosm and only nine were carried out in a field setting. Perhaps not surprisingly the experimental organisms used in laboratory tests were almost exclusively nematodes and copepods. The animals were clearly chosen for their ability to survive in culture conditions and local availability, and as such were confined to a very small number of taxa, the nematodes represented by *Enoplus*, *Monhystera*, *Diplolaimella* (*Diplolaimoides*) and *Caenorhabditis elegans*, while the harpacticoids *Tisbe*, *Tigriopus* and *Nitocra* were the

only three copepod genera tested. Despite the minimal number of taxa, some useful insights into the effects of metals have emerged, much of it concerning ontogenic disruptions on specific life stages or fecundity

1.4.2. Laboratory Studies

Many investigations have taken the form of a simple lethal toxicity test, expressed as an LC_{50} derived over a stated experimental time. Veriopoulos and Moraitou-Apostolopoulou (1989) selected *Tisbe holothuriae* for investigations into the acute effects of zinc on different life stages, concentrating on the copepodite, adult female and two female reproductive stages. The adult showed the least sensitivity with an LC_{50}48h of 1.150 ppm Zn, while the copepodite stage was most sensitive at 0.421 ppm Zn. The two female reproductive stages, with ovigerous bands or with egg sacs, displayed similar levels of response but were significantly more sensitive than the non-reproductive adult with LC_{50}48h values of 0.783 and 0.713 ppm Zn respectively. In a similar investigation with copper and cadmium (Verriopoulos and Moraitou-Apostolopoulou, 1982), the range of life-stages was extended to include one and five day old nauplii, with the copepodite age specified at ten days. For both metals the sensitivity from naupliar to copepodite stages steadily decreased, but the two female reproductive stages exhibited more variable responses, being similar to (Cd), or more sensitive than (Cu) the copepodite. From these and other studies it seems clear that in terms of acute toxicity, the higher the metal concentration the lower the survival rate. Coull and Chandler (1992) noted that this was true for most metals and taxa used, and with most of the life-history stages.

Observations of lethal effects allow a numerical value for single-species toxicity which can be compared with other species; however, the levels commonly used are unlikely to be a reflection of concentrations occurring in the field. In addition, it has been shown that the outcome of an LC_{50} test can be dependent on the time period over which the experiment takes place. In 96 hour tests on some brackish water nematodes, a high degree of resistance to cadmium was reported. However, when the time period was extended to 312 hours the LC_{50} value decreased by almost 80% (Vranken *et al.*, 1985). Sundelin and Elmgren (1991) comment that some metals (such as cadmium) are

excreted from marine species relativily slowly and toxicity should therefore be evaluated over a long period if an accurate assessment is to be made. In addition, the use of different life stages (particularly for copepods) can provoke erroneous comparisons if the stages are inadequately identified (O' Brien *et al.*, 1988).

Clearly, mortality is only one (albeit an important one) of many effects that may be observed during a pollution event, but in the majority of cases, the levels of metals actually found in the marine environment fall far below the ranges tested in LC_{50} experiments. Chronic effects may have drastic consequences, particularly at the population level, but these are often overlooked, or prove too difficult to measure (Dawson, 1979). Bryan (1971) suggests three areas of investigation for sublethal effects: (1) morphological change; (2) inhibitory effects such as modification of growth rate or sexual development; (3) Behavioral changes, resulting in reduced competitive ability or impaired capacity to escape predators.

Most chronic studies have concentrated on ontogenic effects, or observations of the viability of subsequent generations. Verriopoulos and Moraitou-Apostolopoulou (1981) using chromium, and Verriopoulos and Hardouvelis (1988) with zinc, demonstrated a correlation between increasing metal concentration and the ability of the copepod *Tisbe holothuriae* to produce eggs. Interestingly, the individual effects of the metals appeared to be subtly different, with the chromium not inhibiting the production of egg sacs, but preventing their development, while zinc-treated animals produced only a small number of egg sacs, and the number doing so was much reduced. Brand *et al* (1986) reported reductions in the population of *Tisbe* as the concentration of cadmium was increased, noting that at the highest concentration (159 $mg.l^{-1}$), there was a scarcity of advanced copepodites despite continued viable nauplii production by the surviving founder females. Even at the lowest concentration tested (2.3 $mg.l^{-1}$) there was a 25% reduction in population growth. Although this concentration is low, it is still higher than levels encountered in urban polluted areas. Nevertheless, it does highlight the possible mode of action of some pollutants in the field.

In contrast, a study of the effect of waste effluent containing titanium oxide on *Nitocra spinipes* failed to show any effects on fecundity until the concentration was at saturation

and a precipitate began to form (Lehtinen *et al.*, 1984). Further increases in concentration then began to drastically affect fecundity, suggesting a strong toxic and/or mechanical effect from the precipitate, later confirmed using pure and dried precipitate.

Vranken and Heip (1986) applied both chronic and acute exposures of copper, mercury and lead to the nematode *Diplolaimella* sp. and found it to be relatively insensitive when compared to copepods. In fact, the LC_{50} concentrations derived for copper and lead both exceeded seawater solubility levels (*Diplolaimella* sp. was obtained from polyhaline ponds) but reductions in fecundity occurred at an order of magnitude less than the LC_{50}.

In a rather novel approach, Sundelin and Elmgren (1991) combined an investigation of cadmium toxicity with the effect of a macrobenthic amphipod *Pontoporeia affinis*, considered to be an important predator on the meiobenthos. They reasoned that some observed toxic effects may be due to indirect impacts on predators rather than the meiofaunal component. The major effect appeared to be on nematode populations, which were observed to attain significantly larger sizes relative to controls in two of the cadmium + amphipod treatments, suggesting that the amphipods did indeed exert an influence. There were no differences in the high cadmium treatments because the amphipods were unable to survive in a concentration apparently non-critical for the relatively cadmium-resistant nematodes.

Vranken *et al* (1989) attempted to integrate the influences of temperature and food into the effects of chromium on the larvae of the marine bacteriverous nematode *Monohystera disjuncta*. They found that larval mortality increased with increasing temperature in the presence of chromium, and also both temperature and food quality influenced chromium effects on larval development. Although the physiological processes involved in these interactions are unknown, this does indicate that other factors may conspire to make comparisons of different laboratory studies difficult.

Hexavalent chromium was used in the above study because it is this form that is known to be absorbed from seawater. This raises an important point: if the laboratory experiments are to be a reflection of field conditions, how much of the testing is done with metallic forms found in the actual field setting? Are the metals incorporated in

benthic sediments in bioavailable forms? Moreover, much of the domestic and industrial waste released into the estuarine or coastal environment are complex effluents, not single toxicants. Vranken *et al* (1988) examined the effect of paired metal mixtures on *M. disjuncta*, concluding that mortality was lower when exposed to mixtures than with each metal alone. This is in direct contrast to results of similar exposures to the copepods *Nitocra spinipes* (Barnes and Stanbury, 1948) and *Tigriopus japonicus* (D'Agostino and Finney, 1974). Clearly, further studies are needed to determine whether the responses are taxon specific.

Coull and Chandler (1992) pointed out that the great majority of laboratory studies have been conducted by introduction of aqueous solutions of metals into a test chamber containing the test species. Many of these experiments - greater than 90% of those reviewed by Coull and Chandler (1992) - did not incorporate a sediment compartment, thereby ignoring a potentially important natural interaction. Austen *et al* (1994) introduced zinc, copper and cadmium-dosed sediments into laboratory microcosms containing entire meiobenthic communities obtained from both sandy and muddy environments. Analyses were restricted to nematode diversity and abundance as other taxa were not considered to be present in sufficient numbers. Their results were broadly in agreement with Tietjen (1980), with toxicity greatly enhanced in the sand when compared with mud communities. However, the comparative level of toxicity between zinc and copper was different between the sediment types, with zinc more toxic than copper in mud, but within sand the converse was true. These observations were attributed to differential binding to organic materials, the levels of which vary greatly between the sediments. Cadmium (added at levels much greater than that found in British estuaries) did not exhibit any obvious toxic effect.

Subsequently, Austen and Somerfield (1997) employed the same techniques to evaluate the effects of metal-contaminated sediments obtained from locations at varying distances from the influence of mining activity. Discrimination between nematode communities from low and high treatment concentrations was possible, although, in general, it was those subjected to very high combined zinc and copper (1.2-2.5 $mg.g^{-1}$ Copper and 1.4-4.5 $mg.g^{-1}$ Zinc) loading which exhibited a statistically significant response, perhaps suggesting a meiofaunal threshold response.

Clearly, studies of this type need further evaluation over a range of locations, conditions and sediment types, but they do appear to hold some promise as a potential reflection of the field situation.

1.4.3. Field and Mesocosm Studies

The small number of investigations in both field and mesocosm settings have yielded somewhat contradictory conclusions, some indicating trace metal-mediated assemblage disturbance, while others have been unable to discern any effect at all.

What is clear is that meiofaunal organisms from heavily polluted sites contain more metals in their tissues than animals from non-polluted sites (Howell, 1982). Frithsen (1984), by introduction of metallic radionuclides (^{7}Be, ^{109}Cd, ^{134}Cs, ^{58}Co, ^{51}Cr, ^{59}Fe, ^{54}Mn, ^{203}Hg, ^{233}Pa, ^{113}Sn and ^{65}Zn) into a large-scale mesocosm, was able to determine that meiofaunal feeding behaviour and vertical sediment distribution were the major factors governing uptake. Meiofauna were able to incorporate a similar amount of radionuclide to macrofauna despite a greater macrofaunal standing stock biomass. The dominant route appeared to be *via* adsorption and ingestion of particulate matter, with the surface-dwelling fauna incorporating more radionuclides than deeper animals. This holds important implications for the trophic cycling of metals as most predators are thought to feed in the oxygenated surface layers of the sediments, and indeed, calculations suggest that 1% of tin and almost 50% of cadmium may be cycled annually within the benthos (Frithsen, 1984).

In two field investigations of the impact of metal-contaminated sediments on nematode populations (Tietjen, 1977; Tietjen, 1980) no evidence could be found for changes in overall abundance at contaminated sites against clean sites. However, in a particular sediment type (medium sands), species diversity was significantly inversely correlated with increasing concentrations of chromium, copper lead and zinc (Tietjen, 1980). No such correlation could be found for silty sands, although in both sediment types some families were found to be reduced in contaminated conditions.

McLachlan (1977) could find very little effect of iron and manganese dust on a sandy South African beach. Copepods appeared to be slightly reduced and it was suggested that this may be due to interstitial clogging, affecting the heating, cooling and oxygen diffusion rates of the sand.

The impact of pollution transported by the Western Scheldt river into Belgian coastal waters was investigated by Heip *et al* (1984). Diversity was suppressed on all taxonomic levels, and nematodes were the most abundant group. The number of nematode species present was found to be correlated with trace metal concentration in the water column, and only non-selective deposit feeders were found to flourish near the mouth of the river.

Ellison *et al* (1986) were able to trace the movements of the foraminiferan *Ammobaculites crassus* in metal contaminated areas of Chesapeake Bay (USA). Because dead organisms left identifiable empty tests, it was possible to relate records of pollution levels to test remains in cores from different sites. They showed that there was a migration downstream associated with increasing levels of zinc, vanadium and chromium, and an eventual establishment of a migrational limit at the edge of the species tolerance level.

Clearly, most studies so far described have relied on univariate methods of disclosure. There are, in fact, very few published attempts to apply multivariate methods to what, after all, may prove to be rather subtle community effects, particularly as the pollutants are usually introduced as a cocktail of widely differing substances with possibly conflicting effects.

Sandulli and De Nicola-Giudici (1990) applied Multidimensional Scaling (MDS) to a field investigation of an urban coastal area in the Bay of Naples. Their most polluted site, with high measured levels of copper and zinc was spatially very distinct on the MDS ordination from the other sites. Warwick *et al* (1990), using multivariate methods were able to discount water depth and sediment type as influences on macro- and meiobenthic community structure in favour of anthropogenic variables, in particular tributyl tin (TBT) concentration. This study was within a harbour area, and so TBT

concentrations may be expected to be high enough to exert a substantial influence, although this was not evident from the companion univariate data.

Warwick *et al* (1988) has pointed out that, at present, the ability of multivariate methods to differentiate in some conditions is limited, but since there is currently a paucity of field-orientated metal pollution impact studies, it is perhaps too early to pass judgement.

1.4.4. Metal Uptake and Possible Tolerance

There is some evidence to suggest that some aquatic organisms are more capable of handling natural fluctuations in the availability of trace metals, and this may assume particular importance under heavily contaminated conditions. The degree of protection may vary from species to species, providing a mechanism for the success of some taxa in a given condition.

The uptake of metals may take the form of absorption from solution by passive diffusion of a soluble complex, mediated by adsorption at the body surface layer (cuticle, mucus layer), and subsequent binding of constituents of the surface cells, body fluids and internal organs (Bryan, 1976). This route has been proposed for polychaetes (Bryan and Hummerstone, 1973) and decapod crustaceans (Bryan, 1971). There is no evidence that any aquatic organism can reduce their permeability to metals, but animals such as bivalve molluscs may temporarily arrest absorption by simply closing their shell.

The degree of tolerance to a particular metal may be related to the organism's overall permeability. Studies with the polychaete *Nereis diversicolor* have shown a correlation between the rates of absorption of metals and their acute toxicity (Bryan, 1976). This is supported by investigations where *Artemia salina* was exposed to mercuric chloride and N-amyl mercuric chloride (Corner and Rigler, 1958). N-amyl mercuric chloride proved to be the more toxic, and it was concluded that this was due to its more rapid penetration rather than its toxicity in the tissues. The enhanced penetration rate seems likely to be related to the lipid soluble nature of N-amyl mercuric chloride, enabling it to permeate the epidermal cell membranes more rapidly (Corner and Sparrow, 1957).

The form of the metal in the aquatic environment (e.g. soluble ion, complex, compound, chelate or particulate) is only one of many factors which may attenuate or strengthen a toxic effect. The presence of other metals or toxicants (very likely in the field setting) may promote synergistic or antagonistic interactions, while physical factors such as salinity, pH, temperature and dissolved oxygen could modify the pollutant or predispose some organisms. Starvation and ontogenic status have also been shown to be critical in evaluating metal toxicity. In addition, body size or biomass has been shown to influence short-term metal toxicity in copepods (Reeve *et al.*, 1976), where larger sizes both within and between species appear to be less affected by toxicants than smaller sizes.

Studies of trace metal uptake and possible tolerance mechanisms in meiofaunal organisms are very few, particularly so for copepods. O'Brien *et al*, (1988) concluded that the comparative tolerance of the copepod *Tigriopus californicus* to copper was, in part, due to its ability to survive in the highly variable splashpool environment, where frequent extremes of temperature and salinity induce natural changes in metal bioavailability. This however, adds nothing to our understanding of the underlying mechanism of tolerance, except perhaps to suggest the role of genetic selection.

Some hints may come from the observations of naupliar sensitivity. It is known that in adult crustaceans, absorbed metals are concentrated in the carapace rather than in the body tissues (Bertine and Goldberg, 1972), and thus it seems likely that for larval stages, the carapace 'sink' is unavailable (Verriopoulos and Hardouvelis, 1988). The mode of feeding is, of course, important and adults feeding by particle ingestion will absorb substantial amounts of metals. At high levels even essential metals may become enzyme inhibitors. There is currently no information on copepod detoxifying systems, but the crayfish *Procambarus clarkii* is reported to have the ability to sequester copper and iron granules in the cells of the hepatopancreas (Ogura, 1959), and *Nereis diversicolor* from heavily contaminated areas stores copper as fine granules in the epidermal cells.

The uptake of metals by nematodes was examined by Howell (1983). In studies using copper and zinc, concentrations in six tissues were determined before and after uptake. Most of the metals were found to be associated with the cuticle, strongly implicating a process of surface absorption. Howell (1983) suggested that the nematode external

cortical layer is the sight of metal binding by incorporation into collagen, a substance known to contain metal-binding disulphide and sulphydryl groups. In further studies, Howell and Smith (1983) identified two proteins associated with the binding of copper and cadmium and speculated that the biochemical components suggested some similarities with metallothionine-like proteins. When the same species of nematodes, taken from two sites differing in metal pollution levels were tested for acute toxicity of lead, copper, zinc, cadmium and mercury, the nematodes from the polluted area showed a very small increase in survivorship (Howell, 1984).

In conclusion, although there is now good evidence for trace metal uptake and subsequent detoxification for a number of organisms, many of them aquatic, very little is known about meiofaunal strategies, and indeed whether there is such a thing as meiofaunal tolerance at all. If meiofaunal communities are to be used as an evaluation tool for environmental pollution, then further work is necessary to understand how the individual components of the community deal with such inputs.

1.5. Aims of the Present Study

In general, studies on the effects of metals on meiobenthic communities can still be considered to be at the preliminary stages of development. Both early and contemporary toxicological studies with single species are unlikely to reflect a chain of events that may occur during a real contamination event, even if sediments are included in the culture chamber. Field experiments, as we have seen, are relatively sparse and have largely produced inconclusive or conflicting results, often providing a tantalising hint of an effect, but without the all-important statistically valid supporting evidence.

The focus of this study was entirely directed towards the effects of a contaminant on community structure. Particular emphasis was placed on the response of the Harpacticoida, since they are numerically abundant in most sediments but have received a much-reduced level of attention when compared to the Nematoda.

Copper was selected as the test contaminant because it continues to be a major constituent of both domestic and industrial marine discharges world-wide, and therefore constitutes an anthropogenically relevant assessment.

The practical aspect of this study was orientated towards the following objectives:

- An assessment of the effects of a common source of copper contamination (antifouling paint) in the marine environment.

- The development of a fully replicated and controlled contamination assay for use in the field.

- A direct comparison of the resolution capabilities of univariate and multivariate statistical techniques.

- An examination of the use of laboratory-maintained harpacticoid communities in ecotoxicological studies and their relevance to events in the field.

- Examination of contaminated communities for evidence of species tolerance.

- The influence of sediment granulometry on the severity of copper contamination.

Sequentially, a field experiment to determine the impact of a copper-based antifouling preparation was performed first, since it constituted a preliminary study, providing both an indication of ecologically relevant sediment copper levels and an opportunity to become familiar with the meiofaunal community structure. While samples from this experiment were being processed, two laboratory-based experiments were performed to establish the practical possibilities of using sand communities maintained in microcosms for contamination assays. Finally, a second, greatly refined, field experiment was carried out incorporating copper concentrations applied in the previous experiments, allowing direct comparisons between communities, experimental techniques and sediment characteristics.

CHAPTER 2

THE USE OF LABORATORY MICROCOSMS IN THE EVALUATION OF THE EFFECTS OF SEDIMENT COPPER CONCENTRATION ON A MEIOBENTHIC SAND COMMUNITY

2.1. INTRODUCTION

Observation of marine ecosystems at the population and community level presents unique problems not encountered when studying most terrestrial environments. Even now, many of the critical components that define and control a given marine biotope are unknown because the important interactions are difficult to view *in situ*. Yet further problems can be expected when attempting to study the essentially invisible systems that function below sedimentary surfaces.

Although removal by remote grab, trawl and core equipment may provide 'snapshot' impressions of benthic assemblages, these methods are widely regarded as too destructive for many types of ecological studies. The process of recovery from depth does not guarantee the maintenance of benthic infaunal community structure and therefore may not provide the level of control and precision that is required for the accurate evaluation of a chemical or physical disturbance effect. Moreover, manipulative or disturbance impact studies, by their nature, are confined to defined areas which are often very difficult and expensive to sample to any degree of accuracy and confidence.

Single-species toxicity tests using a wide variety of marine organisms have been under continuous development for a considerable length of time. The rationale for these tests often centre around the premise that selection of the most sensitive species will provide the most conservative reflection of overall community sensitivity. Aside from the difficulties of identifying a given 'sensitive species', there has been some evidence that this approach can work. However, the uncertainty that remains due to the exclusion of interspecies, environmental and other interactions must always prompt considerable caution when attempting to extrapolate to the field situation. For this and other reasons single-species tests (frequently as LC/LD_{50}) have largely been considered as a comparative rather than a predictive tool (Graney *et al.*, 1995).

The establishment and maintenance of a complete marine ecosystem in a contained and controlled environment is clearly the obvious and logical way to resolve the major practical difficulties. However, emulation of entire self-sustaining marine communities has proved extremely difficult, precisely because of the lack of knowledge of the essential physical and biological components mentioned above.

Superficially, large mesocosms, in which wild communities are translocated to land-based enclosures, or somehow enclosed within the field environment, appear to offer the best compromise. A wide variety of macrofaunal studies have been performed in these conditions, with a high degree of environmental realism reportedly achieved. However, these installations are expensive to build and maintain, and there are persistent problems with precision of replication (Chandler *et al.*, 1997), prompting Kraufvelin (1999) to conclude that the 'ecological realism' of most mesocosms is not sufficient to allow direct extrapolation of contamination results to field conditions. One of the major problems with the maintenance of macrofaunal taxa in mesocosms is that the scale and dynamics of even the largest enclosures are rarely sufficient to support the entire life-cycles of all of the constituent taxa. It is perhaps because of this that mesocosms have often been used to study impacts of contaminants on selected macrofaunal life-stages, or interspecific interactions between a limited number of species, often accepting a reduction in the spatial confines of the experiment such that they might be best described as microcosms.

The term 'microcosm' is widely used for systems that are of a comparatively reduced scale, although Austen (1989) points out that the distinction between mesocosm and microcosm, within the experimental context, has never been rigidly defined.

The use of macrofauna in microcosms has met with some success but persistent experimental problems have restricted their use. Apart from the problem of scale, the largely seasonal reproductive cycle of macrofauna provides constraints on the time of year bioassays can be performed. In addition, recruitment from field supply is likely to be highly variable (Austen and Somerfield, 1997).

Meiofauna, in contrast to macrofauna, are particularly well-suited to the microcosm environment. The small size of meiofaunal taxa means that entire communities can theoretically be maintained without the need for large holding facilities. Indeed, Chandler *et al* (1997) suggested that a meiofaunal microcosm of only 100 cm^2 may be considered to be equivalent to a 1-5 m^2 macrofaunal mesocosm in both scale and the number of species and individuals. Therefore, because of the high numerical abundance of the meiobenthos, experimental assays may be performed in relatively small volumes with a degree of statistical precision that is largely unobtainable with macrofaunal communities. In addition, there are no recruitment problems because unlike many macrofaunal species which have planktonic larvae, most meiofaunal groups are direct developers. The associated short generation times means that effects on communities can be measured over a time and scale that can be realistically maintained in a laboratory (Austen, 1989).

Unfortunately, the practicalities of maintaining captive meiofaunal communities, particularly harpacticoid copepods, are still, to some extent, under development. Early successes in rearing harpacticoids tended to be with planktonic or semi-planktonic species such as *Longipedia*, *Tigriopus* and *Tisbe* supplied with particulate organic matter. Experiments that did include benthic harpacticoids were small in scale, often consisting of little more than a small amount of sediment and sea water in a petri dish with, perhaps, an added algal food source (Hockin, 1981). Sediment-dwelling, in contrast to planktonic copepods have proved to be harder to rear under experimental conditions and this seems to have been largely due to the specific food preferences of individual species (Hardy, 1978). These problems may, to some extent, be mitigated by increasing the scale of the microcosm, such that a functional community can be maintained.

Of the microcosm studies using meiofauna, most have concentrated on intertidal communities since these carry the obvious advantage of ease of collection. In addition, Austen and McEvoy (1997) commented that because of the extreme variability of conditions inherent in intertidal habitats these communities are likely to be relatively robust and therefore particularly suitable for laboratory manipulation. However, although subtidal communities inhabit a more stable environment and therefore may be

more sensitive to laboratory conditions, they may also be more susceptible to chemical and physical disturbance. Clearly, if this is true then a bioassay incorporating such a community is desirable for studies on the effects of chronic contamination.

Laboratory studies of the effects of sediment-associated metals on the meiobenthos are sparse. Coull and Chandler (1992) noted that prior to 1992, all but two of the laboratory studies were conducted using aqueous solutions of metals rather than contaminating sediments directly. Moreover, Chandler *et al* (1997) observed that of those studies that were concerned with sediment-associated contaminants the majority were performed as a spiked sediment bioassay on a single meiobenthic species and comments that these probably underestimate contaminant toxicity for some meiofauna while overestimating for others.

The direct application of metal-contaminated sediments to microcosm communities has only received experimental attention relatively recently. Table 2.1 lists all of the published studies to date. Of the five that were concerned with meiofaunal taxa only one investigates (in part) the effects on harpacticoid communities (Sundelin and Elmgren, 1991), while the remaining four concentrate entirely on nematode assemblages.

Although harpacticoids are not typically the dominant meiobenthic taxa, they are, in general, abundant and diverse in most sediment types. The current study was designed to evaluate the suitability of harpacticoid communities for laboratory microcosm bioassays, while establishing *a priori* concentrations for observable copper toxicity effects at a community level for the development of future field experiments. This was considered important since the levels of copper that have been reported to produce measurable responses in previous studies appear to vary enormously.

It was decided that a shallow subtidal sand community would provide a suitable test assemblage, partly because this was easily obtainable locally, but also because the design of the experiment required rapid separation of demonstrably live animals from the sediment. In addition, sand communities have been reported to remain closer to field assemblages in laboratory conditions (Schratzberger and Warwick, 1998).

Two experiments were performed, the first to determine effects over a wide copper range, the upper concentration chosen to be of a comparative level with the upper limit of previous studies. The second experiment was, to some extent, dependent on the outcome of the first, and primarily designed to study the effects (if any) at much lower copper contamination levels.

2.2. EXPERIMENT 1: HIGH COPPER RANGE

2.2.1. METHODS

2.2.1.1. The Collection Site

Seacliff is a semi-enclosed north-facing bay situated at the mouth of the Firth of Forth (charted location: 56^{o} 03.32' N, 02^{o} 37.65' W), approximately 2.3 km south of Bass Rock, and 4 km east of North Berwick. With a relatively small, sandy beach bounded and protected by cliffs to the south, and rocky promontories to the west and east, the location is a popular local bathing area during the summer months. A local lobster creel fishery operates from a small harbour cut into the rock of the west promontory, from which catches are hoisted to the top of the cliff by means of a small winch.

The proximity of the site to Edinburgh and hence Heriot-Watt University, coupled with the ease of shoreline access was an important selection factor for the collection of a live meiofaunal community. Equally important was the absence of any sources of metal pollution. The south-eastern coastline of the Firth of Forth is agricultural in nature and does not support the level of heavy industry that can be found further along the Forth Estuary. Consequently, coastal sediments along these shores are considered to be relatively uncontaminated.

2.2.1.2. Meiofauna collection

A subtidal meiofaunal sand community was obtained by diver from Seacliff on the 17th September, 1996 from a depth of 4.8 m below chart datum.

The collection station was just deeper than the shoreward rippled sand, centrally between the rocky headlands, and was characterised by a moderately dense population of *Lanice conchilega*. Previous exploratory sampling had demonstrated that surface sediments from this location yielded a statistically acceptable nematode and copepod abundance.

A 5-litre plastic bucket was used to scoop the surface layer to a depth of approximately 3 cm. When almost full, the water-tight lid was placed on the bucket before transport to the surface. Using a number of buckets, approximately 62 litres of sediment was collected and transported as quickly as possible to the laboratory, where it was homogenised by hand-mixing in a large plastic dustbin.

After homogenisation, the sand was dispensed in roughly equal amounts to three plastic trays of dimensions 59.5 cm x 35 cm x 15 cm (total surface area per tray: 2082.5 cm^2). The sediment was added to a depth of 10 cm with 3 cm of overlying seawater remaining. The trays were then left to settle for twenty-four hours with aeration in a constant temperature room maintained at 10 ^oC.

2.2.1.3. Experimental Microcosm Design

Each of the three sediment trays were overlaid with a 15 x 8 grid and small cores (1.9 cm diameter) were taken to a depth of 5 cm and transferred to 500 ml glass bottles. The cores were extracted from randomly generated co-ordinates within any one of the three trays, and pooled to a total of eight in each bottle. Three replicates were set up for each of four treatment concentrations. In addition, a further replicate was included for sediment metal determination at the conclusion of the experiment. Finally, 250 ml of 45 μm sieved seawater was then added, and the bottles were capped and left to acclimate overnight at 10 ^oC with gentle aeration through a diffuser stone (these were found to clog easily and were later replaced with pipette tips).

To reduce the effect of an initial metal 'pulse', copper was added to an aliquot of sediment prior to introduction into the microcosm. Dried azoic sediment, collected from the same site, but at an earlier date was cored separately, with two cores being placed in each of a number of acid washed plastic bottles. This sediment was supplemented with a copper chloride solution ($CuCl_2.2H_2O$; May and Baker Ltd., U.K.) made up in seawater. The final nominal microcosm copper concentrations were calculated on the basis of a previous experimental determination of dry weight values for freshly-extracted cores and were as follows: 0 (control), 200, 800, 1600 $\mu g.g^{-1}$. The amended sediment was stored at 2 ^oC overnight.

The following morning, the amended sediment was thoroughly mixed into the microcosm bottles by gentle inversion and resuspension until all of the sediment was washed into the water column. The microcosms were then left at 10 ^{o}C with gentle aeration until harvesting (Figure 2.1). To stimulate diatom growth a continuous lighting regime of alternating 12 hours light and darkness was maintained.

2.2.1.4. Sample Processing

Three entire microcosm replicates of each concentration were harvested at 1, 6 and 18 day time points. The metal determination bottles were simply frozen (-20 ^{o}C) after 18 days, and analysed at a later date.

The microcosm contents were washed into a 1 litre stoppered glass measuring cylinder with chilled 45 μm-sieved seawater and made up to the 1litre mark. The sediment was thoroughly resuspended by repeated inversion and then left to settle for 30 seconds before decanting though 1 mm and 45 μm sieves. Cold magnesium chloride solution (75.25 g.l^{-1} $MgCl_2.6H_2O$; Sigma Chemical Co., U. K., in distilled water) was added to the remaining sand to the 1 litre level, and the decantation procedure repeated. The process was repeated twice more with magnesium chloride, after which the material retained on the 45 μm sieve was rinsed with chilled sieved seawater into a beaker and made up to 140 ml with seawater. After vigorous agitation, a 35 ml (1/4) subsample was taken and the meiofauna examined under a dissecting microscope. Dead copepods were removed from the subsamples, while simultaneously counting the number of live nematodes. After counting had been completed, the subsamples were fixed in 4 % formalin in sieved seawater, allowing the enumeration of the total number of nematodes and the identification of the copepods that were alive prior to preservation.

Dead copepods were also removed from the remainder of the sample, which was then fixed in 4 % formalin in sieved seawater. All of the remaining (live) copepods were removed, mounted in lactic acid and identified to species level (where possible) using a compound microscope. Difficult or unusual specimens were dissected before mounting.

2.2.1.5. Metal Content Analyses

2.2.1.5.1. Acid Digestion

The frozen samples were slowly thawed and homogenised. Three replicate 25 g samples of wet sediment were removed from each bottle and oven-dried at 60 ^{o}C for 4 days. 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. 5 ml of deionised distilled water was added to the sediment followed by 5 ml of concentrated nitric acid (trace metal analysis grade; Fisher, U. K.). After the initial reaction had subsided the sample tubes were capped and placed in a water bath at 70 ^{o}C for 4 hours, with agitation by inversion every 30 minutes. After acid digestion, the tubes were removed and allowed to cool before adding a further 40 ml of deionised distilled water. The samples were then left to settle before instrumental analyses.

Replicate reagent blanks were simultaneously taken through the entire process.

2.2.1.5.2. Instrumental Analyses

Copper concentrations were determined on a dry weight basis by atomic absorption spectroscopy (Instrumental Laboratories S11 oxy-acetylene). Copper standards were made up in deionised distilled water, to cover the range 0-50 $\mu g.ml^{-1}$ (985 μg Cu/ml in 1% HNO_3; Sigma Chemical Co.). However, it was subsequently established that a linear response lay within the range 0-10 $\mu g.ml^{-1}$, and so all samples were diluted, where necessary, to give an absorbance value within this range.

2.2.1.6. Particle Size Analyses

Sediment for particle size analysis was obtained by replicate diver-extracted core. The sample was dried in an oven (500 ^{o}C) until a constant initial dry weight was obtained. The sediments were then passed though a sieve stack directly and the weight of fractions retained determined. The particle size classes were separated by the following sieve mesh sizes (in mm): 4.00, 2.80, 2.00, 1.40, 1.00, 0.710, 0.500, 0.355, 0.250, 0.180,

0.125, 0.090, 0.063, <0.063. The stony fraction retained on the 4 mm sieve was not included in any of the subsequent analysis.

The particle size classes were then plotted against cumulative percentage composition and median particle size, sorting coefficient and quartile skewness were derived using the following calculations (Holme and McIntyre, 1971):

Median particle diameter (Md_{ϕ}) was taken as the 50% ($\phi 50$) point of cumulative scale on graph.

Sorting Coefficient: $QD_{\phi} = \frac{\phi 75 - \phi 50}{2}$

Quartile Skewness: $Sk_{\phi} = \frac{\phi 75 - \phi 50}{2} - Md_{\phi}$

2.2.1.7. Data Analyses

Differences in microcosm abundance were examined using both one-way and two-way ANOVA on $\log_e(1+x)$-transformed data. Significant between-concentration and between-time differences were derived from a Tukey test matrix ($p<0.05$). Bartlett's and Levene's tests were first performed to confirm homogeneity of variance.

2.2.2. RESULTS

2.2.2.1. Particle Size Analysis

The data derived from the particle size analysis is given in table 2.2. The values indicate a very well-sorted fine sand.

2.2.2.2. Metal Concentration

Table 2.3 indicates that the assay values for sediment copper concentration are close to the intended (nominal) values.

2.2.2.3. Copepod Community Response

The one-way ANOVA results (table 2.4) indicate that without copper contamination the microcosm copepod community has remained at a stable abundance, with no significant changes in numbers throughout the experiment.

The charts suggest that there is a steady copper-related reduction in the number of copepods (figure 2.2), although there appears to be an anomalous increase in the abundance of the medium concentration samples after 6 days. However, the one-way ANOVA analysis (table 2.4) indicates that at 6 days there is no effect at any of the concentrations. After 18 days, though, all of the contaminated samples had undergone a significant and considerable reduction in abundance.

Further one -way analyses (table 2.5) to determine the effects over time, confirms that continuous exposure at all of the concentrations causes a maintained reduction in copepod abundance. This is further supported by the two-way ANOVA results (table 2.6) with a significant interaction between time and concentration, indicating that prolonged exposure probably causes continued and perhaps increasing mortality rates.

2.2.2.4. Individual Species Response

The effects on selected specific components of the copepod community are largely consistent with the overall effect.

Rhizothrix minuta was the numerically dominant species throughout the experiment and the elevated abundance may, to some extent, have obscured effects on the remaining species. The population appeared to be robust and well-adapted to the microcosm environment, maintaining a statistically stable abundance in the control samples (table 2.7). The species response tended to parallel that of the community. After 6 days there was no detectable effect at any of the concentrations, although the chart implies a clear downward trend (figure 2.3), supported by the response at 18 days, when all of the concentrations exhibited a significant population reduction (table 2.8). In contrast to the community response there does not appear to be a consistent time-related effect (table 2.7). Two-way ANOVA results (table 2.6) support only a concentration-mediated effect, although this may simply be due to the interfering influence of the suspected aberrant 6 day medium concentration point, or a statistical inability to resolve the trend over the relatively short time period.

By removal of *R. minuta* from the data set one can gain some impression of how the response of the community is modified by the dominant taxon. Figure 2.4 and tables 2.9 and 2.10 show that although there are some minor differences, the remaining taxa respond in a similar way to the contaminant levels. The major influence of *R. minuta* appears to lie with the maintenance of the control abundance. It seems that *R. minuta* is perhaps the only species in the sand assemblage that both maintains a stable population abundance and is entirely unaffected by microcosm conditions.

The pattern of response for the second most abundant species, *Halectinosoma herdmani* varies considerably from the underlying trend (figure 2.5). This species was suspected of some sensitivity to the microcosm conditions, but the difference between sampling times is not significant (table 2.11). The low copper concentration samples, although having a generally lower abundance, show a similar trend to the controls and are not significantly different from them (table 2.12), implying, at least at the early stages of the

experiment, little or no effect. In contrast, at both the medium and high concentrations almost the entire population had collapsed after only a single days' exposure.

Two-way ANOVA (table 2.6), indicates that there is both a significant *H. herdmani* abundance reduction over time and a response to copper concentration, although there appears to be no effect associated with the length of time of exposure.

2.2.2.5. Nematode Community Response

Nematode control communities displayed no evidence of sensitivity to microcosm conditions, and indeed the numbers were substantially elevated in the 6 day cultures. This rise was statistically significant, and may perhaps be due to a coincidental seasonal recruitment event, or a generalised response to initial sampling disturbance. Whatever the cause, it did not effect the ability of the assay to detect a contamination effect, since the response was both strong and rapid (figure 2.6). After 1 day the community exposed to the high concentration was significantly reduced (table 2.13), and at 6 days all of the exposed communities were demonstrably affected. By the 18 day time point there was close to 100% mortality in all of the contaminated samples. Analysis by two-way ANOVA (table 2.14) indicates that, as with the copepod community, the severity of the effect is significantly aggravated by the time of exposure.

2.2.2.6. Effect of Live Selection

The selection and enumeration of live meiofaunal specimens does appear to substantially modify the perceived effect. When the charts for total nematode abundance (figure 2.7) and live individuals only (figure 2.6) are compared it is immediately obvious that a very large number of dead but well-preserved animals remain at all stages of the experiment. If, as is common in this type of study, all of the animals in an acceptable state of preservation were assumed to have been living at the point of sampling then statistically significant effects may be erroneously missed (tables 2.15 and 2.16).

There is a major effect on the representation of the control communities, with a steady and significant decline in numbers which would probably have been interpreted as an indication of sensitivity to the microcosm environment. The active selection of live specimens give a very different view, with no such decline, and thus no evidence of a 'microcosm effect'.

It is interesting to note that when the numbers of dead nematodes in the control sample are examined, there appears to be very high initial mortality (figure 2.8). There are a number of possible explanations for this, the simplest of which is that the deaths are due to an initial community-wide reaction to microcosm conditions. Alternatively, there may have been selective mortality of disturbance-sensitive species, although this cannot be confirmed because identification to species level was not carried out.

The charts do, however, strongly imply a copper-dependent effect on grazing of the dead individuals (figure 2.8). After 6 days, very few nematode remains could be found in the control samples, but in the copper-contaminated samples high numbers of dead nematodes remain in all of the microcosms, perhaps with a slight reduction over time. Clearly, the mortalities that are, for the most part, due to copper contamination tend to remain longer because the metal is also reducing the ability of meio- and microbenthic scavengers to assimilate dead individuals.

2.3. EXPERIMENT 2: LOW COPPER RANGE

2.3.1. METHODS

2.3.1.1. The Microcosm

The meiofaunal sand community was collected from Seacliff on the 11th March, 1997, from a depth of 7.7 m below chart datum, and microcosms set up as previously described. The nominal copper concentrations were introduced at: 0 (control), 50, 100, 200 $\mu g.g^{-1}$ dry weight of sediment.

During the previous experiment a potential problem with salinity increases due to evaporation from the microcosm bottles was identified. Consequently, all of the microcosm bottles were monitored regularly over the duration of the experiment, and where necessary distilled water was added to maintain a salinity level of 36 – 38 $^{o}/_{oo}$.

2.3.1.2. Sample Processing

Microcosm replicates of each concentration were harvested at 1, 5 and 20 days, and processed as previously described. Only live nematodes were enumerated in this experiment.

2.3.1.3. Copepod Diversity

A range of diversity indices were calculated for the copepod communities in each microcosm. These were: the total number of species, species richness (Margalef's index), Shannon-Wiener index, Simpson's dominance index and Pielou's eveness. All diversity indices were derived using the PRIMER (Plymouth Routines in Multivariate Ecological Research) computer package, and where logarithms are used in the calculations $\log_2$ was selected.

2.3.1.4. Vertical Distribution of Copper

To determine the distributional fate of the introduced copper within the microcosm sediment an experiment was set up to mimic microcosm settling and culture conditions.

Sediment was collected from the Seacliff site on 17th of November 1997, and allowed to acclimate with aeration at 10 ^{o}C for 2 days. Three 500 cm^2 beakers of a similar base diameter to the microcosm bottles were obtained and 16 cores of fresh sediment were placed in each. Preliminary experiments suggested that the increase in sediment volume was necessary to allow extraction of cores that could be adequately sectioned. Three sets of two cores were amended with 1646.4 mg $CuCl_2.2H_2O$ to give a final target concentration of 1600 μg.g^{-1}, and added to the uncontaminated sediment, rinsed with 5 ml of seawater and homogenised within the beaker with vigorous mixing and agitation. No further addition of seawater was considered necessary. Each of the beakers were double-sealed with a single central aeration line, and left in identical conditions to that of the microcosms for 15 days. After this time a single sediment core was taken from the centre of each beaker and immediately frozen at -20 ^{o}C. Immediately prior to preparation for metal analysis the cores were partially thawed and gently extracted from the coring tubes, where they were cut in three sections of approximately 1 cm each using an acid-washed plastic scalpel. Metal analysis was performed as previously described with the exception that the entire sample was processed.

2.3.2. RESULTS

2.3.2.1. Microcosm Metal Concentration

As in the previous experiment the measured microcosm copper concentrations are very close to the intended values (table 2.17).

2.3.2.2. Copepod Community response

The very much lower levels and the reduced concentration range of copper in this experiment has, perhaps unsurprisingly, produced a rather more subtle copepod response. The community is again statistically stable throughout the experiment in the control microcosms (table 2.18), demonstrating that, at least at this level, there is no observable 'microcosm effect'.

The charts (figure 2.9) are consistent with a graduated response to copper contamination, with the medium and high concentrations exhibiting a significant reduction (one-way ANOVA) in copepod numbers at 20 days (table 2.19). Additionally, the continued exposure is a significant factor, and the abundance in the high and medium concentrations are significantly affected in the period between the 5 and 20 day sample times (table 2.19). These conclusions are strongly supported by two-way ANOVA results (table 2.20)

Importantly, the communities subjected to the lowest concentration appear to be statistically indistinguishable from the controls, thus suggesting that the threshold for a measurable community effect may lie between copper concentrations of 50 and 100 $\mu g.g^{-1}$ dry weight sediment.

2.3.2.3. Individual Species Response

As in the initial experiment *Rhizothrix minuta* is the dominant species in all of the microcosm samples, although in this experiment *R. minuta* has a greater abundance, and thus has an even greater tendency to influence the overall pattern of community response. A comparison between the charts of total copepod community (figure 2.9) and

R. minuta response (figure 2.10) reveals very little difference, and, in fact, the ANOVA results are almost identical (table 2.21 and 2.22)

The second most abundant species is again *Halectinosoma herdmani*, and this experiment confirms the results of the first study, in that this species tends to exhibit a decline that is not attributable to copper exposure (figure 2.11). Again there is a rapid and significant drop in abundance that seems to be largely due to an inability to survive in a closed microcosm system (table 2.23). Two-way analysis, although not quite significant, does, however, hint at a possible antagonistic role for copper (table 2.20).

2.3.2.4. Mesobenthic Species Response

At least five mesobenthic or interstitial species were found in the microcosm sediments. As a group, these highly adapted taxa often comprised over 10% of the total harpacticoid abundance, and so these were examined to see if there was a collective trend. Figure 2.12 shows the combined response of the mesobenthic species at the different copper exposure levels. An inexplicable drop in abundance in the 5-day control samples adds some confusion to the general picture, but overall it can be seen that there is a downward trend in abundance over time. Two-way ANOVA confirms that this trend is significant ($p = 0.002$), but does not support a significant copper-mediated effect.

2.3.2.5. Copepod Diversity Response

In general, changes in copepod diversity (figures 2.13 - 2.17) are attributable to increases in dominance. The one-way ANOVA of the control time-points does imply that the number of species and Species Richness remains stable throughout with no significant differences, although the other indices indicate a significant difference between 1 day and 20 days ($p<0.05$).

Time-mediated effects on the copper exposed communities, as determined by one-way ANOVA, do not fit into a clear pattern. Although there are some significant reductions in diversity, the lack of consistency perhaps suggests that this does not merit further examination.

The two-way ANOVA results (table 2.24) provide a simplified, but clearer picture and confirms that all of the diversity indices are affected by microcosm conditions. However, the Shannon-Wiener index, Simpson index and Evenness are also significantly modified by copper exposure, with Evenness exhibiting an interaction between copper level and exposure time

2.3.2.6. Nematode Community Response

Unlike the copepod community response there is no obvious differentiation between the copper exposure ranges (figure 2.18). Indeed, the overall pattern of abundance decline is largely similar to the previous experiment, although the reduction in abundance at 5 days is clearly not as marked as the 6 day effect with the higher concentrations. This is reflected in the in the one-way ANOVA results (tables 2.25 and 2.26) where there are significant differences between all of the time-points suggesting a relatively protracted, rather than rapid, community decline. The two-way ANOVA result (table 2.27) strongly reinforces this view with a highly significant time and time-concentration interaction effect.

An indication of the relative severity of the cumulative nematode exposure effect may lie with the end-point of the experiment. While the high copper levels resulted in very near community collapse after 18 days (5-15 individuals remaining), the lower range, while still producing a major decline, has a correspondingly elevated number of survivors at 20 days (95-55 individuals remaining). The subtleties of this pattern are further clarified by displaying the abundance of each time point as a percentage of the corresponding control (figure 2.19).

This result clearly indicates that nematode sand communities are highly susceptible to exposure to relatively low concentrations of copper.

2.3.2.7. Vertical Distribution of Copper

The results of the study of sediment copper distribution within the microcosms clearly show that the metal is not evenly dispersed. Despite homogenisation by means of stirring and vigorous agitation such that the sediment is briefly held in suspension, the greater proportion of the metal is retained in the surface sector after 15 days (table 2.28). The low degree of variability between replicates confirms that this is not an artefact created by inadequate and inconsistent mixing during the initial construction of the microcosm. In addition, the mean concentration for each of the cores suggests that if even metal distribution had been achieved then the actual concentration would have been extremely close to the target concentration as has been evident in the experimental systems used for meiofaunal exposure.

Figure 2.20 displays these results as a percentage copper content. Approximately 70% of the sediment copper is found in the top one-centimetre (approx.) layer, while less than 20% is bound in the middle layer, and only around 12% in the bottom section.

2.4. DISCUSSION

The major purpose of this study was to simulate the impact of a range of copper concentrations on a shallow subtidal sand community by the use of enclosed laboratory microcosms. The results are summarised in figure 2.21, where it can be seen that there is a clear and consistent nematode and copepod community abundance response to all of the sediment copper concentrations.

Overall, it is the nematode community that appears to exhibit the greatest sensitivity to copper contamination with a significant response after 5 days, even at a concentration as low as 57 $\mu g.g^{-1}$. This confirms the results of Tietjen (1980) which suggested that in sandy field sediments, metals in the range of 50 $\mu g.g^{-1}$ may alter nematode population structure.

Although the copepod community may not appear as sensitive it is still affected by the lowest concentration, but it requires an exposure of some 20 days or greater to begin to produce a visible decline in the community. It is clear from this that for copepod communities a given contamination effect is highly dependent on both the concentration of the contaminant and the length of time of exposure. This presents a number of implications for both laboratory and field experiments, and perhaps goes some way to explain the widely varying dose-responses that have resulted from other studies. Clearly, without a comparative and reasonably long exposure period, no two similar studies are likely to produce equitable results.

The time period of 18-20 days used in this study was selected for both practical and biological reasons. Most meiobenthic copepods species are though to have an egg to egg lifecycle of between 15 and 25 days and can produce multiple generations per year (Hicks and Coull, 1983). A bioassay of around 20 days provides the opportunity for the eggs to hatch and grow to at least the fourth of six copepodite stages. Similarly, the nematode *Monhystera disjuncta* has a generation time of 18 days at 9 ^{o}C, although, most other species can be expected to exceed this (Warwick *et al.*, 1988), (Vranken and Heip, 1986).

A common practice is to perform the microcosm assay at a rather higher temperature than would be encountered in the wild. Austen and Somerfield (1997) maintained microcosms in the dark for two months, gradually raising the temperature at 1-2 ^{o}C per day until 20 ^{o}C was reached. The (nematode) community exposure to metal was performed at this temperature to optimise metabolic activity, species reproduction and turnover as indicated by Heip *et al* (1985). This practice may be valid for intertidal sediments where such temperatures are commonly reached, particularly in mud communities which are confined to the sediment surface. However, subtidal communities, even in shallow waters, are less likely to experience such temperatures and the associated rapid rate of change. Little is known about the potential interaction between temperature increase, sediment-metal reactivity, and changes in biological and biochemical activity. Additionally, complicating factors such as deeper burrowing in coarse sediments, perhaps as a thermotaxic response would prove difficult to simulate or control in microcosm conditions.

There is some disagreement about the validity of integrating ontogenic considerations into the experimental approach. Kovatch *et al* (1999) investigated the effect of a field-collected sediment contaminated with a cocktail of contaminants on two copepod species. The two species were maintained in separate cultures and also exposed to a relatively clean sediment as a comparison. The populations were assayed for significant differences at both 14 and 21 days. The authors concluded that the 21 day assay did not provide any greater resolution of an effect than the 14 day assay. This may, however, reflect the different requirements of a community rather than a single-species-based approach.

What is beyond dispute is that a microcosm-based assay is a cost-effective, convenient and potentially powerful tool in the field of marine ecotoxicology. An important consideration, though, is the ability to repeat experiments with the same or similar community. In this respect the selection of a shallow subtidal sand community is appropriate. Although by its nature the assemblage will undergo periodic weather disturbance, the components are both well-described and common throughout Britain.

For this study collections were completed at different times of year and in different years (17/9/96 and 11/3/97). However, between-collection abundance and species composition remained remarkably consistent for copepods although *Rhizothrix* was more abundant in the second experiment. The temptation may have been to attribute this to sampling or experimental variability, but the similarity of *H. hermani* and nematode numbers suggests that this was a real elevation in abundance and if absolute reproducibility is required (perhaps where routine comparison between experiments is required) a study of the community dynamics of the source site may be appropriate.

In contrast, mud communities are known to be highly spatially and temporally variable, and field experiments performed as part of this study strongly confirm this (*c.f.* field communities in Chapters 3 and 4). Repeated collection of similar mud assemblages for prolonged studies or a series of experiments would probably not be possible on a regular basis.

Chandler *et al* (1997) amended microcosms constructed from natural mudflat sediment with a known number of the benthic copepod *Amphiascus tenuiremis* thereby determining the response of a sentinel population. Here, the addition of a spiked flocculent layer to a specified depth (2 cm) ensured the movement of most of the meiofaunal organisms into the" oxic horizon" of the contaminated sediment. This is a potentially useful assay for mud-dwellers where sublethal effects can be tested. However, the subsequent harvesting required fixing in formalin and staining with rose bengal before counting could commence, so possible problems with lingering dead individuals remains a problem here.

The potential modification of results from counting dead animals as alive is a largely unexplored problem. Experimental strategies that include enumeration of live individuals are rare in meiobenthic community studies. The additional effort and time required to maintain such discrimination falls outside the scope of most investigations. It has been a long-held, but little considered, assumption that individuals that are dead or dying as a result of experimental contamination are quickly degraded by bacterial action or remove by predation. Although this may arguably be the case in field experiments, where recruitment and scavengers may play a role, an enclosed or semi-enclosed system

under contamination stress might not support the rate of turnover that is required for rapid removal of dead material.

This study has shown that, in the case of nematodes, dead individuals may persist for a considerable time and in such numbers that might cause substantial modification of the final result, perhaps even to the point of masking a significant impact effect. This effect is particularly acute when the levels of the toxicant is such that it may reduce or halt the bacterial degradation process.

It is probable that, for the most part, this problem is ignored or just considered not to have a significant influence on the outcome of an experimental result. Austen *et al* (1994) in their microcosm experiments commented that it was frequently difficult to determine whether nematode specimens were dead or alive at the time of sampling. An animal was considered to have been dead if there was clear visible disintegration or decomposition of internal organs. If there was an element of doubt, a 'conservative approach' was adopted and the animal was *included as alive*! However, subsequently Austen and McEvoy (1997) reported the preservation of nematode communities in high dose levels of TBT. Similarly, Austen and McEvoy (1997) attributed the apparent high nematode survival rates to exposure to zinc and copper to a reduction in the decomposition activity of the bacterial and fungal organisms imported with the microcosm sediments. Austen and McEvoy (1997) suggested that this provides a strong argument for the use of whole sediment communities since the removal of microbial activity represents a catastrophic reduction in meiofaunal food supply which would, in turn, cause a major change in community structure.

The 'live selection' approach is unfortunately only feasible in clean sediments. Muddy sediments may be tested only if the experimental design does not require enumeration of live animals, because any live separation technique is likely to be too long and cumbersome to efficiently and reproducibly recover active meiobenthic taxa. There may be some room for the application of vital stains, but at present they are known to be rather unreliable with staining efficiency known to vary even between families within the same phyla (Ogiga and Estey, 1974; Dressel *et al.*, 1972).

An important and rather unexpected aspect of this study was the discovery of the tendency of introduced contaminants to form a vertical concentration gradient. Despite considerable efforts to achieve complete homogenisation, around 70% of the introduced copper was found to reside in the top centimetre of sediment. This has also been observed in a field experiment involving sediment dosed with zinc (Watzin and Roscigno, 1997), where the metal concentration was found to be an order of magnitude higher in the top 2 cm than in the 2-6 cm depth.

The mechanism by which this occurs can only be the subject of speculation, although the nature of the experiment would suggest that the gradient structure is formed rapidly and at the initial setting-up of the microcosm. The most likely explanation is that the majority of copper was quickly bound to the organic detrital matter recovered with the sediment during field collection. During the homogenisation process the heavier sediment particles settled first and the lighter organic-bound metal settled at a slower rate promoting the formation of a surface layer.

With only three one-centimetre segments the resolution of this experiment is rather low, and it seems likely that the gradient is rather smoother than these data might imply. Indeed, one could reasonably expect an exponential increase in copper concentration towards the sediment surface, such that the doubling of the mean (nominal) concentration found in the surface section in this experiment is, in turn, a dilution of the higher concentrations found at smaller scales.

In practical terms, this carries considerable implications for relating contaminant concentration to effect on the benthos. It is probable that sediment metal concentration assays are prone to over-simplification unless sophisticated micro-spatial analysis is employed. In real terms, the measured concentration within an experimental system may not reflect the true exposure on any given community component.

Moreover, there must now be some concern for the way in which samples are taken for metal analysis. For the current exposure studies the contaminated sediments were mixed before relatively large replicate samples were taken for eventual assay. This resulted in an accurate ‘mean’ concentration. If, as is often the case, a sample had been removed

from the surface layer a very much higher concentration would have been recorded. Perhaps examination of vertical distribution of contaminants should be considered an essential feature for this type of experiment.

The uneven spatial distribution of the contaminant is clearly a complicating factor when attempting to define a community effect. A vertical concentration gradient may have a differential impact on a community which in itself is vertically structured. This may act in two ways. Firstly, burrowing or interstitial species may be at a sufficient sediment depth, or may retreat to deeper levels, to avoid the zone of high copper concentration, and this may be one explanation for the apparent insensitivity of *Rhizothrix minuta* (although this cannot be invoked for mesobenthic species). The second and most direct route of contamination may be through the grazing of the surface-bound detrital matter (or the microbiota associated with it). Clearly, feeding within this zone might involve high exposure levels, both externally and by ingestion.

The role of food as a potential route of contaminant uptake has been addressed by a number of other studies. Meador *et al* (1997) investigated the toxicity of TBT to three macrofauna species with detritivorous, predatory or non-selective deposit feeding modes. The level of toxicity to all three decreased with increasing organic carbon content suggesting that, regardless of mode of feeding, TBT is taken up in dissolved form *via* the pore water. Austen and McEvoy (1997) suggest that in organic-rich fine-grained sediments ingestion of particulates is likely to be a major pathway for macrofaunal exposure to TBT. However, examination of nematode feeding guild response to TBT contamination failed to demonstrate that food selectivity promoted mortality to a greater or lesser degree in particular guilds. In contrast, Frithsen (1984) related the vertical sediment concentration of metallic radionuclides introduced into a large-scale mesocosm to direct uptake by surface dwelling fauna, noting that label incorporation was similar for both macro- and meiofauna despite the former having a larger standing stock biomass. In this experiment the labelled metals could be directly observed to be rapidly removed from the water column to the sediments where the dominant faunal route of uptake was judged to be most likely through the ingestion of particulate matter.

Additionally, one perhaps cannot dismiss the possibility that the surface metal contamination may be of a sufficient level to substantially reduce the availability of food items such that epibenthic grazers may simply starve.

During the course of both of the experiments it became very apparent that copepod (and to some extent nematode) locomotive activity was visibly correlated with copper concentration and length of time of exposure. At the start of the experiments, and throughout in the controls, physical activity was rapid and largely continuous. However, individuals when physically disturbed became slower with greater periods of inactivity in a manner consistent with increasing copper exposure. In the high copper range experiment, the medium and high exposure regime rendered the remaining copepods at 18 days almost comatose, with stimulation using a tungsten wire necessary to establish condition. Although this was not investigated further, it seems this may provide an additional assessment tool in the future. This form of assessment has previously been applied to impact effects on other crustacean species. Pynnönen (1996) used physical movement (burrowing activity) as an indicator of toxic effects of copper and cadmium on the isopod *Saduria* (*Mesidotea*) *entomon*. Similarly, Kemp and Swartz (1988) used the ability of the amphipod *Rhepoxynius abronius* to rebury itself as an indicator of sublethal effects of cadmium.

Although community abundance in the uncontaminated microcosms was statistically maintained the integrity of the assemblage was compromised by some species that were unable to withstand the microcosm environment. The inability of some species to survive is commonly reported and is perhaps the strongest argument against the use of microcosms.

Austen and Somerfield (1997) reported that the conditions in their microcosms clearly suited some nematode species better than others leading to an overall “microcosm effect”. They went on to suggest that the species that survived better in microcosms were more physiologically robust and hence more resistant to physiochemical stress and disturbance.

Hockin (1981) noted major parallel successional changes in replicate intertidal sand microcosms that were not apparent in the field community. These dissimilarities were attributed to interstitial drainage rate, salinity and sediment depth (5 cm in the microcosm; 1-25 cm on the beach sampled). These differences aside, Hockin (1981) did however claim to have been able to maintain a diverse community (mean number of species ranged between 3 and 9) within a microcosm environment for some 168 days. In fact, there are strong similarities between this and the present study. Not least, the fact that Hockin (1981) too found that ectinosomatids and interstitial species such as *Paraleptastacus espinulatus* did not persist in microcosms.

Most microcosm studies have suffered from an inability to maintain or exceed starting densities of the majority of the captive taxa (Chandler *et al.*, 1997). One cannot, therefore, escape the fact that there must be additional factors impinging on the established microcosm community, such that an element of caution must be accepted when making direct comparisons between field and microcosm results.

One of the most likely limiting factors is the availability and maintenance of meiofaunal food organisms and ontogenic- and species-specific survival may be directly attributable to the suitability and availability of meiofaunal food organisms.

Both Austen *et al* (1994) and Schratzberger and Warwick (1998) noted a decline in microcosm-maintained nematode abundance and commented that this was probably due to a reduction in food availability since there was no nutrient input from external sources. Austen (1989), by controlling light levels, was able to selectively reduce the availability of diatoms and concluded that copepods are less flexible in their nutritional requirements and it is this that causes the microcosm community to become dominated by a few species. Nematodes appear to possess greater flexibility and hence are less prone to dominance.

To counter this effect, many laboratory experiments have included the addition of specific food sources such as yeast suspensions, diatoms, bacteria, detritus and decaying algae. Hockin (1981) attributed the successful maintenance of a relatively diverse

microcosm community to the provision of a range of food items, such as microbial nutrients to encourage bacterial growth and a light source for epipsammic algae.

Other feeding-related events may play an important role in the reduction of selective species. Bacterial slimes have been implicated as a factor in the failure of some copepod cultures (Hardy, 1978), where they drastically reduce the ability to move or feed. Antibiotics have been employed to combat this problem, but Hardy (1978) comments that this is only suitable for feeding regimes that use only algal sources and the removal of natural bacterial flora may give rise to unnatural conditions. Moreover, some common sand-dwelling species are known to feed directly on sand-encrusting bacteria rendering the use of antibiotics undesirable.

The change in the environmental physical regime may also modify and restrict the ability of some species to feed in a controlled environment. Decho (1988) demonstrated that some species exhibit a feeding activity regime that is stimulated or controlled by tidal cycling, with an optimum at a fixed tidal level. Austen (1989), suggests that the conditions in a microcosm may simulate a permanently low tide, and thus the feeding stimulus is disrupted or food items are not available at a particular sediment horizon.

Other important factors may include the subtle modifications of field sediments during the transfer and re-establishment of benthic structure. Austen and Somerfield (1997) suggests that homogenisation may serve to disrupt natural sediment structures such as biogenic features and small-scale chemical gradients which might define natural micro-scale distributions. However, Hansen *et al* (1996) claimed that biochemical gradients and complex microhabitats associated with geochemical and biological processes were quick to form in their flow-through microcosms.

Salinity appeared to be a major community structuring factor in the microcosm communities maintained by Austen (1989), and this may have been a reflection of the tolerances of the diatom and bacterial food organisms. Salinity-linked changes in nematode feeding morphology appeared to support this view.

The problems of microcosm conditions aside, Austen *et al* (1994) argued a strong case for the use of captive meiofaunal communities in the determination of the effects of localised pollution on soft sediments. They suggested that a laboratory bioassay incorporating a complex and fully interactive community obtained from the location under examination could be used to predict the nature and scale of a disturbance effect. Austen and Somerfield (1997) examined this possibility by exposing a nematode community obtained from a relatively clean site to sediments which had undergone extensive and prolonged metal contamination from local mining activity. The resulting changes in the microcosm communities appeared to be correlated to the levels of metals in the test sediments, and tended to reflect the communities that existed at the sites from which the contaminated sediments were originally obtained. Thus the primary goal of a captive community assay, that of providing an accurate predictive tool seems to have been broadly satisfied.

In conclusion, one has to accept that the use of microcosm systems does not provide a straightforward answer to all of the complexities of community impact assessment. There are still many problems associated with the unknown interactions that define a meiobenthic community. However, the range of results from both this and other microcosm studies are encouraging, and although we cannot yet claim to be able to recreate a functional replica of a marine ecosystem in a laboratory, there are good grounds for accepting that the results obtained so far are a good, but incomplete, representation of the field situati

Reference	Sediment Type	Taxa	Metal	Metal Concentrations and Details[a]
Sundelin, 1983	"Fine sediment"	Single amphipod species (*Pontoporeia affinis)*	Cadmium	<0.2, 6.3-6.5, 41-46, 127 $\mu g.l^{-1}$ Effects on mating, fertilisation, sexual maturation and reproductive success.
Sundelin and Elmgren, 1991	Mud	Foraminifera, Turbellaria, Kinorhyncha, Nematoda, Oligochaeta, Copepoda, Ostracoda, Polychaeta, Mollusca	Cadmium	0, 6, 41-46, 125 $\mu g.l^{-1}$. Combined with the effect of predation of the amphipod *Pontoporeia affinis*.
Austen *et al*, 1994	Mud and sand	Nematoda	Cadmium Copper Zinc	Mud: 0.16, 0.9, 1.19, 2.89. Sand: 0.02, 0.54, 0.9, 1.4. Mud: 245.8, 749.32, 1427.02, 1631.04. Sand: 2.51, 491.09, 896.77, 1445.07. Mud: 427.8, 917.04, 1399.58, 1788.56. Sand 12.56, 593.44, 1149.49, 1315.14.
Hansen *et al*, 1996	Mud	Macrobenthos	Cadmium	0-7, 8-10, 28-157, 28 000-174 $\mu g.l^{-1}$[b] Effect on recolonisation of defaunated sediment.
Austen and McEvoy, 1997a	Mud, sand and muddy sand	Nematoda	Tributyltin	Mud: .0.4, 0.27, 0.48, 0.95 Sand: Values not determined Muddy sand: 0.01, 0.19, 0.53, 0.92
Austen and McEvoy, 1997b	Mud	Nematoda	Cadmium Copper Lead Zinc	2.524 55, 794, 1270, 1480 56, 247, 1343, 1580 156, 757, 1376, 1530
Austen and Somerfield, 1997	Mud	Nematoda	Cadmium Copper Lead Tributyltin Zinc	0.015, 0.261, 0.817, 0.874, 1.325, 3.029 239.0, 299.5, 388.2, 679.6, 1158.1, 2523.6 118.3, 181.3, 122.7, 154.3, 188.2, 240.9 0.024, 0.038, 0.047, 0.075, 0.097, 0.148 387.2, 390.0, 648.2, 952.9, 1384.2, 4462.3
Hall and Frid, 1997	Mud (?)	Macrobenthos	Copper	111.33, 411.13 Simulated contamination and recovery cycle.

[a]Concentrations are $\mu g.g^{-1}$ dry weight sediment unless otherwise stated
[b]Determined as concentration in interstitial pore water.

Table 2.1 Summary of published microcosm studies into the effects of sediment metal contamination.

	Replicate 1		Replicate 2		Replicate 3	
	ϕ	mm	ϕ	mm	ϕ	mm
Median Particle Diameter (Md_{ϕ})	2.37	0.193	2.36	0.195	2.37	0.193
Sorting Coefficient (QD_{ϕ})	0.315		0.315		0.320	
Quartile Skewness (Sk_{ϕ})	+0.005		+0.005		+0.010	

Table 2.2 Summary of results of particle size analyses for sediment from Seacliff sampling.

Copper Level	Sediment Copper Concentration ($\mu g.g^{-1}$)	
	Nominal	Actual
Control	0	2.9
Low	200	188.2
Medium	800	1103.6
High	1600	1977.4

Table 2.3. Nominal and measured mean sediment copper levels in the high copper range microcosm experiment.

Cu	Time								
	1 Dav			6 Davs			18 Davs		
	Control	Low	Medium	Control	Low	Medium	Control	Low	Medium
Control									
Low							✓		
Medium	✓						✓		
High							✓		

Table 2.4 Results of between-copper level one-way ANOVA (Tukey multiple comparison test) for each sampling time: total copepod abundance. ✓ denotes significant difference ($p<0.05$).

Time	Time							
	Control		Low		Medium		High	
	1 d	6 d	1 d	6 d	1 d	6 d	1 d	6 d
1 d								
6 d								
18 d			✓		✓	✓	✓	

Table 2.5 Results of between-time one-way ANOVA (Tukey multiple comparison test) for each copper concentration: total copepod abundance. ✓ denotes significant difference ($p<0.05$).

	Variable	p
Total Copepod Community	Time	0.000
	Concentration	0.000
	Interaction	0.012
Rhizothrix minuta only	Time	0.093
	Concentration	0.000
	Interaction	0.118
Helectinosoma herdmani only	Time	0.017
	Concentration	0.000
	Interaction	0.074

Table 2.6 Results of two-way ANOVA applied to total copepod, *Rhizothrix minuta* and *Halectinosoma herdmani* abundance.

	Time							
	Control		Low		Medium		High	
Time	1 d	6 d	1 d	6 d	1 d	6 d	1 d	6 d
1 d								
6 d			✓					
18 d			✓			✓		

Table 2.7 Results of between-time one-way ANOVA (Tukey multiple comparison test) for each copper concentration: *Rhizothrix minuta* only. ✓ denotes significant difference ($p<0.05$).

	Time								
	1 Day			6 Days			18 Days		
Cu	Control	Low	Medium	Control	Low	Medium	Control	Low	Medium
Control									
Low							✓		
Medium							✓		
High							✓		

Table 2.8 Results of between-copper level one-way ANOVA (Tukey multiple comparison test) for each sampling time: *Rhizothrix minuta* only. ✓ denotes significant difference ($p<0.05$).

	Time								
	1 Day			6 Days			18 Days		
Cu	Control	Low	Medium	Control	Low	Medium	Control	Low	Medium
Control									
Low							✓		
Medium	✓	✓							
High	✓						✓		

Table 2.9 Results of between-copper level one-way ANOVA (Tukey multiple comparison test) for each sampling time after removal of *R. minuta*. ✓ denotes significant difference ($p<0.05$).

	Time							
	Control		Low		Medium		High	
Time	1 d	6 d	1 d	6 d	1 d	6 d	1 d	6 d
1 d								
6 d								
18 d	✓		✓	✓			✓	

Table 2.10 Results of between-time one-way ANOVA (Tukey multiple comparison test) for each copper concentration after removal of *Rhizothrix minuta*. ✓ denotes significant difference ($p<0.05$).

Time	Time							
	Control		Low		Medium		High	
	1 d	6 d	1 d	6 d	1 d	6 d	1 d	6 d
1 d								
6 d								
18 d			✓	✓				

Table 2.11 Results of between-time one-way ANOVA (Tukey multiple comparison test) for each copper concentration: *Halectinosoma hermani* only. ✓ denotes significant difference ($p<0.05$).

Cu	Time								
	1 Day			6 Days			18 Days		
	Control	Low	Medium	Control	Low	Medium	Control	Low	Medium
Control									
Low									
Medium	✓	✓		✓					
High	✓	✓		✓					

Table 2.12 Results of between-copper level one-way ANOVA (Tukey multiple comparison test) for each sampling time: *Halectinosoma herdmani* only. ✓ denotes significant difference ($p<0.05$).

Cu	Time								
	1 Day			6 Days			18 Days		
	Control	Low	Medium	Control	Low	Medium	Control	Low	Medium
Control									
Low				✓			✓		
Medium				✓			✓		
High	✓			✓	✓		✓		

Table 2.13 Results of between-copper level one-way ANOVA (Tukey multiple comparison test) for each sampling time: Nematode community. ✓ denotes significant difference ($p<0.05$).

	Variable	p
Live Nematodes Only	Time	0.000
	Concentration	0.000
	Interaction	0.002
Combined Live and Dead	Time	0.000
	Concentration	0.000
	Interaction	0.022
Dead Nematodes Only	Time	0.090
	Concentration	0.000
	Interaction	0.092

Table 2.14 Results of two-way ANOVA applied to nematode abundance.

	Time								
	1 Day			6 Days			18 Days		
Cu	Control	Low	Medium	Control	Low	Medium	Control	Low	Medium
Control									
Low							✓		
Medium				✓			✓		
High				✓			✓		

Table 2.15 Results of between-copper level one-way ANOVA (Tukey multiple comparison test) for each sampling time: total enumerated nematodes (live + dead). ✓ denotes significant difference ($p<0.05$).

	Time							
	Control		Low		Medium		High	
Time	1 d	6 d	1 d	6 d	1 d	6 d	1 d	6 d
1 d								
6 d								
18 d	✓	✓						

Table 2.16 Results of between-time one-way ANOVA (Tukey multiple comparison test) for each copper concentration: total enumerated nematodes (live + dead). ✓ denotes significant difference ($p<0.05$).

Copper Level	Sediment Copper Concentration ($\mu g.g^{-1}$)	
	Nominal	**Actual**
Control	0	3.8
Low	50	56.8
Medium	100	123.8
High	200	214.9

Table 2.17 Intended and measured sediment copper levels in the second microcosm (low copper range) experiment.

	Time							
	Control		Low		Medium		High	
Time	1 d	5 d	1 d	5 d	1 d	5 d	1 d	5 d
1 d								
5 d								
20 d					✓	✓	✓	✓

Table 2.18 Results of between-time one-way ANOVA (Tukey multiple comparison test) for each copper concentration (low copper range): total copepod abundance. ✓ denotes significant difference ($p<0.05$).

	Time								
	1 Day			5 Days			20 Days		
Cu	Control	Low	Medium	Control	Low	Medium	Control	Low	Medium
Control									
Low									
Medium							✓		
High							✓		

Table 2.19 Results of between-copper level one-way ANOVA (Tukey multiple comparison test) for each sampling time (low copper range): total copepod abundance. ✓ denotes significant difference ($p<0.05$).

	Variable	p
Total Copepod Community	Time	0.000
	Concentration	0.000
	Interaction	0.025
Rhizothrix minuta only	Time	0.000
	Concentration	0.011
	Interaction	0.006
Helectinosoma herdmani only	Time	0.000
	Concentration	0.052
	Interaction	0.639

Table 2.20 Results of two-way ANOVA applied to total copepod, *Rhizothrix minuta* and *Halectinosoma herdmani* abundance: low copper range exposure.

	Time							
	Control		Low		Medium		High	
Time	1 d	5 d	1 d	5 d	1 d	5 d	1 d	5 d
1 d								
5 d								
20 d					✓	✓	✓	✓

Table 2.21 Results of between-time one-way ANOVA (Tukey multiple comparison test) for each copper concentration: *Rhizothrix minuta*. ✓ denotes significant difference ($p<0.05$).

	Time								
	1 Day			5 Days			20 Days		
Cu	Control	Low	Medium	Control	Low	Medium	Control	Low	Medium
Control									
Low									
Medium							✓		
High		✓					✓		

Table 2.22 Results of between-copper level one-way ANOVA (Tukey multiple comparison test) for each sampling time: *Rhizothrix minuta*. ✓ denotes significant difference ($p<0.05$).

	Time							
	Control		Low		Medium		High	
Time	1 d	5 d	1 d	5 d	1 d	5 d	1 d	5 d
1 d								
5 d			✓					
20 d	✓		✓		✓		✓	✓

Table 2.23 Results of between-time one-way ANOVA (Tukey multiple comparison test) for each copper concentration: *Halectinosoma herdmani*. ✓ denotes significant difference ($p<0.05$).

	Variable	p
No. of Species	Time	0.000
	Concentration	0.355
	Interaction	0.168
Species Richness	Time	0.001
	Concentration	0.167
	Interaction	0.435
Shannon Index	Time	0.000
	Concentration	0.002
	Interaction	0.142
Evenness	Time	0.012
	Concentration	0.001
	Interaction	0.013
Simpson Index	Time	0.000
	Concentration	0.002
	Interaction	0.085

Table 2.24 Results of two-way ANOVA applied to microcosm copepod diversity: low copper range exposure.

Time	Time							
	Control		Low		Medium		High	
	1 d	5 d	1 d	5 d	1 d	5 d	1 d	5 d
1 d								
5 d					✓		✓	
20 d			✓	✓	✓	✓	✓	✓

Table 2.25 Results of between-time one-way ANOVA (Tukey multiple comparison test) for each copper concentration: nematode response to low-level copper exposure. ✓ denotes significant difference ($p<0.05$).

Cu	Time								
	1 Day			5 Days			20 Days		
	Control	Low	Medium	Control	Low	Medium	Control	Low	Medium
Control									
Low				✓			✓		
Medium				✓			✓		
High				✓			✓		

Table 2.26 Results of between-copper level one-way ANOVA (Tukey multiple comparison test) for each sampling time: nematode response to low-level copper exposure. ✓ denotes significant difference ($p<0.05$).

Variable	p
Time	0.000
Concentration	0.000
Interaction	0.000

Table 2.27 Results of two-way ANOVA applied to nematode abundance: low copper range exposure.

Core 1

		Concentration ($\mu g.g^{-1}$)	Mean Concentration ($\mu g.g^{-1}$)
Top	Section 1	3256.3	
	Section 2	775.5	1551.0
Bottom	Section 3	621.3	

Core 2

		Concentration ($\mu g.g^{-1}$)	Mean Concentration ($\mu g.g^{-1}$)
Top	Section 1	3927.5	
	Section 2	916.1	1780.1
Bottom	Section 3	496.6	

Core 3

		Concentration ($\mu g.g^{-1}$)	Mean Concentration ($\mu g.g^{-1}$)
Top	Section 1	3419.6	
	Section 2	870.8	1635.7
Bottom	Section 3	616.8	

Table 2.28 Copper distribution in the cores extracted from the contaminated microcosm sediment.

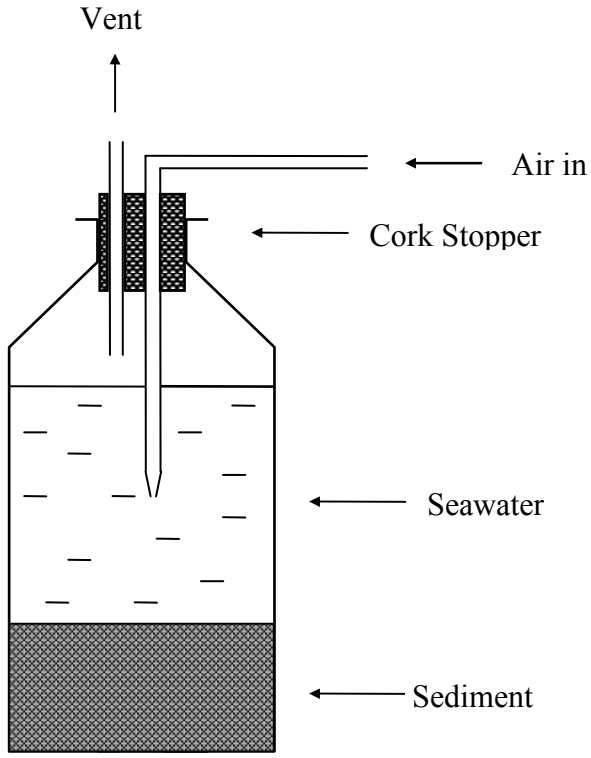


Figure 2.1 Detail of microcosm culture bottle.

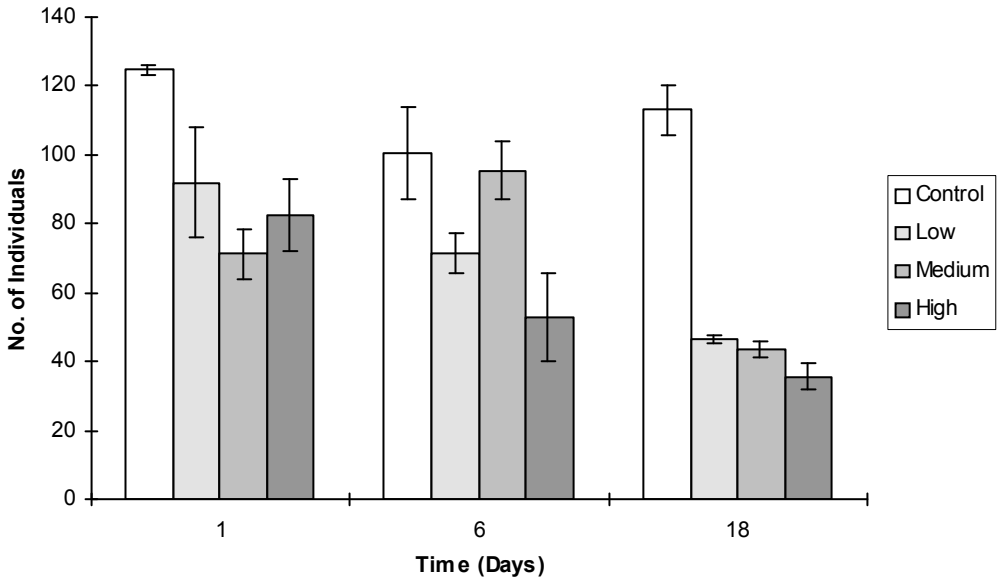


Figure 2.2 Microcosm copepod community response to high experimental copper range. Error bars denote standard error.

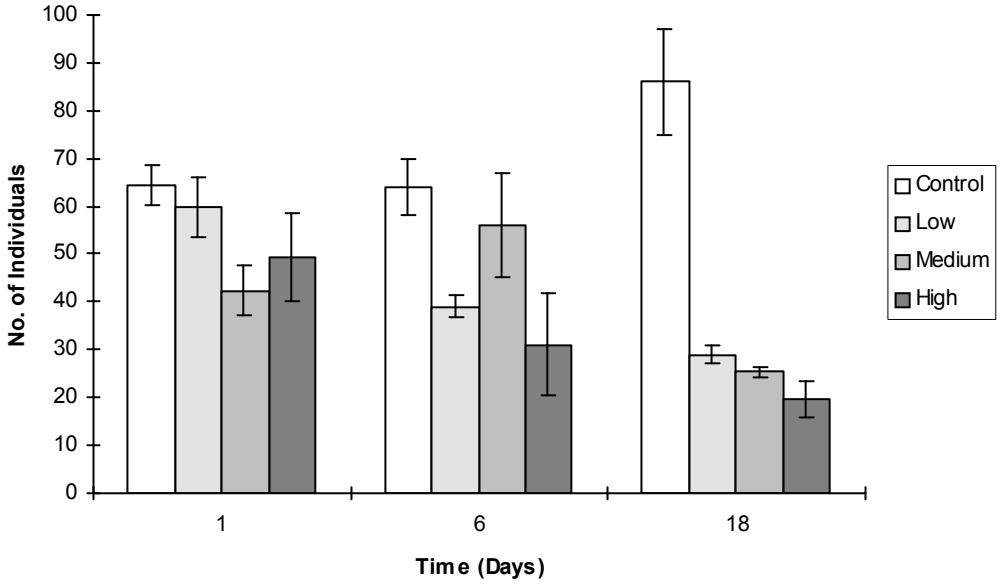


Figure 2.3 *Rhizothrix minuta* response to high experimental copper range. Error bars denote standard error.

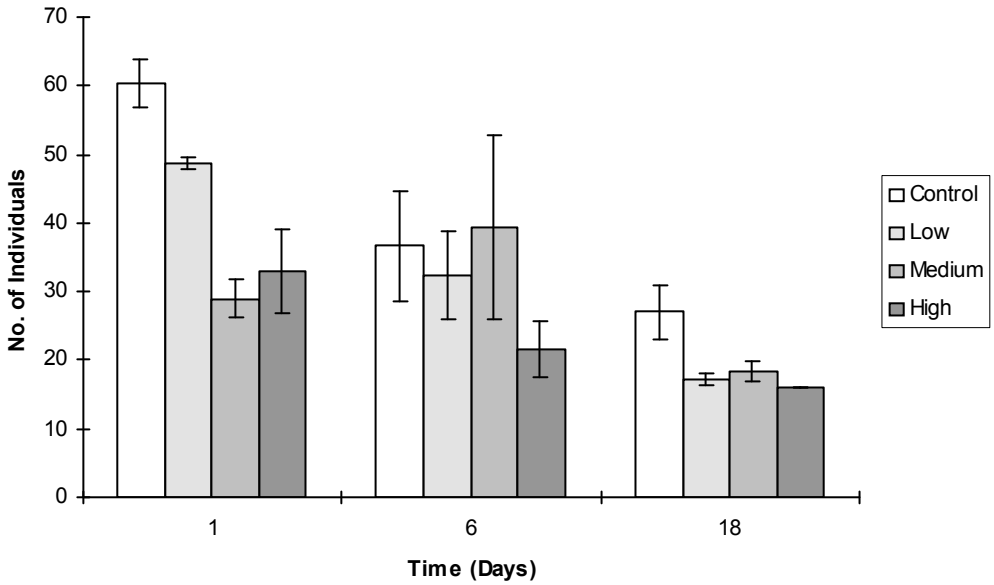


Figure 2.4 Copepod community response to high experimental copper range after removal of *Rhizothrix minuta*. Error bars denote standard error.

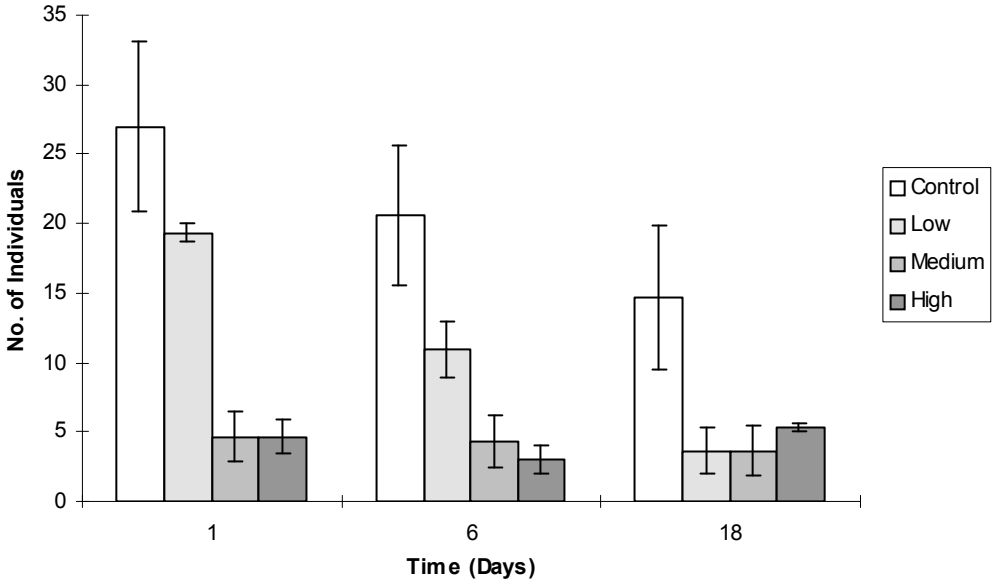


Figure 2.5 *Halectinosoma herdmani* response to the high experimental copper range. Error bars denote standard error.

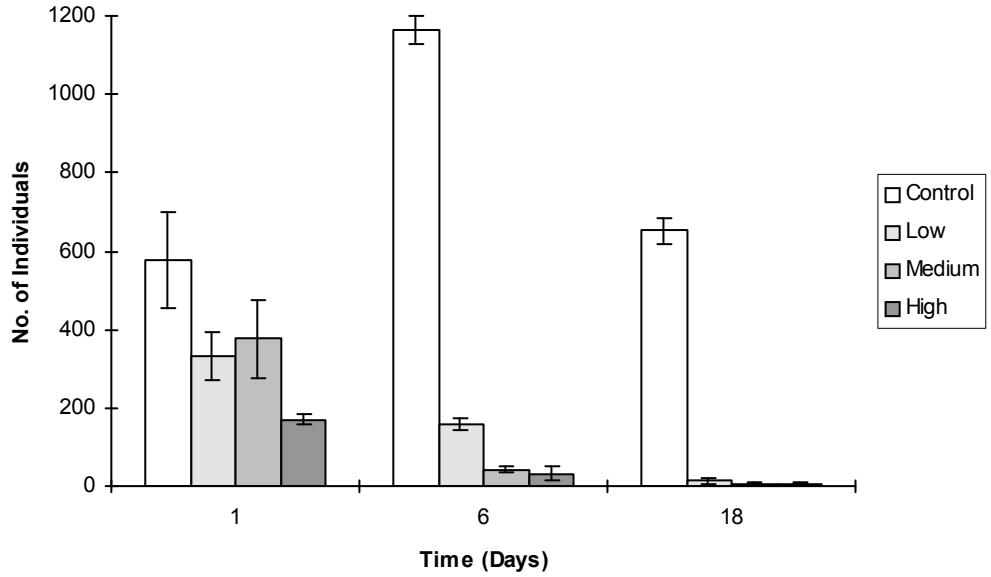


Figure 2.6 Nematode response (live only) to high experimental copper range. Error bars denote standard error

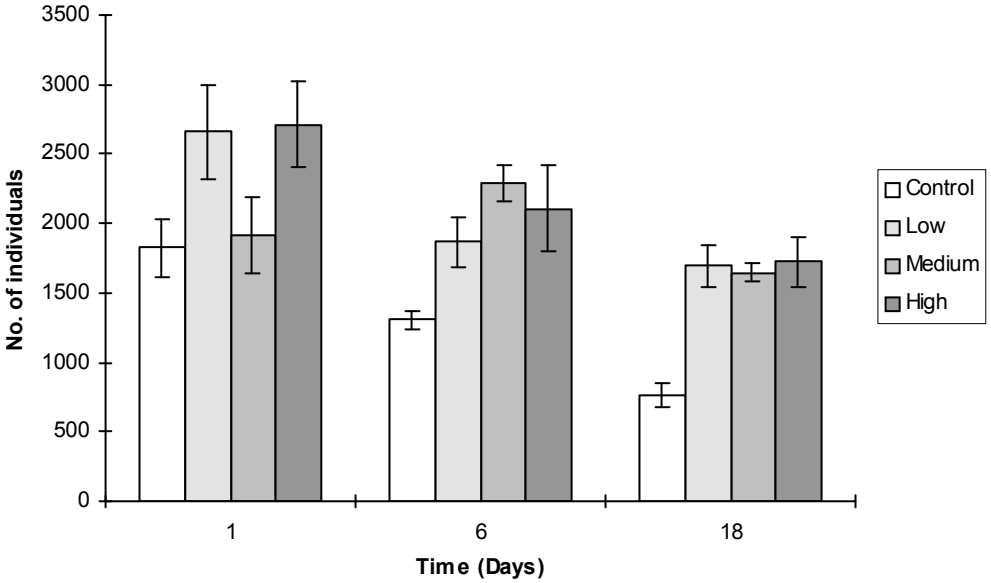


Figure 2.7 Total enumerated nematode (live + dead) response to the high experimental copper range. Error bars denote standard error.

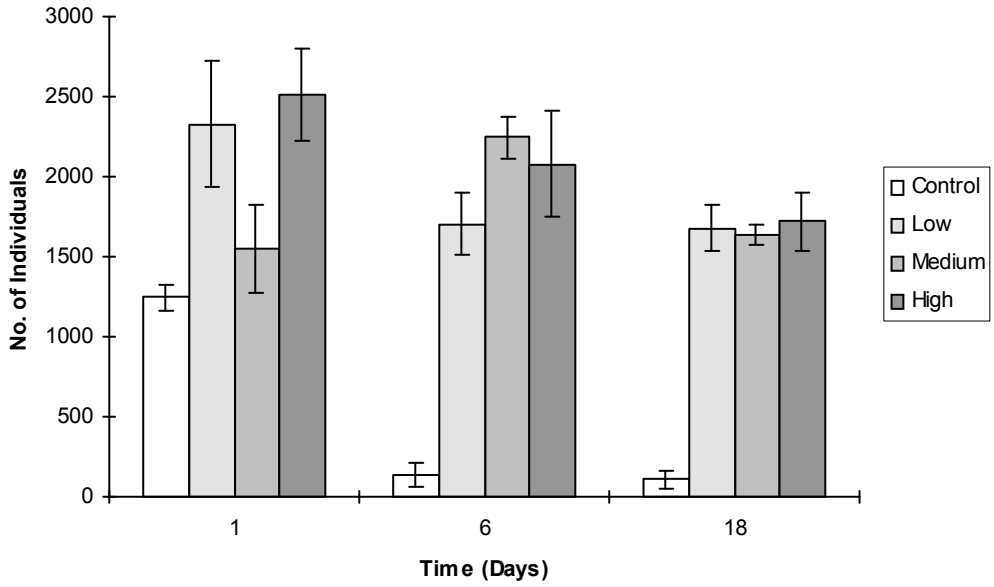


Figure 2.8 Incidence of dead nematodes in the microcosms exposed to the high experimental copper range. Error bars denote standard error.

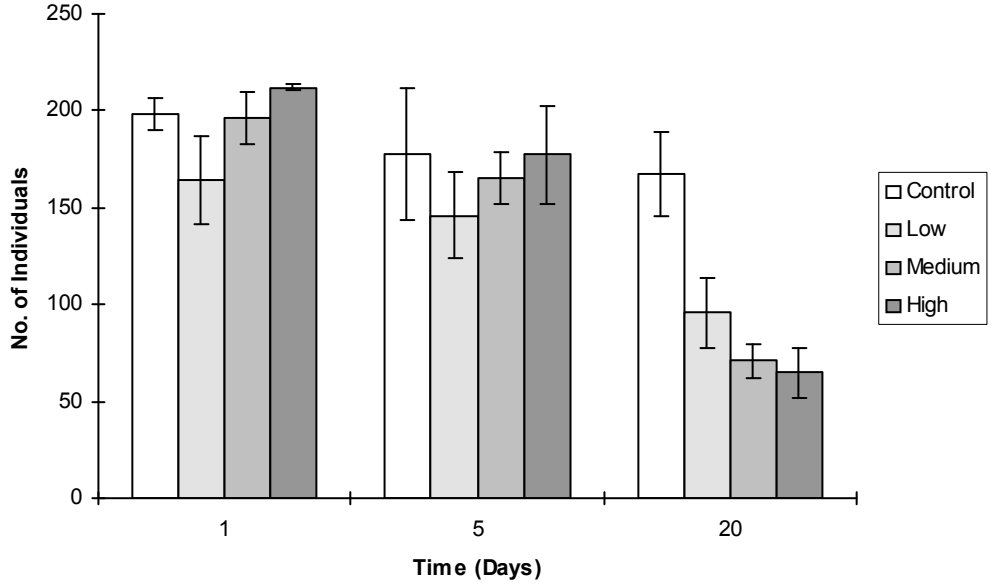


Figure 2.9 Microcosm copepod community response to low experimental copper range. Error bars denote standard error.

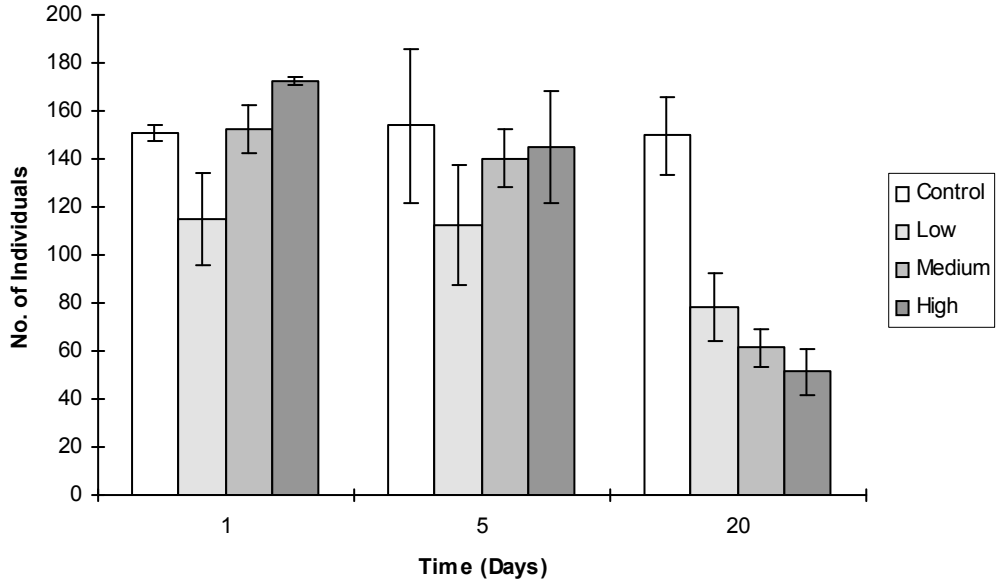


Figure 2.10 *Rhizothrix minuta* response to low experimental copper range. Error bars denote standard error.

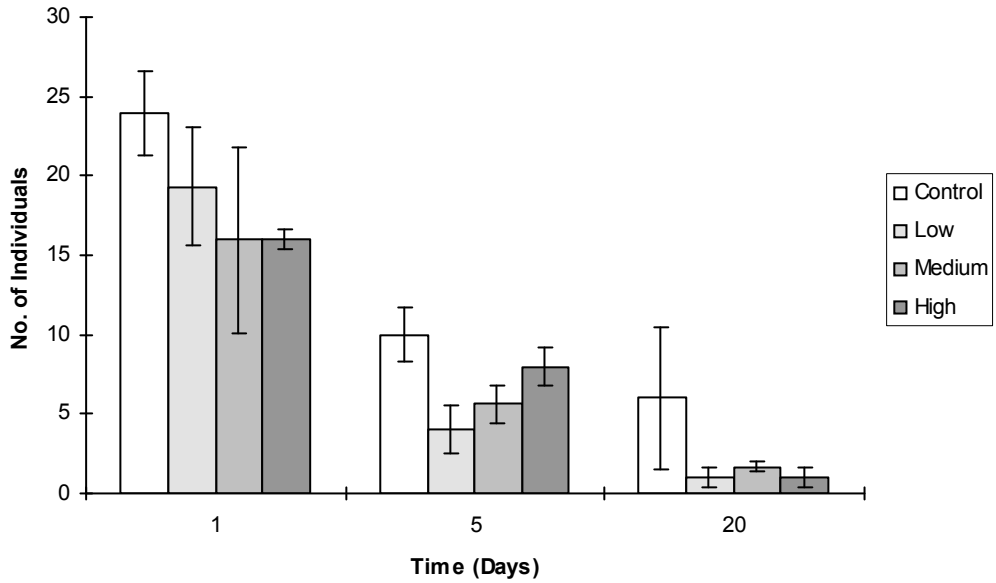


Figure 2.11 *Halectinosoma herdmani* response to low experimental copper range. Error bars denote standard error.

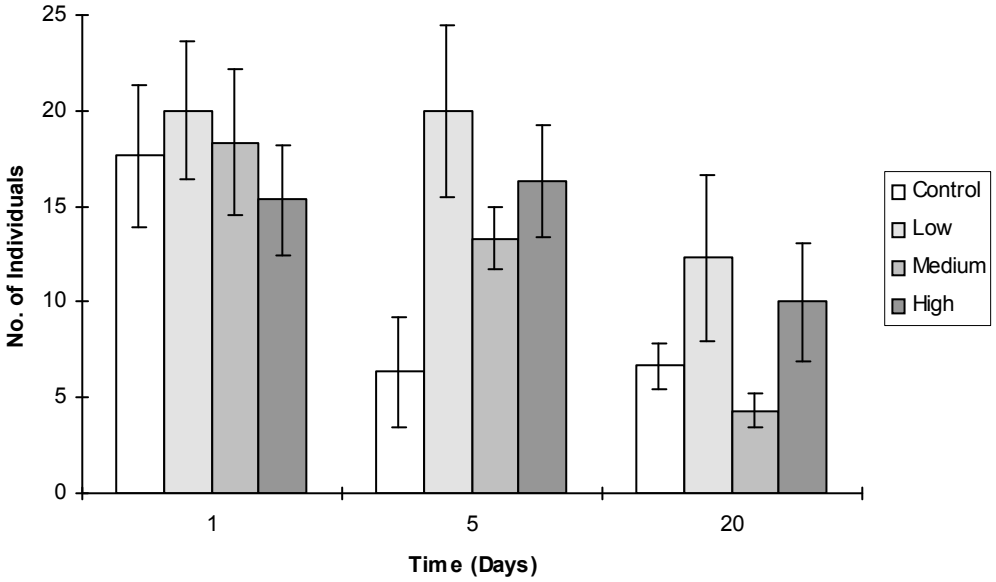


Figure 2.12 Mesobenthic copepod response to low experimental copper range. Error bars denote standard error.

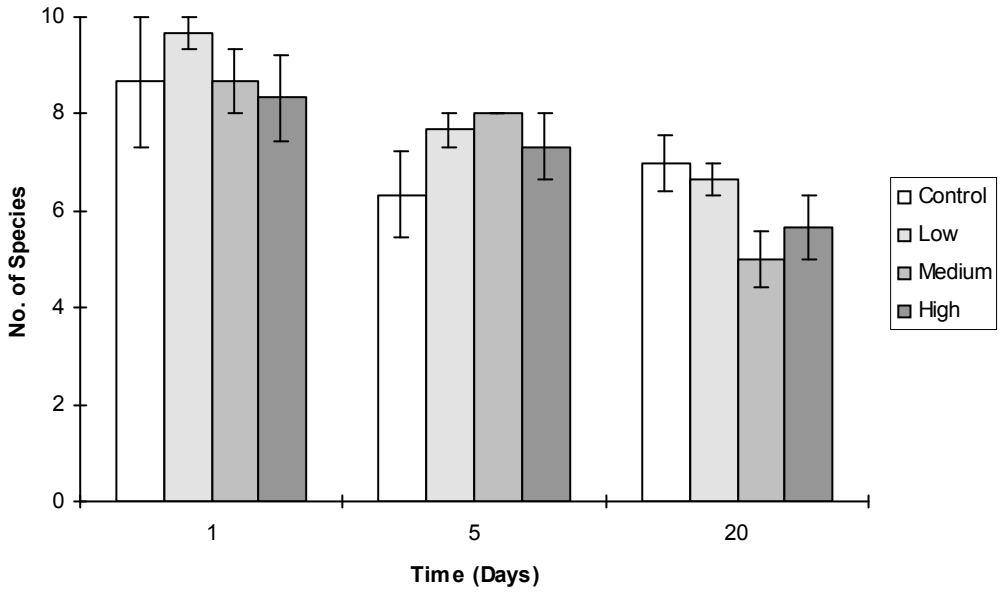


Figure 2.13 Effect of microcosm copper contamination on copepod diversity: number of species. Error bars denote standard error.

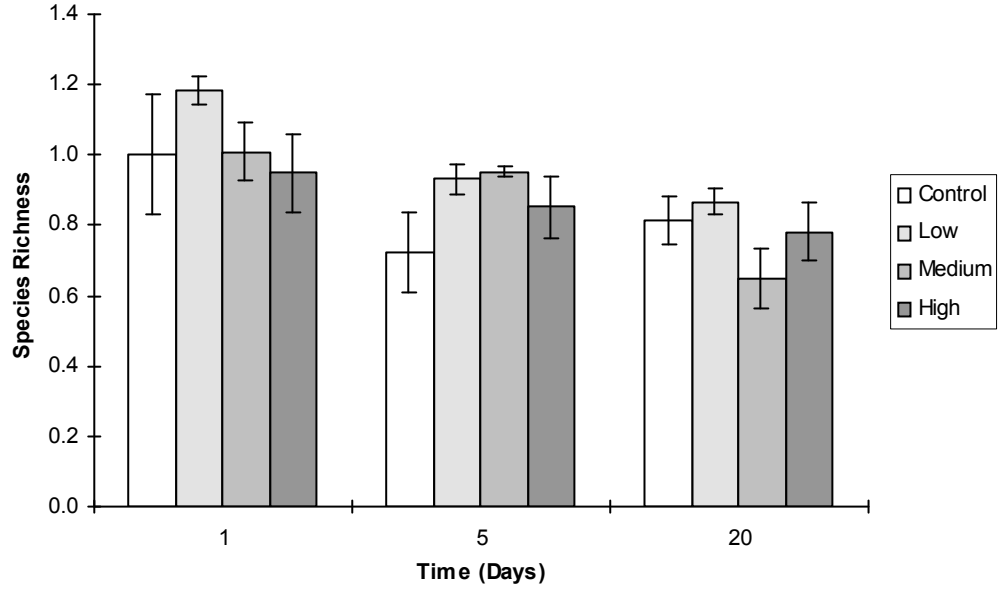


Figure 2.14 Effect of microcosm copper contamination on copepod diversity: Species Richness (Margalef's index). Error bars denote standard error.

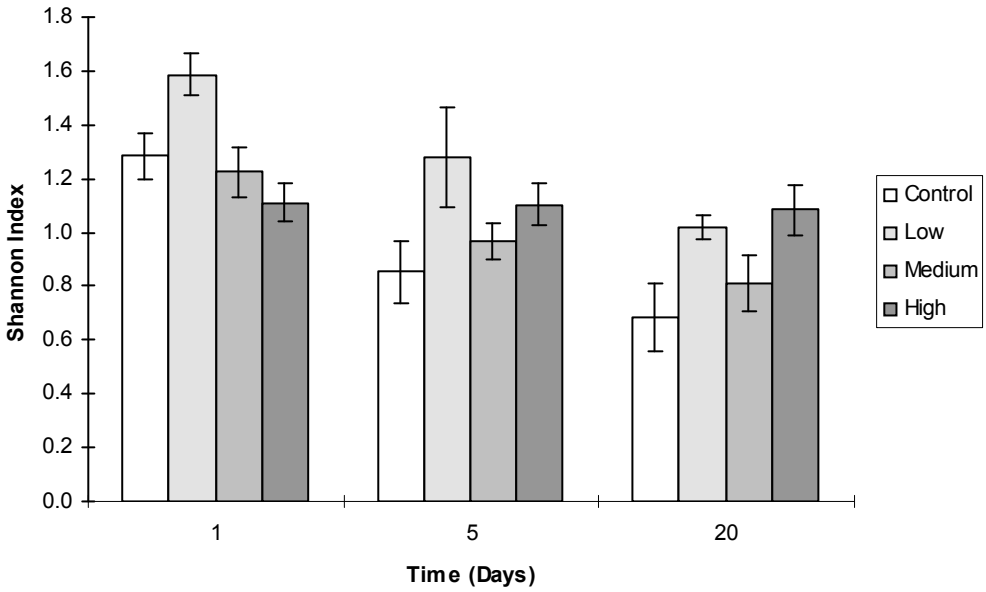


Figure 2.15 Effect of microcosm copper contamination on copepod diversity: Shannon Index. Error bars denote standard error.

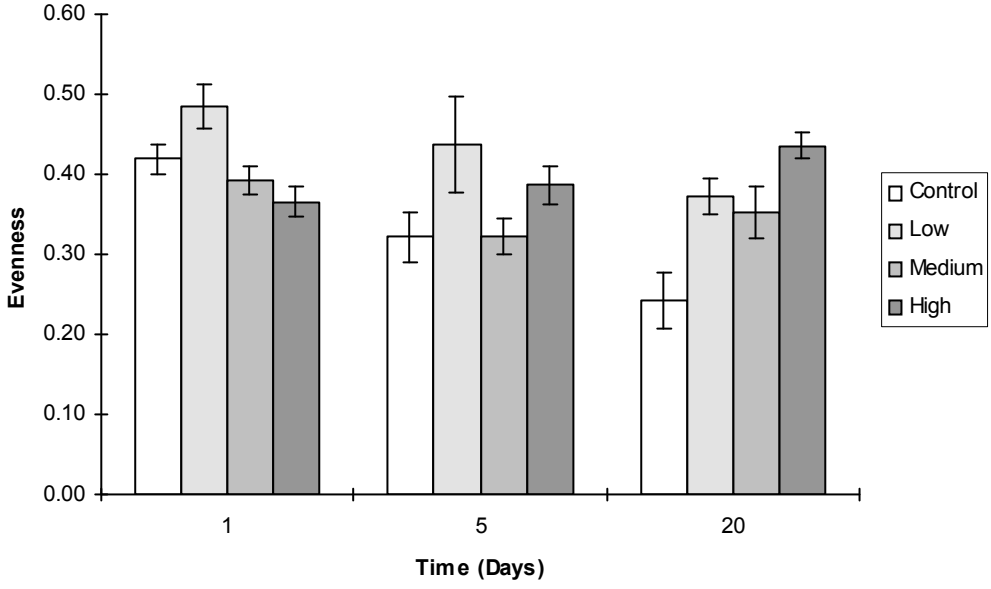


Figure 2.16 Effect of microcosm copper contamination on copepod diversity: Evenness. Error bars denote standard error.

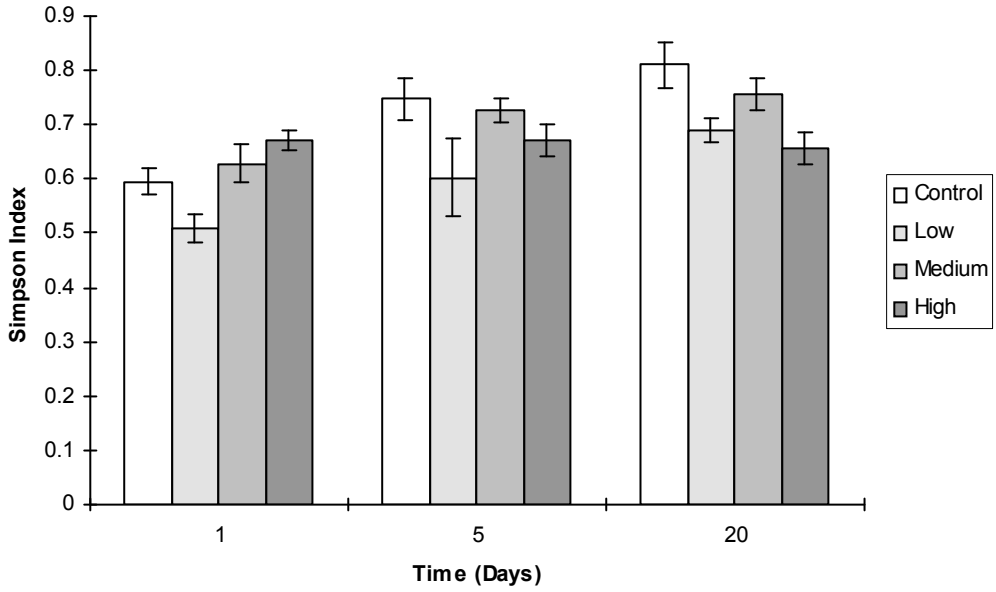


Figure 2.17 Effect of microcosm copper contamination on copepod diversity: Simpson Index. Error bars denote standard error.

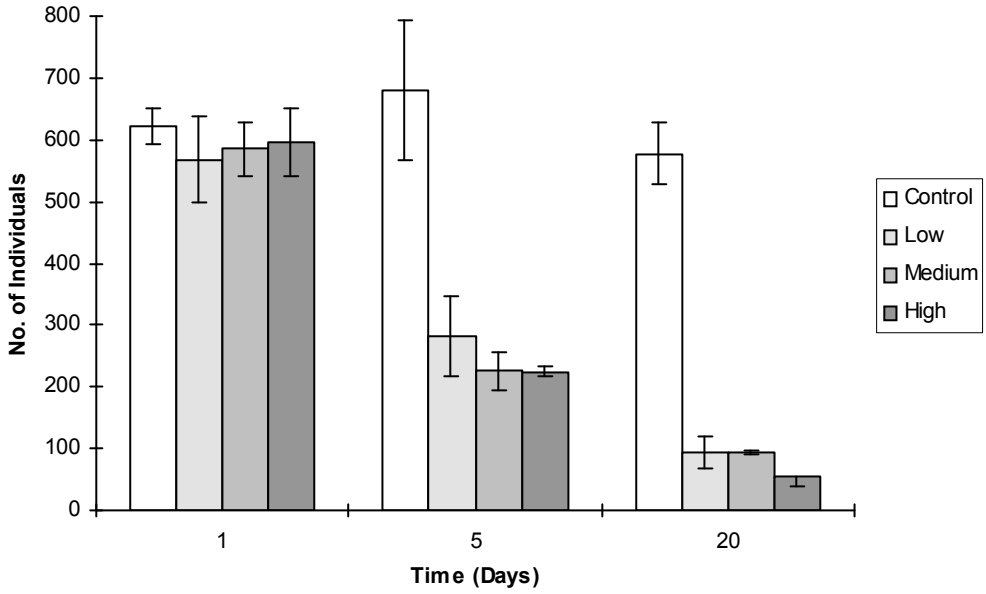


Figure 2.18 Nematode community response to low experimental copper range. Error bars denote standard error.

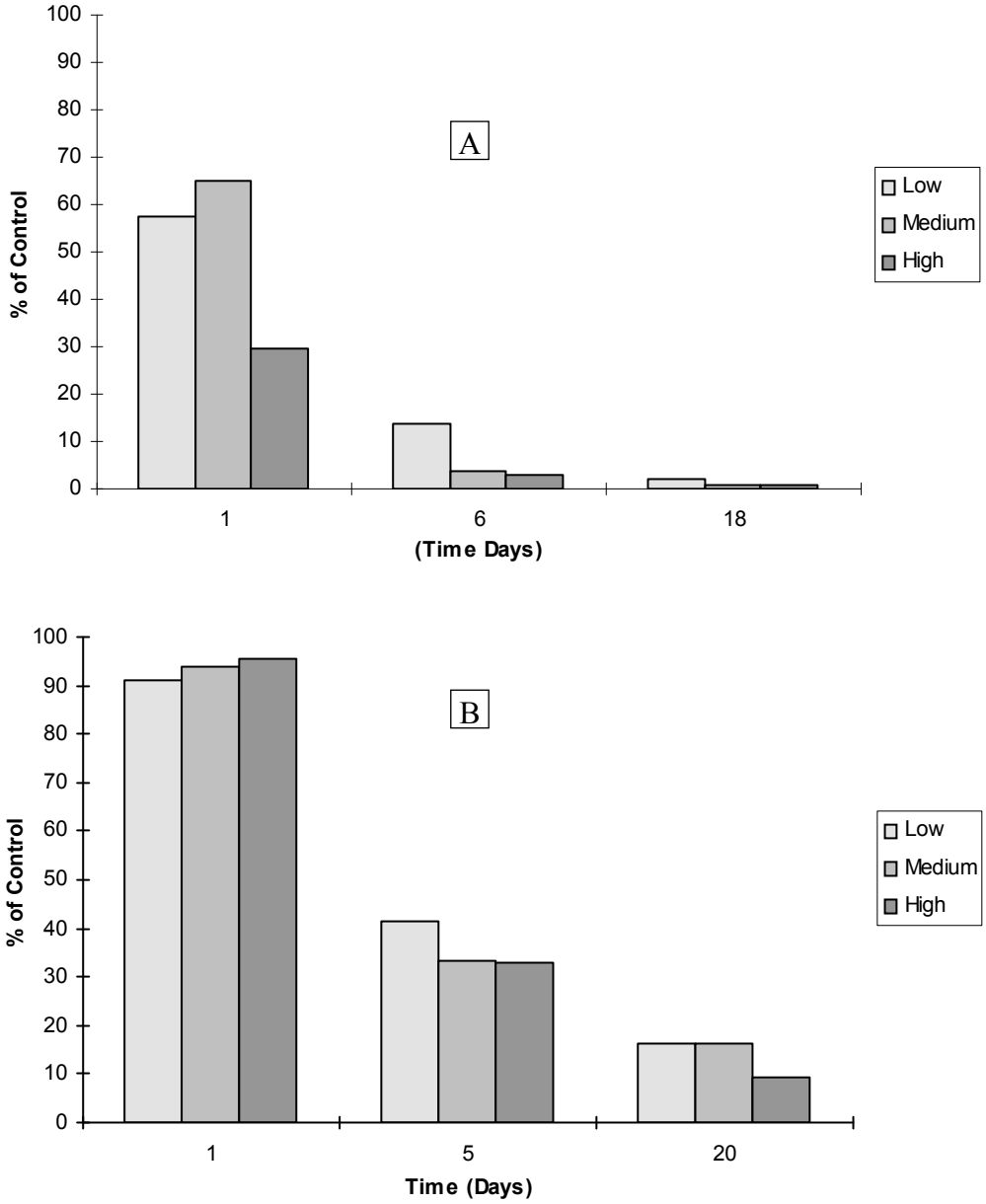


Figure 2.19 Nematode community response to microcosm copper contamination represented as a percentage of the control abundance. A: high copper range, B low copper range.

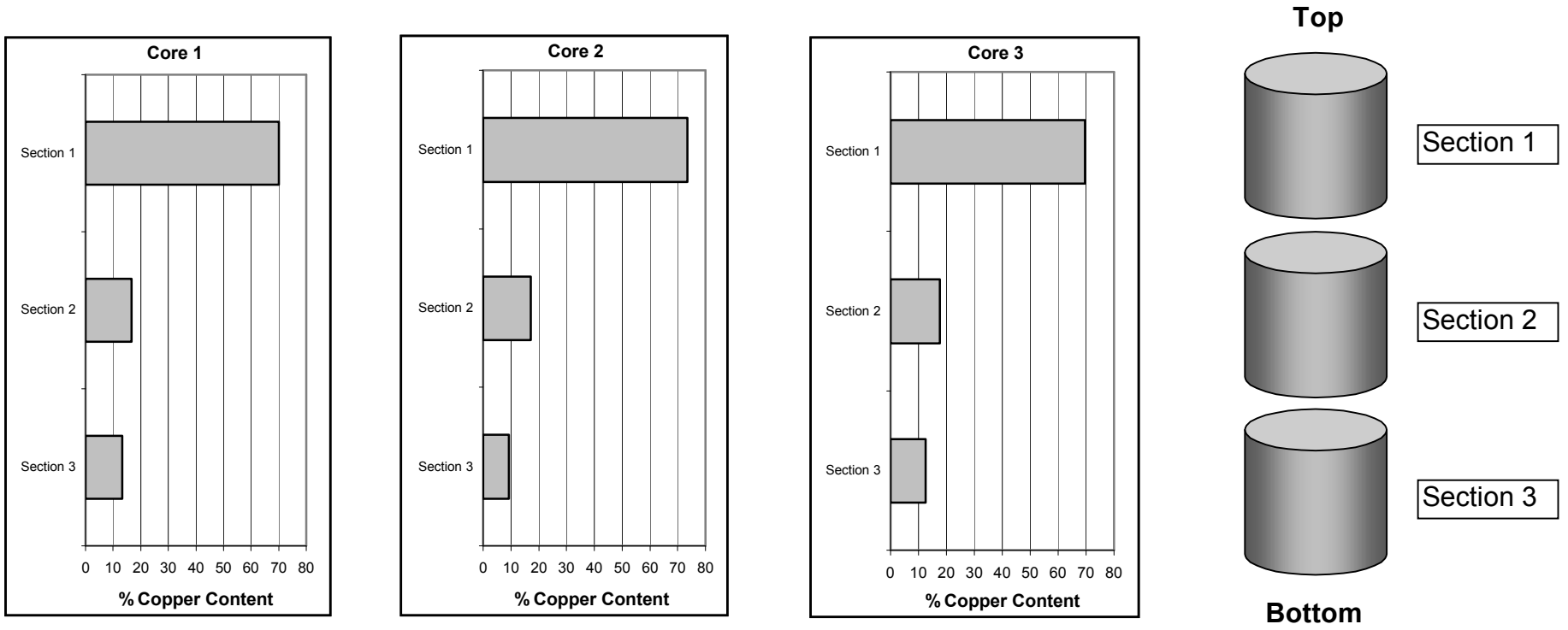


Figure 2.20 Distribution of copper contaminant within microcosm sediments.

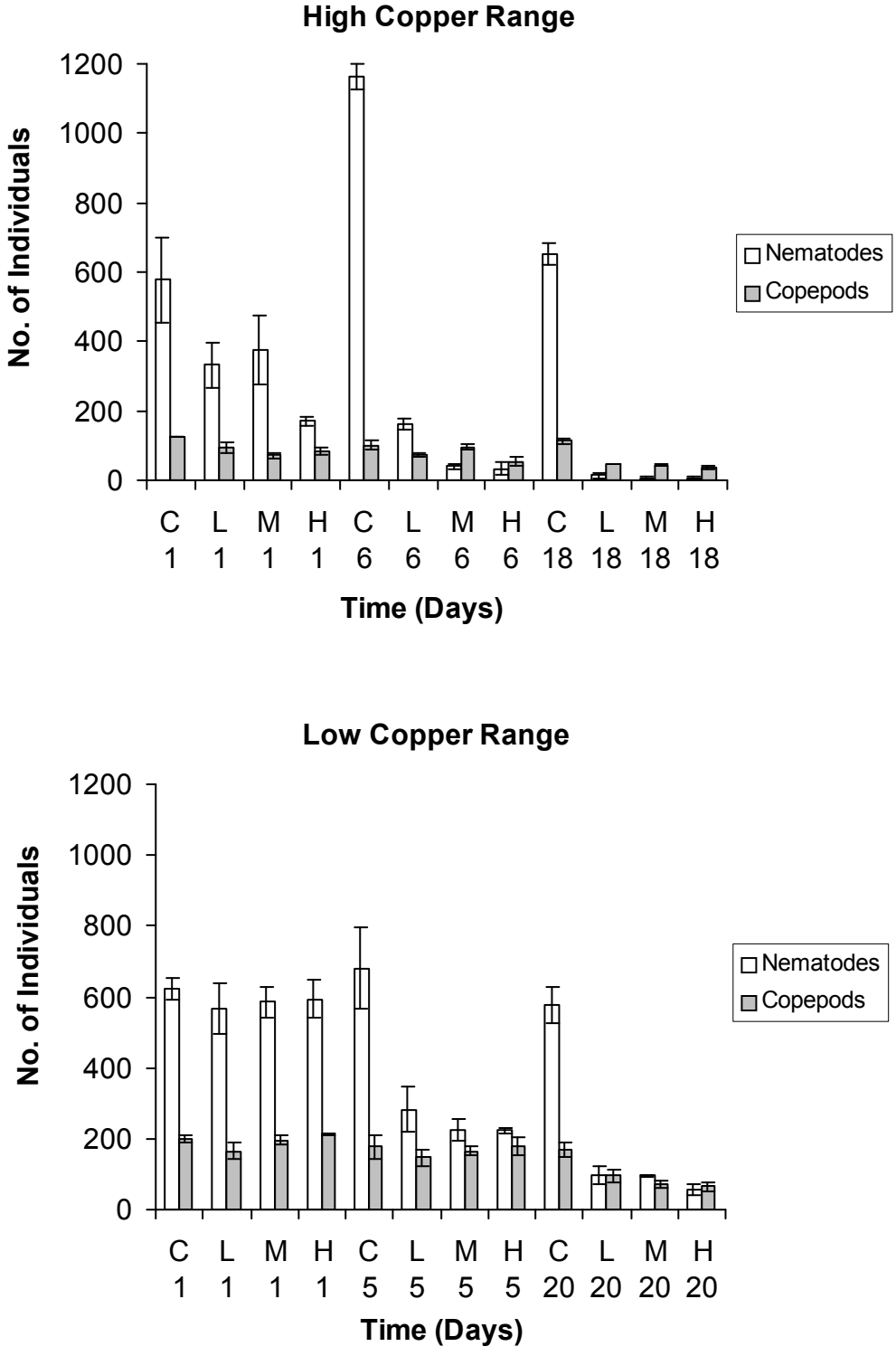


Figure 2.21 Summary of nematode and copepod abundance in each treatment microcosm. C: control, L: low, M: medium, H: high. Error bars denote standard error.

CHAPTER 3

FIELD STUDY 1: THE EFFECT OF A COPPER-BASED ANTIFOULING PAINT ON A MEIOBENTHIC COPEPOD COMMUNITY

3.1. INTRODUCTION

The use of metals in the control or prevention of marine fouling has its origins in the days of wooden sailing vessels. Copper was the first documented metal to be used in this way, when it underwent trials with HMS *Alarm* in the mid-1700's as an attempt to prevent hull damage by shipworm (Swain *et al.*, 1982). The trials were judged a great success since not only was the incidence of shipworm much reduced but the build-up of attached marine communities was also greatly slowed, giving these vessels the advantages of increased speed and manoeuvrability. For wooden-hulled ships the copper was simply applied as sheathing, but the introduction of iron-hulled vessels saw the discontinuation of this method due to the damaging galvanic interaction between the two metals.

Metal preparations in paint form were subsequently developed to circumvent this problem, and they remain the most popular form of fouling prevention to this day. However, much in the way of refinement has taken place since their introduction. Commercial preparations incorporating a variety of metals as their base have been available in the past but most of these, such as those containing arsenic, cadmium, lead and mercury, have been restricted for environmental reasons.

One of these, Tributyltin (TBT), was first introduced as an antifoulant in the 1960's and has remained the most commonly used antifouling biocide. Its effectiveness on all forms of fouling organism is beyond dispute, but its mode of action is entirely dependent on its high level of toxicity, prompting Goldberg (1992) to comment that it is "…perhaps the most toxic substance deliberately introduced into the environment by man." It was found to degrade quickly in laboratory tests and was therefore expected to present minimal environmental problems. However, its widespread and continued use, combined with the distinctive chemical and physical conditions within estuaries, contributed to a high level of unexpected persistence, and its effects at remarkably low concentrations started to become apparent.

The problem was first recognised in the late 1970's in France, where TBT leaching from boats berthed at marinas adjacent to oyster beds was implicated in the shell malformation and lack of spatfall of the Pacific oyster (*Crassostrea gigas*). Later, it was found to be a serious problem in gastropods, particularly the dogwhelk *Nucella lapillus*, where reproductive failure due to imposex, the imposition of male sexual characteristics on females, was found (Gibbs *et al.*, 1991).

The implications of these and other effects prompted a number of countries to impose controls on the use of TBT, and in 1987 the UK imposed a ban on the use of such paints for vessels below 25 m in length (below 12 m for fishing vessels). In addition, the larger commercial shipping is currently restricted to paints of the comparatively expensive self-polishing copolymer type, which promote a constant and slow release of the toxicant.

These measures have, of course, significantly reduced the overall TBT input into the marine environment, but its continued use for commercial shipping means that certain areas such as major shipping routes and harbours will continue to receive substantial amounts. This has been estimated to be of the order of some 35 tonnes distributed around the major industrial maritime sites of the UK (Davies *et al.*, 1998).

In contrast, areas of predominantly recreational boating activity now receive very little or no TBT loading, and should be undergoing a process of remediation, although hazardous levels may still exist in some locations (GESAMP, 1990).

The abandonment of TBT for small vessels has meant the re-emergence of copper as a preferred, less toxic alternative. However, although copper does not appear to have the high level of toxicity attributed to TBT, it may still present its own problems when released in a localised way, such as may occur around commercial moorings and marinas.

The current general perception is that copper represents a somewhat benign alternative, perhaps typified by the yachting press where it has been stated that: "…most dissolved

copper from the AF should rapidly become precipitated and end up harmlessly in sediments" (Hill, 1991). In fact, there has been no examination of the impacts of copper-based antifouling treatments on marine ecosystems, and if indeed it were true that copper was rapidly and continuously incorporated into adjacent sediments, then it seems likely that it may eventually accumulate to levels that are harmful to the benthos. Claisse and Alzieu (1993) have detected an increase in copper levels in oysters from the same beds in southern France that first gave rise to concern about TBT. This area, surrounded by marinas and moorings, appears to have undergone a steady rise in oyster body-burden of copper since 1982, when the TBT ban forced a switch to the copper alternative. To date, this is the only study to suggest a potential pollution effect from copper-based paints.

The present study was performed as a pilot investigation into the effects of antifoul-coated structures on adjacent sediment communities. Here, the short-term effects of a copper-based paint on a copepod assemblage was examined using an approach never previously undertaken. A structure representing a surface area of approximately 10 m^2 was completely coated in a commercial antifouling paint and placed on the surface of a fine sediment. The effect of the structure on the meiobenthos was monitored over a three-month period following deployment.

3.2. METHODS

3.2.1. Loch Creran

Loch Creran is a fjordic sea-loch, consisting of two interconnecting basins 12.8 km in combined length, located on the west coast of Scotland. The total high water area of the Loch is 15.1 km^2 (Edwards and Sharples, 1986), of which the main basin constitutes 11.49 km^2 (Gage, 1972). The upper basin (maximum charted depth 36 m) sustains relatively high freshwater input which is reflected by the presence of some brackish water faunal elements, but the lower basin (maximum charted depth 49 m) is considered to be fully saline (Connor, 1990). The physical influences of faunal distribution, in contrast to nearby Loch Etive, where it is largely correlated with temperature and salinity gradients, are probably dependent on a complex combination of wave exposure, sediment type and infrequent fluctuations in salinity caused by freshwater run-off (Gage, 1974).

The Loch as a whole is well protected by the surrounding terrain, and has been catagorised as "very sheltered" or "extremely sheltered" by Connor (1990). Tidal cycles conform to a normal semi-diurnal regime, and the annual water temperatures range between a low of approximately 6 ^oC in February-March, to a high of 13-15 ^oC in August-September (Gage, 1972).

Human activity within, and around, the Loch has led to localised areas of pollution or physical disturbance. Waste effluent created in the industrial extraction of alginate from algae has resulted in gross organic enrichment of a substantial area of the seabed towards the head of the lower basin. The factory itself ceased operating in 1997, but the effects are likely to remain for many years to come.

Well established fish farming activity has similarly resulted in organic enrichment, specifically in those areas where salmon cages are, or have been moored. All of the known sites have, to date, been in the lower basin. Some minor physical disturbance has also been reported as a result of associated anchor and mooring installations (Moore, 1996).

The deployment of trawl nets by small fishing vessels has occasionally been observed, usually at night. This is probably considered as a bad-weather option by the fishermen, with the target species likely to be *Nephrops norvegicus* in the deeper muddy sediments.

Adjacent to the seaweed processing plant lies a commercial yacht mooring. Although there are a few single recreational and work boat moorings scattered around the edge of the Loch, the greater majority of moored vessels are confined to this area.

From a perspective of meiofaunal community ecology Loch Creran is perhaps unique amongst Scottish Lochs, in that a number of studies, largely emanating from Heriot-Watt University, have focused primarily on meiofaunal taxonomic groups, with experiments performed at a range of sites around the Loch. Macrofaunal-meiofaunal interactions were the subject of the studies of Olafsson (1989), Olafsson and Moore (1990, 1992) and Olafsson *et al* (1990), while Harries (1995) investigated the effects of disturbance by demersal fishing gear. As a consequence of these studies, the local meiobenthic community structure (within the confines of inter-site variability) is relatively well-known – an important factor, given the difficulties associated with the taxonomy of these groups.

3.2.2. The Study Site

The major site selection criterion was that the location be sufficiently remote from potentially interfering anthropogenic influences, particularly sources of trace metals, such as jetty structures or large numbers of moored vessels, where metal-based antifouling paints may have been applied. Other requirements included a relatively flat and shallow bottom, for transect station reproducibility, coupled with feasible bottom-time for sample collection by SCUBA, and, less critically, shore access, in the event of boat unavailability.

The site used for the present study was located in an embayment on the southern side of the lower basin (figure 3.1) at 56^{o} 31.397' N, 05^{o} 19.980' W (position established by differential GPS). Although a single mooring was present close to the study area, it

appeared to be for the use of a single small yacht and was very infrequently occupied, and hence judged unlikely to be a significant source of sediment trace metal contamination.

The sediment of the area is composed of sandy mud and is interspersed with large calcareous tube structures and scattered shell debris which may, to some extent, contribute to the visibly rich epibenthic community. All sampling stations were at approximately 9 m below chart datum, which was outside the influence of the low salinity run-off surface layer that may form during extended periods of rainfall.

3.2.3. Core Size and Spatial Variability

Meiobenthic taxa are known to be spatially variable or patchy in their distribution. This 'clumping' may occur at various taxonomic levels and over different scales (Findlay, 1981; 1982), presenting potential problems for manipulative experiments that may rely on observations of subtle changes in (say) species composition. One of a set of core replicates placed within a small area of high meiobenthic density may greatly modify statistical variances, rendering between-site (or between-treatment) comparisons inaccurate or misleading.

In practical terms, sampling strategy is often a compromise between examination of as large an area as possible and the constraints imposed in processing. However, experimental design may preclude sampling over large areas. Examination of the effects of a contaminant emanating from a point source may require sample stations contained within narrow distance boundaries so that points along a gradient may be reliably defined. Moreover, it has been suggested that a large number of small samples may provide a better estimate of densities than a smaller number of larger samples (Elliott, 1977; Fleeger *et al.*, 1988). Rutledge and Fleeger (1988) tested the efficacy of using cores of area 5.3, 25.5 and 86.8 cm^2 and concluded that with sufficient care the smaller corers were no less efficient than the larger.

Meiobenthic samples are commonly taken using perspex tube corers with an internally-defined cross-sectional area of between 10 and 20 cm^2 (Giere, 1993), the lower value

recommended as the minimum by Wells (1971). Accordingly, previous sampling of subtidal meiobenthos in Loch Creran has been achieved with three replicate cores of surface areas 11.34 cm^2 (Olafsson, 1989) or 12.57 cm^2 (Harries, 1995).

For the present study, in which sampling was required within discrete areas adjacent to a structure discharging copper, smaller cores were considered to hold some advantages. Therefore a preliminary field experiment was conducted to compare meiofaunal abundances obtained in cores of surface area 12.57 cm^2 and 2.72 cm^2 (sediment depth 5 cm). Four replicate large cores and twenty small cores were taken in the vicinity of the proposed study site at an approximate depth of 9 m below chart datum on 5th May 1994. Five of each of the small cores were combined to make four randomly pooled replicate samples. Each sample was fixed with formalin and the meiofauna extracted with Ludox (described elsewhere). The number of nematodes and copepods in each sample were determined and the values standardised to number of individuals per 10 cm^2. The results obtained (table 3.1), in general, confirm the observations of Rutledge and Fleeger (1988), with no conspicuous differences between core sizes.

Core Size	No. of nematodes per 10 cm^2	Mean with 95% confidence limits	No. of copepods per 10 cm^2	Mean with 95% confidence limits
12.57 cm^2	1878, 1106, 1572, 1327	1471 ± 528	154, 220, 162, 116	163 ± 68
2.72 cm^2	1686, 1230, 1264, 1943	1531 ± 549	192, 140, 166, 172	168 ± 34

Table 3.1. Effect of core size on nematode and copepod abundances in diver-obtained samples.

Two-sample t-tests were performed on these data, and the null hypothesis – that there is no significant difference in mean abundance between core sizes - could not be rejected for either nematodes ($p = 0.81$) or copepods ($p = 0.85$). The 95% confidence limits for each taxon were somewhat variable, and it appears that for these sets of replicates at least, there is little attenuation of within-replicate variation. However, there is no real

evidence of patchiness, and so the advantages of the "homogenisation" effect of randomly pooling small cores is not evident here. Given that the absence of obvious patchiness in these samples is unlikely to be an indication of future spatial variability, it was decided that the modest increase in time needed to take smaller cores was substantially outweighed by the potential benefits of increased replicate precision.

3.2.4. Construction and Deployment of Experimental Array

An experimental structure consisting of a frame designed to securely hold steel plates in an upright position on the Loch bottom was constructed (figure 3.2). The day before deployment forty steel plates of dimensions 50x25x0.4 cm were completely coated using 2.5 litres of copper-based TRAWLER antifoul paint (International Paints, Southampton). On 14th of June, 1994 the array structure was positioned with lifting-bags such that the longest dimension was at 90^{o} to prevailing currents and a preconstructed station guideline (running south-west from the array; figure 3.3).

3.2.5. Sample Collection

Samples were taken at 0 and 10 m from the array, with both of the stations at a depth of 9 m below chart datum. Twenty 2.72 cm^2 cores (5 cm sediment depth) were taken from each station for fauna samples and subsequently randomly pooled to make five samples as previously described. Each sample was immediately preserved in 4% formalin in filtered seawater. Sampling at each station took place at 1, 10, 30 and 90 days after deployment of the array.

Prior to the deployment of the array, the designated 0 m station was presampled for particle size and metal content analysis, each with a single 12.5 cm^2 core (5 cm sediment depth). These were repeated on each subsequent sampling occasion for both the 0 and 10 m stations.

3.2.6. Sample Processing

The meiofauna was extracted from the sediment by decantation followed by two density separation steps utilising Ludox-TM (Du Pont, Stevenage, UK), a colloidal silica polymer.

Each sample was washed into a 1 litre glass stoppered measuring cylinder and made up to the litre mark with fresh water. The sediment was then fully resuspended by agitation and inversion and then allowed to settle for 30 seconds. The supernatant was poured slowly and carefully through 1mm and 45 μm sieves to remove macrofaunal taxa. The remaining sediment was re-extracted for a further three times. After removing excess water by draining the underside with a paper towel, the fauna and sediment retained on the 45 μm sieve was washed into a 2-litre conical flask with Ludox solution (diluted to achieve a density of 1.115 with 4% formalin in fresh water). Once all of the material had been transferred to the flask, further Ludox solution was added, with stirring to completely resuspend sediment and fauna. The flask was filled to just beyond the shoulder, covered to prevent evaporation and left at room temperature for 24 hours. The following day, the material which had migrated to the top of the Ludox was slowly poured onto a 45 μm sieve and washed into a sample bottle with 4% formalin in seawater for storage prior to examination. The remainder of the sample was again resuspended by stirring and a second Ludox extraction was carried out for each sample.

Copepods only were removed from each sample and identified to species level where possible.

3.2.7. Data Analyses

The data were analysed using both univariate and multivariate techniques.

3.2.7.1. Univariate Analyses

Total copepod abundances and effects on individual taxa were examined by two-way analysis of variance (ANOVA). Prior to performing ANOVA all abundance data were

log-transformed i.e. $Log_e(1+x)$, and Bartlett's and Levene's tests were applied to confirm homogeneity of variance.

Differences in community structure were determined by performing two-way ANOVA on a variety of diversity indices, including;

Total number of species
Species richness (Margalef's index)
Shannon-Wiener index
Simpson's dominance index
Pielou's eveness

All diversity indices were derived using the PRIMER (Plymouth Routines in Multivariate Ecological Research) computer package, and where logarithms are used in the calculations log_2 was selected. Before obtaining diversity indices, values referring to copepods groups not identified to species level were removed from the data matrix. These were mostly early-stage copepodites which could only be assigned to a genus with any degree of certainty.

3.2.7.2. Multivariate Analyses

Multivariate analyses were performed on the data at both species and genus level. Before applying multivariate treatments the data matrix was examined for the unidentified species groups referred to above. The numbers within each group, where possible, were redistributed amongst their genera in proportion to the existing species occurrence for each sample. The small numbers of individuals that could not be identified to species or genus level were removed from the data set.

The data were subjected to detrended correspondence analysis (DECORANA) achieved with the MVSP computer software package. To determine whether the resulting eigenvectors were correlated with either time or distance from the experimental array Spearman's rank correlation coefficient was calculated. A non-parametric statistic was selected since it is not known whether DECORANA axes scores are normally distributed.

Non-parametric multidimentional scaling (MDS), a second ordinal multivariate method, was also applied to the same data, together with a two-way analysis of similarities (ANOSIM) test performed on the groups (sampling time and distance from array) of community samples (defined *a priori*). Both MDS and ANOSIM results were obtained using the PRIMER software package.

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

3.2.8. Particle Size Analyses

The samples were removed from the storage bottles, placed on trays and dried in an oven until a constant dry weight was obtained. The dried sediment was then placed in a beaker with fresh water and 50 ml of 6.2 $g.l^{-1}$ aqueous sodium hexametaphosphate $(NaPO_3)_6$ (BDH Chemicals Ltd., Poole, UK) and physically broken up with a glass rod. A further volume of fresh water was added to make 1 litre and the sediment slurry was stirred vigorously with a magnetic stirrer for 15 minutes, after which it was left to stand at room temperature overnight. The following morning the sediment was again stirred for 15 minutes and then washed through a 63 μm sieve several times until all of the sub-63 μm particles had been removed. The retained fraction was placed on trays and dried until a constant weight was obtained. The sediments were then passed though a sieve stack and the weight of fractions retained determined. The particle size classes were separated by the following sieve mesh sizes (in mm): 4.00, 2.80, 2.00, 1.40, 1.00, 0.710, 0.500, 0.355, 0.250, 0.180, 0.125, 0.090, 0.063.

3.2.9. Sediment Metal Concentration

Determination of sediment metal concentration was made exactly as described in Chapter 2.

3.3. RESULTS

3.3.1. General Observations

General *in situ* observations made during the course of the experiment revealed the appearance of red particulate material settling in the immediate vicinity of the array structure (figure 3.4). This was not visible during the 10 day sampling, but was prominent by 30 days. The extent of the coloration was initially judged to extend to between 0.5 and 1.0 m from all sides of the structure and appeared to be little changed by 90 days. Examination of the 0 m cores obtained at 30 and 90 days immediately after extraction revealed red particles well mixed into the sediment to a depth of approximately 1 cm. This was not seen in any of the cores obtained from the 10 m station.

No other disturbance (or potential source of disturbance) was observed during the course of the experiment.

3.3.2. The Meiofaunal Community

The composition of the meiofaunal communities obtained during the course of two field experiments in Loch Creran was broadly comparable to previous studies, with densities within the ranges found elsewhere for similar sediment types. Heip *et al* (1985) noted that nematodes were always the dominant taxon in muddy sediments, often with very high numbers, while harpacticoids are usually the second most dominant but can comprise 4-95% (Hicks and Coull, 1983). In former Loch Creran studies Olafsson (1989) and Harries (1995) both found nematodes to comprise around 85%, with harpacticoids constituting between 7-11%. The compositional range for copepods is largely due to Olafsson's (1989) observations of seasonal fluctuations within his study site, where abundances were markedly higher in summer.

In the present and later experiment (Chapter 4) which were both carried out in the summer months, the background and control samples were found to comprise 77-87% nematodes and 3-7% copepods. However, what appeared to be a single unidentified foraminiferan species was also, perhaps unusually, very common, constituting 5-7% of

the total. Of the remaining taxa (5-10%), the most important were polychaetes (2-5%) and kinorhynchs (1-2%).

Overall densities were also unremarkable and within the ranges found by others. In the background samples, nematodes achieved an abundance of 3000-4000, and copepods 100-350 individuals per 10 cm^2. Similarly foraminiferans were found to reach 200-300 per 10 cm^2.

3.3.3. Particle Size Analysis

Particle size analysis confirmed the sediment to be a sandy mud with a high silt-clay content comprising between 55-64% at the 0 m station, and 47-55% at 10 m from the array (table 3.2). The sandy components (>0.063 μm) remained similar, both over time and between stations, but the silt-clay fraction (<0.063 μm) exhibited some variation across both parameters (figure 3.5). Although only 10 m apart, the station distal to the array was consistently lower in sediment silt-clay than the proximal station, by a mean difference of approximately 8 %. In addition, the silt-clay content at both stations sustained a detectable drop in the short time between pre- and 1-day sampling, followed by an increase to a maximum at 30 days.

It is likely that short-term fluctuations may, in part, be due to sampling disturbance but the changes over a longer period probably represent a natural process of silt transport and settling. Taken overall, the differences between stations are not considered sufficient to constitute a major modifier of the copepod community.

3.3.4. Metal Concentration

The results of the chemical analysis was somewhat surprising, with only a slight rise in the sediment copper content within the first thirty days of the experiment (table 3.3, figure 3.6). However, on the last sampling at 90 days, the copper level was measured at 431.6 $\mu g.g^{-1}$ dry weight. This was unexpected since the rate of copper release from all of the most common antifouling preparations is at its maximum on initial entry into the water (figure 3.7) and in terms of Cu_2O should represent some 20-40 $\mu g.cm^{-2}.day^{-1}$.

Given that the total surface area of the array was of the order of 10 m^2, one should have expected a total initial output of some 2-4 $g.day^{-1}$! Clearly there are a number of other factors that will determine where, and how the metal will be distributed, making predictions for sediment concentrations within the sampling area difficult, but it is hard to reconcile these with the single high loading at 90 days. Moreover, a supplementary sample, also taken at 90 days, and at the same distance from the array but on the other side yielded a sediment copper concentration of 56.2 $\mu g.g^{-1}$ dry weight. This presents the possibility that the sediment-bound copper may be grossly uneven in its distribution around the array. What is now clear, and with the benefit of hindsight, is that a greater number of smaller samples, taken over more frequent time intervals were required for a more complete assessment of the fate of the released copper.

3.3.5. Copepod Abundance

Perhaps as might be expected, temporal changes are the most obvious variation in the community throughout the course of experiment. All of the samples were obtained in the summer months to take advantage of naturally elevated copepod abundance, but the appearance of copepodites tended to affect the overall abundance trends. Figure 3.8 shows mean percentage copepodites from each set of replicate samples. The graph suggests a common trend at both 0 m and 10 m from array, with the proportion of copepodites reducing during the experiment. The graph of the absolute numbers of copepodites (figure 3.9) confirms this trend, while the numbers of adults (figure 3.10) appear to be increasing, suggesting recruitment of new adults into the community. Clearly, this experiment was performed after a peak of copepodite production.

These observations imply, at least at the community level, that the normal process of egg production and adult recruitment is unaffected by the level of copper input close to the array. The results of the two-way ANOVA (table 3.4), however, does suggest significant differences between the stations for the total number of copepods and adults only ($p=0.013$ and 0.003 respectively). Unfortunately, these differences, when examined concurrently with the graphical evidence (figures 3.10 and 3.11) could be attributable to natural variation between the populations at each station.

Comparisons by between-station one-way ANOVA for each individual time point (table 3.7) offers some basis for believing that spatial variation may not be the only factor involved. The source of the two-way variation appears to be due to the higher copepod numbers in the 0m- 1 day and 90 day samples. Clearly, if the samples are significantly different after only 1 day there would be grounds for dismissing a copper effect, particularly since the subsequent 10 and 30 day samples are essentially very similar. However, the differences between stations after 1 day for both total and adult-only values are not significant (p=0.055 and 0.074 respectively), while the converse is true at 90 days (p=0.040 and 0.038; no significant difference for copepodites).

This is undoubtedly a rather subtle difference, and a degree of caution should be attributed to this result. Examination at lower taxonomic levels presents a more complex picture, with the overall community characteristics severely dominated by a single species, *Stenhelia gibba*. On day 1, *S. gibba* abundance (both adults and copepodites) is substantially higher at the 0 m station (table 3.9, figure 3.12), and since throughout the experiment this species comprises 20-56% of the total community, this initial disparity tends to distort the statistical analysis. The reason(s) for this early difference is unknown, but abundance remains similar for all subsequent samples, demonstrating that *Stenhelia*, at least, remains unaffected by the copper contamination.

At the family level, two-way ANOVA analyses of those with the greatest number of representatives reveals no station-dependent differences, (table 3.5) although the graphs (figures 3.13-3.16) perhaps imply a minor impact on the Cletodidae after 90 days. However, examination by one-way ANOVA reveals no significant impact (p=0.08; table 3.8).

Examination of the most abundant individual species or genera by two-way ANOVA (table 3.6) gives station-dependent significant differences for *Halectinosoma angulifrons*, and *Haloschizopera* spp. A brief glance at the graphs (figures 3.17-3.21), however, is enough to see that the differences are simply a matter of population sizes, and that the overall trends at both stations are identical.

Longipedia spp., although showing no effect with the two way ANOVA, displays an obvious divergence at 90 days (figure 3.17). This is caused by a marked increase in the 0m population which proves to be significant when tested with one-way ANOVA ($p=0.008$). This is the only potential indication of an impact at a level of discrimination beyond the total number of copepod individuals.

A significant difference between stations when applying two-way ANOVA may not in itself provide adequate evidence for a copper contamination effect, but a significant interaction between stations and time may imply a more subtle cumulative or exposure-dependent response. This is not observed in any of the above analyses, although the null hypothesis for the total copepod abundance was barely upheld at $p=0.058$.

3.3.6. Diversity

There appeared to be no major differences in diversity, however defined, between the 0 m and 10 m stations throughout the experiment (figures 3.22-3.27). Indeed for diversities derived from the data for all copepods (figures 3.22-3.24), the greatest variation is exhibited between the samples taken at the start of the experiment (1 day), again perhaps partly attributable to the dominant influence of *Stenhelia*. Calculation of the same indices using adult data only does little to change the relationship between sites (figures 3.25-3.27).

Statistical comparisons by two-way analysis of variance (table 3.10) revealed only time-dependant significant differences, which are likely to be the result of intrinsic copepodite recruitment.

3.3.7. Detrended Correspondence Analysis

Detrended correspondence analysis ordination was largely consistent with the univariate trends.

When performed on the data for all copepod age groups, the primary axis appears to be strongly related to sampling time (figure 3.28). This trend, although maintained, is not

as obvious with $\log_e$-transformed data (figure 3.29). Spearman's rank correlation analysis confirms a significant correlation ($p<0.05$) between sampling time and axis 1 scores (table 3.11) for both non- and $\log_e$-transformed data. The second axis is similarly significantly correlated with sampling distance from the array.

When this analysis is repeated with adult-only data (figures 3.30 and 3.31) the segregation of groups are not as well maintained as in the total abundance ordinations. In addition, axis 1 is now significantly correlated with both sampling time and distance with both non- and $\log_e$-transformed data, while only axis 2 of the log-transformed data continues to be correlated with distance (table 3.12).

The axis correlations, although indicative of important trends, do not in themselves provide adequate evidence for a copper-mediated disturbance effect. The spatial displacement between replicate groups from the 0 and 10 m stations in the total copepod ordinations is largely similar within the sampling time groups, and can therefore only be considered as an indication of reoccurring faunal differences between the two sampling locations.

Removal of the considerable copepodite influence does, however, produce a comparative axis 1-displacement of the 90 day - 0 m replicates with both non- and transformed data. Unfortunately, any assessment at this level of discrimination can only be subjective at best, and without the benefit of a clear and unequivocal statistic one cannot present an entirely convincing argument for an effect on the 90 day samples based on these ordinations alone.

3.3.8. MDS

The total copepod MDS ordinations (figure 3.32) show a migrational pattern very similar to the DECORANA plots although it should be noted that since MDS is not axes dependent, the orientation has been, wherever possible, adjusted to aid visual comparison. Within the context of replicate groups, there does seem to be a progressive convergence, in terms of similarity, from day 1 to day 30, broken by the striking dissimilarity of the 90 day samples. The significance of this is somewhat tempered by

the relatively high stress values of all but the ordination derived from non-transformed data, indicating the potential for erroneous interpretation.

Clarke (1994) suggests that in situations where MDS ordinations do not give clear segregation it is prudent to cross-check suspected groupings with alternative techniques such as cluster analysis. Group average clustering from Bray-Curtis similarities on non-transformed data (figure 3.33) produces two major clusters, in one of which the putatively polluted 90 day – 0m replicates form a distinct subgroup, perhaps allowing the tentative proposition that some form of perturbation has caused a slight, but discernible change in the structure of this community.

A two-way ANOSIM test performed on the total abundance (table 3.13) serves to add further detail to the severity of the community changes over time, with significant differences detectable between all of the sampling times, although community changes, particularly at the level of species composition is very much reduced between 10 and 30 days. Of greater interest is the station difference component of this analysis. Through all levels of transformation, station differences persist and ideally each sample time should be tested by one-way ANOSIM for significant station differences. However, the nature of the ANOSIM technique is such that with only three replicates for sampling group there is a high risk of incurring a statistical Type 1 error (Clarke and Warwick, 1994).

Examination of the copepodite population in isolation gives perhaps the best representation of what has been hinted at by the previous analyses. Figure 3.34 shows an MDS plot of the untransformed copepodite data with the replicate groups clearly conforming to a pattern obviously attributable to sampling time only. A cluster analysis dendrogram (figure 3.35), constructed from the same data serves to further substantiate the temporal groupings, while also confirming that copepodite stages exhibit no detectable response to the elevated copper level. Even with transformations up to the highest level of severity (presence/absence) the integrity of the time-dependent groups are maintained (not shown), perhaps demonstrating the potential for copepodite abundance fluctuations to hide the more subtle effects of pollutants on adult populations.

MDS ordinations derived from adult-only data (figure 3.36) are, in fact, similar in overall configuration to the total abundance plots and although the 90 day-0 m replicates appear displaced to some extent, the stress values on the ordinations where this is most marked are high enough to consider this evidence to be, at best, only a somewhat tenuous indication of a copper impact.

3.4. DISCUSSION

Within the constraints of the present study, it appears that if there is a copper contamination effect it is evident, for the most part, only at the gross community level with statistical differences in abundance of adult-only and total numbers of copepods. It does seem that, to a certain extent, the often-encountered problems associated with the use of 'control' sites is a confounding factor here. A cursory examination of the graphs (figures 3.10 and 3.11) reveals an apparent (although not quite statistically significant; p=0.075 and 0.055 respectively) difference in the population at the start of the experiment, inevitably casting doubt on the ability of this study to show a clear copper contamination effect. This dissimilarity between the initial 1-day communities is unfortunate, since the equally uncontaminated 10- and 30-day samples appear very similar, leading one to more easily accept that the elevated copper level at 90 days was indeed the cause of the observed community differences.

The root of the disparity between the stations at the 1-day time point can be found at the species level, providing an insight into the potential causes of between-station variability. *Stenhelia gibba* was the dominant species in all of the samples, and the source of the 1-day variability can be solely attributed to the overwhelming effect of this species, which in itself does not seem to be affected by the copper contamination. It appears that a naturally-elevated abundance of adult *S. gibba* at the 0 m station had been correspondingly amplified by the production of copepodites, thus giving rise to the observed population dissimilarity (figure 3.12). Removal of *S. gibba* from the data set restores a response consistent with the known pattern of contamination (figure 3.37, table 3.14). This experiment, then, provides clear evidence for the need to retain the ability to remove components of the data set at both the species and ontogenic level if inconsistencies due to natural spatial variability can be reliably demonstrated.

If we now accept the statistical evidence that at the community level there is a significant response, then the nature of that response can be examined. On initial inspection, the direction of the abundance differences are perhaps not as one might expect, with the impacted station exhibiting an increase in the number of individuals, a response commonly attributed to organic enrichment events, although Giere (1993)

notes that this type of response is not uncommon shortly after contamination from a wide range of pollutants.

This aside, most studies examining the effect of metals on meiofaunal taxa have, in general, reported a reduction in abundance with increasing metal dose. However a small number of studies that have included more detailed analyses have also, under some conditions, found significant population increases. In most of these cases, where comparisons can be made, the metal doses applied are similar to the final concentration of the present experiment.

The mechanism of this type of response does not appear to have an underlying universal pattern, but is probably ultimately influenced by complex community attributes such that species-dependent tolerance, predator susceptibility and increased mortality of food items, amongst other factors, may combine to provide (perhaps transient) conditions favourable for population increase.

Watzin and Rosigno (1997) observed large numbers of newly-recruited gastropods in some of their field-deployed containers of zinc-contaminated sediment, and suggested that a taxon specific ability to regulate accumulation of the chemical may have lead to an attraction to the site. However, turbellarians, generally found in low abundance in the experimentally-contaminated sediments were observed to increase in only those samples with high gastropod recruitment. Subsequent examination of the turbellarian gut contents strongly suggested a movement into the site in order to feed on the gastropods.

There is very little direct evidence for pollution-induced changes in predation patterns directly impinging upon meiofaunal population densities. Sundelin and Elmgren (1991), in a laboratory experiment, observed an increase in turbellarian and harpacticoid (but not nematode and ostracod) numbers when exposed to cadmium in the presence of a predatory amphipod, and concluded that changes in competitive and predatory relationships within the meiobenthos coupled with modifications of macrofaunal abundance and activity may be a major modifier of a pollution-stressed community structure.

Austen and McEvoy (1997) investigated the effects of TBT in a microcosm experiment on nematode communities from a range of sediment types and observed a rise in the number of individuals subjected to their intermediate concentrations. Unlike the present study, though, there was a corresponding drop (although not statistically significant against any of the other treatments) in the number of species.

Perhaps the simplest and most readily observed mechanism for this type of response is that of a direct increase in the abundance of a small number of species. In the case of a toxic substance such as a metal this is likely to require a species-specific tolerance to such conditions, or a mode of feeding that minimises exposure to the toxicant. In the above study it was suggested that the mode of action of TBT is different to copper, or that nematodes respond in a different way, perhaps because of the lack of epibenthic opportunists (Austen and McEvoy, 1997).

Austen *et al* (1994), again in a microcosm experiment, found that, in general, nematode abundance decreased with increasing metal content. However, their lowest muddy sediment zinc concentration (917 $\mu g.g^{-1}$) produced a substantially elevated overall abundance. Further analysis at the species level revealed that two nematode species were able to proliferate at their lowest experimental sediment loading of both zinc and copper. Experimental evidence appears to suggest that some nematode species possess, or are able to develop, an inherent tolerance to a metal-rich environment (Somerfield *et al.*, 1994; Millward and Grant, 1995; Millward, 1996). However, there is little to indicate that meiobenthic copepod species are able to acquire a similar immunity, except, perhaps in the case of those species that inhabit sites of extreme environmental fluctuations, such as those that may be found in intertidal splashpools (O' Brien *et al.*, 1988).

Warwick *et al* (1988), maintaining a mesocosm contaminated with a mixture of diesel oil and copper, noted that, overall, copepods exhibited a greater sensitivity than nematodes despite a copepod population increase with increasing copper concentration. A corresponding decrease in diversity pointed to a progressive dominance by a small number of species most notably *Tisbe* spp.. This and other studies may indicate that species such as *Tisbe* spp. are able to migrate and feed in a contaminated area in the

manner of an epibenthic opportunist, perhaps, therefore, avoiding direct contact with the sediment-bound or pore water-entrained metal.

The present study does not show a marked change in diversity or a contaminant-dependent dominance by a small group of taxa. Intriguingly there is though a demonstrable single significant response by way of an increase in the abundance of *Longipedia* spp (figure 3.17). Olafsson (1989) observed that in Loch Creran *Longipedia helgolandica* (the most common *Longipedia* species found in the present study) is an important epibenthic coloniser, although the experiments of Harries (1995) demonstrate that it is not always present in large numbers. Although *Longipedia* spp. do not attain a high level of abundance, this experiment suggests that either this is the only taxon able to exploit the consequences of copper contamination, or that there may be a reduction in predation pressure brought about by the presence of the metal. Probably due to its large size and epibenthic habit, *Longipedia helgolandica* is known to be preferentially selected as a food item by some fish species (Alheit and Scheibel, 1982), and representatives of the genus are commonly found in the gut of gobies trawled from Loch Creran (personal observation). It is, therefore, conceivable that avoidance by predatory fish species may have contributed to the elevated numbers at the contaminated station.

The large number of juvenile individuals in the samples allowed an assessment of the effect of contamination on pre-adult development. Copepodites in the present study constituted 19-63% of the total abundance, with the lowest proportions, perhaps fortunately, in the later samples. Laboratory studies have provided us with somewhat conflicting information regarding the ontogenic effect of metals on harpacticoids. D'Agostino and Finney (1974), Hutchinson *et al*(1994) and Verriopoulos and Moraitou-Apostolopoulou (1982) have all recorded clear effects on naupliar mortality, but similar or concurrent studies do not provide a consensus for copepodite sensitivity. Brand *et al* (1986), using cadmium and Verriopoulos and Moraitou-Apostolopoulou (1982), with copper and cadmium, found copepodites of *Tisbe holothuriae* to be relatively insensitive compared to adults, while Verriopoulos and Moraitou-Apostolopoulou (1989) found a strong copepodite susceptibility to zinc. Field observation on the effect of zinc on a harpacticoid community has, however, yielded no clear differences in response for adult, copepodite and nauplii (Watzin and Roscigno, 1997). Similarly, the

present experiment produced no evidence that copepodite stages were affected in any way by the copper released from the paint.

Within this study there are indeed clear statistical differences between the contaminated and non-contaminated stations, but, on balance, the nature of these differences are not unequivocally consistent with the observed pattern of copper contamination. An additional factor that must be considered is that of the role of sediment characteristics. It is possible that faunal abundance may be more allied to the granulometric dissimilarity, or more specifically the silt-clay content, rather than metal input. Although particle size/sediment type is thought to be a major influence in the structuring of the meiofaunal community, particularly for the Harpacticoidea, it is not known whether a difference of some 8% in the silt-clay content of sites only 10 m apart is enough to account for at least some of the differences in community structure. (Somerfield *et al.*, 1994) found that copepod community response in the Fal estuary was best explained by a combination of sediment copper concentration and silt-clay content. Nematodes, although influenced to some extent by silt-clay content were however considered more responsive to copper concentration alone. Similarly, Gee *et al* (1992) could do little more than infer metal contamination as a modifiers of meiobenthic community structure in the German Bight, because sediment structure and depth tended to obscure the relationship between meiofauna and sediment pollutants.

In practical terms, the anticipation of a clear overall differentiation between an impact or non-impact effect was perhaps somewhat optimistic, resulting in a potentially flawed experimental design. The primary problem springs from an old and reoccurring ecological dilemma; that of the identification and establishment of an unaffected or undisturbed control (or reference) site in a field impact study. *A posteriori* definition of control sites is common in pollution studies, but is widely recognised as an undesirable, but necessary, compromise (Coull and Chandler, 1992). The inherent spatial variability of meiofaunal taxa presents an additional critical complicating factor in the comparison of samples from non-contiguous sites. In somewhat analogous circumstances to the present study Eskin and Coull (1984) were forced to abandon an experiment designed to examine the effect of oil contamination on an intertidal mudflat because they were

unable to establish *a priori* that the nematode community structure was sufficiently similar at the putative test and reference sites.

The chemistry of an antifouling preparation is complex, since it is designed to be simple and easy to apply to a range of surfaces, and when immersed it is then required to release the matrix-suspended copper at a constant ideal rate of 10-20 $\mu g.cm^{-2}.day^{-1}$ (Swain *et al.*, 1982) over a time span of a year or greater. A variety of commercially available copper-based paints are currently produced and their copper release characteristics tend to be centred around the type and speed of the vessel to which they are to be applied.

In this study a conventional preparation, produced specifically for slow moving vessels, was used because it was anticipated that the site in which the array was deployed was not subjected to a great deal of water movement; conditions similar to, say, that of a locally moored vessel. Unfortunately, the expected slow, but constant, transfer of copper to the sediment proximal to the experimental array did not occur during the early part of the experiment, and the samples taken up to one month after deployment were not contaminated to any appreciable level. The reason for this is unknown, but it seems likely that there was an extended 'lag' period before the copper was able to escape from the paint matrix. The efficiency of copper release from antifouling paints is known to be affected by a range of factors, such as variation in localised pH brought about through surface conditioning by settling marine organisms (Swain *et al.*, 1982), or the formation of insoluble compounds such as copper hydroxychlorides (Swain *et al.*, 1982; Price *et al.*, 1991). The former is unlikely to have played a role at such an early stage of deployment, but a form of chemical retardation is conceivable given the static nature and close sediment proximity to the array.

Metallic antifouling paints pose particular problems in estuarine or brackish environments where the solubilised metals will tend to complex with humic acids. The resulting colloids are destabilised by increasing salinities, causing some to flocculate. Most flocculation is complete by the time mixing has elevated salinities to 15 $^{0}/_{00}$ (Libes, 1992) and the flocculated humic acid-metal complex will eventually settle in the sediment. This process may also hold some significance for semi-enclosed, but fully-

saline locations such as lochs and fjords, where prolonged periods of rain or melting snow leads to a persistent surface layer of fresh or brackish water, often with a distinctive colour caused by suspended detrital material and dissolved humic compounds (Gage, 1974). Clearly, in areas where these conditions combine with heavy boat traffic or concentrations of moored vessels there will be a rapid and highly localised sediment loading of metals originating from the hull treatments. Moreover, these weather-mediated inputs are probably supplemented by the seasonal routine of boat maintenance. Past studies of sediment TBT levels have revealed a seasonal peak corresponded to the refloating of newly-painted boats in spring and early summer, often with later, secondary peaks, when the process of hosing and scrubbing fouled hulls is traditionally practised (Bryan and Langston, 1992). Future studies should perhaps establish that the same pattern is indeed true for copper, and if confirmed, whether these peaks hold implications for specific taxa regarding the timing of meiofaunal egg production and naupliar development.

Despite the initial low release rate, this study has demonstrated that a substantial copper input can be imparted to sediments within as little as three months from a treated surface area equivalent to a single vessel. The observed effect on the copepod community appeared subtle at best, and the experiment would clearly have benefited from further sampling if time had permitted. However, it is clear that the metal remained resident in the sediment and that the concentration was likely to continue to increase, as is probably the case with the sustained use of copper-based paints. The introduction of TBT into the aquatic environment produced concentrations (measured as tin) ranging from 1 $ng.l^{-1}$ in boat-free areas to 600 $ng.l^{-1}$ near marinas (Langston and Spence, 1994). Since there are no other known sources of this compound, one can only conclude that the contamination is derived entirely from boat movements, perhaps giving some indication of the potential problem for continued use of copper.

In fact, as long ago as 1970 it was reported that the vessel-derived copper concentration in the Suez canal was seven times higher than the threshold for deterioration of water quality (Brady *et al.*, 1989), while more recently, inputs from pleasure boats were estimated to be the major single source of copper pollution in Californian (Stephenson and Leonard, 1994) and Swedish coastal waters (Andersson and Kautsky, 1996). The

latter is, perhaps, not too surprising since there is a current vogue for establishing boating amenities in areas of high natural beauty, many previously considered as somewhat remote.

Concern about the release of all paint-based toxicants is growing, particularly with the emergence of evidence that copper may induce effects similar in nature to imposex by inhibiting the transformation of testosterone into estradiol (Alzieu, 1998). Recently, Sweden declared its intention to ban the use, within Swedish waters, of all toxic marine paints on recreational boats in 1999 (Anon., 1997), while in Scotland the salmon farming industry have been warned that localised sediment copper attributed to antifoul-treated cage structures may prompt similar measures (Anon., 1998).

Although manufacturers are attempting to develop non-toxic paints that prevent organisms from gaining secure attachment to hull surfaces, it is likely that it may yet be some considerable time before there are effective and economically acceptable substitutes to the paints currently in use on both recreational and commercial vessels.

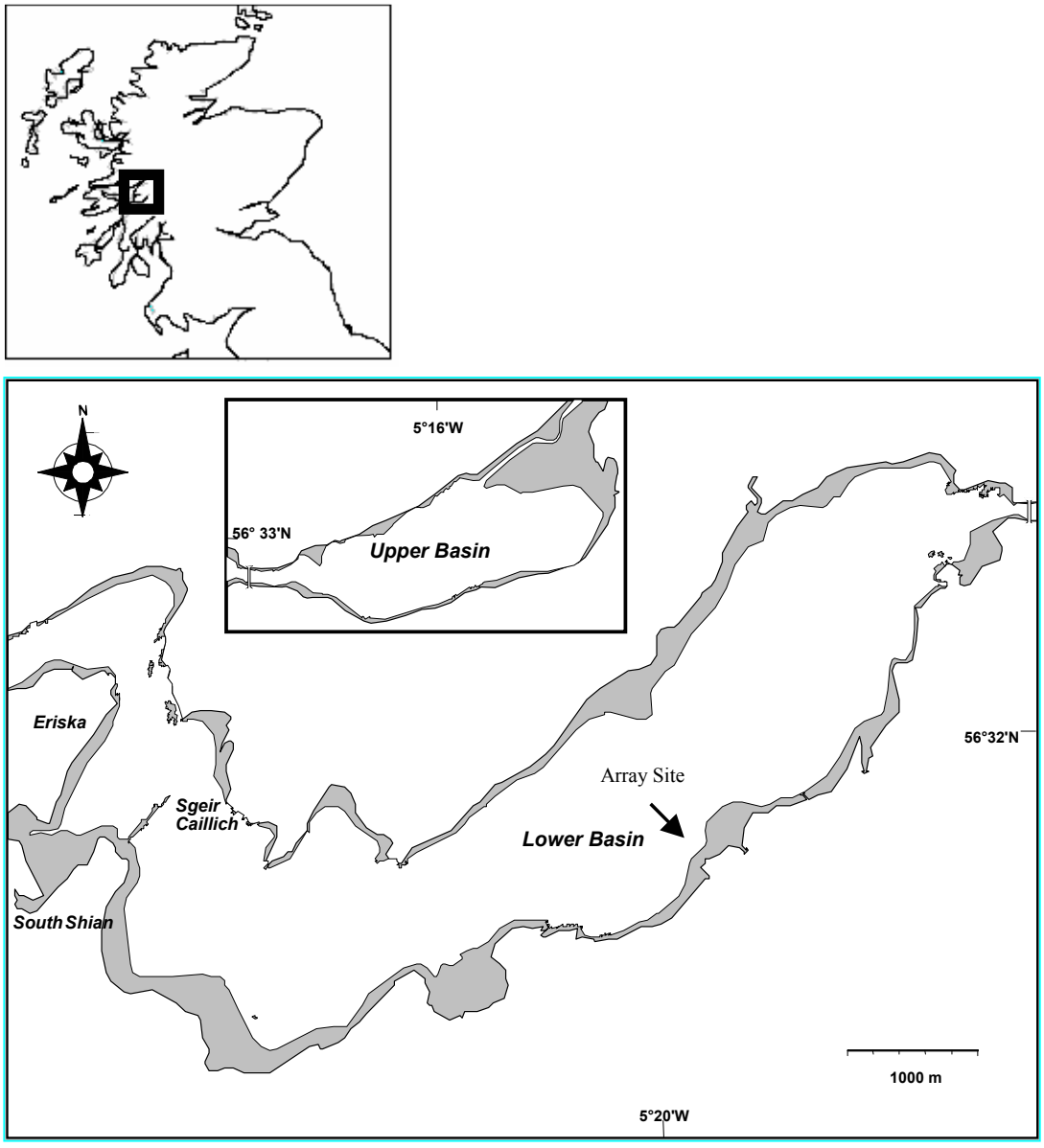


Figure 3.1 Loch Creran and the location of the experimental array site.

Figure 3.2 The completed array structure with antifoul-coated plates inserted.

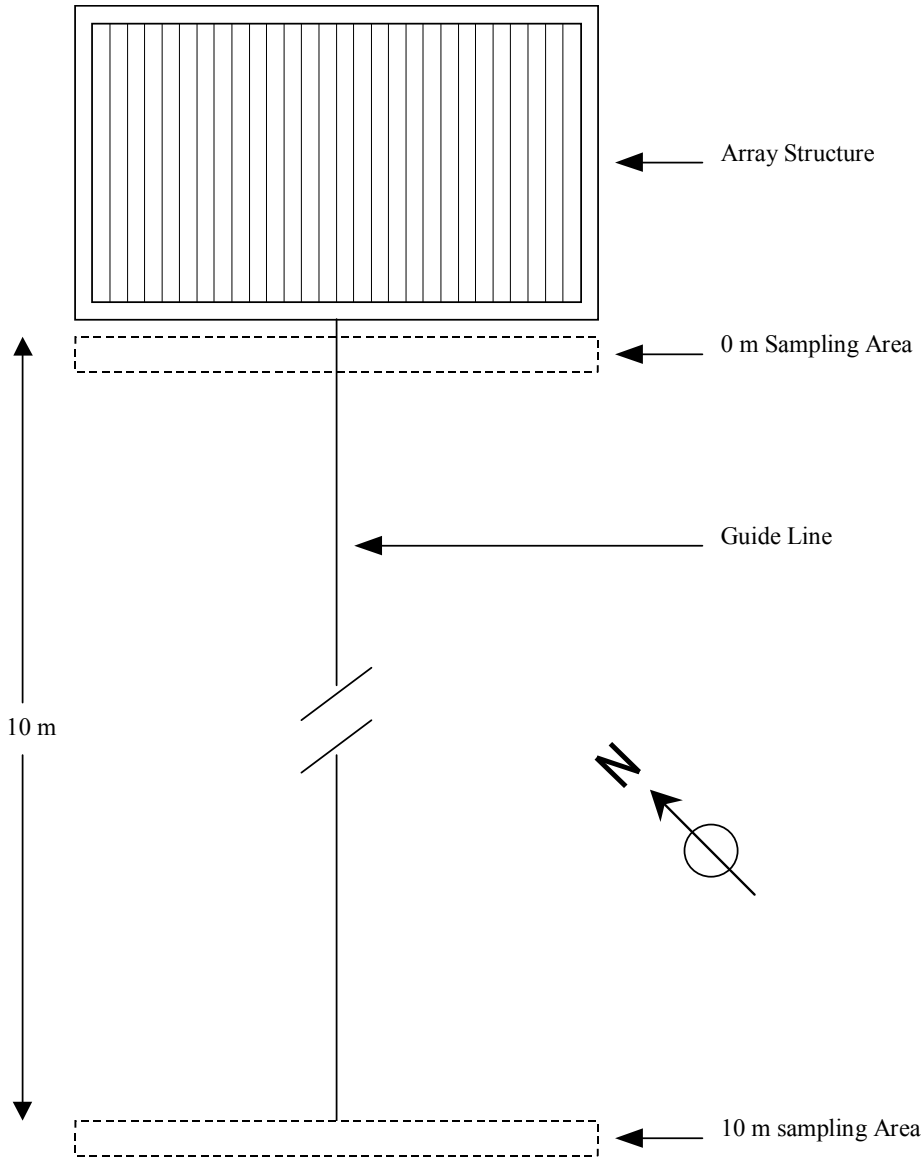


Figure 3.3 Plan view of array positioning and the sample station layout.

Figure 3.4 Array structure *in situ* after 90 days.

Mesh Size		Presample		1 Day		10 Day		30 Day		90 Day	
(mm)	ϕ	0m	10m	0m	10m	0m	10m	0m	10m	0m	10m
2.800	-1.50	1.5	1.8	1.3	0.7	1.2	2.2	0.4	0.9	1.1	1.3
2.000	-1.00	1.8	1.7	2.0	1.9	1.4	2.7	0.6	1.4	1.4	1.9
1.400	-0.50	2.4	2.6	2.3	3.1	2.2	3.6	0.8	1.3	2.5	2.6
1.000	0.00	2.5	2.1	2.1	3.0	2.2	2.8	1.1	1.6	2.4	2.2
0.710	0.50	2.6	2.4	2.3	3.4	2.6	3.1	1.7	2.1	2.9	2.9
0.500	1.00	2.4	2.3	2.2	2.9	2.3	2.6	1.6	1.9	2.9	2.4
0.355	1.50	2.4	2.5	2.2	3.0	2.4	2.4	1.8	1.9	2.7	2.6
0.250	2.00	2.5	3.1	3.0	3.2	2.8	2.8	2.0	2.4	2.7	2.9
0.180	2.50	2.1	2.9	2.7	2.8	2.2	2.4	2.2	2.2	1.9	2.3
0.125	3.00	4.7	6.2	6.1	6.0	4.6	5.4	4.9	5.9	3.9	5.7
0.090	3.50	8.2	12.0	9.3	10.6	8.5	9.7	8.7	12.5	7.8	9.9
0.063	4.00	8.9	10.7	9.8	11.9	8.5	9.1	9.7	10.9	9.8	11.0
<0.063	>4.00	58.0	49.6	54.5	47.4	59.2	51.1	64.4	54.9	58.0	52.4

Table 3.2 Percentage composition of size-sorted sediment fractions.

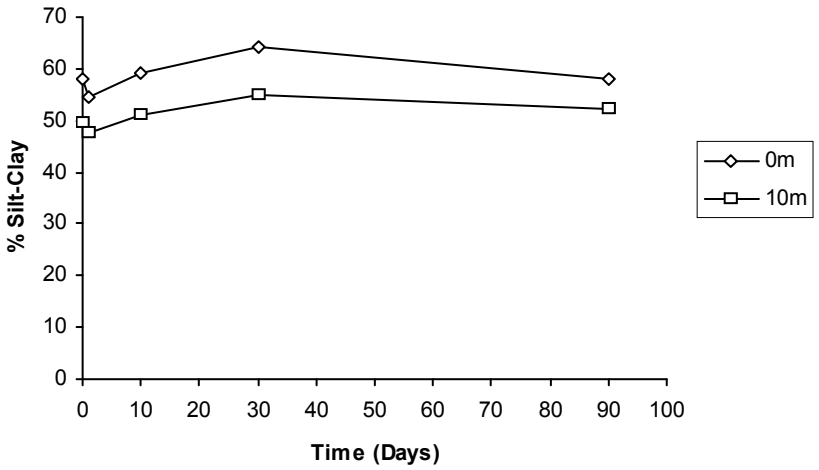


Figure 3.5 Temporal variation of silt-clay content at the 0 and 10 m sample stations.

Time (Days)	Mean Copper Concentration ($\mu g.g^{-1}$)	
	0 m	10 m
0	15.1	14.4
1	15.3	13.6
10	17.5	13.3
30	16.9	13.9
90	431.6	14.4

Table 3.3 Sediment concentration of copper at each sampling station.

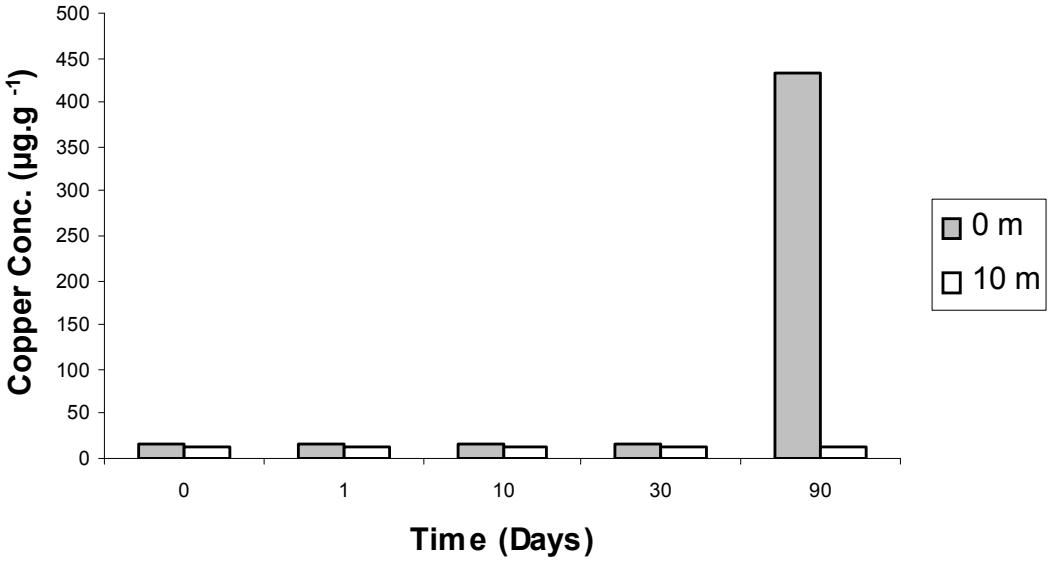


Figure 3.6 Bar chart of sediment concentration of copper at each sample station.

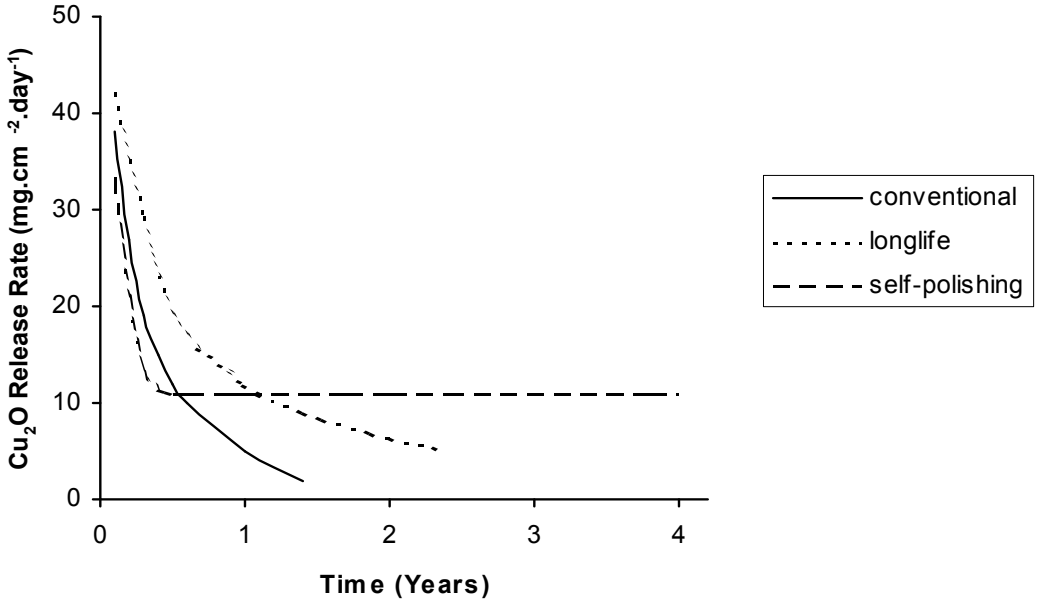


Figure 3.7 Characteristics release rates of the common commercial antifouling paint preparations.

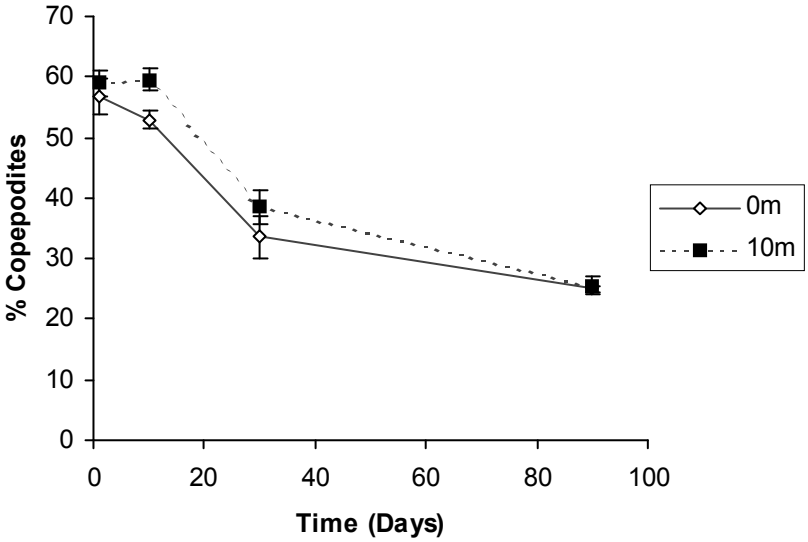


Figure 3.8 Mean percentage proportion of copepodites in core replicates. Error bars denote standard error.

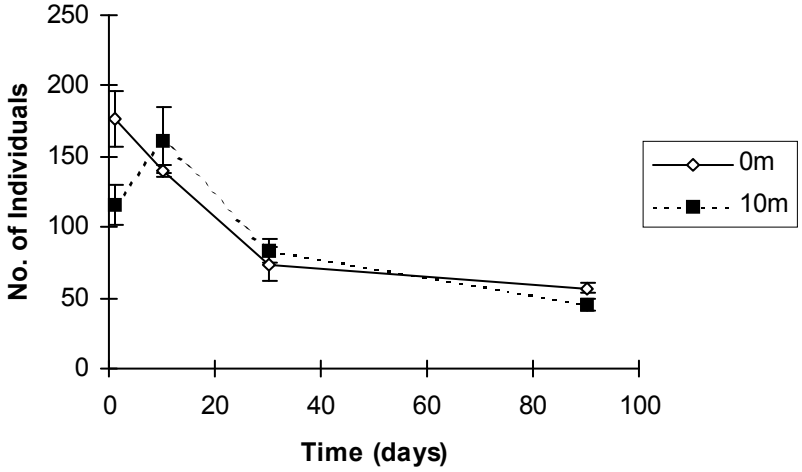


Figure 3.9 Mean copepodite abundance in replicate cores from the 0 and 10 m sample stations. Error bars denote standard error.

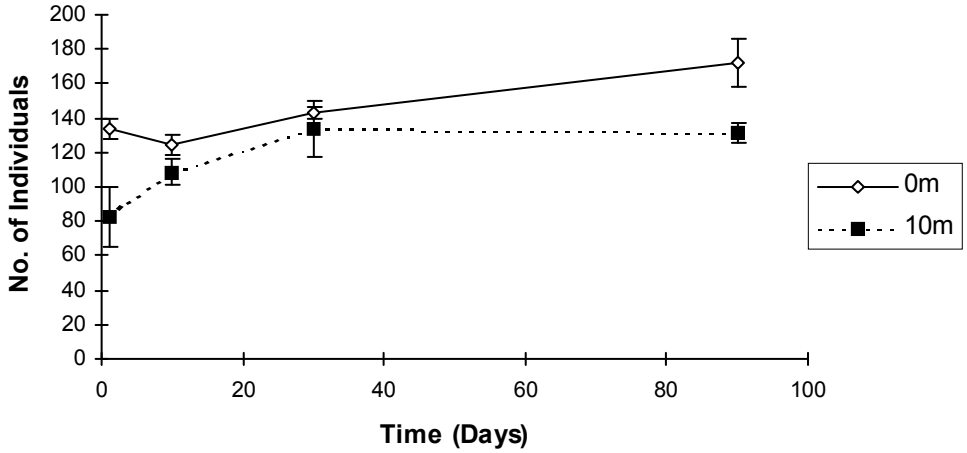


Figure 3.10 Temporal changes in adult abundance at the 0 and 10 m stations. Error bars denote standard error.

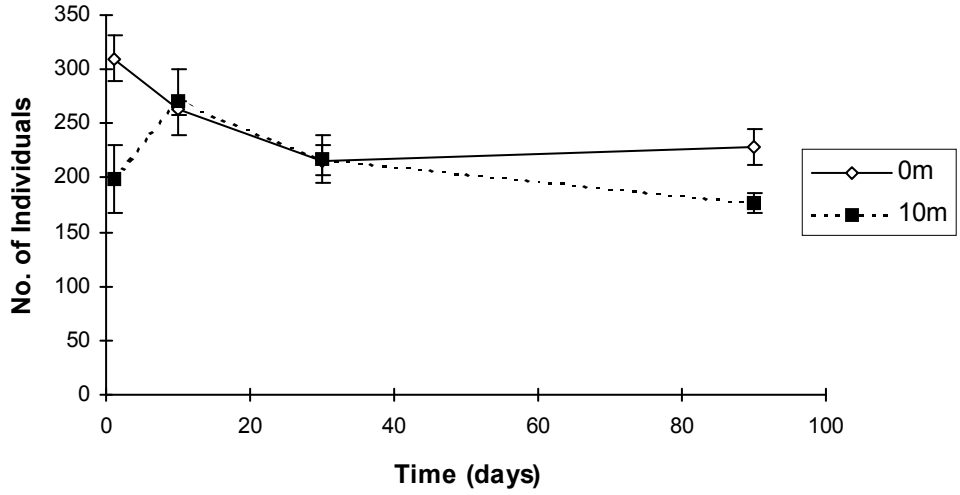


Figure 3.11 Temporal changes in total copepod abundance at the 0 and 10 m stations. Error bars denote standard error.

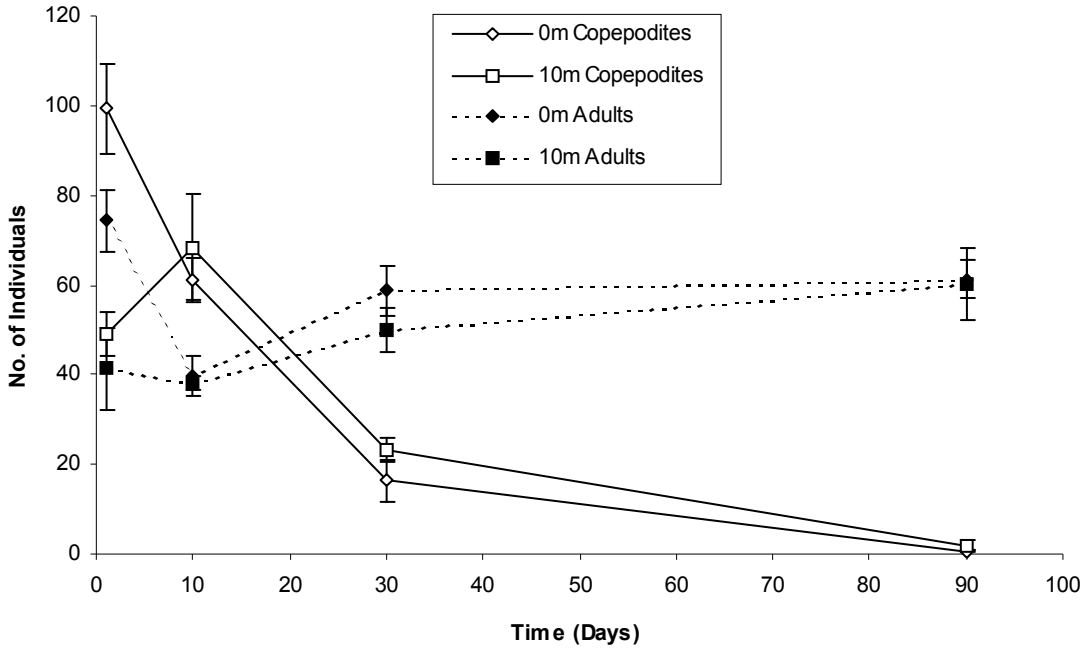


Figure 3.12 Mean abundance plots of adult and copepodite *Stenhelia gibba* at the 0 m and 10 m stations. Error bars denote standard error.

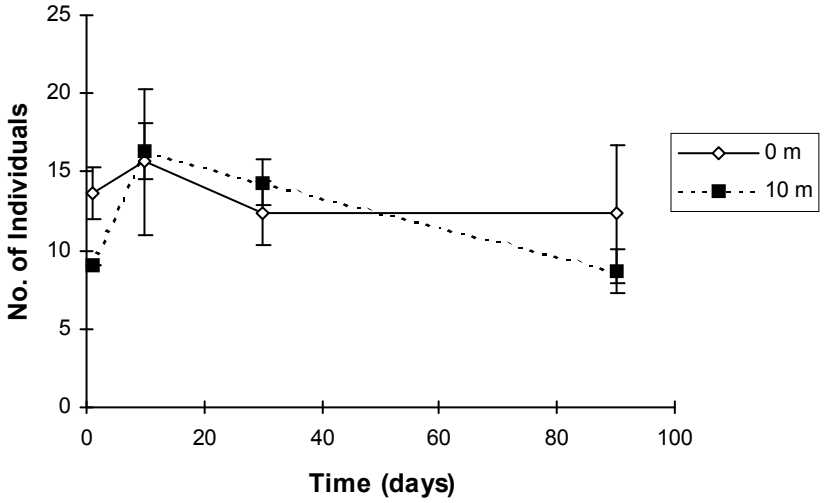


Figure 3.13 Mean abundance of Ameiridae at the 0 m and 10 m stations. Error bars denote standard error.

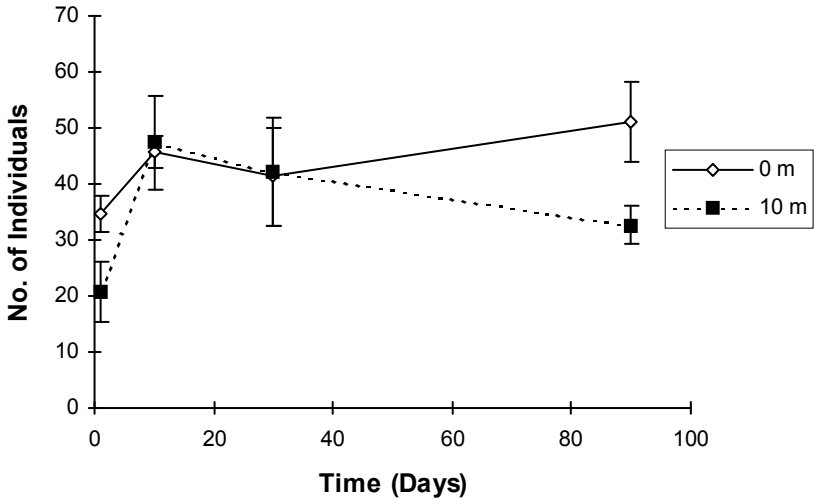


Figure 3.14 Mean abundance of Cletodidae at the 0 m and 10 m station. Error bars denote standard error.

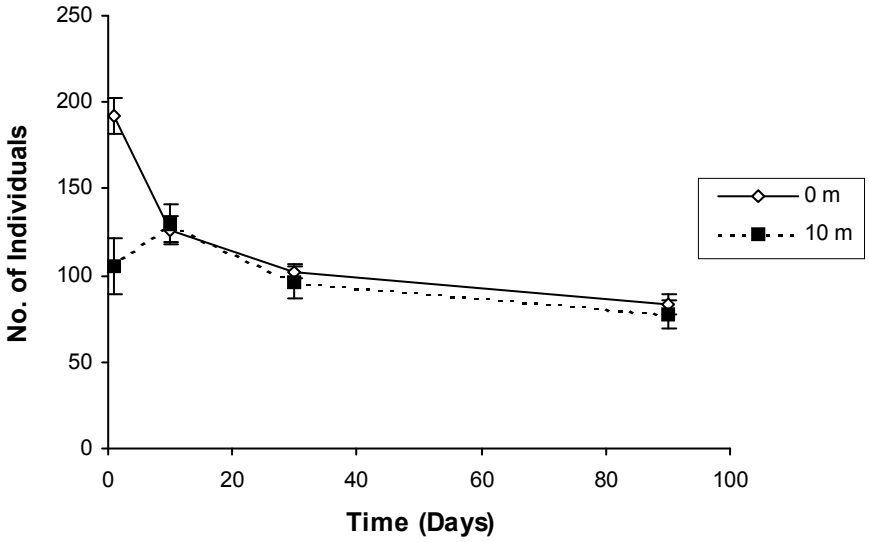


Figure 3.15 Mean abundance of Diosaccidae at the 0 m and 10 m station. Error bars denote standard error.

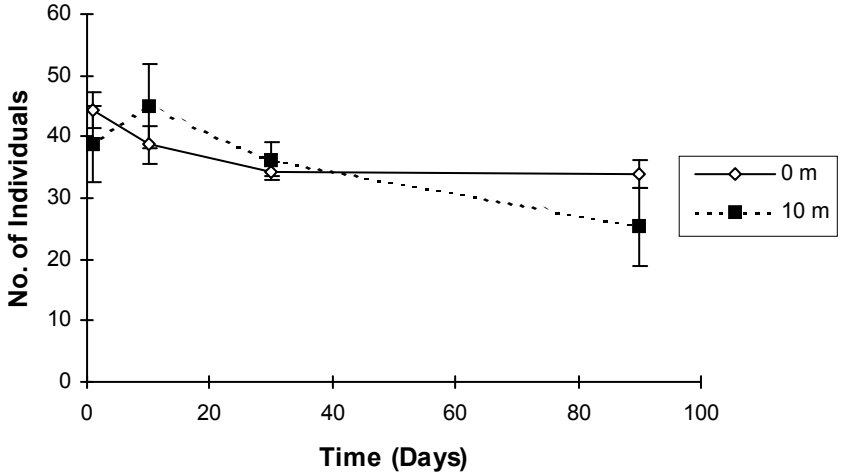


Figure 3.16 Mean abundance of Ectinosomatidae at the 0 m and 10 m station. Error bars denote standard error.

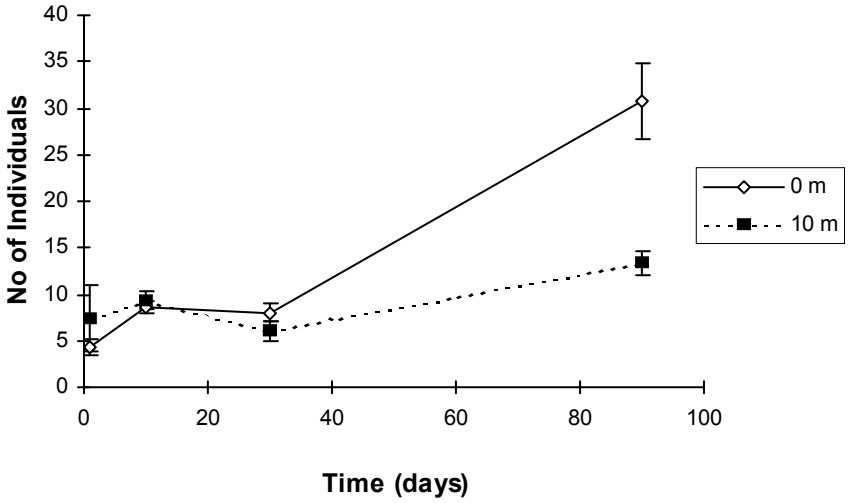


Figure 3.17 Mean abundance of *Longipedia* spp. at the 0 m and 10m sample stations. Error bars denote standard error.

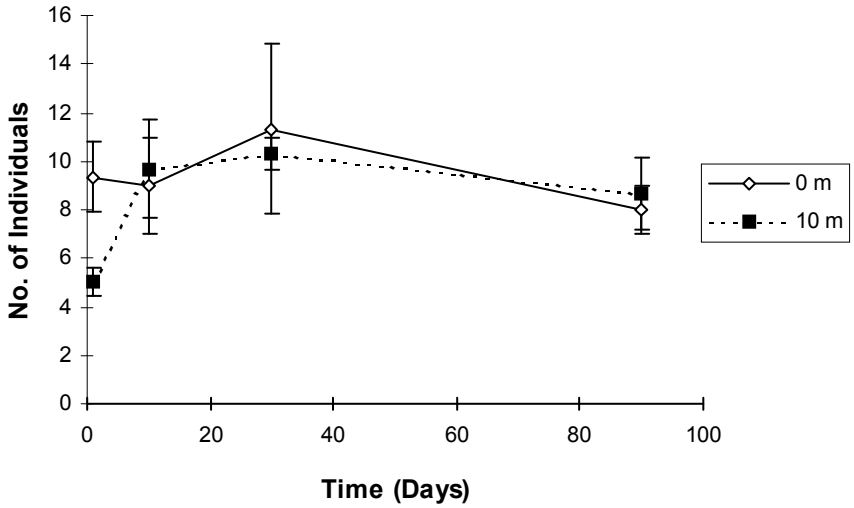


Figure 3.18 Mean abundance of *Heteropsyllus* spp. at the 0m and 10 m sample stations. Error bars denote standard error.

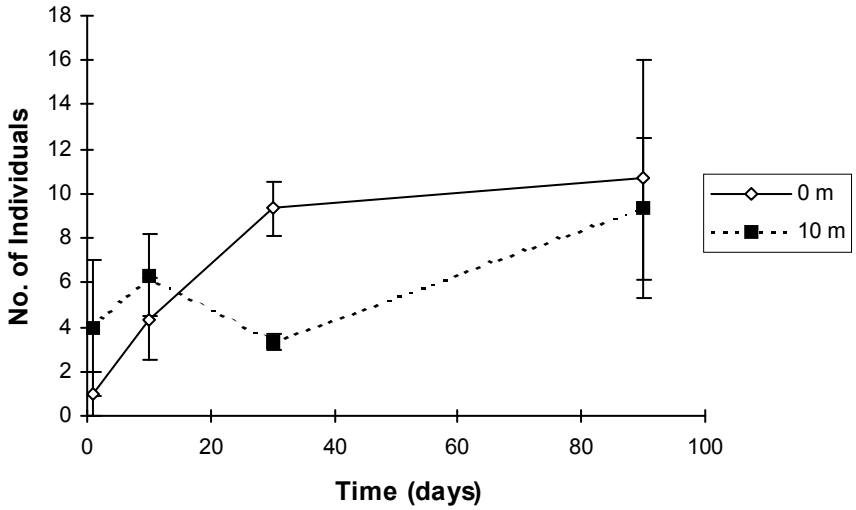


Figure 3.19 Mean abundance of *Cletodes longicaudatus* at the 0 m and 10 m sample stations. Error bars denote standard error.

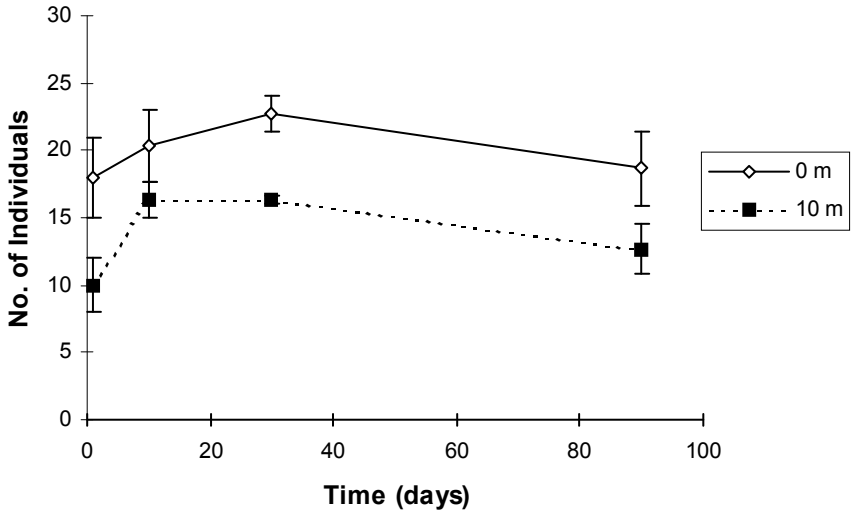


Figure 3.20 Mean abundance of *Haloschizopera* spp. at the 0 m and 10 m sample stations. Error bars denote standard error.

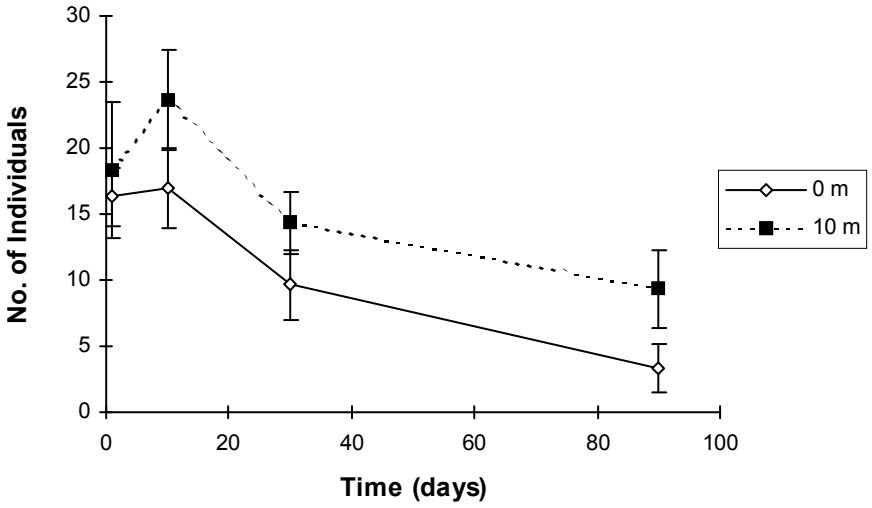


Figure 3.21 Mean abundance of *Halectinosoma angulifrons* at the 0 m and 10 m sample stations. Error bars denote standard error.

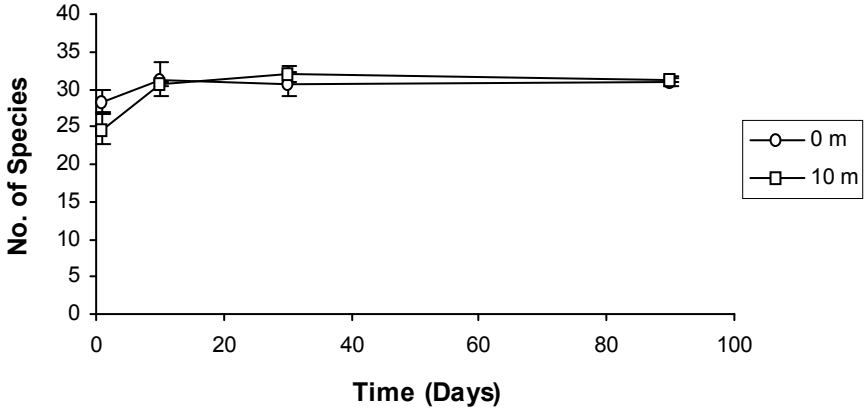


Figure 3.22 Temporal changes in the number of species at the 0 m and 10 m sample stations. Error bars denote standard error.

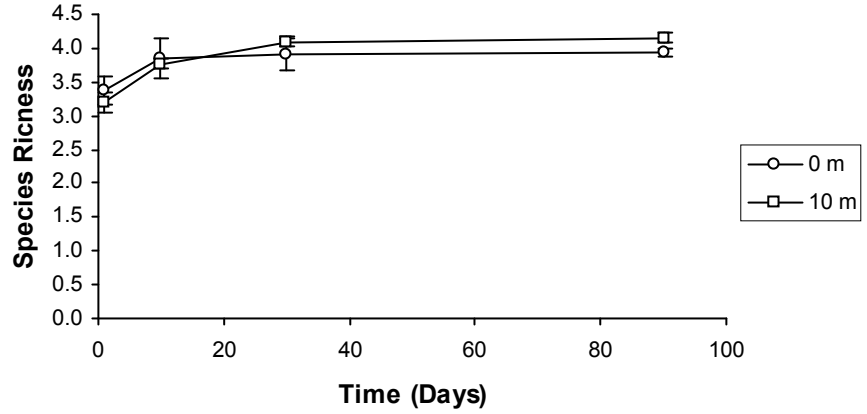


Figure 3.23 Temporal changes in Species Richness at the 0 m and 10 m sample stations. Error bars denote standard error.

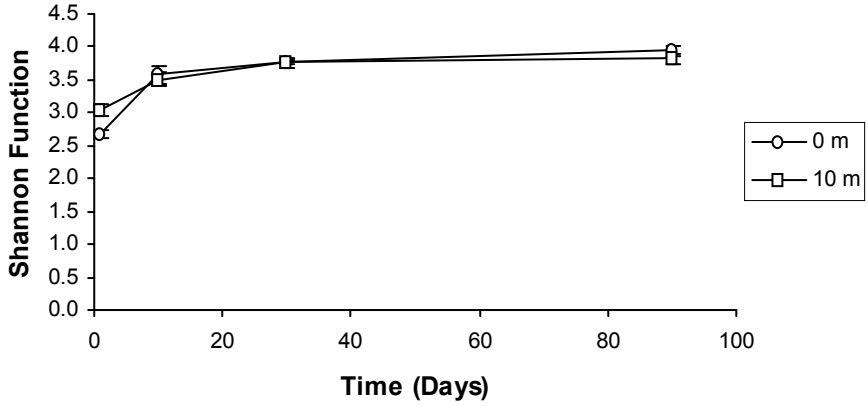


Figure 3.24 Temporal changes in the Shannon-Wiener Index at the 0 and 10 m sample stations. Error bars denote standard error.

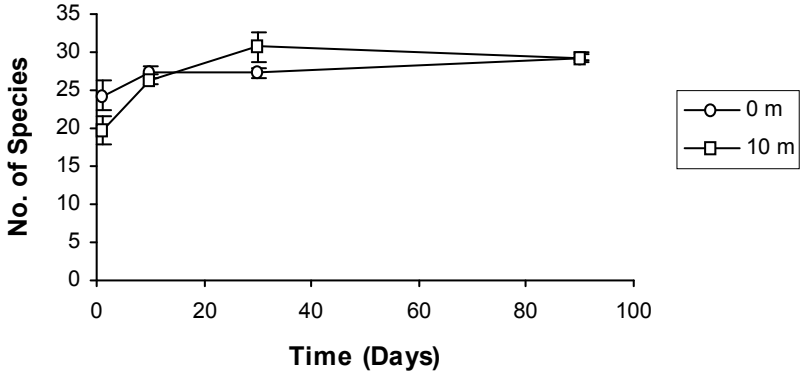


Figure 3.25 Diversity index from adults only: number of species. Error bars denote standard error.

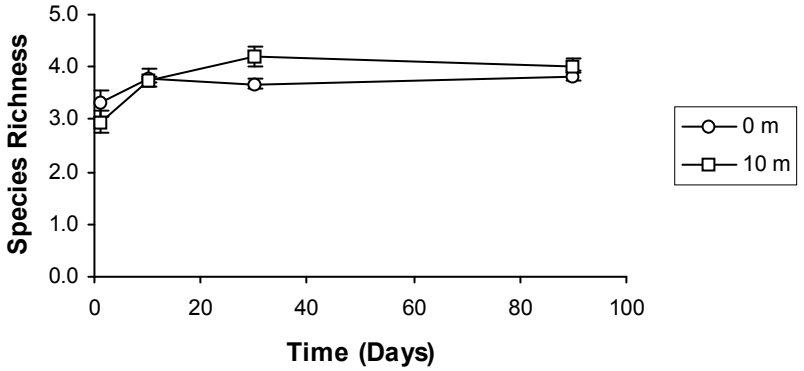


Figure 3.26 Diversity index calculated from adults only: Species Richness. Error bars denote standard error.

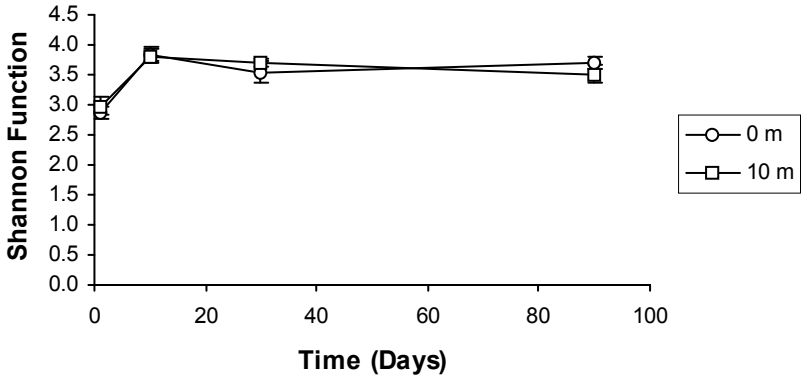


Figure 3.27 Diversity index calculated from adults only: Shannon-Wiener Index. Error bars denote standard error.

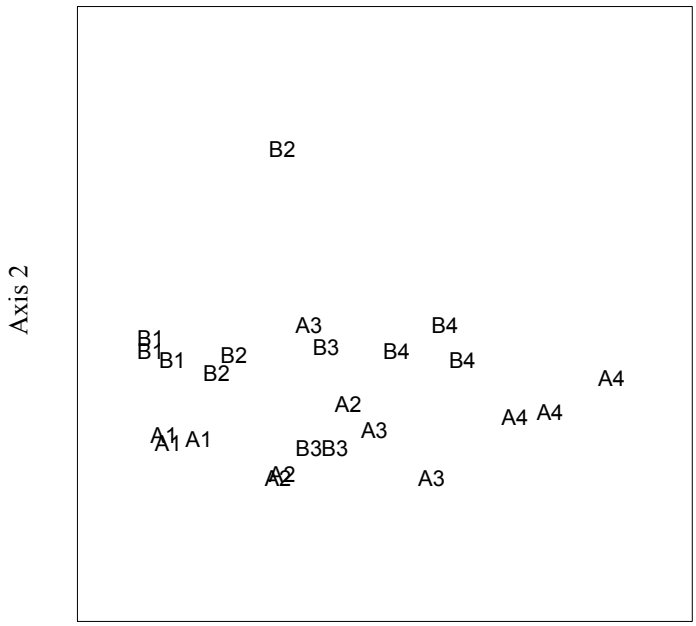


Figure 3.28 DECORANA ordination of species abundance data for copepods (all age groups): non-transformed data. Stations defined by A=0 m, B=10 m. Sample times are: 1=1day, 2=10 days, 3=30 days, 4=90 days.

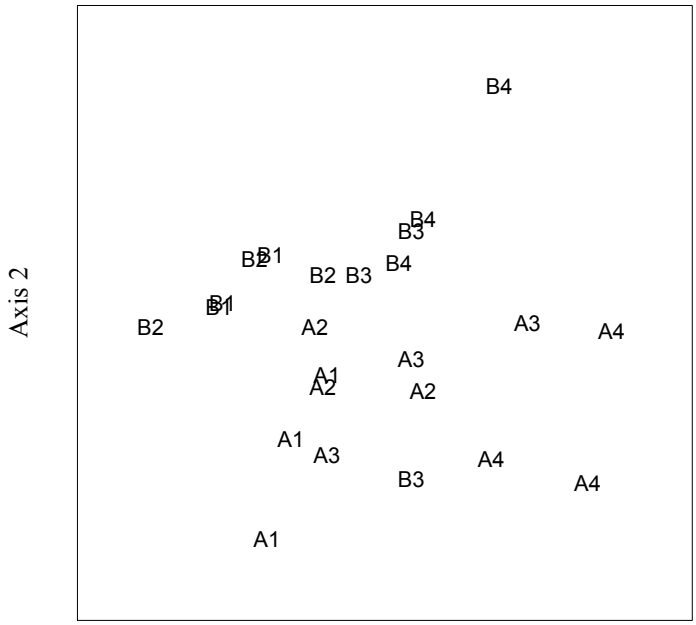
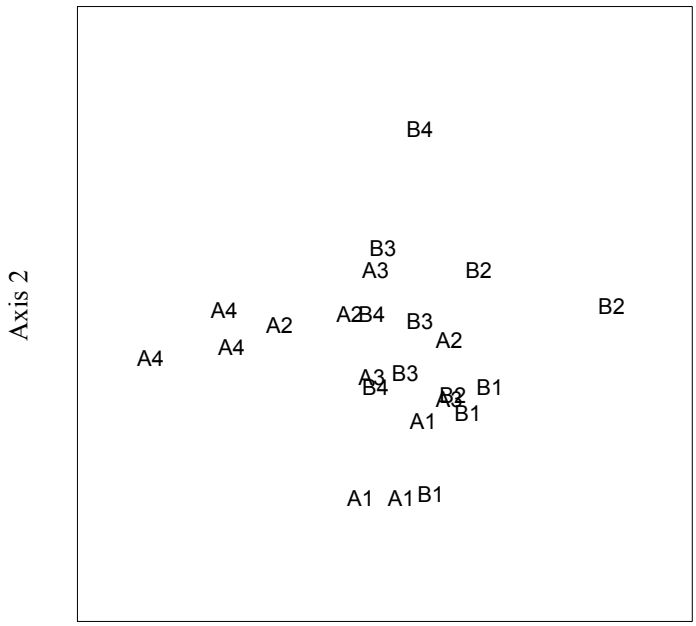


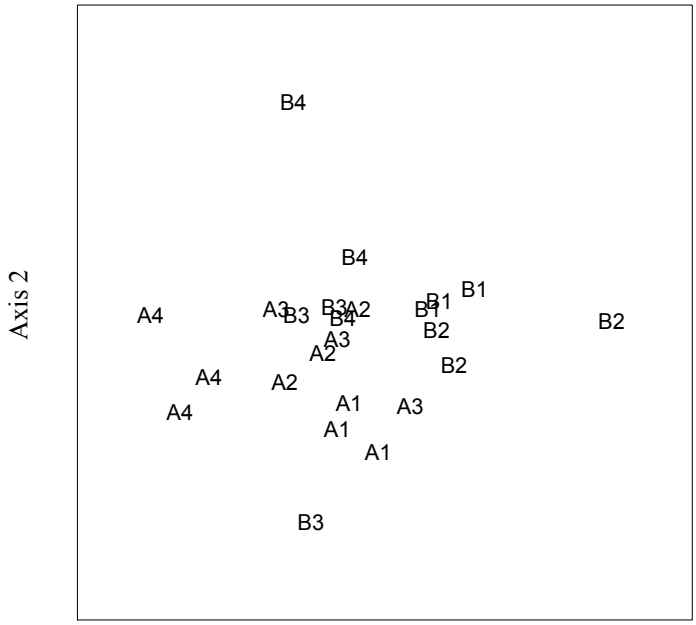
Figure 3.29 DECORANA ordination of species abundance data for copepods (all age groups): $\log_e$-transformed data.



Axis 2

Axis 1

.**Figure 3.30** DECORANA ordination of species abundance data for copepods (adults only): non-transformed data.



Axis 2

Axis 1

Figure 3.31 DECORANA ordination of species abundance data for copepods (adults only): $\log_e$-transformed data.

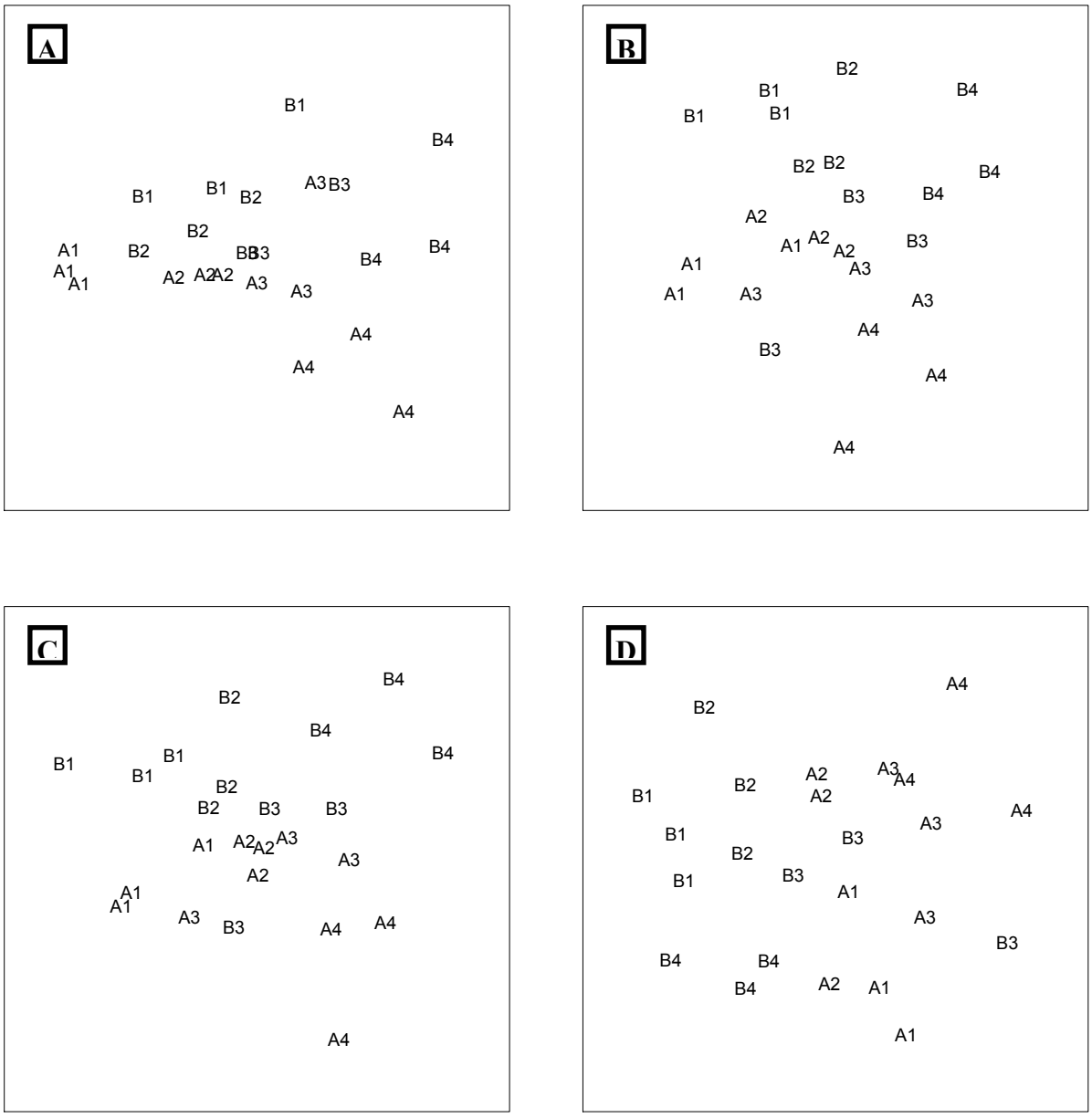


Figure 3.32 MDS ordinations of species abundance data for copepods (all age groups). A: non-transformed, B: 4th root-transformed, C: Log$_e$-transformed, D: presence/absence. Stress values are 0.12, 0.22, 0.21 and 0.24 respectively.

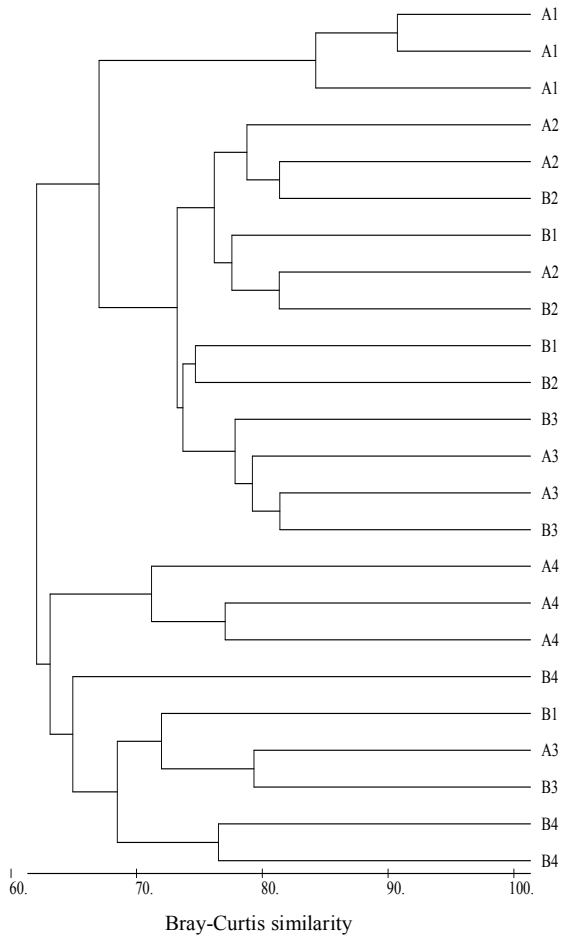


Figure 3. 33 Dendrogram of samples using Bray-Curtis similarity on non-transformed abundance.

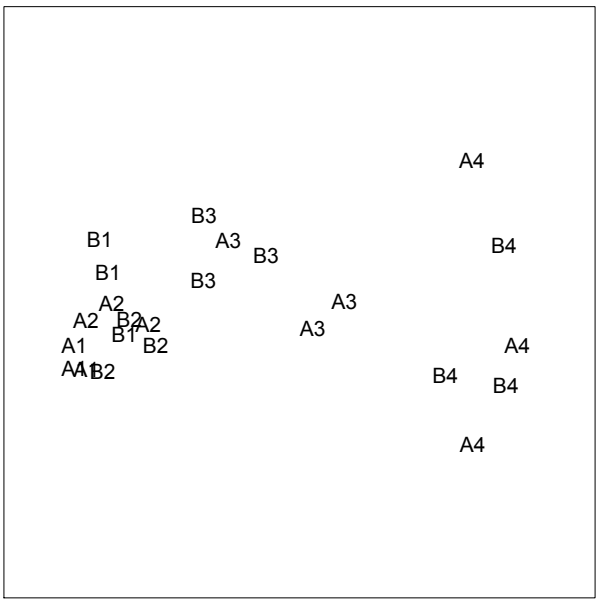


Figure 3.34 MDS ordination of copepodite-only abundance on non-transformed abundance data (stress = 0.07).

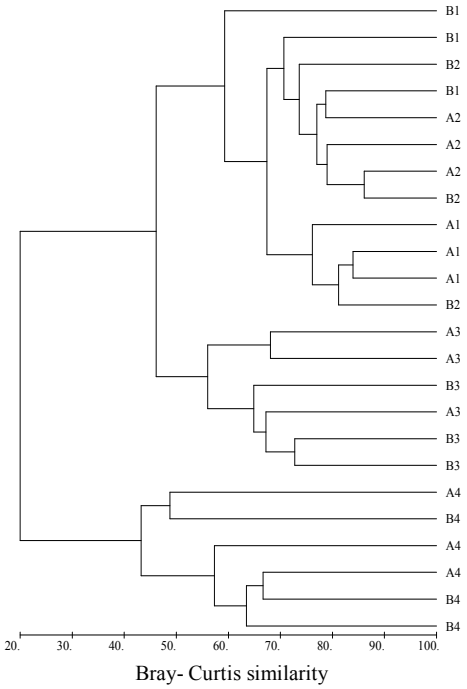


Figure 3.35 Dendrogram of samples using Bray-Curtis similarity on non-transformed copepodite abundance.

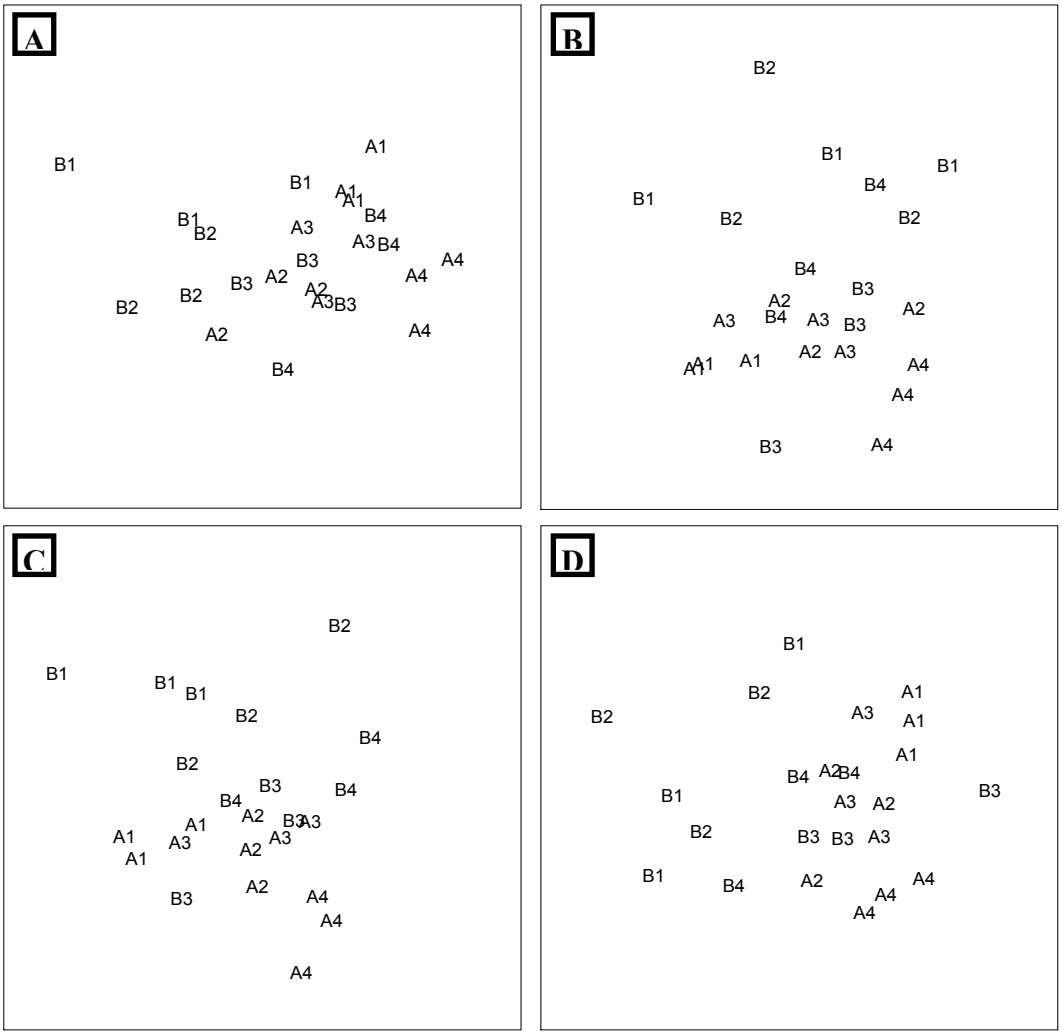


Figure 3.36 MDS ordinations of adult-only copepod abundance. A: non-transformed, B: 4th root-transformed, C: Log$_e$-transformed, D: presence/absence. Stress values are 0.16, 0.20, 0.20 and 0.20 respectively.

	Variable	p
Total Copepods	Time	0.031
	Station	0.013
	Interaction	0.052
Adult Copepods	Time	0.007
	Station	0.003
	Interaction	0.159
Copepodites	Time	0.000
	Station	0.240
	Interaction	0.058

Table 3.4 Two-way analysis of variance results for total, adult and copepodite abundance.

Family	Variable	p
Ameiridae	Time	0.120
	Station	0.469
	Interaction	0.378
Cletodidae	Time	0.044
	Station	0.083
	Interaction	0.324
Diosaccidae	Time	0.000
	Station	0.009
	Interaction	0.009
Ectinosomatidae	Time	0.046
	Station	0.404
	Interaction	0.298

Table 3.5 Results of two-way analysis of variance for the most abundant copepod.

Taxon	Variable	p
Longipedia spp.	Time	0.000
	Station	0.224
	Interaction	0.085
Heteropsyllus spp.	Time	0.231
	Station	0.431
	Interaction	0.294
Cletodes longicaudatus	Time	0.094
	Station	0.711
	Interaction	0.434
Halectinosoma angulifrons	Time	0.002
	Station	0.050
	Interaction	0.404
Stenhelia gibba	Time	0.000
	Station	0.016
	Interaction	0.009
Haloschizopera spp.	Time	0.053
	Station	0.002
	Interaction	0.595

Table 3.6 Results of two-way analysis of variance for the most abundant copepod genera or species.

	p			
	1 Day	**10 Days**	**30 Days**	**90 Days**
Total Copepods	0.055	0.918	0.991	0.040
Adult Copepods	0.074	0.179	0.580	0.038
Copepodites	0.650	0.410	0.530	0.096

Table 3.7 Results of the between-station one way ANOVA for each sampling time: total, adult and copepodite abundance.

Family	p			
	1 Day	**10 Days**	**30 Days**	**90 Days**
Ameiridae	0.027	0.740	0.439	0.515
Cletodidae	0.141	0.953	0.995	0.080
Diosaccidae	0.011	0.838	0.592	0.567
Ectinosomatidae	0.432	0.483	0.603	0.257

Table 3.8 Results of the between-station one way ANOVA for each sampling time: most abundant families.

Species	p			
	1 Day	**10 Days**	**30 Days**	**90 Days**
Longipedia spp.	0.612	0.583	0.252	0.008
Heteropsyllus spp.	0.035	0.822	0.987	0.774
Cletodes longicaudatus	0.446	0.411	0.004	0.779
Halectinosoma angulifrons	0.938	0.233	0.217	0.198
Stenhelia gibba	0.016	0.915	0.932	0.447
Haloschizopera spp.	0.109	0.233	0.217	0.198

Table 3.9 Results of the between-station one way ANOVA for each sampling time: most abundant genera and species.

Diversity Index	Variable	p
No. of Species	Time	0.010
	Station	0.519
	Interaction	0.362
Species richness (Margalef's index)	Time	0.002
	Station	0.834
	Interaction	0.610
Shannon-Wiener index	Time	0.000
	Station	0.485
	Interaction	0.045
Simpson's dominance index	Time	0.000
	Station	0.087
	Interaction	0.002
Pielou's evenness	Time	0.000
	Station	0.215
	Interaction	0.003

Table 3.10 Results of two-way ANOVA applied to sample diversities derived from species abundance data for all copepod age groups.

	Time	Distance
Axis1	✓	
Axis 1 (Log_e)	✓	
Axis 2		✓
Axis 2 (Log_e)		✓

Table 3.11 Spearmans Rank Correlation test for DECORANA axis scores derived from total abundance data (from ordinations using non- and Log_e-transformed data). ✓ denotes significant correlation ($p<0.05$).

	Time	Distance
Axis1	✓	✓
Axis 1 (Log_e)	✓	✓
Axis 2	✓	
Axis 2 (Log_e)		✓

Table 3.12 Spearmans Rank Correlation test for DECORANA axis scores derived from adult abundance data (from ordinations using non- and Log_e-transformed data). ✓ denotes significant correlation ($p<0.05$)

Transformation	Group Comparison	Significance Level (%)
None	1 Day: 10 Day	5.0
	1 Day: 30 Day	3.0
	1 Day: 90 Day	1.0
	10 Day: 30 Day	4.0
	10 Day: 90 Day	1.0
	30 Day: 90 Day	1.0
	0 m: 10 m	0.8
4^{th} Root	1 Day: 10 Day	4.0
	1 Day: 30 Day	1.0
	1 Day: 90 Day	1.0
	10 Day: 30 Day	53.0
	10 Day: 90 Day	1.0
	30 Day: 90 Day	4.0
	0 m: 10 m	0.2
Log_e	1 Day: 10 Day	1.0
	1 Day: 30 Day	1.0
	1 Day: 90 Day	1.0
	10 Day: 30 Day	29.0
	10 Day: 90 Day	1.0
	30 Day: 90 Day	2.0
	0 m: 10 m	0.3
Presence/absence	1 Day: 10 Day	17.0
	1 Day: 30 Day	3.0
	1 Day: 90 Day	1.0
	10 Day: 30 Day	64.0
	10 Day: 90 Day	3.0
	30 Day: 90 Day	9.0
	0 m: 10 m	0.1

Table 3.13 ANOSIM results of pairwise tests for differences between samples using different levels of transformation.

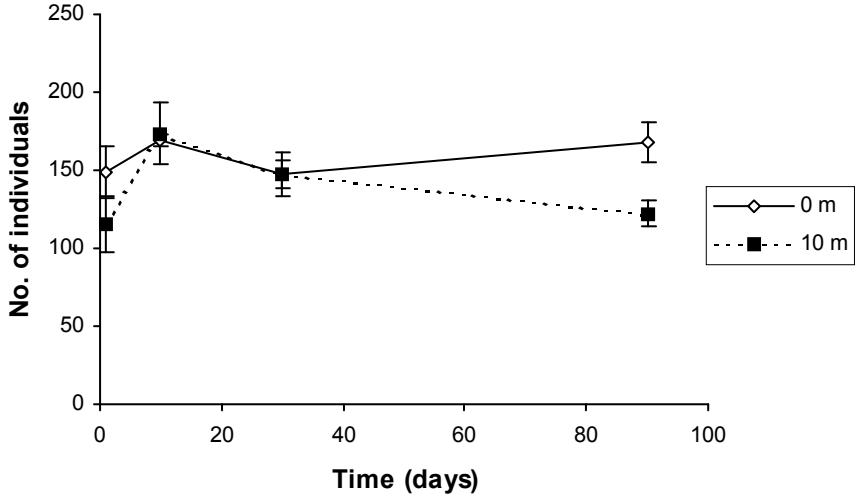


Figure 3.37 Mean abundance plots of copepod population at the 0m and 10 m stations after removal of *Stenhelia gibba*. Error bars denote standard error.

	p			
	1 Day	**10 Days**	**30 Days**	**90 Days**
Stenhelia gibba copepodites	0.008	0.675	0.235	0.451
Stenhelia gibba adults	0.057	0.811	0.322	0.853
Stenhelia gibba removed	0.250	0.923	0.950	0.035

Table 3.14 Results of the between-station one-way ANOVA for adult and copepodite-only *Stenhelia gibba* abundance, and after removal from data set.

CHAPTER 4

FIELD STUDY 2: THE EFFECTS OF COPPER CONTAMINATION ON ENCLOSED MEIOBENTHIC COMMUNITIES

4.1. INTRODUCTION

In the previous chapter the confounding effects of meiobenthic spatial variability on the use of control or reference sites in field conditions was highlighted. It was shown that these effects are likely to be more than sufficient to obscure otherwise measurable contamination effects at the community level.

These confounding factors can take many forms such as; depth, oxygen levels, salinity and temperature fluctuation, patchiness of food source, current regime and, of course granulometric variation. Coull and Chandler (1992) noted that field studies evaluating the effect of pollution on meiofauna are invariably one of two types: (1) monitoring 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 uncontaminated. In the first approach, the selection of control or reference sites may amount to a 'best guess' as to what may constitute an environmentally equivalent area. The second strategy, by its very nature, provides for a greater degree of control with precontamination sampling, but *in situ* manipulation and controlled contamination of subtidal sediments, particularly those with a high silt-clay content, is notoriously difficult. In addition, the contaminated plot may have 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 minimise 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 localised sites. But a reduced separation distance presents a 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 under test, and then returned to the site for sampling at a later date. The contained sediments are therefore theoretically no different to that surrounding them, and should also be under the same local environmental influences.

However, the application of this approach has been largely orientated towards investigations into the dynamics of settlement and recolonisation and so the test sediment are usually rendered azoic by drying or repeated freezing and thawing before placing on the seabed. Both Hall and Frid (1997) and Olsgard (1999) investigated the effect of copper on recolonisation patterns of subtidal macrobenthic species with some degree of success. Hall and Frid (1997) found rapid but sporadic recolonisation over a 24 hour period by relatively few species, many of which appeared to be attracted to the remains of the individuals killed in the freezing 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 $\mu g.g^{-1}$ on selected polychaete species. The majority of taxa, though, exhibited no response even at the highest concentration of approximately 2000 $\mu g.g^{-1}$.

Similar studies using meiofauna are surprisingly rare. Alongi *et al* (1983) examined the colonisation process within a shallow subtidal meiobenthic sand community subjected to oil contamination at a range of concentrations. After 16 days 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 was slower to occur.

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), where the attraction of organic remains are suspected to be implicated in abundance increases, are common. In the more extreme treatments where all organisms are removed, the converse situation can occur with the colonisation process retarded due to loss of sedimentary organic binding properties or 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.

However, experience with the relocation of sandy sediments to laboratory microcosms (Chapter 2) coupled with preliminary tests with sediments extracted directly from the site of the previous field experiment (Chapter 3) suggests that most meiofaunal taxa can withstand the rigours of displacement and some degree of physical manipulation. In this experiment the feasibility of live-community manipulation in field conditions is investigated while being simultaneously applied to the evaluation of the effects of a range of copper concentrations on a muddy subtidal meiobenthic community. Replicated individual enclosed community units were treated by mixing with copper powder and inserted into the sediment. 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 meiofauna are rare and therefore this study probably represents a new approach to the evaluation of the effects of metals on meiobenthic communities.

4.2. METHODS

4.2.1. The Study Site

The site selection criteria for this experiment was considered to be identical to the previous field study, and so it was therefore decided that a location proximal to the uncontaminated 10 m array sample site provided a suitable option. This could be justified on the basis of familiarity of the meiobenthic community, known absence of copper contamination and the established presence of moorings and underwater guide-lines, minimising the need for further disturbance. Thus a full description of the survey site can be found in Chapter 3.

4.2.2. Experimental Preparation and Deployment

Initially, an exploratory dive was performed to establish the condition of the site and a preparatory sediment sample was collected. This fresh sample was weighed while wet, then dried to constant weight to determine water content to allow calculation for the nominal sediment concentration in $\mu g.g^{-1}$ dry weight of sediment.

A 3 metre x 3 metre grid with thirty-six 0.25 m^2 sectors was constructed *in situ* by diver with garden stakes and polypropylene string at a depth of 9.5 m below chart datum. The orientation of the grid was roughly in a north-south direction.

On 05/06/97 sediment was diver-collected by gently scooping the surface layer into five-litre buckets. The collection site was within 4-5 m of the grid location to preserve the sediment parity, but was sufficiently distant to cause negligible disturbance prior to the start of the experiment. The recovered sediment was taken to the surface where it was kept cool and out of direct sunlight to minimise meiobenthic community mortality.

The sediment was homogenised in a single 20 litre bucket by gentle manual stirring. Aliquots were taken and made up to a predetermined weight in one-litre acid-washed plastic beakers. Copper powder (-200 mesh, 99%, Sigma-Aldrich, Pool, U.K.) was added to achieve nominal dry weight concentrations of 50, 500 and 5000 $\mu g.g^{-1}$. Each

preparation was again gently but thoroughly homogenised before dispensing to 100 cm^3 screw-cap polycarbonate bottles. 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 four control replicates, treated identically, but without the addition of copper. Both the bottles themselves and the lids were carefully labelled with indelible marker. This procedure took approximately 1.5 hours. Once capped, the bottled were placed in buckets containing cold seawater and transported with the minimum of agitation to the site of deployment.

Each bottle was placed within the centre of a grid square. The location within the grid was determined by random allocation (figure 4.1). The 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 (06/06/97), four background core samples for meiofauna and one each for metal content and particle size analyses were taken from randomly allocated squares within the grid (figure 4.1). 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.

On 04/07/97 the bottles were gently but securely recapped and returned to the surface, where they were immediately preserved in formalin. After this was completed repeat background samples for meiofauna, metal content and particle size analyses were taken from randomly allocated squares not previously sampled.

4.2.3. Sample Processing

The meiobenthos was extracted from the sediment with Ludox as previously described in Chapter 3. In addition, the processing residues were retained from two of the control samples and all of the remaining meiofaunal animals were counted to obtain an estimate of extraction efficiency.

The Ludox-extracted copepods were examined and identified to species wherever possible. All other meiofaunal groups were only identified to major taxa (except the inadequately preserved soft-bodied fauna; these are referred to as Unidentified Soft Fauna). For some of these taxa, particularly the Foraminifera, it was necessary to distinguish between long-dead or empty tests by the addition of Rose Bengal stain.

4.2.4. Statistical Analyses

To determine statistical differences between the community sample means, one-way ANOVA was performed on $\log_e(1+x)$-transformed numerical data. One-way ANOVA was also applied to diversity indices derived from adult abundance, using (where appropriate) $\log_2$ in the index calculation.

Multivariate analyses methods was applied with ordinations derived from both detrended correspondence analysis (DECORANA) and multidimensional scaling (MDS). These were obtained using either the MVSP or the PRIMER computer packages respectively. A range of transformations were applied to reduce the influence of dominant taxa.

To determine whether the treatment community eigenvectors were correlated with copper level Spearman's rank correlation coefficient was calculated.

Significant differences between replicate groups were determined by one-way analysis of similarities (ANOSIM). The contribution of individual taxa to these differences was investigated by the calculation of similarity percentages on $\log_e(1+x)$-transformed data by means of the SIMPER program. In addition, the relationship between copper

contamination and multivariate dispersion of replicates was examined by application of the MVDISP program.

4.2.5. Particle Size Analysis

Particle size analysis was performed as previously described in Chapter 3.

4.2.6. Copper Analyses

Copper analyses were performed as previously described in Chapter 3.

4.3. RESULTS

4.3.1. Particle Size Analysis

The results of the particle size analysis shows that there was very little change in any of the sieve fractions throughout the course of the experiment (table 4.1). The silt-clay composition did appear to change slightly (figure 4.2), but the magnitude of the change was not sufficient to effect meiobenthic community structure.

4.3.2. Metal Concentration

Table 4.2 shows the nominal and chemically determined sediment concentration of copper in the core and bottle-confined samples. Background and control concentrations did not vary between the start and the end of the experiment despite the close proximity of high concentration sample,. and the introduced metal remained largely bound in the sediment matrix, with minimal loss *via* solubilisation.

The measured concentrations were substantially higher than intended, but were within an experimentally relevant range. The concentration excess was probably due to a greater liquid content in the experimental sediment compared to the preliminary test sediment used to determine the wet weight:dry weight ratio.

4.3.3. Copepod Community Effects: Univariate Analyses

4.3.3.1. Combined Age Group Abundance

The mean total (all age groups) copepod abundance in the copper contaminated sediments are shown in figure 4.3. There appears to be little or no effect at the low and medium concentrations in comparison to the control abundance. The high concentration exposure did, however exhibit a marked reduction in numbers, and the mean abundance was significantly different from the control, low and medium level communities, but not from the background samples (table 4.3).

Examination of the effects at the level of individual taxa reveals a more complex pattern of response, with an elevated abundance in either the low or medium treatments common. Figures 4.4, 4.5 and 4.6 show a range of typical responses by three of the most abundant species in the combined treatments. An additional frequent feature for most of the species was the clear reduction in abundance in the high concentration exposure, and this was largely supported by the results of one-way ANOVA performed on the species with sufficient abundance for such analysis (table 4.4). It can be seen that one species, *Cletodes longicaudatus*, achieved a significant abundance increase in the high copper level treatment.

The overall pattern of effect is broadly maintained at the higher taxonomic levels of genera (table 4.5 and figure 4.7) and family (table 4.6 and figure 4.8), with the majority of taxa undergoing a significant abundance drop corresponding to the high copper contamination. However, the Cletodidae (figure 4.8), predominantly species of *Cletodes* (figure 4.7), continue to collectively maintain elevated abundance at the greatest contamination level although this is not statistically significant ($p<0.05$).

When the most abundant individual genera are ranked and grouped by the treatment in which they reached their highest abundance (table 4.7) there is 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 is replaced by *Cletodes*, *Laophonte* and *Stenhelia* in the higher concentration treatments. This trend is largely maintained at the family level (table 4.8) with the highest mean abundance of Ectinosomatidae and Tisbidae found in the control or low treatments, while the higher copper concentrations promoted a peak abundance of the Cletodidae. The diosaccids were less well defined, but tended towards a tolerance of higher copper levels.

The cyclopoids, although constituting an entirely different taxonomic order, are included in table 4.8 since they were a common constituent in all of the samples. Figure 4.9 displays the cyclopoid abundance in all of the treatments and although there is an appearence of an abundance increase there is no statistical evidence to support this.

4.3.3.2. Adult abundance

In order to investigate the copepod community response in greater detail, the abundance of adult and copepodites were examined independently. Figure 4.10 displays the mean abundance of both adult and copepodites in the treatments. It is clear that the overall pattern of effect is different, with the adult component exhibiting an abundance reduction corresponding to the increasing copper, while the copepodite numbers clearly do not conform to a similar pattern. The ANOVA results for the adult abundance confirm a significant difference between the communities subjected to the highest copper level and those in the control, low and background sediments respectively (table 4.9).

Further examination of adult sensitivity is difficult at the species level because of low individual species abundance. Only *Tachidella minuta* adults are present in sufficient numbers to warrant statistical attention, and the chart (figure 4.11) reveals a clear effect at the high copper concentration, which is supported by the ANOVA results (table 4.11). This differs from the results incorporating all age groups in the ability to detect a significant difference between the medium and high concentration exposures.

The number of genera represented by adults are also reduced when compared to the combined age groups. Figure 4.12 displays the genera with the greatest number of adult representatives. A visual comparison with the corresponding combined all age group charts (figure 4.7) does not suggest any major changes in the extent and nature of the community response, except some minor shifts in the peak abundance profiles for *Haloschizopera* and *Halectinosoma*. However, a comparison of the statistical resolution of the adult (table 4.12) and combined age groups (table 4.4) indicates that while the response of *Longipedia* and *Pseudameira* remains the same, the difference between the control and high exposure means are improved for *Haloschizopera*. Conversely, the treatment differences are lost in the *Halectinosoma* and *Stenhelia* adult abundance.

The modification effect of the removal of juvenile individuals is also apparent at the family level. The dominant adult family groups are displayed in figure 4.13. Again there are differences in the abundance profiles when compared to the all age group charts

(figure 4.8). A comparison of the ANOVA results (tables 4.13 and 4.6) again shows changes characterised by both losses and increases in statistical resolution. The detectable response of the Diosaccidae and Ectinosomatidae are reduced (greatly so for the Ectinosomatidae), while there is an increase in resolution (additional detectable difference between the medium and high treatments) for the Tisbidae. The Ameiridae response remained unchanged.

The ranked species community composition for adults is displayed by treatment in table 4.14 and clearly shows a copper-correlated reduction in the dominance of *Tachidiella minuta* replaced by cyclopoid species and *Rhizothrix curvata*. Also of note is the loss of *Longipedia helgolandica* and the appearance of *Cletodes longicaudatus* as a major component of the high concentration exposure community.

4.3.3.3. Copepodite abundance

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 (figure 4.10). 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 the copepodite numbers reached greatly elevated numbers in the low and medium copper concentrations, giving rise to significant differences between these treatments and the high copper exposure (table 4.10).

The proliferation of copepodites in these contaminated sediments does not seem to be confined to particular species. Figure 4.14 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 the copepodite taxa are able to thrive in the low and medium concentrations, and that it is this population increase that largely gives rise to the observed statistically significant effects (table 4.15). Only *Amonardia* and *Longipedia* copepodites display a response that could be said to approach a negatively correlated relationship with copper concentration

(figure 4.14), and thus show a significant population reduction between the control and high metal concentration.

Examination of the differences in copepodite community composition between the treatments (table 4.16) reveals a pattern of modification similar to that exhibited by the adult species (table 4.14), with the dominance of *Tachidiella* in the low concentration sediments replaced by cyclopoids *Cletodes* and *Rhizothrix*.

Of incidental interest is the absence of *Amonardia* species in the background samples (figure 4.14), while maintaining a proportionately high presence in the treatments. This appears to be the only taxon that exhibits a clear attraction for the treatment enclosures.

4.3.3.4. Diversity

Of the suite of diversity indices that were applied to the copepod community it appears that it is only those dependent on the number of taxa present rather than individual abundance that are able to adequately display a visible effect (figure 4.15). The communities subjected to the low copper concentration appear enhanced in terms of number of species and genera and Species Richness, but they are not markedly different from the control communities. However the communities exposed to the high concentration have undergone a clear decline when compared to the controls.

The ANOVA results provide confirmation of these effects, although the level of sensitivity varies for each index (table 4.17). The Shannon-Weiner, Simpson and evenness indices show 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. However, it is the simple enumeration of species and genera that provide the clearest indication of copper impact.

4.3.4. Copepod Community Effects: Multivariate Analyses

4.3.4.1. DECORANA Ordinations

The DECORANA ordinations derived from untransformed data incorporating all age groups (figure 4.16A) appear to exhibit a degree of segregation that results in a general concentration-related trend along axis 1. This pattern is less visible, although largely maintained when the data are Log_e-transformed (figure 4.16B). Rank correlation analysis indicates a statistically significant correlation with copper level along axis 1 in both cases (table 4.18).

Ordinations derived from adult-only abundance fall into a similar pattern (figure 4.17), although the strength of the correlations are significant but reduced (table 4.19).

A clear feature in all of the ordinations, although less well defined by the adult community, is the spatially distinct axis 1 grouping of the background samples. The polarisation is such that an affinity with the control samples is inferred, but there is clearly a component that differentiates both the 1 day and 30 day core-extracted samples from the treatment enclosures.

4.3.4.2. MDS Ordinations

The MDS ordinations are spatially similar and essentially consistent with the DECORANA results. As in the previous chapter the ordinations have been reorientated, where required, to aid comparison.

Figures 4.18 A-D display the results of MDS applied to all age group data. It is clear that the communities that were exposed to the high concentration remain spatially segregated, despite a comprehensive range of data transformations. The maintenance of the integrity of this segregation in the binary (presence/absence) transformation strongly implies that the nature of the community difference is taxon-dependent; thus confirming the conclusions drawn from the univariate diversity analyses. The communities exposed to the lower concentrations are, however, less distinct in their response, although the

medium concentration treatments do appear to exhibit a partial segregation from the more tightly clustered control-low grouping.

When the same analysis is performed with adult-only abundance data (figure 4.19 A-D), the overall response is largely unchanged, although the integrity of the cluster groups is visually reduced.

In both of these sets of ordinations there are clear indications of differences between the non-enclosed sediment as represented by the background cores and the treatment communities. The all-age group ordinations imply a close similarity between the core-extracted assemblages at start of the experiment and those taken 30 days later, but perhaps less so with the control (and low concentration) treatment communities. The adult ordinations, however, suggest that the control and low concentration communities are similar to the temporally equivalent core assemblage, but that there may have been some change over the space of time between the core extractions. In effect, this suggests that if there is an enclosure treatment effect the greatest influence comes from the copepodite component of the community. If the ordinations are repeated with copepodite only abundance data (figure 4.20) the co-segregation of the core samples does indeed appear somewhat tighter.

4.3.4.3. ANOSIM

Statistical examination of the spatial difference of the replicate groups provides another, less subjective, level of detail. The ANOSIM results for the all-age-group data (table 4.20) supports the results obtained by univariate analyses, but does not provide an improved level of sensitivity, in that it is only the communities exposed to the high concentration that are significantly different from the controls and other treatments. This remains true for the adult results (table 4.21) but with an increased sensitivity in the presence/absence-transformed data.

The copepodite results (table 4.22) also indicate the divergence of the high concentration communities, but here there is also evidence, after transformation, that the medium concentration exposure is also modifying the species composition of the

juvenile component, with significant differences when compared against both the control and low exposures. This is the only firm statistical indication of an effect for any of the concentration levels below that of the high concentration.

Significant differences can also be seen between the control and background communities confirming the presence of an 'enclosure effect', although the results do not provide strong enough evidence to support a differential life stage response.

4.3.4.4. SIMPER Analyses

The average Bray Curtis dissimilarities are shown for all age groups in table 4.23 and for adults only in table 4.24. It is clear that in both cases there was a steady decrease in similarity with the controls concurrent with increasing copper level. Accordingly, the high concentration samples consistently obtain the highest dissimilarity values in pairwise comparisons with the other replicate groups over a range of transformations.

The most important species contributing to the group dissimilarities are shown in table 4.25 for adult only and 4.26 for all-age-group data. In general, the differences between the control and treatments were largely due to decreases in abundance of *Tachidiella minuta* in the contaminated sediments accounting for between 5 - 10% of the dissimilarity. Other contributing species that responded in a similar manner include *Longipedia helgolandica*, *L. scotti*, *Amonardia normani* and a number of species of *Halectinosoma*. In contrast, in the low and medium concentrations there were also notable increases in abundance of *Pseudobradya similis*, *Laophonte longicaudata* and *Stenhelia gibba*. The severity of the high concentration effect is reflected in a reduction in relative abundance of all species except that of a single cyclopoid.

4.3.4.5. Discrimination at Higher Taxonomic Level

Figure 4.21 shows MDS ordinations derived from abundance of copepod genera. A comparison between these and the species-level equivalents (figure 4.18) reveals very little difference in the level in the degree and character of the group aggregations.

Indeed, a further comparison between the ANOSIM results (tables 4.27 and 4.20) serves to consolidate this conclusion.

MDS ordinations for family data (figure 4.22) show considerably less resolution, but the underlying dissimilarity of the high concentration samples is retained, even with log-transformation. The ANOSIM results (table 4.28) support a continued statistically significant segregation of the high concentration-exposed communities from the other samples.

4.3.4.6. Multivariate Dispersion

The within-group variability, as represented by the relative dispersion of the replicate groups in the MDS ordinations appears to display a pattern that is related to the level of contamination. Table 4.29 presents the groups in increasing order of within-group variability calculated as Relative Dispersion. A consensus can be seen to emerge across the range of transformations that indicates an increase in dispersion with increasing copper exposure. The transition from lowest to highest dispersion is predominantly:

low < 30 day background < 1 day background < control < medium < high

This relationship is maintained without exception when repeated with adult-only abundance data.

4.3.5. Effects on Other Meiobenthic Taxa

A total of six major taxonomic groups, other than the Copepoda were found in sufficient abundance to justify examination for contamination effects, these were; Foraminifera, Nematoda, Kinorhyncha, Priapulida (larvae), Polychaeta and Halacaridae. All of these groups follow a similar pattern of response to the copepod community, although there are differences in the peak abundance concentration and the scale of the response (figure 4.23). Peak mean treatment abundance was achieved in the low copper concentration by the foraminiferans, nematodes, polychaetes and halacarid mites, while the kinorynchs

and priapulid larvae were markedly more abundant in the medium concentration exposure.

Significant effects (table 4.30) were detected for all but the halacarids, although the polychaete difference was not due to a within-treatment effect, but rather the result of elevated background abundance. All of the differences from the major taxa control communities were attributable to abundance increases in contaminated sediments, but, unlike the copepod response, the highest concentration did not cause a significant reduction in numbers.

4.4. DISCUSSION

An important basic requirement for the success of this experiment was that the integrity of the sediment-copper concentration was maintained throughout the experimental period. The previous field experiment (Chapter 3) demonstrated that a substantial proportion of the metal, after transfer from the water column, remained immobile within the sediment matrix. Similarly, in this study there is no evidence for any appreciable copper transfer to the overlaying water column. Other studies, however, have reported substantial losses by diffusion. Hall and Frid (1995) in a contaminated microcosm experiment with 30% of water replaced at 2-week intervals (silt-clay content comparable to present experiment at about 59%) found that copper was exported from the sediment at the rate of 19.4 $\mu g.m^{-2}.day^{-1}$ over a one-year period. This rate however did not fully reflect the exponential nature of the transfer with a loss of 161 $\mu g.g^{-1}$ of copper in the first week of the experiment.

Similarly Watzin and Roscigno (1997) found that the introduction of zinc-contaminated sediments to field conditions resulted in a concentration reduction in the surface 2 cm of greater than half the original value over a one week 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 may have caused a higher-than-intended copper loading. This indeed was the case since the measured concentration was almost exactly double the intended start concentration after a month in the field. However, if the diffusion rates indicated by the above studies were applied here, the start 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 overlaying water column. It is perhaps 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 $\mu g.g^{-1}$ dry weight of sediment) had clearly given rise to conditions that many taxa were not only able to comfortably tolerate but that actively stimulated an increase in abundance. It appears that the plotting of a generalised taxon response such as the total number of copepods does not wholly reflect the complexities of the underlying pattern of effect on the component species. Within the community there were a few species that exhibited a high degree of sensitivity to relatively low copper concentrations (*Longipedia* spp., *Tachidiella minuta*) and those that appeared highly copper tolerant (*Cletodes* spp. *Stenhelia* spp., Cyclopoida spp.).

These results largely confirm the less clear-cut observations derived from antifoul paint contamination (Chapter 3) where the sediment copper concentration falls between the low and medium concentrations of this study at 431.6 $\mu g.g^{-1}$. Exposure at that level also stimulated a significant increase in abundance, and both *Stenhelia* and the Cletodidae were notable for their insensitivity to the contaminated conditions.

There was however, a divergent response between the two experiments for *Longipedia* species. The indications from the present experiment suggest a greater sensitivity than that demonstrated in the previous study.

This aside, there is clear evidence that the majority of the copepod taxa normally present within uncontaminated sediments are able to survive and even flourish in what might be considered as moderately contaminated conditions. This pattern of intermediate exposure level community enhancement is also maintained for all of the other common meiobenthic taxa, with an associated implication of similar complex within-group community modification.

Detailed analysis of the copepod species data clearly shows 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 that emphasise changes in the number of taxa that most comprehensively defines the shifts in community structure.

This phenomenon of species number increase 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 have been applied to established communities such as those found in rain forest or on coral reefs. In these systems it is suggested that physical disturbance plays an important role in maintaining diversity by preventing competitively dominant species from excluding others, with diversity maximised at an optimum level of disturbance frequency and intensity. At the 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 specialised 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 recreating conditions in which such a diversity increase has been seen to occur, and identifying incidences where 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 these studies the predictions of the intermediate disturbance hypothesis were considered to be 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 to be

equally applicable to the present study. Here, a stable sandy mud community has undergone contamination that may have removed, or reduced the abundance of, copper-sensitive competitively dominant species at the low and medium copper levels. At the 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 level 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 the median contamination levels.

The dynamics of the community restructuring process involved in sediment contamination may be obtained by examining the sample communities' species composition 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 involved a degree of physical disturbance. In addition, one must also the take into account the consequences of community confinement within enclosure structures.

To some extent these issues have been addressed by way of a number of essentially similar approaches. In the present study, an attempt was made to preserve natural community structure, but other studies have documented the recovery of an extreme situation, that of recolonisation of defaunated sediment. Evidence from these experiments tend to suggest that since the test sediments were replaced in the same

location from which they were taken, the community was 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 abundance of suspended communities have been shown to be equivalent (Sibert, 1981) or in excess (Fegley, 1988) of that of the sediment. Chandler and Fleeger (1983) demonstrated that recolonisation by suspension and resettlement was at least as important as the through-sediment route in muddy sediments, and Scheibel (1974) found that both nematodes and copepods were rapid colonisers of azoic sediment 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.

Recolonisation experiments within Loch Creran have been performed by Olafsson (1989) and Olafsson and Moore (1992). In these experiments they noted a disproportionate abundance of *Tachidiella minuta*, *Laophonte longicaudata* and *Pseudobradya similis* in the early stages of colonisation. *T. minuta* clearly appeared to be a successful early coloniser in azoic sediments, but Harries (1995) observed no such dominance in studies on dredging disturbance, again in Loch Creran. In the present study *T. minuta* does achieve dominance, both as copepodites and adults, in the exposure treatments, but it is also present in relatively high abundance in the background communities, such that there is 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 forms the greatest contribution to treatment differences and is thus one of the major copper-mediated effects.

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

Many of the species that contribute to the overall community are epibenthic foragers and it is possible that these species, particularly at the intermediate concentrations are able to gain advantage 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 cyclopoid species achieve overall dominance. This taxon, together with *Amonardia normani* is rarely found in the background samples and appear to have a positive association with the enclosure treatments. However unlike the Cyclopoida, *A. normani*, mostly represented as copepodites, is clearly sensitive to copper at the high concentration.

Most of these taxa probably constitute opportunist species. Warwick *et al* (1988) found that the substantial diversity reduction in response to increasing levels of a mixture of oil and copper was almost entirely due to elevated abundance of *Tisbe* and *Danielssenia typica* and an unidentified diosaccid. They concluded that since opportunistic copepods such as *Tisbe* had higher colonising potential than nematodes, the response was general in nature due to organic enrichment brought about by addition of hydrocarbons and 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) suggests 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' (the circular lip of the enclosure would form a small depression) 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 (1999) during an experiment in which trays of sediment were placed in site close to the location of the present experiment

The high proportional abundance of *Cletodes*, *Stenhelia* and *Rhizothrix*, all endobenthic species, in the high concentration samples, at first sight, tends to rather contradict much of what has previously been proposed. However, *Stenhelia* 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 the 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, while epibenthic species such as *Tachidius*, *Pseudobradya*, and *Microarthridon* were common. Some endobenthic tube-building species, including *Stenhelia*, however, were still present.

The sand-dwelling *Rhizothrix minuta* was found to be comparatively copper-tolerant in the microcosm experiments reported in Chapter 2, 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 colonise (Olafsson and Moore, 1992), is perhaps a good indicator of the maturity of the enclosure communities. This group is though to disperse only by movement within the sediment and is known to be robust, with a thick and heavy cuticle, and this may resist contaminant diffusion into the 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 $\mu g.g^{-1}$ 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 $\mu g.g^{-1}$) were representative of 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 and highlighted the

need for a pragmatic approach to these types of field data. The elevation of species and abundance 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 community attribute detail and the number of treatments. If this experiment had been performed with only the control and high treatments 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 abundance 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 within the species present in the adult community (from which the indices were calculated), it is the fluctuation in the number of taxa which largely defined the treatment differences. Commonly-used indices such as Shannon-Wiener did not provide sufficient discrimination to determine diversity changes even in the communities exposed to the highest concentration.

The abundance patterns of all of the other major taxa were broadly consistent with the copepod result. The Foraminifera, Nematoda, Polychaeta and Halacaridae exhibited clear abundance peaks in the low concentration, while the Kinorhyncha and priapulid larvae appeared less sensitive and had a peak abundance in the medium concentration. Unlike the microcosm experiments with sand communities the muddy sediment nematode communities displayed a diminished effect when compared to the copepods.

Somewhat surprisingly it was the foraminiferan community that displayed the greatest discrimination within the low and medium copper range because both of these treatments resulted in greatly enhanced abundance when compared to the control and high treatments. Foraminiferan abundance was unexpectedly high in the sediment samples at approximately 200-300 per 10 cm^2. This figure should, however, be approached with some caution. Although the Foraminifera have occasionally been

reported to achieve densities of greater than 1000 per 10 cm^2 in fine sediments such as in mud flats, where they are most common, the distinction between specimens that were alive or dead during sampling is notoriously difficult (Gooday, 1988).

In order to distinguish between live individuals and empty tests, samples were stained with Rose Bengal before counting. The staining of the internal protoplasm with Rose Bengal is recognised as a standard method for this taxon. Unfortunately, this method is known to have severe limitations and the reliability of abundance counts derived from its use must be considered questionable. Indeed, the only method considered completely reliable is to carefully observe fresh specimens for signs of life (Arnold, 1974), an option not considered practical for this study

Multivariate ordination of copepod data, in general terms, did not provide any greater detail than species abundance charts, except to indicate a subtle divergence between the treatment and background assemblages (discussed above). The eigenvalues for the DECORANA ordinations were relatively low but there was statistical evidence that at least the first axis was correlated with increasing copper concentration.

ANOSIM tests on adult and all-age-group data did not greatly improve on the discrimination obtained with univariate ANOVA results, mainly reiterating the differences of 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 be largely due to the abundance peaks of copepodites of *Rhizothrix*, *Pseudobradya* and *Stenhelia* at this concentration.

The application of both univariate and multivariate analyses on species data aggregated to higher taxonomic level has been advocated by a number of authors. The benefits of reduced identification effort are particularly attractive for the 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 to species. Warwick (1988) and Warwick *et al* (1990) analysed 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 significant reduction of 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 undertook 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 the other treatments was largely maintained throughout. Indeed, aggregation was beneficial or even necessary in some instances since many individual taxa contributed such low abundance that analysis at lower taxonomic levels was inappropriate. Multivariate analyses applied to the same data gives similar results, with ANOSIM discrimination virtually identical between species, genus and family.

This experiment very much concurs with Somerfield and Clarke (1995) in their belief that in situations where community change due to any form of disturbance is well defined the impact will be discernible at almost all taxonomic levels.

Shifts and disruptions in the taxonomic composition of impacted communities may also act in another way. Warwick and Clarke (1993) drew attention to the increase in variability between replicate samples collected from disturbed sites when compared to undisturbed or control samples. They presented examples where meiobenthic, macrobenthic and coral reef communities were each subjected to one of a range of different types of disturbance. Similarly, Schratzberger and Warwick (1998) found that the variability within replicates taken from a microcosm tended to increase with an increase in the frequency of physical disturbance (shaking) and suggested that this was indicative of community stress.

Warwick and Clarke (1993) noted that replicate variability is reflected in their spatial configuration in two-dimensional MDS ordination, and proposed a comparative measure of the spatial dispersion between selected groups termed the Index of Multivariate Dispersion (IMD). This was further refined by Clark and Warwick (1994) to allow a measure of replicate disimilarity as a relative dispersion value. For the current data, although there is an increase in replicate disimilarity across control, medium and high treatments the existence of an overall trend cannot be confirmed since the relationship is not maintained for the control to low concentration transition, and indeed it seems that the community enhancement effect at the lower intermediate concentration may serve to increase replicate similarity. The nature of the dissimilarities appears to be mainly due to the presence and absence of low abundance species, although the patchiness of transient epibenthic opportunists may also be implicated.

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 cm^3 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 for statistical relevance to be maintained. In this experiment a single sampling time of one month 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 characterised and eliminated in any subsequent studies.

Mesh Size (mm)	ϕ	Samples			
		1d Background	1d Bottle	30d Background	30d Bottle
2.800	-1.50	1.2	2.2	1.4	3.3
2.000	-1.00	2.5	3.2	2.1	3.1
1.400	-0.50	2.6	3.5	3.3	3.9
1.000	0.00	2.6	2.9	3.2	3.2
0.710	0.50	3.2	2.8	3.2	2.9
0.500	1.00	2.8	2.4	2.8	2.5
0.355	1.50	3.0	2.7	3.4	2.4
0.250	2.00	3.2	3.1	3.6	3.1
0.180	2.50	3.7	2.2	2.6	1.9
0.125	3.00	4.8	5.1	5.5	5.0
0.090	3.50	9.0	9.8	10.9	10.5
0.063	4.00	8.4	8.6	9.6	9.6
<0.063	>4.00	53.6	52.1	49.0	49.2

Table 4.1 Percentage composition of size-sorted sediment fractions.

Copper Level	Sediment Copper Concentration ($\mu g.g^{-1}$)	
	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

Table 4.2 Nominal and measured mean sediment copper levels.

	Background	Control	Low	Medium
Background				
Control				
Low				
Medium				
High		✓	✓	✓

Table 4.3 Results of one-way ANOVA comparison between mean copepod abundance in treatment and background samples: all age groups. ✓ denotes a significant difference ($p<0.05$).

Taxon	Treatment With Highest Mean Abundance	Significant Treatment Differences ($p<0.05$)
Pseudameira sp. 1	Medium	Control and Background Control and Medium Low and Background Medium and High High and Background
Heteropsyllus major	Low	None
Cletodes longicaudatus	High	Control and High Low and High Medium and High
Stenhelia gibba	Medium	Medium and High
Amonardia normani	Medium	Control and Background Low and Background Low and High Medium and Background Medium and High
Haloschizopera sp. A	Control	Control and High Low and High
Halectinosoma cooperatum	Control	None
Laophonte longicaudata	Low	None
Normanella incerta	Medium	Medium and High
Rhizothrix curvata	Medium	None
Tachidiella minuta	Low	Control and High Low and High High and Background

Table 4.4 Results of between-treatment ANOVA for copepod species: all copepod age groups.

Genus	Treatment With Highest Mean Abundance	Significant Treatment Differences ($p<0.05$)
Longipedia	Control	Control and High Low and High Medium and High Background and High
Pseudameira	Medium	Low and High Medium and High Background and High
Heteropsyllus	Low	None
Cletodes	High	Control and High Low and High
Stenhelia	Medium	Medium and High
Haloschizopera	Low	Background and High
Ectinosoma	Low	Low and High
Bradya	Low	Low and High
Halectinosoma	Low	Control and High Low and High
Normanella	Medium	Medium and High
Zosime	Low	Background and High

Table 4.5 Results of between-treatment ANOVA for all copepod age groups: genera.

Family	Treatment With Highest Mean Abundance	Significant Treatment Differences ($p<0.05$)
Longipedidae	Control	Control and High Low and High Medium and High Background and High
Ameiridae	Low	Control and High Low and High Medium and High Background and High
Canthocamptidae	Low	None
Cletodidae	High	None
Diosaccidae	Medium	Control and High Low and High Medium and High Background and High
Ectinosomatidae	Low	Control and High Low and High Medium and High
Harpacticidae	Control	None
Laophontidae	Low	None
Normanellidae	Medium	None
Thalestridae	High	None
Tisbidae	Low	Control and High Low and High Background and High

Table 4.6 Results of between-treatment ANOVA for all copepod age groups: family.

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

Table 4.7 The ten most abundant Genera ranked by percentage mean abundance for each treatment: all age groups.

	Background		Control		Low		Medium		High	
Rank	**Family**	**% Mean Abun.**	**Family**	**% Mean Abun.**	**Family**	**% Mean Abun.**	**Family**	**% Mean Abun.**	**Family**	**% Mean Abun.**
1	Ectinosomatidae	20.73	Ectinosomatidae	20.30	Ectinosomatidae	20.35	Diosacchidae	23.92	Cyclopoida	31.15
2	Diosacchidae	19.11	Diosacchidae	19.97	Tisbidae	19.62	Ectinosomatidae	17.13	Cletodidae	14.23
3	Tisbidae	13.01	Tisbidae	15.68	Diosacchidae	19.19	Cyclopoida	11.57	Diosacchidae	11.92
4	Rhizothricidae	10.98	Longipedidae	12.87	Cyclopoida	5.96	Tisbidae	11.27	Ectinosomatidae	11.15
5	Longipedidae	9.55	Cyclopoida	8.09	Longipedidae	5.67	Rhizothricidae	8.64	Rhizothricidae	8.46
6	Ameiridae	6.91	Rhizothricidae	4.29	Rhizothricidae	4.80	Normanellidae	6.02	Laophontidae	8.46
7	Cyclopoida	4.47	Cletodidae	3.96	Laophontidae	4.80	Longipedidae	5.71	Thalestridae	4.62
8	Cletodidae	4.27	Ameiridae	3.80	Ameiridae	4.65	Cletodidae	4.32	Normanellidae	2.69
9	Normanellidae	3.66	Normanellidae	2.81	Normanellidae	3.92	Ameiridae	3.86	Tisbidae	1.92
10	Canthocamptidae	2.24	Thalestridae	2.48	Canthocamptidae	3.05	Laophontidae	2.78	Ameiridae	1.54

Table 4.8 The ten most abundant copepod families* ranked by mean percentage abundance for each treatment: all age groups.

*Abundance of cyclopoids (Order) are also included.

	Copper Level			
	Background	**Control**	**Low**	**Medium**
Background				
Control				
Low				
Medium				
High	✓	✓	✓	

Table 4.9 Results of between-treatment ANOVA for mean combined adult abundance. ✓ denotes significant difference ($p<0.05$).

	Copper Level			
	Background	**Control**	**Low**	**Medium**
Background				
Control				
Low				
Medium				
High			✓	✓

Table 4.10 Results of between-treatment ANOVA for mean combined copepodite abundance. ✓ denotes significant difference ($p<0.05$).

	Copper Level			
	Background	**Control**	**Low**	**Medium**
Background				
Control				
Low				
Medium				
High	✓	✓	✓	✓

Table 4.11 Results of between-treatment ANOVA for adult *Tachidiella minuta*. ✓ denotes significant difference ($p<0.05$).

Genus	Treatment With Highest Mean Abundance	Significant Treatment Differences ($p<0.05$)
Longipedia	Control	Control and High Low and High Medium and High Background and High
Pseudameira	Medium	Low and High Medium and High Background and High
Stenhelia	Medium	None
Haloschizopera	Control	Control and High Background and High
Halectinosoma	Control	None

Table 4.12 Results of between-treatment ANOVA for adult copepods: genera.

Family	Treatment With Highest Mean Abundance	Significant Treatment Differences ($p<0.05$)
Ameiridae	Low	Control and High Low and High Medium and High Background and High
Diosaccidae	Low	Low and High Medium and High Background and High
Ectinosomatidae	Control	None
Tisbidae	Control	Control and High Low and High Medium and High Background and High

Table 4.13 Results of between-treatment ANOVA for adult copepods: family.

	Background		Control		Low		Medium		High	
Rank	**Species**	**% Mean Abun.**	**Species**	**% Mean Abun.**	**Species**	**% Mean Abun.**	**Species**	**% Mean Abun.**	**Species**	**% Mean Abun.**
1	*Tachidiella minuta*	12.15	*Tachidiella minuta*	19.86	*Tachidiella minuta*	21.54	*Tachidiella minuta*	17.39	Cyclopoida sp. 3	16.49
2	*Pseudameira* sp. 1	10.93	*Halectinosoma cooperatum*	11.64	*Rhizothrix curvata*	5.77	*Rhizothrix curvata*	6.96	*Rhizothrix curvata*	10.31
3	*Longipedia helgolandica*	9.72	*Longipedia helgolandica*	6.51	*Haloschizopera* sp. A	5.38	*Pseudameira* sp. 1	6.96	Cyclopoida sp. 1	10.31
4	*Haloschizopera bulbifera*	6.88	*Haloschizopera sarsi*	6.16	*Longipedia helgolandica*	4.62	*Stenhelia gibba*	6.96	*Halectinosoma cooperatum*	9.28
5	*Rhizothrix curvata*	6.48	*Rhizothrix curvata*	4.11	*Amonardia normani*	4.23	*Longipedia helgolandica*	4.78	*Laophonte longicaudata*	9.28
6	*Haloschizopera sarsi*	5.67	*Halectinosoma pygmeum*	3.77	*Halectinosoma angulifrons*	3.85	*Amonardia normani*	4.78	*Cletodes longicaudatus*	4.12
7	*Halectinosoma angulifrons*	4.45	*Halectinosoma angulifrons*	3.42	*Heteropsyllus major*	3.08	*Pseudobradya similis*	4.35	*Pseudameira* sp. 1	3.09
8	*Heteropsyllus major*	4.45	Cyclopoida sp. 1	3.42	*Laophonte longicaudata*	3.08	Cyclopoida sp. 2	4.35	*Stenhelia gibba*	3.09
9	*Stenhelia gibba*	4.45	Cyclopoida sp. 2	3.08	Cyclopoida sp. 1	2.69	Cyclopoida sp. 1	3.91	Cyclopoida sp. 2	3.09
10	*Halectinosoma pygmeum*	4.05	*Longipedia scotti*	3.08	*Pseudameira* sp. 2	2.69	*Halectinosoma angulifrons*	3.04	*Harpactacus obscurus*	3.09

Table 4.14 The ten most abundant adult copepod species ranked by mean percentage abundance for each treatment.

Taxa	Treatment With Highest Mean Abundance	Significant Treatment Differences ($p<0.05$)
Longipedia	Control	Control and High
Cletodes	High	Low and High
Stenhelia	Medium	None
Amonardia	Low	Control and High Low and High Medium and High Control and Background Low and Background Medium and Background
Haloschizopera	Low	None
Bradya	Low	None
Pseudobradya	Medium	Control and Medium Medium and High Low and Background Medium and Background
Halectinosoma	Low	Low and High Background and High
Laophonte	Low	Control and Low
Normanella	Medium	Low and High Medium and High
Rhizothrix	Medium	None
Tachidiella	Low	Low and High

Table 4.15 Results of between-treatment ANOVA for mean copepodite abundance within each of the most important genera.

	Background		Control		Low		Medium		High	
Rank	**Species**	**% Mean Abun**	**Species**	**% Mean Abun**	**Species**	**% Mean Abun**	**Species**	**% Mean Abun**	**Species**	**% Mean Abun**
1	*Halectinosoma*	13.40	*Amonardia*	14.40	*Tachidiella*	18.76	Cyclopoida	13.11	Cyclopoida	32.80
2	*Rhizothrix*	12.77	*Longipedia*	12.53	*Halectinosoma*	12.56	*Amonardia*	10.30	*Cletodes*	15.87
3	*Haloschizopera*	8.41	*Tachidiella*	11.73	*Amonardia*	9.12	*Stenhelia*	8.61	*Rhizothrix*	7.41
4	*Bradya*	8.10	Cyclopoida	10.40	*Ameira*	9.09	*Rhizothrix*	8.05	*Laophonte*	6.88
5	*Tachidiella*	7.17	*Halectinosoma*	7.47	Cyclopoida	6.88	*Normanella*	7.87	*Stenhelia*	5.82
6	*Normanella*	6.54	*Bradya*	6.40	*Normanella*	6.02	*Tachidiella*	7.49	*Halectinosoma*	3.70
7	*Stenhelia*	6.54	*Stenhelia*	5.60	*Bradya*	5.51	*Pseudobradya*	5.81	*Paradactylopodia*	3.70
8	Cyclopoida	6.23	*Normanella*	4.53	*Laophonte*	4.99	*Halectinosoma*	5.43	*Amonardia*	3.17
9	*Zosime*	5.61	*Rhizothrix*	3.73	*Longipedia*	4.30	*Bradya*	5.43	*Normanella*	2.65
10	*Longipedia*	4.98	*Haloschizopera*	3.20	*Haloschizopera*	4.13	*Laophonte*	3.93	*Tachidiella*	2.65

Table 4.16 The ten most abundant copepodite Genera* ranked by mean percentage abundance for each treatment.

*Abundance of cyclopoids (Order) are also included.

Diversity Index	Treatment With Highest Mean Abundance	Significant Treatment Differences ($p<0.05$)
No. of Species	Low	Control and High Low and High
No. of Genera	Low	Control and High Low and High Medium and High
Species Richness	Low	Low and High
Shannon-Wiener Index	Low	None
Evenness	High	None
Simpson Index	High	None

Table 4.17 Results of between-treatment ANOVA for copepod diversity indices.

Axis	Transformation	r_s	Significant Correlation ($p<0.05$)
Axis 1	None	0.764	✓
Axis 2	None	0.206	
Axis 1	Log_e	0.740	✓
Axis2	Log_e	0.030	

Table 4.18 Spearman's Rank Correlation analysis of the relationship between the DECORANA ordination axes and copper level: all age groups (critical value = 0.503).

Axis	Transformation	r_s	Significant Correlation ($p<0.05$)
Axis 1	None	0.576	✓
Axis 2	None	0.655	✓
Axis 1	Log_e	0.618	✓
Axis2	Log_e	0.206	

Table 4.19 Spearman's Rank Correlation analysis of the relationship between the DECORANA ordination axes and copper level: adults only (critical value = 0.503).

Transformation		Treatment				
		Control	Low	Medium	High	1 d Backgr.
None	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓	✓	✓	
	30 d Backgr.	✓	✓	✓	✓	
Square Root	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓	✓	✓	
	30 d Backgr.		✓	✓	✓	✓
4^{th} Root	Low					
	Medium					
	High	✓	✓			
	1 d Backgr.		✓	✓	✓	
	30 d Backgr.		✓	✓	✓	✓
Log_e	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓	✓	✓	
	30 d Backgr.		✓	✓	✓	✓
Presence/absence	Low					
	Medium					
	High					
	1 d Backgr.		✓			
	30 d Backgr.		✓	✓	✓	✓

Table 4.20 ANOSIM results for all age group abundance. ✓ denotes a significant difference ($p<0.05$).

Transformation		Treatment				
		Control	Low	Medium	High	1 d Backgr.
None	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓	✓	✓	
	30 d Backgr.	✓	✓		✓	✓
Square Root	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓	✓	✓	
	30 d Backgr.	✓	✓		✓	✓
4^{th} Root	Low					
	Medium					
	High	✓	✓			
	1 d Backgr.	✓	✓	✓	✓	
	30 d Backgr.		✓	✓		✓
Log_e	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓	✓	✓	
	30 d Backgr.	✓	✓		✓	✓
Presence/absence	Low					
	Medium					
	High	✓	✓			
	1 d Backgr.	✓	✓	✓	✓	
	30 d Backgr.					

Table 4.21 ANOSIM results for adult copepod abundance. ✓ denotes a significant difference ($p<0.05$).

Transformation		Treatment				
		Control	**Low**	**Medium**	**High**	**1 d Backgr.**
None	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓	✓		
	30 d Backgr.	✓	✓	✓	✓	✓
Square Root	Low					
	Medium	✓	✓			
	High	✓	✓	✓		
	1 d Backgr.	✓	✓		✓	
	30 d Backgr.	✓	✓	✓	✓	✓
4^{th} Root	Low					
	Medium		✓			
	High	✓	✓	✓		
	1 d Backgr.		✓		✓	
	30 d Backgr.	✓	✓	✓	✓	✓
Log_e	Low					
	Medium	✓	✓			
	High	✓	✓	✓		
	1 d Backgr.	✓	✓			
	30 d Backgr.	✓	✓	✓	✓	✓
Presence/absence	Low					
	Medium					
	High		✓			
	1 d Backgr.		✓			
	30 d Backgr.	✓	✓		✓	

Table 4.22 ANOSIM results for copepodite abundance. ✓ denotes a significant difference ($p<0.05$).

Transformation		Treatment				
		Control	**Low**	**Medium**	**High**	**1 d Backgr.**
None	Low	45.32				
	Medium	56.09	51.07			
	High	73.17	73.02	69.65		
	1 d Backgr.	62.94	59.26	61.91	73.15	
	30 d Backgr.	54.56	53.14	59.96	73.48	51.32
Square Root	Low	37.23				
	Medium	44.38	41.34			
	High	60.31	59.97	57.85		
	1 d Backgr.	48.48	47.21	48.88	61.61	
	30 d Backgr.	44.00	42.63	48.89	61.19	39.82
Log_e	Low	47.17				
	Medium	55.25	54.00			
	High	71.56	70.79	70.46		
	1 d Backgr.	60.18	56.03	64.44	74.60	
	30 d Backgr.	51.14	49.10	56.66	71.37	54.4

Table 4.23 Average Bray Curtis dissimilarity between copepod communities (all age groups) in replicate groups of treatments and background cores.

Transformation	Treatment					
		Control	Low	Medium	High	1 d Backgr.
None	Low	51.11				
	Medium	63.21	59.92			
	High	79.68	77.60	77.25		
	1 d Backgr.	69.35	63.31	70.35	79.73	
	30 d Backgr.	57.44	55.23	62.83	79.76	60.19
Square Root	Low	46.68				
	Medium	54.86	53.56			
	High	70.83	69.94	69.69		
	1 d Backgr.	59.61	55.47	63.92	73.54	
	30 d Backgr.	51.1	48.93	56.79	70.08	53.95
Log_e	Low	47.17				
	Medium	55.25	54.00			
	High	71.56	70.79	70.56		
	1 d Backgr.	60.18	56.03	64.44	74.60	
	30 d Backgr.	51.14	49.10	56.66	71.37	54.40

Table 4.24 Average Bray Curtis dissimilarity between adult copepod communities in replicate groups of treatments and background cores.

	Control	Low	Medium	High
Low	*Halectinosoma cooperatum* *Stenhelia giesbrechti* *Laophonte longicaudata* *Halectinosoma pygmeum* Cyclopoida sp. 2 *Tachidiella minuta* *Longipedia scotti* *Ectinosoma aff. californicum* *Paradactylopodia* sp. 1 *Stenhelia gibba*			
Medium	*Halectinosoma cooperatum* *Tachidiella minuta* *Haloschizopera sarsi* *Pseudobradya similis* *Longipedia helgolandica* *Pseudameira* sp. 2 Cyclopoida sp. 2 *Longipedia scotti*	*Tachidiella minuta* *Haloschizopera sarsi* *Pseudameira* sp. 2 *Stenhelia gibba* *Ectinosoma aff. californicum* Cyclopoida sp. 2 *Stenhelia giesbrechti* *Pseudobradya similis* *Halectinosoma angulifrons*		
High	*Tachidiella minuta* *Longipedia helgolandica* *Halectinosoma cooperatum* *Haloschizopera sarsi* *Longipedia scotti* *Pseudameira* sp. 2	*Tachidiella minuta* *Longipedia helgolandica* *Haloschizopera sarsi* *Amonardia normani* *Pseudameira* sp. 2 *Stenhelia giesbrechti* *Halectinosoma angulifrons*	*Tachidiella minuta* *Amonardia normani* *Pseudobradya similis* *Stenhelia gibba* *Pseudameira* sp. 1 Cyclopoida sp. 3 *Longipedia helgolandica*	
30 day Backgr.	*Halectinosoma cooperatum* Cyclopoida sp. 1 *Pseudameira* sp. 1 *Haloschizopera bulbifera* *Pseudameira* sp. 2 *Haloschizopera sarsi* *Halectinosoma angulifrons* *Tachidiella minuta*	*Amonardia normani* *Haloschizopera bulbifera* *Pseudameira* sp. 2 *Pseudameira* sp. 1 *Halectinosoma angulifrons* *Tachidiella minuta* *Stenhelia gibba* *Pontopolites typicus* Cyclopoida sp. 1	*Amonardia normani* *Pseudobradya similis* *Longipedia helgolandica* *Haloschizopera bulbifera* *Tachidiella minuta* *Haloschizopera sarsi* Cyclopoida sp. 1 Cyclopoida sp. 2	*Tachidiella minuta* *Longipedia helgolandica* *Pseudameira* sp. 1 *Haloschizopera bulbifera* *Haloschizopera sarsi* *Longipedia scotti*

Table 4.25 Species contributing to the differences between treatments based on SIMPER analysis of square-root-transformed data: adults only. The species listed account for approximately 30% of the overall dissimilarity and are ranked in order of importance of their contribution to the dissimilarity. Underlined species are those which have increased in relative abundance in the treatments represented by the row label.

	Control	Low	Medium	High
Low	*Halectinosoma cooperatum* *Longipedia scotti* *Laophonte longicaudata* *Tachidiella minuta* Cyclopoida sp. 1 *Normanella incerta* *Halectinosoma angulifrons* *Enhydrosoma longifurcatum* *Halectinosoma argyllensis* *Amonardia normani* *Brady scotti*			
Medium	*Tachidiella minuta* *Pseudobradya similis* *Halectinosoma cooperatum* *Stenhelia gibba* *Longipedia scotti* Cyclopoida sp. 2 *Haloschizopera sarsi* *Longipedia helgolandica* *Rhizothrix curvata*	*Tachidiella minuta* *Halectinosoma angulifrons* *Stenhelia gibba* *Haloschizopera sarsi* *Ectinosoma aff. californicum* Cyclopoida sp. 2 *Longipedia helgolandica* Calanioda sp. *Bradya scotti* *Halectinosoma cooperatum*		
High	*Tachidiella minuta* *Amonardia normani* *Longipedia helgolandica* *Longipedia scotti* Cyclopoida sp. 3 *Halectinosoma cooperatum* *Haloschizopera sarsi*	*Tachidiella minuta* *Amonardia normani* *Halectinosoma angulifrons* *Longipedia helgolandica* *Haloschizopera sarsi* Cyclopoida sp. 3 Calanioda sp.	*Amonardia normani* *Pseudobradya similis* *Tachidiella minuta* Cyclopoida sp. 2 Cyclopoida sp. 3 *Stenhelia gibba* *Normanella incerta* Cyclopoida sp. 1	
30 day Backgr.	*Amonardia normani* Cyclopoida sp. 1 *Halectinosoma cooperatum* *Haloschizopera bulbifera* *Longipedia scotti* *Pseudameira* sp. 1 *Rhizothrix curvata* *Tachidiella minuta*	*Amonardia normani* *Tachidiella minuta* *Pseudobradya similis* Cyclopoida sp. 1 *Haloschizopera bulbifera* Calanioda sp. *Halectinosoma angulifrons* *Pseudameira* sp. 1 *Bradya scotti*	*Amonardia normani* *Pseudobradya similis* Cyclopoida sp. 1 *Tachidiella minuta* Cyclopoida sp. 2 *Haloschizopera bulbifera* *Halectinosoma angulifrons* *Haloschizopera sarsi*	*Longipedia helgolandica* *Tachidiella minuta* Cyclopoida sp. 1 *Haloschizopera bulbifera* Cyclopoida sp. 3 *Haloschizopera sarsi* *Halectinosoma angulifrons* *Pseudameira* sp. 1

Table 4.26 Species contributing to the differences between treatments based on SIMPER analysis of square-root-transformed data: all age groups. The species listed account for approximately 30% of the overall dissimilarity and are ranked in order of importance of their contribution to the dissimilarity. Underlined species are those which have increased in relative abundance in the treatments represented by the row label.

Transformation		Treatment				
		Control	Low	Medium	High	1 d Backgr.
None	Low					
	Medium					
	High	✓	✓			
	1 d Backgr.	✓	✓	✓	✓	
	30 d Backgr.		✓	✓	✓	
Square Root	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓	✓	✓	
	30 d Backgr.	✓	✓	✓	✓	
4^{th} Root	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.		✓	✓	✓	
	30 d Backgr.	✓	✓	✓	✓	
Log_e	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓	✓	✓	
	30 d Backgr.	✓	✓	✓	✓	
Presence/absence	Low					
	Medium					
	High					
	1 d Backgr.		✓	✓		
	30 d Backgr.	✓	✓	✓		

Table 4.27 ANOSIM results for genera abundance: all age group data. ✓ denotes a significant difference ($p<0.05$).

Transformation		Treatment				
		Control	Low	Medium	High	1 d Backgr.
None	Low					
	Medium					
	High	✓	✓			
	1 d Backgr.	✓				
	30 d Backgr.					
Square Root	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓		✓	
	30 d Backgr.				✓	
4^{th} Root	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓	✓		
	30 d Backgr.	✓		✓	✓	
Log_e	Low					
	Medium					
	High	✓	✓	✓		
	1 d Backgr.	✓	✓		✓	
	30 d Backgr.				✓	
Presence/absence	Low					
	Medium					
	High	✓				
	1 d Backgr.	✓				
	30 d Backgr.	✓		✓	✓	

Table 4.28 ANOSIM results for family abundance: all age group data. ✓ denotes a significant difference ($p<0.05$).

No Transformation		Square-root		4^{th}-Root		Log_e		Presence/absence	
Group Order	**Relative Dispersion**	**Group Order**	**Relative Dispersion**	**Group Order**	**Relative Dispersion**	**Group Order**	**Relative Dispersion**	**Group Order**	**Relative Dispersion**
Low	0.39	Low	0.35	30 d Bgr.	0.43	Low	0.34	30 d Bgr.	0.43
30 d Bgr.	0.85	30 d Bgr.	0.55	Low	0.53	30 d Bgr.	0.71	Low	0.62
1 d Bgr.	1.05	1 d Bgr.	1.05	1 d Bgr.	0.94	1 d Bgr.	0.95	1 d Bgr.	0.91
Control	1.05	Control	1.07	Control	1.08	Medium	1.05	Control	1.03
Medium	1.14	Medium	1.23	Medium	1.23	Control	1.15	Medium	1.21
High	1.52	High	1.74	High	1.78	High	1.79	High	1.79

Table 4.29 Relative dispersion values derived from MVDISP for all-age group data.

Taxon	Treatment With Highest Mean Abundance	Significant Treatment Differences ($p<0.05$)
Foraminifera	Low	Control and Low Control and Medium Low and High Medium and High
Nematoda	Low	Control and Low
Kinorhyncha	Medium	Control and Medium Control and Background
Priapulid larvae	Medium	Control and Medium Control and Background
Polychaeta	Low	High and Background
Halacaridae	Low	None

Table 4.30 Results of between-treatment ANOVA for major taxa.

1 Control (d)	**2** High (b)	**3** PSA (1 Day)	**4** Background 30 day (c) (CORE)	**5** PSA Background 1 Day (CORE)	**6** Background 1 Day (d) (CORE)
7 Control (c)	**8** Low (c)	**9** Low (d)	**10** Low (a)	**11** Metal Analysis: Low	**12** Background Copper: 1 Day (CORE)
13 Medium (d)	**14** Metal Analysis: High	**15** Background Copper 30 Day (CORE)	**16** Unused	**17** Background 1 Day (b) (CORE)	**18** Metal Analysis: Medium
19 Background 1 Day (a) (CORE)	**20** High (d)	**21** Background 30 day (d) (CORE)	**22** PSA (30 Day)	**23** Medium (b)	**24** High (a)
25 Control (b)	**26** PSA Background 30 Day (CORE)	**27** Background 1 Day (c) (CORE)	**28** Unused	**29** Metal Analysis: Control	**30** Low (b)
31 Medium (a)	**32** Background 30 Day (b) (CORE)	**33** Medium (c)	**34** High (c)	**35** Background 30 Day (a) (CORE)	**36** Control (a)

Figure 4.1 Randomly allocated grid positions of sample bottles and core extraction sites.

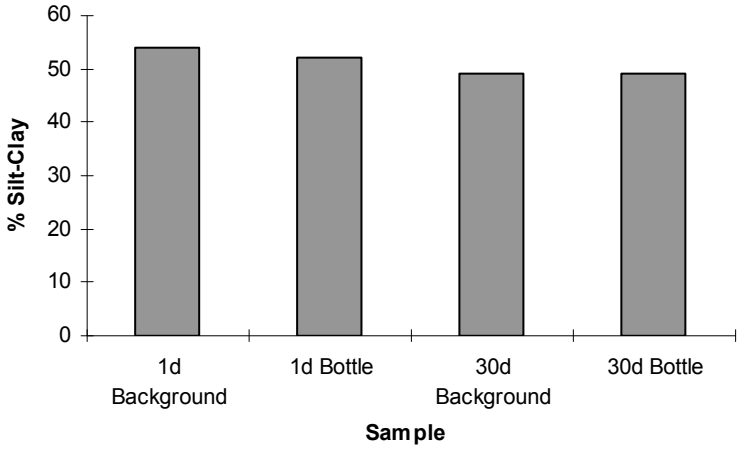


Figure 4.2 Silt-clay composition of the bottle-enclosed and non-enclosed sediment after 1 and 30 days.

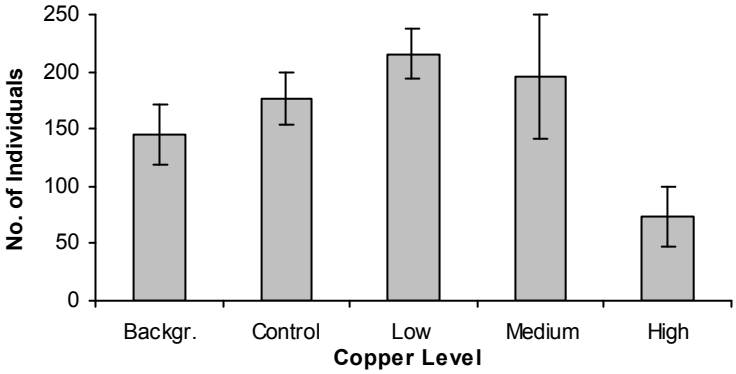


Figure 4.3 Bar chart showing mean abundance of the copepod community (all age groups) in copper treatment bottle enclosures and background samples. Error bars denote standard error.

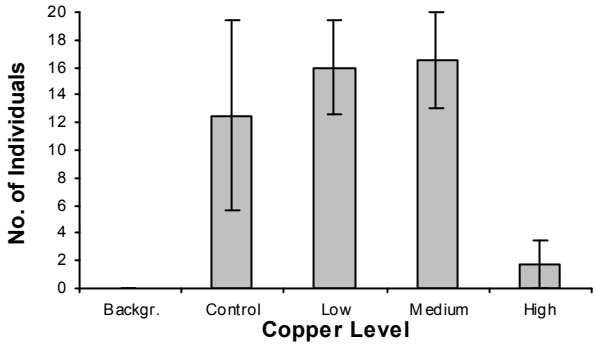


Figure 4.4 Mean abundance of *Amonardia normani* (all age groups) in the enclosure treatments and background cores. Error bars denote standard error.

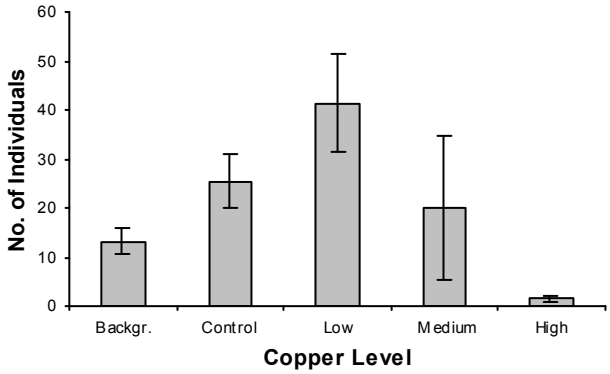


Figure 4.5 Mean abundance of *Tachidiella minuta* (all age groups) in the enclosure treatments and background cores. Error bars denote standard error.

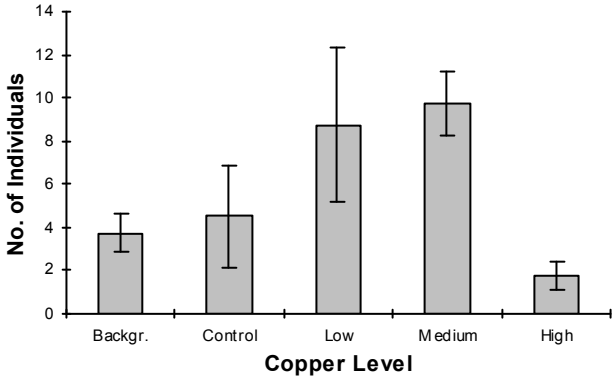


Figure 4.6 Mean abundance of *Normanella incerta* (all age groups) in the enclosure treatments and background cores. Error bars denote standard error.

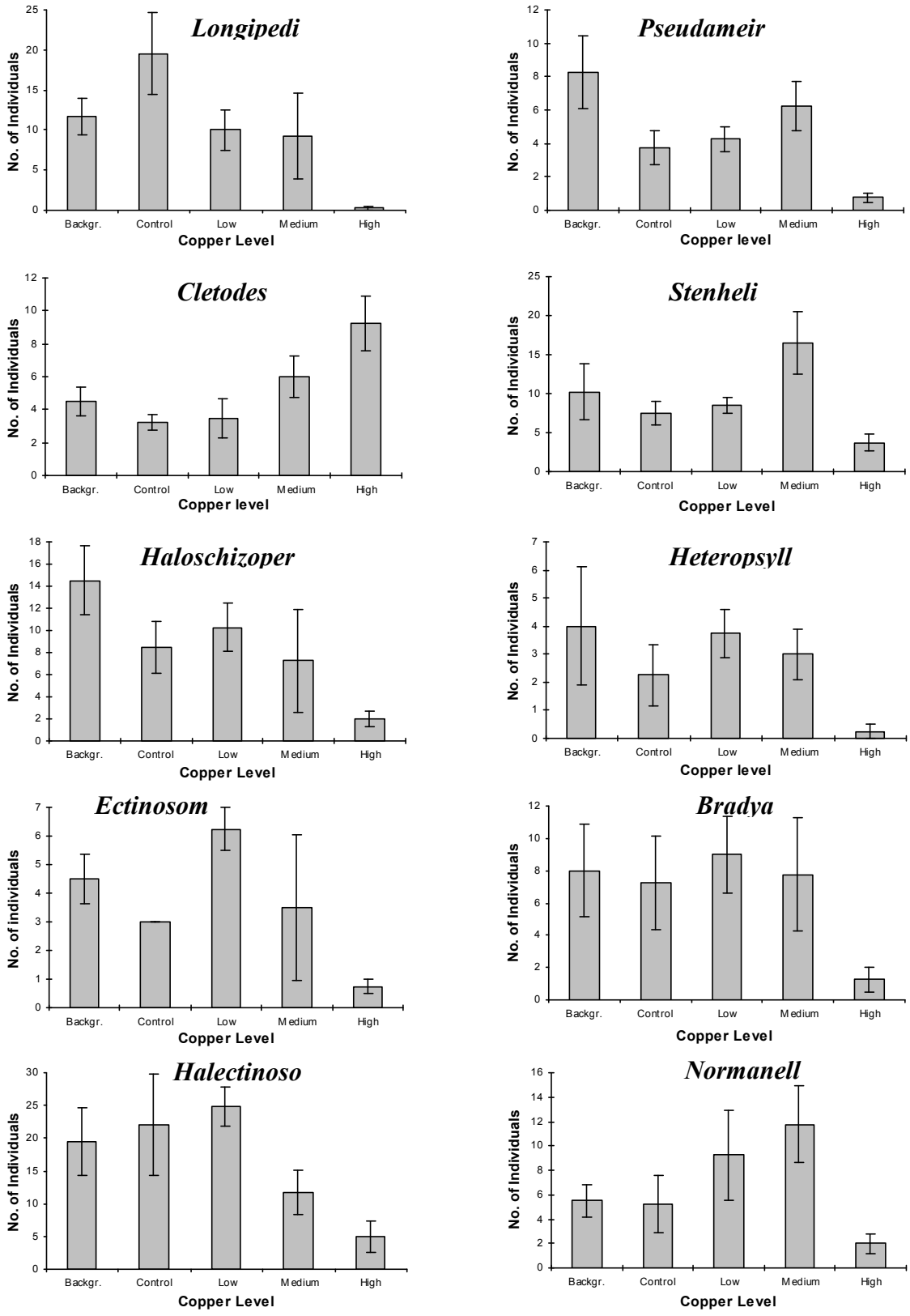


Figure 4.7 Mean abundance of copepods by genera (all age groups) in the enclosure treatments and background cores. Error bars denote standard error.

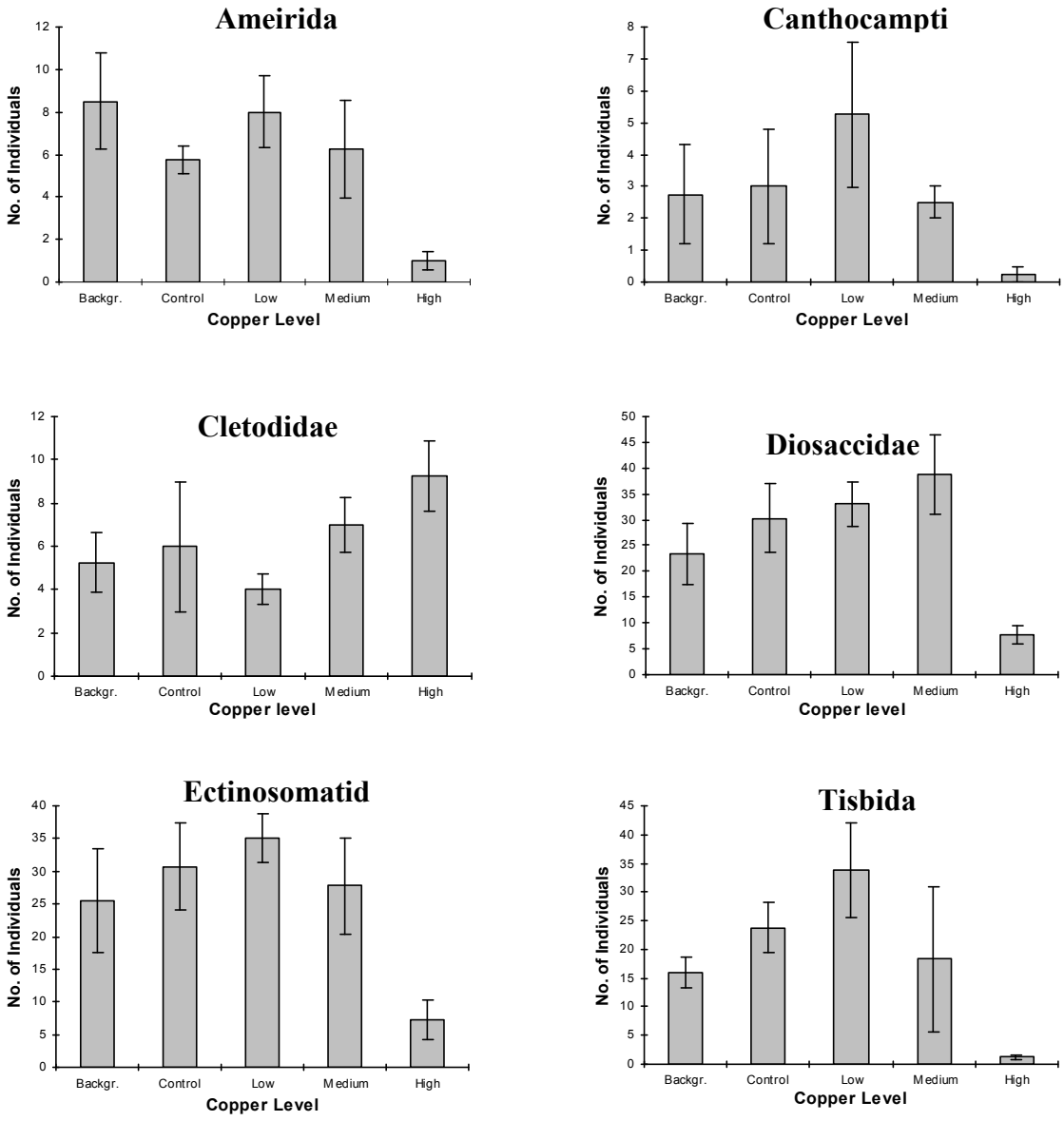


Figure 4.8 Mean abundance of copepods by family (all age groups) in the enclosure treatments and background cores. Error bars denote standard error.

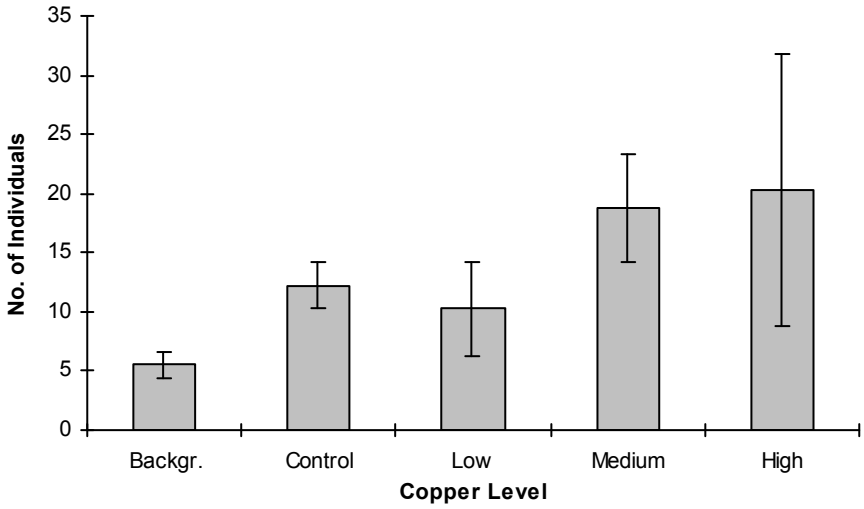


Figure 4.9 Mean abundance of cyclopoid copepods (all age groups) in the enclosure treatments and background cores. Error bars denote standard error.

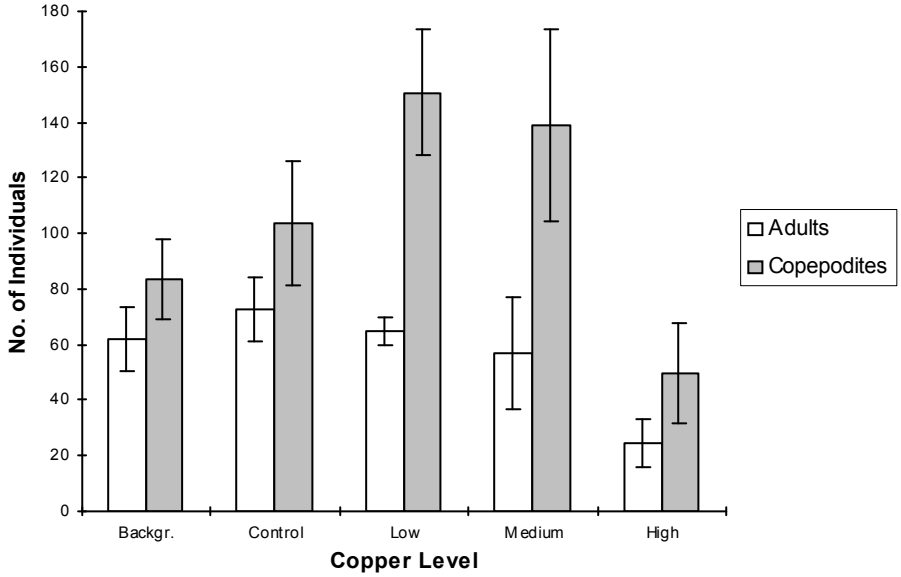


Figure 4.10 Mean abundance of adults and juvenile (copepodite) copepods in the background cores and copper treatment enclosures. Error bars denote standard error.

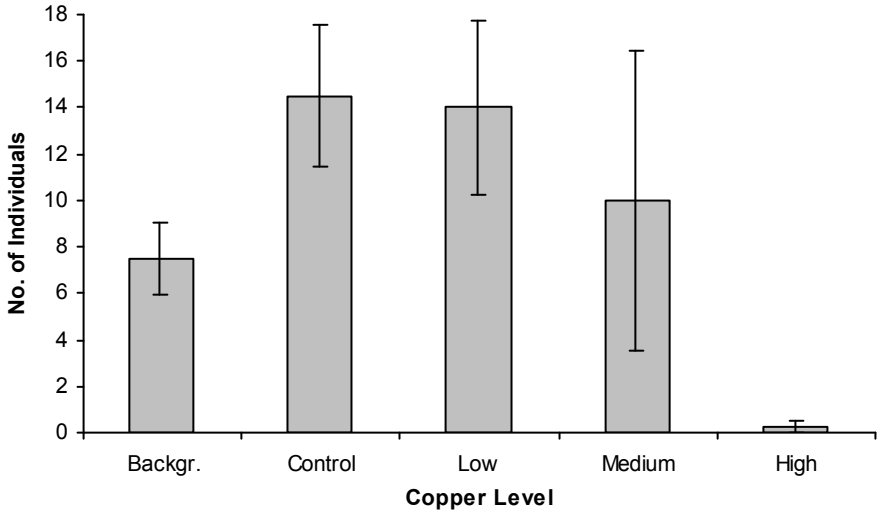


Figure 4.11 Mean adult *Tachidiella minuta* abundance in each treatment. Error bars denote standard error.

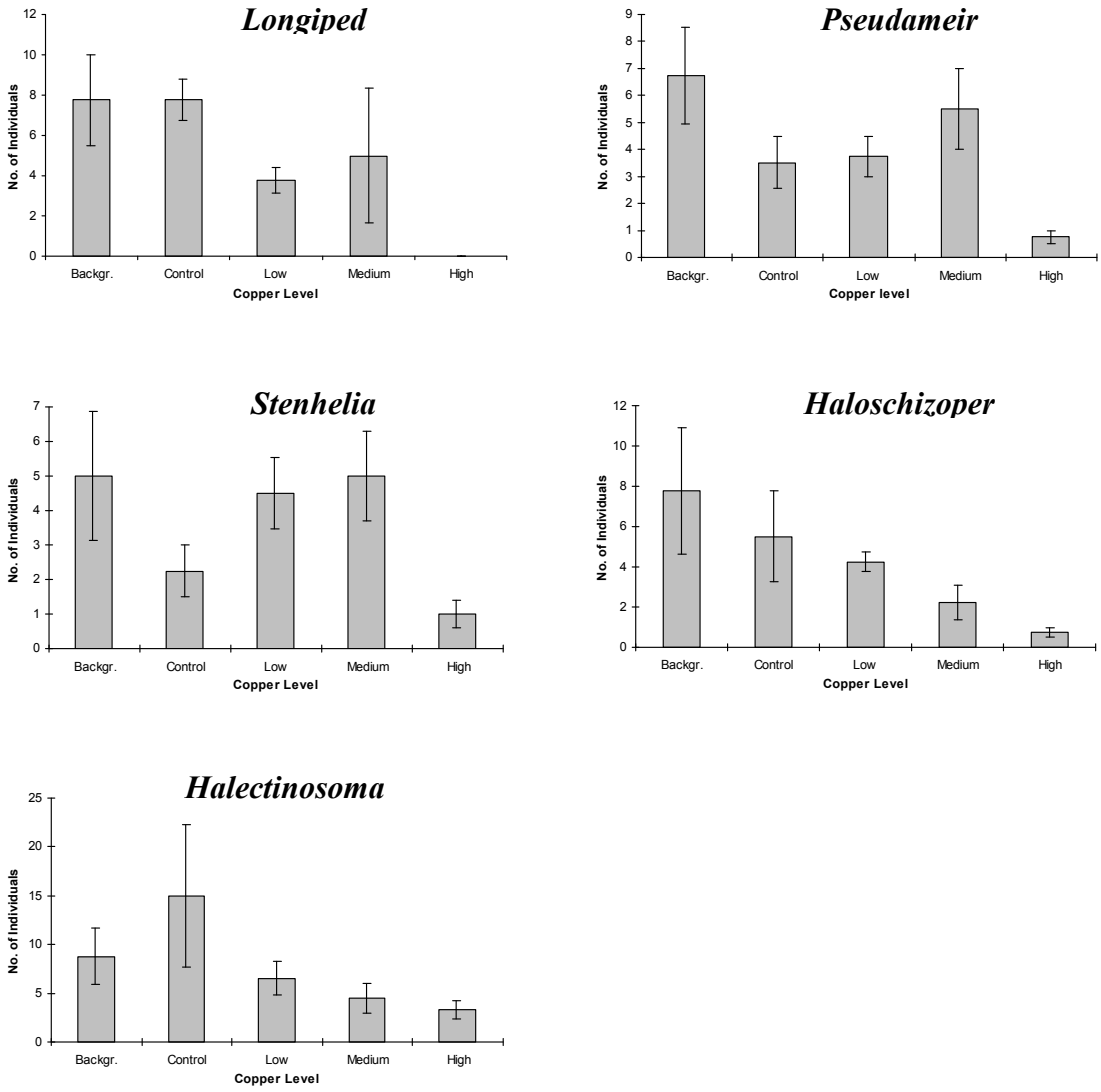


Figure 4.12 Mean adult treatment copepod abundance within dominant genera. Error bars denote standard error.

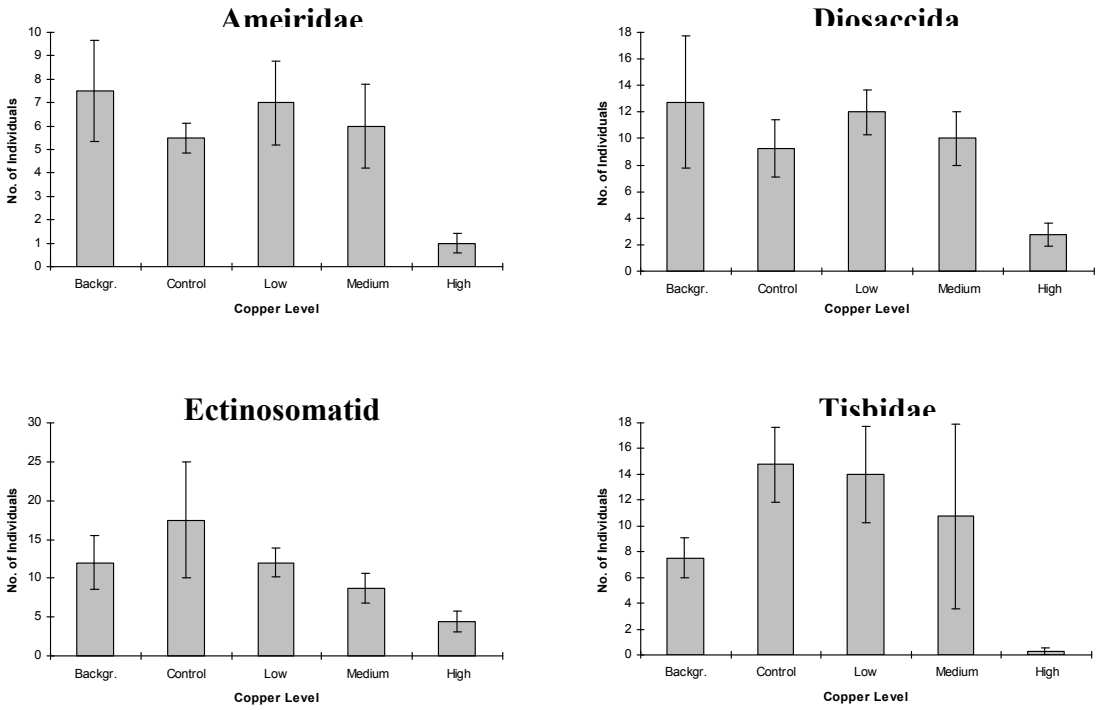


Figure 4.13 Mean treatment adult copepod abundance within dominant families. Error bars denote standard error.

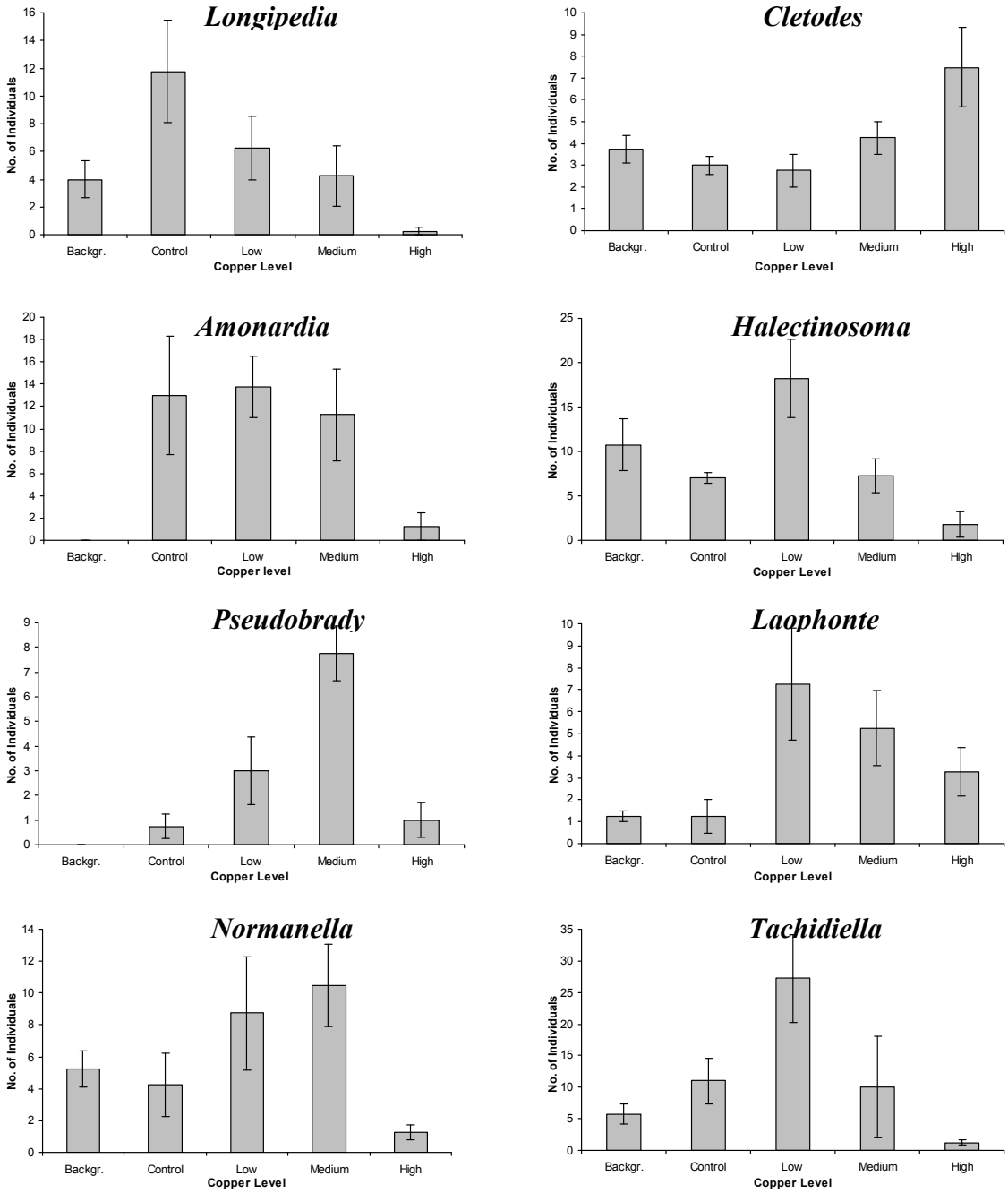


Figure 4.14 Mean copepodite treatment abundance within dominant genera.. Error bars denote standard error.

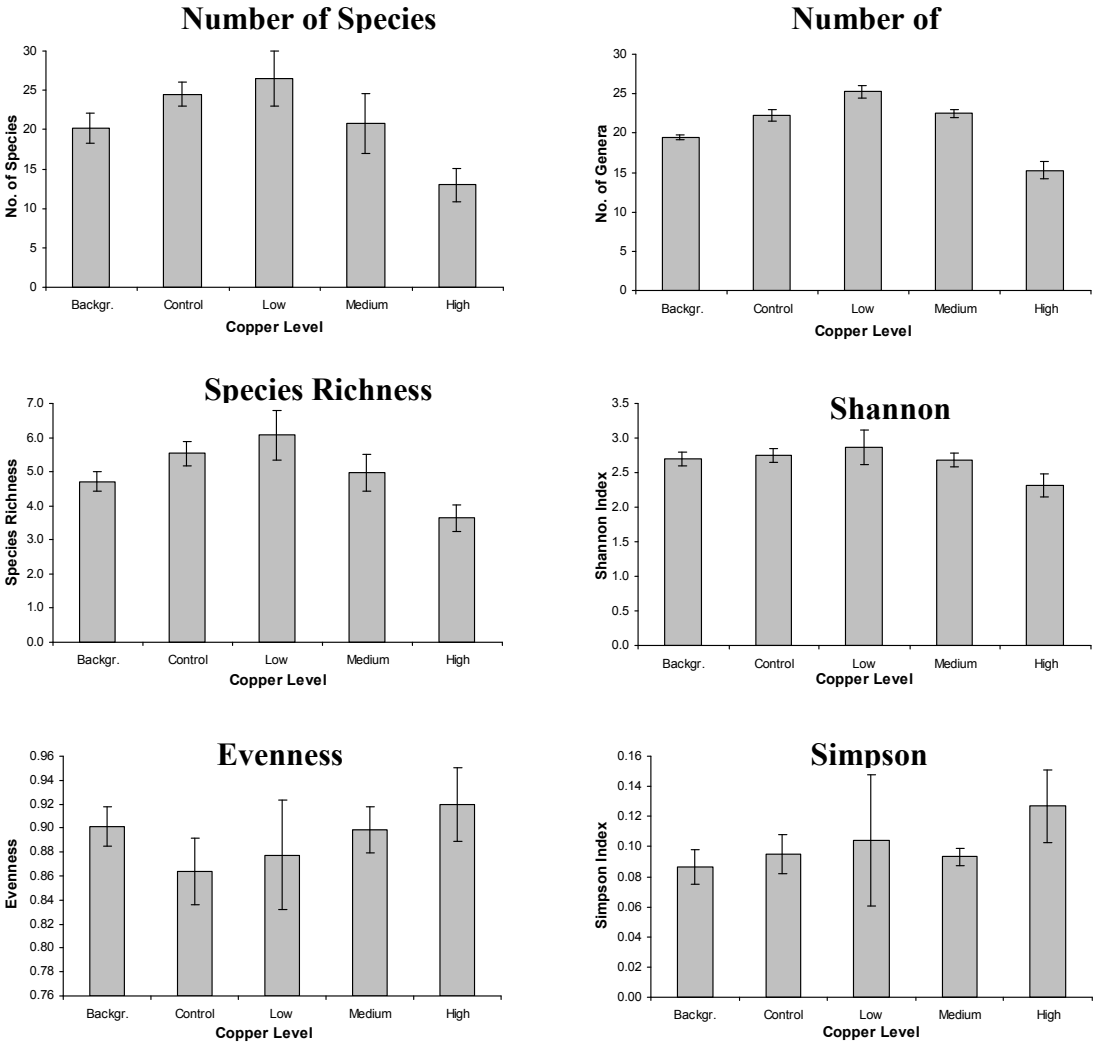


Figure 4.15 Mean copepod community diversity in the background and treatment samples. Error bars denote standard error.

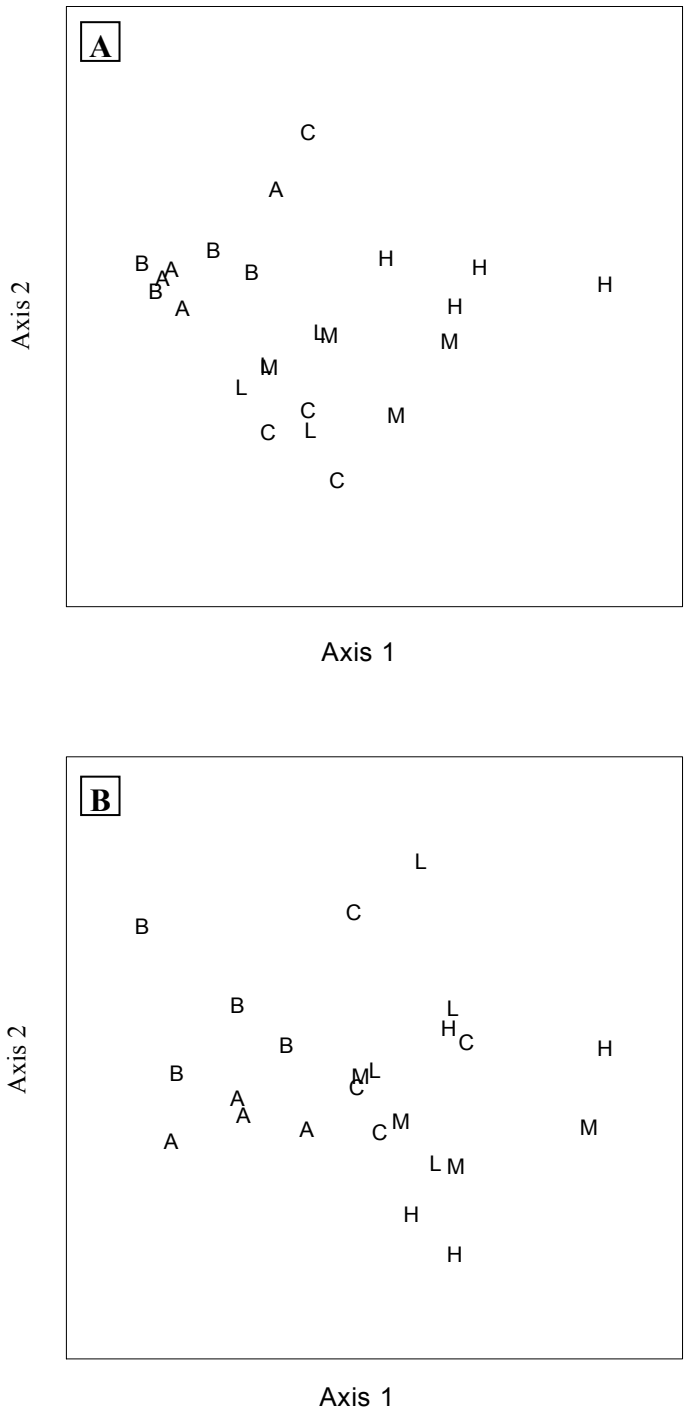


Figure 4.16 DECORANA ordinations of treatment and background samples. A: no transformation, B: Log_e-transformed. Treatment groups are: C = Control; L = Low; M = Medium; H = High. Background cores are: A = 1 day; B = 30 day.

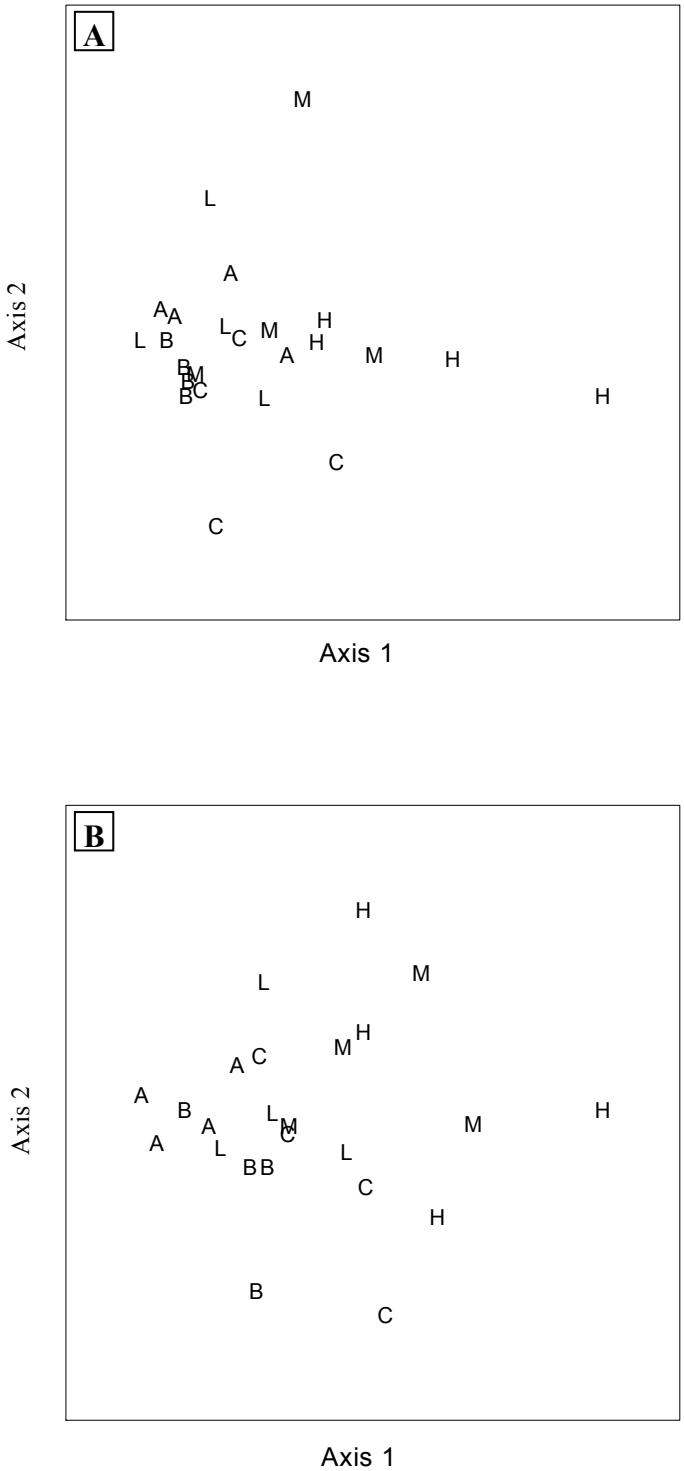


Figure 4.17 DECORANA ordinations of treatment and background samples: adults only. A: no transformation, B: Log_e-transformed.

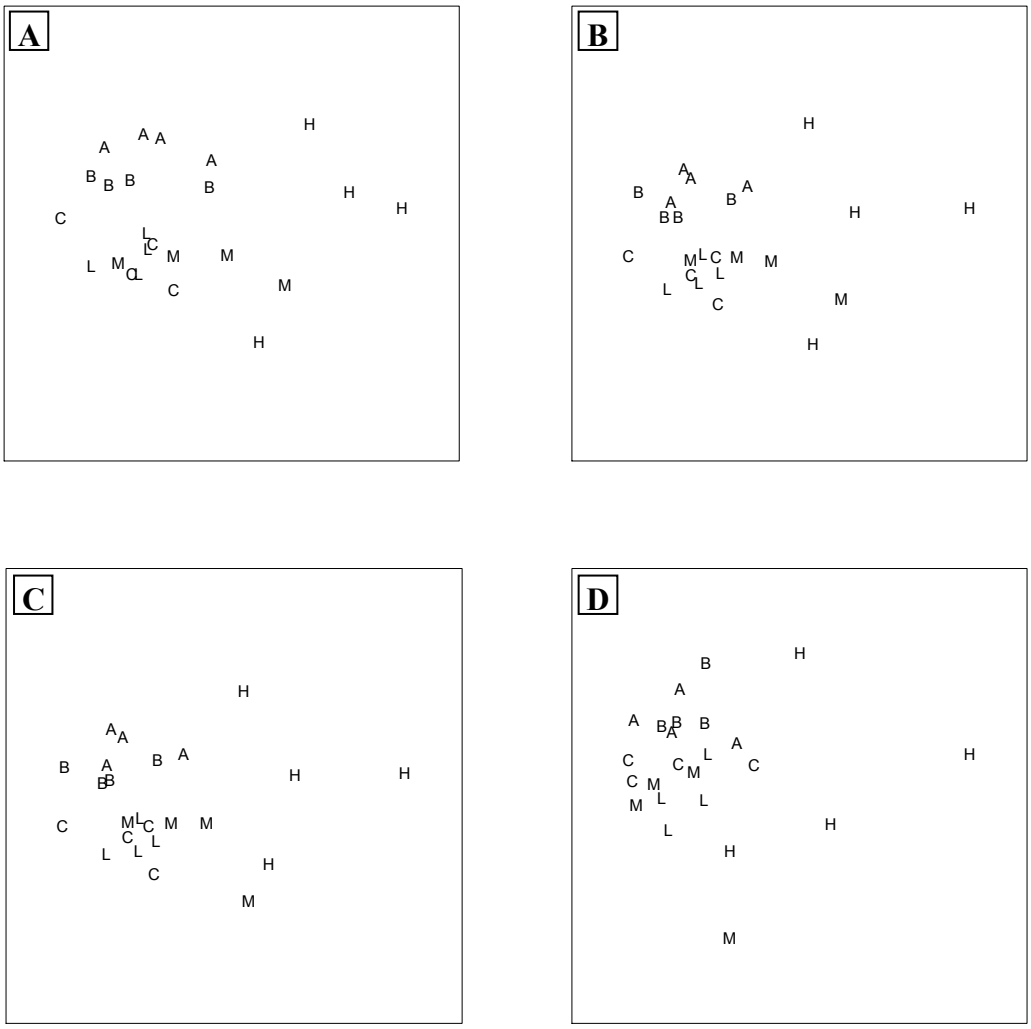


Figure 4.18 MDS ordinations of copepod species (all age groups) from treatment enclosures and background cores. A: non-transformed, B: square root-transformed, C: Log_e-transformed, D: presence/absence. Stress values are 0.15, 0.16, 0.15 and 0.17 respectively.

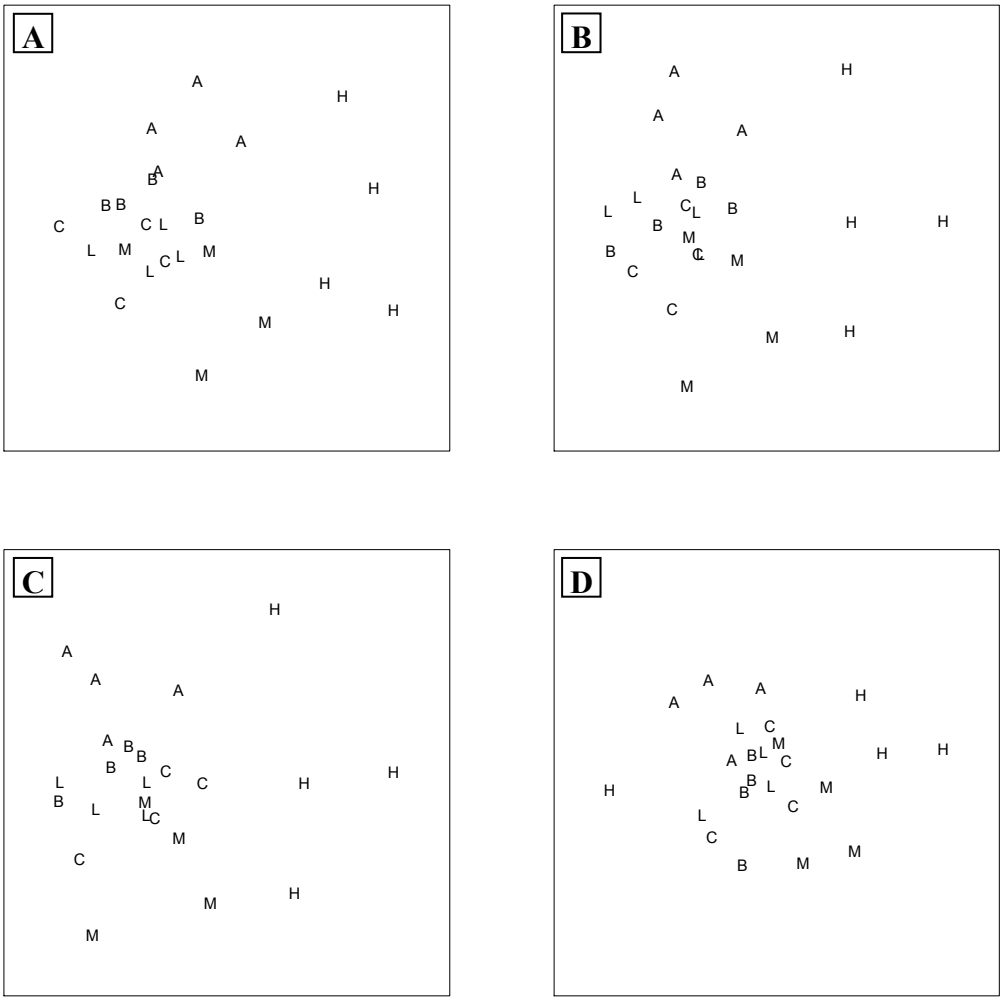


Figure 4.19 MDS ordinations of adult copepod species from treatment enclosures and background cores. A: non-transformed, B: square root-transformed, C: Log_e-transformed, D: presence/absence. Stress values are 0.19, 0.20, 0.20 and 0.22 respectively.

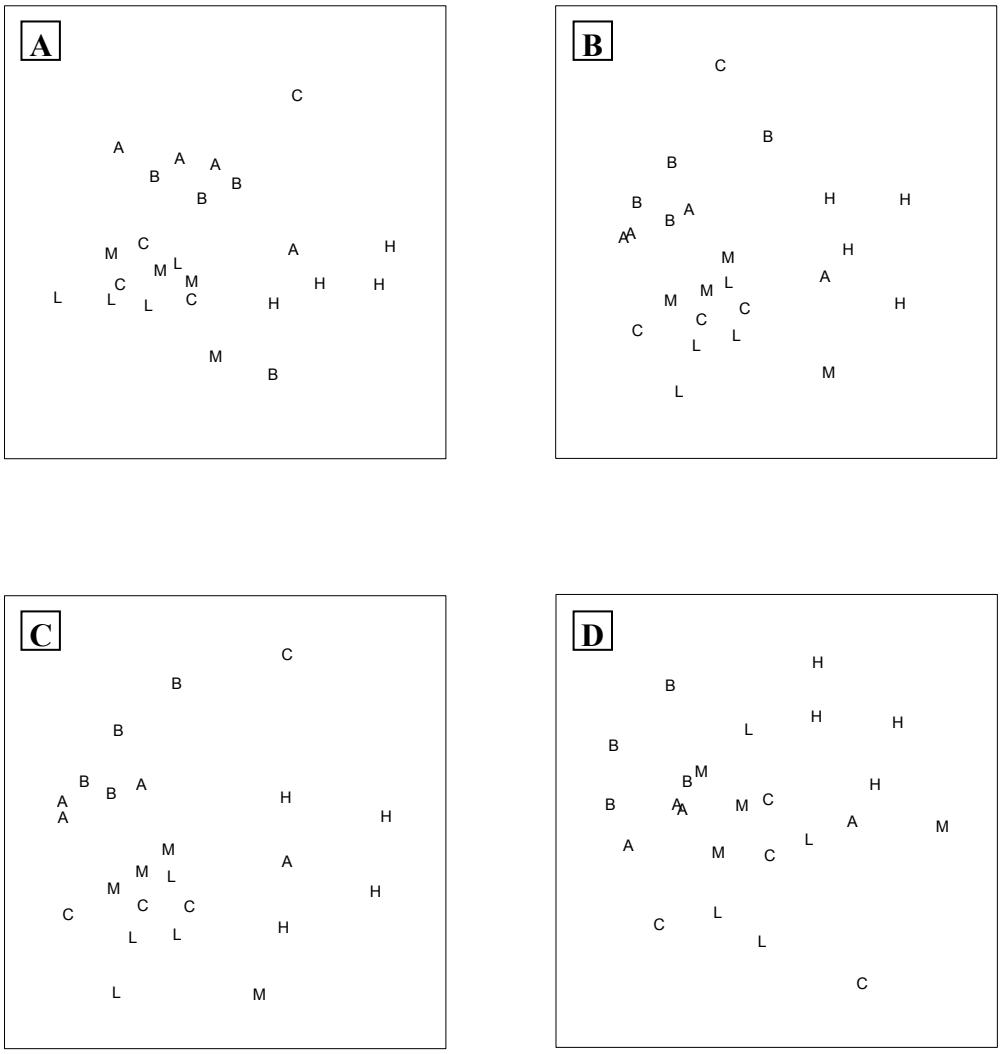


Figure 4.20 MDS ordinations of copepodite species from treatment enclosures and background cores. A: non-transformed, B: square root-transformed, C: Log_e-transformed, D: presence/absence. Stress values are 0.18, 0.19, 0.20 and 0.21 respectively.

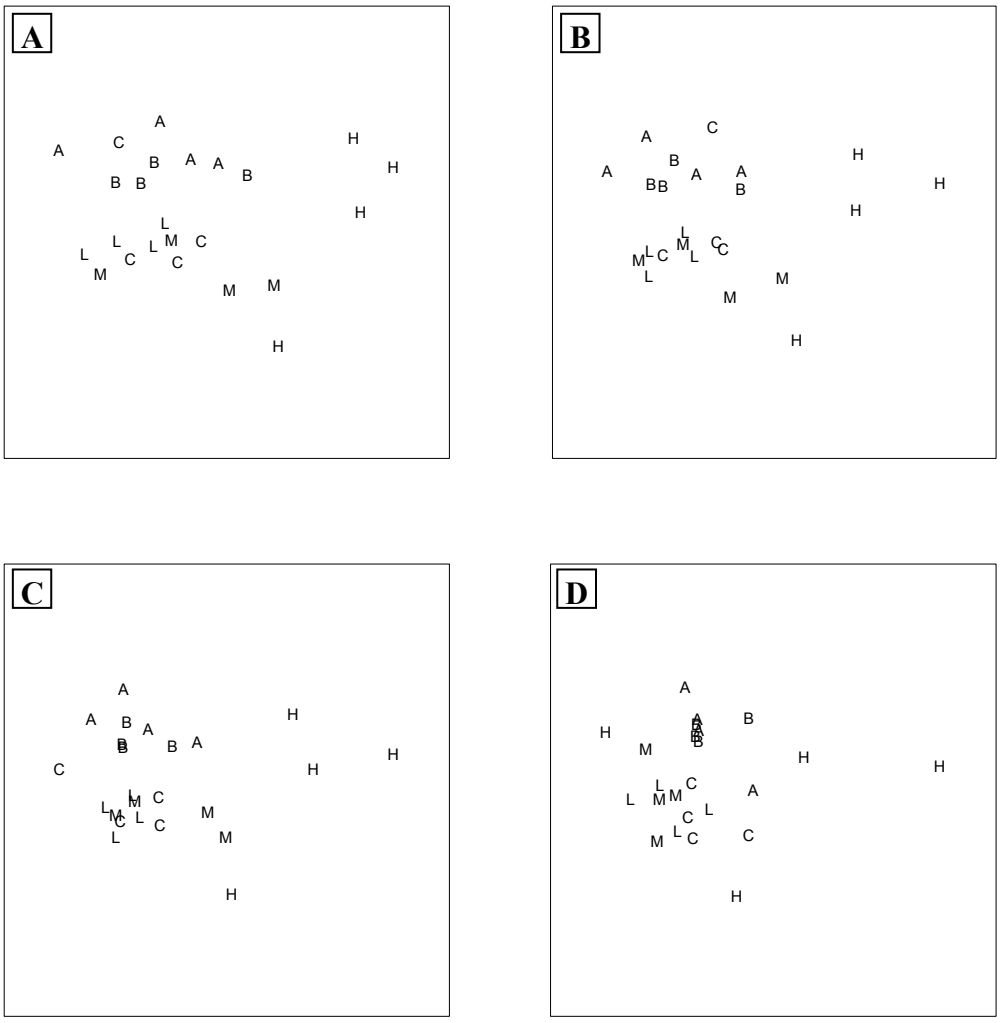


Figure 4.21 MDS ordinations of copepod genera (all age groups) from treatment enclosures and background cores. A: non-transformed, B: square root-transformed, C: Log_e-transformed, D: presence/absence. Stress values are 0.12, 0.15, 0.14 and 0.18 respectively.

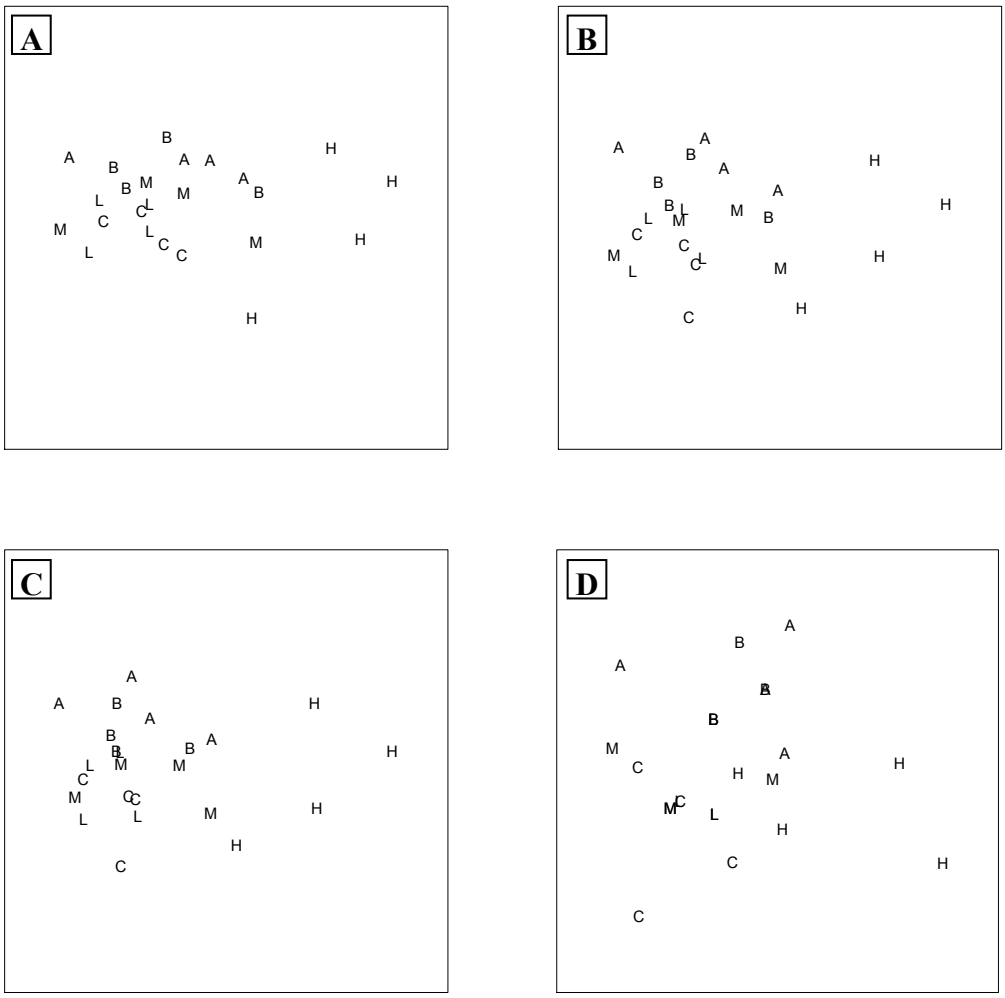


Figure 4.22 MDS ordinations of copepod families (all age groups) from treatment enclosures and background cores. A: non-transformed, B: square root-transformed, C: Log_e-transformed, D: presence/absence. Stress values are 0.12, 0.14, 0.16 and 0.15 respectively.

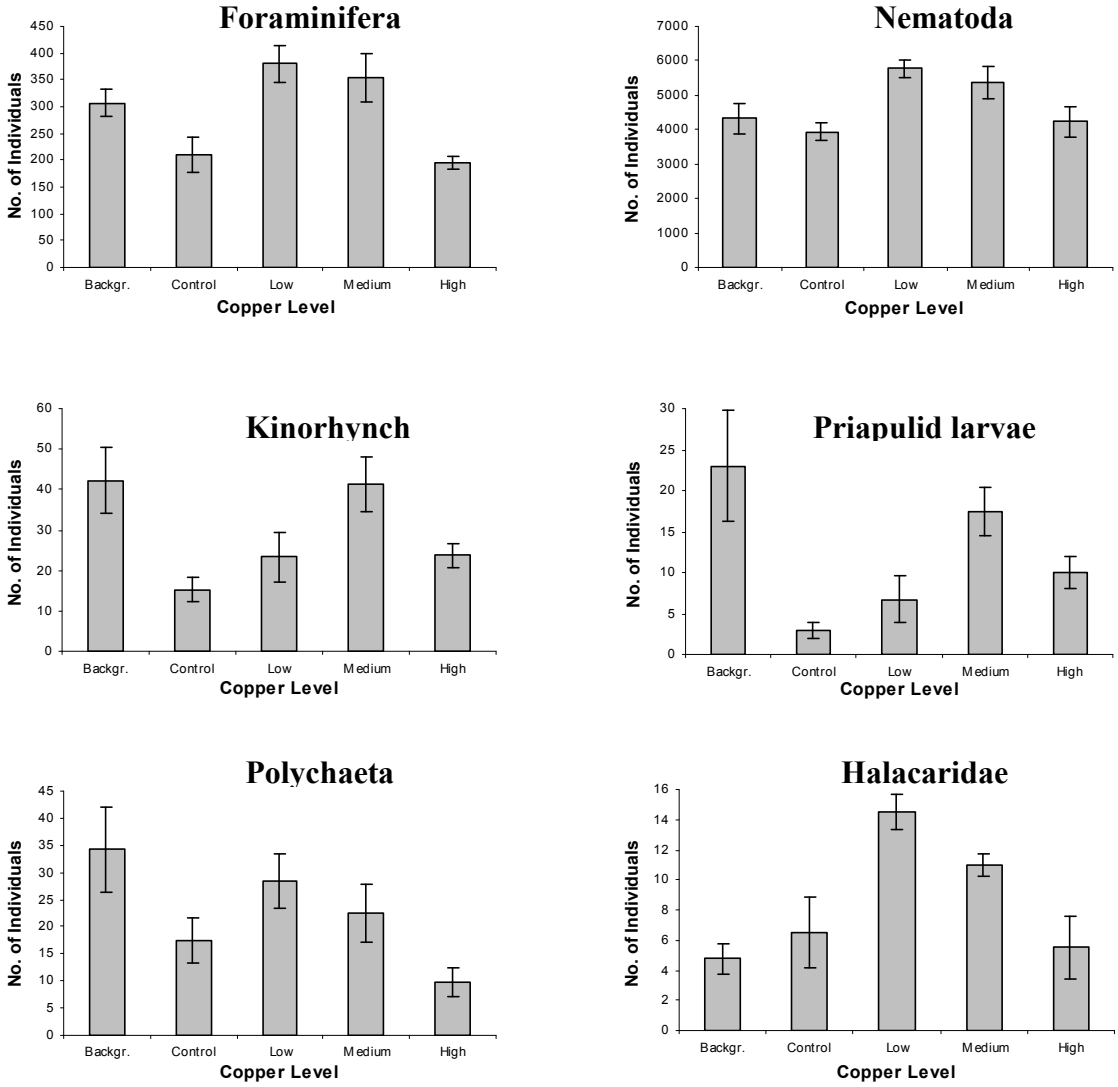


Figure 4.23 Mean abundance of major taxa present in enclosure treatments and background cores. Error bars denote standard error.

CHAPTER 5

FINAL DISCUSSION

5.1. Major Contamination Effects

In both the field and laboratory experiments there was evidence of a taxon-dependent sensitivity range within the meiobenthic copepod community. At the species level this was clearly evident by the persistence and decline of a number of species in the copper contaminated sediments. Strongly insensitive species included *Rhizothrix minuta* in sandy sediments and *R.curvata*, *Stenhelia gibba*, *Cletodes longicaudatus*, *Pseudobradya similis* and a number of cyclopoid species in muddy sediments. In contrast, clear species sensitivity was less evident although *Tachidiella minuta* and *Longipedia* spp. tended to decline with increasing copper.

There have been a number of studies that have attempted to attribute sensitivity or tolerance to particular harpacticoid taxa, although it is those species that tend to achieve numerical dominance in contaminated sediments that have received the most attention. In a review Coull and Chandler (1992) were able to broadly state that *Tisbe* and large epibenthic copepods are often common in polluted areas, and noted that the diosaccid *Bulbamphiascus imus* was particularly associated with organic enrichment. However, specific effects attributable to metal contamination have very rarely been reported, and although community abundance reductions have, on the whole, formed the majority, reports of no effects or abundance increases have not been uncommon. Indeed, Coull and Chandler rightly commented in 1992 that the number of field experiments were so few that it was not surprising that there was no developing paradigm for *in situ* metal toxicity effects. Since their review, little more than ten new field studies have been completed, and although nearly all of them have included an examination of copepod community response they were largely confined to total abundance as part of a broader study. In addition, many of them were attempting to evaluate the impacts across well-established pollution gradients where the presence of non-metallic contaminants and spatial variability of other physical factors have undermined the visualisation of metal-specific effects. It is perhaps, then, not surprising that the statement of Coull and Chandler (1992) still remains largely unchallenged.

Nonetheless, there are some indications that metal-determined community adjustment due to taxon sensitivity or tolerance may take place. Bunker (1999) reported an absence of the copepod families Tisbidae and Ectinosomatidae from within 500 m of a North Sea oil drilling platform, while the family Diosaccidae dominated the community within 300 m of the platform. The dominance was almost exclusively due to three species: *Paramphiascopsis longirostris*, *Bulbamphiascus imus* and *Paramphiascella hyperborea*. However, in field experiments where sediments were experimentally contaminated with crude oil or synthetic drilling muds there was no such effect. This may, perhaps, indicate that the observed effects of platform discharges are largely due to the metal component of the drill cuttings, rather than hydrocarbon input. In a similar study around offshore platforms in the Gulf of Mexico Montagna and Harper (1996) and Peterson *et al* (1996) also concluded that metal-toxicity associated with drill cutting discharge was the main determinant of observed community effects, although it was noted that the use of water-based drilling muds rather than the oil-based type used in the North Sea resulted in a much lower hydrocarbon loading. Interestingly, in this case there was a marked reduction in the abundance and diversity of the ectinosomatid-dominated harpacticoid community, close to the platforms.

From these and other results, Peterson *et al* (1996) argue that a combination of physiological and ecological factors operate at higher taxonomic levels allowing one to generalise when attempting to evaluate disturbance impacts. This is, after all, implicit in the acceptance of the ability to detect effects when taxa are aggregated to higher taxonomic level.

The major taxon-specific effects observed during the present series of studies can be summarised thus:

Longipediidae: The two field experiments gave ambiguous results, but the more controlled enclosure experiment tends to suggest that the copepodites of this epibenthic family are sensitive to relatively low concentrations of copper, while the adults are less so.

Cletodidae: Species of *Cletodes*, prominently *Cletodes longicaudatus*, tended to increase in abundance with increasing copper concentration. The number of adults was generally low, and so this trend was most evident for copepodites.

Diosaccidae: Overall, there was no characteristic or generalised taxon response for this family. *Bulbamphiascus imus*, although present, was only very rarely encountered. Of the most abundant members *Haloschizopera* spp. (particularly the adults) appeared sensitive, with an abundance reduction corresponding to increasing copper, while both *Amonardia normani* and *Stenhelia* spp. displayed a level of tolerance to concentrations of up to 800 $\mu g.g^{-1}$. The ability of *Stenhelia* to tolerate high levels of metals has been reported in at least one other study (Somerfield *et al.*, 1994), and is likely to be due to its ability to build a tube. The insulating structures built by tube-builders are likely to confer a protective advantage for their inhabitants and modification of their architecture may even allow complete isolation from a pollutant. Olsgard (1999) observed that the vertical tubes of a spionid polychaete dominant in copper contaminated sediment were noticeably longer in the highest concentration.

Ectinosomatidae: In the microcosm sand communities, ectinosomatids, principally as *Halectinosoma herdmani* were relatively abundant, However, they were notable in their inability to maintain a steady population in the microcosm conditions. A comparison of the rate of decline in each of the treatments did, though, suggest a possible copper effect. Results from field contamination of muddy communities suggest that for *Halectinosoma* spp. *Ectinosoma* spp. and perhaps *Bradya* spp. there may be limited tolerance to fairly low concentrations, but an abundance decline is seen thereafter. Copepodites may possess a tolerance to higher levels of copper. In contrast, *Pseudobradya* sp. (probably *P. similis*) copepodites were notable for their insensitivity to the relatively high levels of copper.

Rhizothricidae: A relatively high degree of tolerance was exhibited by both *Rhizothrix minuta* in the sand microcosms and *R. curvata* in muddy sediments. In the muddy sediments, their overall abundance, although comparatively low, was maintained across all copper levels, thus causing them to attain minor dominance in the medium and high copper treatments. The reason for the insensitivity of this group may be allied with the

physical (and, perhaps, physiological) similarity with members of the Cletodidae. Indeed, in the past the genus *Rhizothrix* has been included within the Cletodidae (Lang, 1948).

Tisbidae: Representatives of the Tisbidae were only found in the muddy sediments and almost exclusively comprised *Zosime* spp. (predominantly *Z. major*), *Tisbe* spp. and *Tachidiella minuta*. *T. minuta*, commonly found in British sediments, was the only species that attained densities suitable for evaluation. In the second field experiment, *T. minuta* adults dominated in the background and all of the treatment communities except the highest copper concentration where they were almost completely absent. This suggests a fairly high level of tolerance, although a mean abundance drop was evident in the medium concentration. In comparison, the copepodite abundance was greatly elevated in the low concentration but declined substantially in the medium, and was again very low in the high concentration.

5.2. Developmental Sensitivity

A difference between copepodite and adult response appears to be fairly common. Experimental determination of the extent and reason for this was outside the scope of this study, but the overall impression was of a greater metal tolerance in the copepodite stages. At its simplest level this may be just a function of metal accumulation over time, such that by the time adulthood is reached the body burden of copper may cause mortality by direct toxicity or by rendering the individual more susceptible to predation.

However, there was also evidence that *Longipedia* copepodites were more sensitive than adults. Carr *et al* (1996) established that exposure to metal-contaminated pore water extracted from sediments in the vicinity of oil and gas platforms substantially depressed the survival rate of newly-hatched *Longipedia americana* nauplii. Adult Longipedidae are known to be filter-feeders (Nicholls, 1935) and a change in sensitivity may reflect a transition in feeding mode from juvenile to adult. Studies of feeding preferences over entire life-stages are rare, but Hicks and Coull (1983) noted a report where the pelagic nauplii of *Scottolana canadensis* were found to feed on phytoplankton, while the adults adopted a benthic source of nutrition.

5.3. Implications for Temporal Community Changes

Purely for practical reasons both of the field experiments were located at the same site, with the uncontaminated station of the first experiment providing the marker for the second experiment plot. However, although the experiments were carried out at the same time of the year there were considerable differences between the communities, notably the dominance of *Stenhelia gibba* in the first field experiment. This highlights a potential problem outwith that of spatial variability. Temporal variation, either seasonal or between-year, can result in a very different community and thus a potentially different response to a single contaminant in a single sampling site.

Watzin and Roscigno (1997), noted large abundance differences in major meiobenthic groups over a twenty-six month study, and found that this substantially modified the response to zinc contamination, such that there no consistent pattern of response from year to year. They concluded that the species composition within the major groups did not follow precise, repeated, seasonal cycles. Heip (1980) also found large fluctuations in copepod species abundance over a continuous nine-year study. It may, therefore, not always be sufficient for field studies to attempt to observe impacts at the level of total individuals, phyla or class, but instead more emphasis must be placed on the community composition at the species or genus level. This, of course, presents problems when attempting to compare studies from geographically distinct areas. The reality may be that certain species, genera or feeding strategies may be more susceptible to a particular polluting chemical than others, while simultaneously being influenced by naturally fluctuating environmental variables. If this proves to be so then this presents us with the unpalatable possibility that much more needs to be known about the community structure and its dynamics before a valid assessment of an anthropogenic impact can be made. In simple terms, one month may see a community made up of mostly highly susceptible individuals, while the following month all of the taxa may be highly tolerant.

Moreover, this situation is further complicated by the possible differential responses of the naupliar and copepodite stages. Unfortunately, past laboratory experiments have not provided as clear a picture as one would have hoped on the ontogenic effects of metals.

These studies have usually been carried out with rather robust species such as *Tigriopus* or *Tisbe*, and the results have commonly conflicted with that of others, and crucially, have not agreed with the small number of published field experiments. The different seasonal breeding cycles of the constituent species of a community may lead to a rather unpredictable and complex response if there is both a life-stage and species-specific response to contamination.

If there is indeed a life-stage dependent sensitivity to copper then it is, perhaps, important to consider the effects in the context of the timing of the copper release. Given that at least some studies show effects as early as the nauplius (D'Agostino and Finney, 1974; Verriopoulos and Moraitou-Apostolopoulou, 1982; Hutchinson *et al.*, 1994) or even egg production stage (D'Agostino and Finney, 1974; Verriopoulos and Moraitou-Apostolopoulou, 1982), while also suggesting a copepodite insensitivity (Verriopoulos and Moraitou-Apostolopoulou, 1982; Brand *et al.*, 1986), the possibility of a short-term experiment occurring at a favourable or unfavourable time for a particular species seems high. Therefore, this may mean that an identical study, performed at the same location but at a slightly different time, will produce a different community response.

5.4. Which Taxon is Best?

Implicit in much of the literature dealing with meiofaunal pollution response is an argument for and against the virtues of a particular taxonomic group. Unsurprisingly, the emphasis has been placed on the relative merits of either harpacticoid copepods or nematodes because of their numerical dominance, but comparative studies have been unable to arrive at a consensus.

Montagna and Harper (1996) found harpacticoids to be the best indicator of gas platform effects manifested in total abundance, species diversity, population structure and reproductive effort. More gravid females and greater clutch sizes (i.e. greater reproductive effort) were evident near the platform, but fewer copepodites and adults (i.e. reduced recruitment) were present. Conversely, nematode diversity was not

considered a good indicator of contamination but examination of nematode feeding guilds did indicate an increase in non-selective deposit feeders.

On the other hand, Somerfield *et al* (1994) found that nematodes were more responsive to metal concentration than copepods in sediments heavily contaminated by a long-term tin mine discharge. While multivariate ordination of nematode diversity indices and community structure were closely correlated with metal concentrations over several orders of magnitude, copepods showed a detectable response at only at the highest concentration with the absence of endobenthic species.

Peterson (1996) suggested that crustaceans in general seem to be particularly sensitive to metals both as meiofauna (harpacticoids) and macrofauna (amphipods), whilst oligochaetes, polychaetes and nematodes are more sensitive to organic enrichment.

However, such taxonomic generalisations are perhaps not appropriate, at least when considering the effects of metal contamination. In this series of experiments there appears to be a pattern of response that is dependent on the sediment type. In the microcosm sand the nematode community is clearly very sensitive and the abundance response is far greater than that of the copepod community. In the mud assemblages, however, the converse is true with very little deviation from control nematode abundance in the high copper concentration, while there is a significant reduction in copepod abundance.

5.5. Granulometry and Bioavailability

Over the course of this study, a wide range of copper concentrations was applied to two very different sediment types (see Appendix 1 for a summary). Despite the rather different experimental methodology applied to each type it is abundantly clear that granulometry is closely linked to toxicological effect. In the sand, a mean sediment copper concentration of only 56.8 $\mu g.g^{-1}$ produced a nematode abundance decline to less than 50% of the controls in five days, while a concentration of 123.8 $\mu g.g^{-1}$ caused a significant reduction in the copepod community in the same timespan. In contrast, mean treatment concentrations of 91.3 and 893.4 $\mu g.g^{-1}$ had either no effect or appeared to

stimulate abundance *increases* of all of the major meiofaunal groups in muddy sediments.

These results support the observations of other studies, most, if not all of which have been hitherto restricted to nematode communities. Tietjen (1980) found that nematode species diversity in the vicinity of metropolitan New York was repressed in 'silty sands' but not in medium sand. Austen and McEvoy (1997) in their microcosm studies, demonstrated that sand nematode communities were substantially more sensitive to TBT than those from mud or sandy mud, and speculated that the TBT was desorbed from the sandy sediment into the pore water where it may be more toxic. Similarly, Austen *et al* (1994), in another microcosm experiment found that the effect of copper and zinc on nematode communities was substantially greater in sand communities when compared directly against those from mud. Diversity measures, though, were broadly reduced with increasing metal in both sediment types. Increasing copper appeared to produce a drop followed by an increase in the number of species in mud, while similar concentrations in sand produced a significant reduction at all concentrations.

The mechanism for this effect is still the subject of much speculation, with a large body of recent work implicating a complex interaction of organic and inorganic processes both within the sediment and in water column (Boughriet, *et al.*, 1994; Di Toro *et al.*, 1990; Di Toro *et al.*, 1996; Fengler *et al.*, 1994; Fernandes, 1997; Hansen *et al.*, 1996; Hassan *et al.*, 1996; Lee *et al.*, 2000; Liber *et al.*, 1996). In general, estuarine and coastal mud habitats are found in areas of reduced tidal flow where there is an associated continuous settling of detrital and other organic material. This material readily complexes with dissolved metals and the metal-organic complex is deposited in the underlying sediment. In areas such as estuaries this process is further enhanced by particulate flocculation, chemically promoted by an increase in salinity (Libes, 1992).

The vertical structure of the sediment is also a critical component, and further chemical complexation occurs with oxides of iron and the sulphide compounds associated with the reduced oxygen environment of the deeper layer.

All of these processes restrict the bioavailability of the free hydrated form (Cu^{2+}) of copper, the species generally accepted to be the most toxic. In muddy sediments, although there may be a greater input of copper and other metals, the fraction able to induce toxicity is substantially diminished because the majority is particulate-bound and effectively rendered unavailable. Coarser sediments are generally characterised by lower organic content and a much deeper anoxic layer, thus the processes that serve to reduce the toxicity of metals are largely absent.

However, sediment-determined bioavailability may give rise to temporal effects. Hansen *et al* (1996) asserts that the seasonal temperature-dependent anaerobic diagenesis of organic matter and the associated bacterial sulphate reduction causes large fluctuations in the bioavailable state of metals. This essentially implies that the toxicity of a given sediment concentration of a metal may vary temporally at the sediment surface, and the biogeochemistry of a given sediment type may have to be considered when attempting to assess the impact of a toxicant.

5.6. Tolerance

When examining the response of meiobenthic taxa to the effects of copper contamination the species or higher taxonomic groups that have not exhibited detrimental effect with increasing metal concentration have often been referred to as 'tolerant'. However, it is perhaps necessary to examine what is actually meant by this term within the context of this, and indeed, other studies.

The ability of an organism to withstand unnatural levels of chemical disturbance may be due to a range of factors that can ultimately be attributed to one of two categories: (a) simple avoidance or physical insulation from the contaminant, and (b) true physiological or biochemical tolerance.

The acquisition of physiological or biochemical resistance to normally-toxic chemicals is a well-documented occurrence with a growing number of marine examples. Depledge (1990) identified three mechanisms by which tolerance may be achieved: (1) impairment of metal uptake, (2) enhanced excretion of metals following uptake, and (3)

storage of metals in non-toxic forms. In general, it is the processes that involve metal storage or detoxification that has received the most detailed attention in marine invertebrates.

Many metals are important as micronutrients in aquatic organisms and elaborate homeostatic mechanisms have evolved to maintain the optimal internal concentrations while external levels may fluctuate over several orders of magnitude. The transport and metabolism of metals in higher organisms has been widely reported and is known to be dependent on low molecular weight sulphydryl-rich proteins called metallothioneins. Metallothioneins or metallothionein-like proteins have been identified in a range of marine invertebrate taxa including polychaetes, brachyuran crabs (Roesijada, 1980-81) and amphipods (Ritterhoff and Zauke, 1998). Of the meiobenthos, only studies of nematodes have indicated the possible presence of metal-binding proteins (Howell, 1983; Howell and Smith, 1983) or other detoxifying systems such as granule formation (Millward, 1996).

The biochemical mechanisms aside, it is clear that there are grounds for accepting pollution-induced tolerance, in which contaminant exposure stimulates or selects for resistance, which is subsequently transferred to successive generations. This has been demonstrated for both nematodes (Millward and Grant, 1995) and copepods (Miliou *et al.*, 2000). In addition, species that inhabit conditions subjected to naturally fluctuating environmental extremes such as estuaries or rockpools have been shown to be physiologically more robust when subjected to metal contamination (O' Brien *et al.*, 1988; Depledge, 1990).

In the present study the copepod species that were numerically abundant in the contaminated sediments comprised both epibenthic and endobenthic species. Of the epibenthos the most successful were either tube-builders or epibenthic (or semi-planktonic) scavengers, and were thus either isolated from the contaminant, or likely to be translocated *via* the water column. It is only the endobenthic *Cletodes* and *Rhizothrix* that appear to exhibit true physiological tolerance. However, some doubt must remain since we know that in the sand microcosm the copper was concentrated in the surface layer, while in the mud, individuals may have avoided exposure to high levels of

bioavailable metal by migrating away from the oxic horizon. Clearly, future work is needed to determine the effect of metal contamination on the vertical distribution of the meiobenthos.

5.7. Metals and Meiofauna: Ecological Implications

In this study copper was applied to both coarse and fine sediments at concentrations that are relevant to current anthropogenic inputs. Copper is considered to be the most toxic metal to marine organisms with the exception of mercury and silver (Bryan, 1976) and is currently classified as a 'list two' substance under the European Community's dangerous substance directive (Council of European Communities, 1976). Although global marine inputs are declining, the widespread industrial, domestic and maritime use of copper means that it continues to be the most common contaminant in estuarine and coastal waters. Indeed, in areas of heavy boat traffic sedimentary concentrations may even be rising (Claisse and Alzieu, 1993; Stephenson and Leonard, 1994).

Experimental evidence from this and other studies suggests that concentrations currently encountered around British waters are able to modify meiobenthic community structure. Moreover, in the case of sandy sediments even minor inputs may substantially erode a meiofaunal community over the short term.

This study, in common with others, has sought to examine the value of meiofaunal communities in assessing the extent and nature of the effect of a particular anthropogenic contaminant. However, there are other questions that are rarely, if ever asked, such as: should there be concern over the observed pollution impacts on meiofauna? Does it matter if meiofaunal groups are lost to pollution? Clearly, outside of the academic community meiofaunal organisms are never going to command the attention of the larger, more charismatic animals, and it would come as no surprise to know that they have not been considered for, and are never likely to have, any form of conservation status.

However, there is a large and steadily growing list of publications on the widespread utilisation of the meiobenthos as a major food source for macrofaunal and megafaunal

organisms. Coull (1988) commented that since the early 70's over 50 papers have been published documenting the presence of meiofaunal prey in the stomach contents of marine fish and invertebrate predators and concluded that the significance of the meiobenthos in the food chain is beyond doubt. Benthic copepods are overwhelmingly selected over other available prey even though they are rarely the most abundant taxon (Alheit and Scheibel, 1982; Hicks, 1984). In addition, it appears that there may be a preference for particular copepod species, specifically those that are typically surface floc dwellers because they may be easier to catch (Sibert, 1979; Alheit and Scheibel, 1982; Hicks, 1984; Coull, 1988). If, as this study has suggested, metal contamination does substantially modify meiobenthic communities then future work should, perhaps, be directed towards the subsequent impacts on macrobenthic community structure.

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APPENDIX 1
Summary of mean copper concentrations in each experimental treatment

Experiment	Sediment Type	Copper Level	Mean Concentration ($\mu g.g^{-1}$)	Mean Concentration ($\mu mol.g^{-1}$)
Laboratory Microcosm High Copper Range (Chapter 2)	Sand	Control	2.9	0.05
		Low	188.2	2.96
		Medium	1103.6	17.37
		High	1977.4	31.12
Laboratory Microcosm Low Copper Range (Chapter 2)	Sand	Control	3.8	0.06
		Low	56.8	0.89
		Medium	132.8	2.09
		High	214.9	3.38
Field Experiment 1 Antifoul Paint (Chapter 3)	Sandy Mud	Presample - 0m	15.1	0.24
			14.4	0.23
		Presample - 10m	15.3	0.24
		1 day - 0m	13.6	0.21
		1 day - 10 m	17.5	0.28
		10 day - 0m	13.3	0.21
		10 day - 10m	16.9	0.27
		30 day - 0m	13.9	0.22
		30 day - 10m	431.6	6.79
		90 day - 0m	14.4	0.23
		90 day - 10 m		
Field Experiment 2 Enclosure Experiment (Chapter 4)	Sandy Mud	Background (1 day)	16.2	0.25
		Background (30 days)	13.5	0.21
		Control	14.7	0.23
		Low	91.3	1.44
		Medium	893.4	14.06
		High	8662.3	136.32

APPENDIX II
Copepod species abundance matrix for the high copper range microcosm experiment

1 Day Samples

	Copper Level											
	Control			Low			Medium			High		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Longipedia scotti		1										
Longipedia helgolandica												1
Longipedia sp.	1	3	1	1				2				1
Canuella perplexa	2	6	6	6	9	5	1	4	3	5	1	3
Danielssenia typica								1				
Diosacchidae sp.								2				
Halectinosoma herdmani	16	37	28	20	20	18	2	4	8	3	4	7
Halectinosoma pterinum	3	4	4	3	1	1	2	1	3	1		
Halectinosoma sp. F	1		2	1	3							
Halectinosoma sp.	1							1		2		
Asellopsis hispida	3	1	2	2	1	3	1	4		2	1	2
Harpacticus flexus												1
Paraleptastacus espinulatus	14	8	5	5	6	9	8	5	14	12	7	12
Arenocaris bifida	9	6	4	7	7	11	7	1	6	18	8	6
Leptopsyllus sp. 1												1
Rhizothrix minuta	72	58	63	48	13	69	33	51	43	58	59	31
Thompsonula hynaenae	5	1	6	2	3	2	1	4			1	
Zosime sp.			1									

6 Day Samples

	Copper Level											
	Control			Low			Medium			High		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Longipedia scotti											1	
Longipedia sp.	3											
Canuella perplexa	5	2	5	1	1	3	4	2	4	1	6	1
Paramphiascopsis longirostris	1				1	2	1		1	1		2
Pseudobradya sp. 2											1	
Halectinosoma herdmani	28	11	23	9	9	15	3	2	8	4	4	1
Halectinosoma pterinum	1	1	1				2		2	1		1
Halectinosoma sp. F				1								
Halectinosoma sp.	2											1
Asellopsis hispida	2	3	6	2	1	2	1	1	3	2		1
Harpacticus flexus	1							1				
Paraleptastacus espinulatus	2	1	1	4	3	11	6	10	27	9	2	3
Arenocaris bifida		2	5	12	7	10	11	7	21	10	7	5
Leptopsyllus sp. 1							1					
Kliopsyllus constrictus										1		
Rhizothrix minuta	66	53	73	35	43	39	50	77	41	30	50	13
Thompsonula hynaenae	2	1	1	1								
Indet.				1		1						

APPENDIX II continued...

18 Day Samples

	Copper Level											
	Control			Low			Medium			High		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Longipedia sp.			1									
Canuella perplexa	4	2			1			1		1		
Psyllocamptus minutus		1	1									
Cletodes teuipes					1							
Cletodes sp.												1
Paramphiascopsis longirostris						1				1		
Diosacchidae sp.								3	1			
Pseudobradya sp. 1				1								
Halectinosoma herdmani	9	25	10	7	2	2	7	3	1	5	5	5
Halectinosoma pterinum	1		3		1	3	1	1	3	2		1
Halectinosoma sp.												1
Asellopsis hispida	1	1	1	2		1		2	1	2	2	
Paraleptastacus espinulatus	1	1	1	2	6	4	8	3	7	4	3	5
Arenocaris bifida	6	5	4	7	5	6	2	3	7	1	5	3
Kliopsyllus constrictus									1			
Rhizothrix minuta	100	64	94	25	31	31	27	23	26	24	23	12
Thompsonula hyaenae	2		1									

APPENDIX III
Copepod species abundance matrix for the low copper range microcosm experiment

1 Day Samples

	Copper Level											
	Control			Low			Medium			High		
Species	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
Canuella perplexa	2	2	2	4	3	2	1	1	4	2	3	3
Heteropsyllus major sensu Bodin					1							
Danielssenia typica	1			1						1	1	
Halectinosoma herdmani	28	25	19	22	24	12	25	18	5	15	16	17
Halectinosoma pterinum	2	3	3	2	5	6	2	4	2	3	3	3
Halectinosoma sp. F	1		1	1			1	2			1	
Asellopsis hispida			2		1	1	2	2	2	2		2
Harpacticus flexus					1				1			
Leptasticidae sp.						1						
Paraleptastacus espinulatus	8	1	2	2	3	7	3	2	5	4	2	3
Arenocaris bifida	16	12	12	20	9	14	19	9	15	15	8	13
Leptopsyllus sp. 1			1				2					
Kliopsyllus constrictus	1			3	1					1		
Rhizothrix minuta	156	144	152	153	93	99	154	169	134	170	175	172
Rhizothrix reducta										1		
Thompsonula hyaenae	1		1	1		1	1					

5 Day Samples

	Copper Level											
	Control			Low			Medium			High		
	R1	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
Canuella perplexa		3	2	3	6	4	2	3	3	4	5	2
Danielssenia typica						1	1	1	1			
Halectinosoma herdmani	7	13	10	7	3	2	5	4	8	6	8	10
Halectinosoma pterinum	2	6	5	1		3		3	1	3		1
Halectinosoma sp. F		1		2				1				1
Asellopsis hispida		1	1	1	2	4	1			2	4	1
Laophonte longicaudata							1					
Paraleptastacus espinulatus	4			5	2	3	2	4	3	4	2	5
Arenocaris bifida	7	7	1	24	13	13	8	11	11	17	9	12
Kliopsyllus constrictus									1			
Rhizothrix minuta	195	176	91	80	96	161	132	125	164	189	136	110
Thompsonula hyaenae		1			1					1		
Indet.					1							

APPENDIX III continued...

20 Day Samples

	Copper Level											
	Control			Low			Medium			High		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Canuella perplexa	3	3	1	4	1	1					2	
Heteropsyllus major sensu Bodin								1				
Cletodes sp.		1										
Enhydrosoma propinqvum									1			
Halectinosoma herdmani	1	15	2	1	2		2	2	1		1	2
Halectinosoma pterinum	1	2			1	2			3		1	
Halectinosoma sp. F			1							1		1
Asellopsis hispida	2			1		1	2	3		1		
Paraleptastacus espinulatus	3	1		1	8	2		2	4	2	6	3
Arenocaris bifida	2	8	6	7	12	5	3	4		4	9	5
Kliopsyllus constrictus					1	1					1	
Rhizothrix minuta	121	177	151	59	106	71	48	60	76	33	65	56
Thompsonula hyaenae		1	1									
Indet.									1			2

APPENDIX IV

Copepod species abundance matrix for the antifoul field experiment

Column heading letters are as follows: A; adult, C; copepodite, T; total (all age group) abundance.

1 Day Samples

	0 m Station									10 m Station								
	Repl. 1			Repl. 2			Repl. 3			Repl. 1			Repl. 2			Repl.3		
	A	C	T	A	C	T	A	C	T	A	C	T	A	C	T	A	C	T
Longipedia coronata			0			0			0			0			0	2		2
Longipedia helgolandica			0			0			0			0	2		2	2		2
Longipedia minor			0	1		1			0			0			0			0
Longipedia scotti	1		1	1		1			0			0	1		1	2		2
Longipedia spp.		2	2		2	2		6	6		2	2		3	3		8	8
Pseudameira sp. 1	7		7	8	3	11	14	1	15	4	1	5	8		8	6	2	8
Pseudameira sp. 2			0			0			0		2	2	1		1	1		1
Pseudameira sp. 3	1		1			0			0			0			0			0
Sarsameira longiremis			0			0			0			0			0			0
Sarsameira parva	1		1			0	1		1	1	1	2			0			0
Sarsameira sp. 1			0			0			0			0			0			0
Sarsameira sp.			0			0			0			0			0			0
Proameira sp. 1	3		3	1		1	1		1			0			0			0
Eurycletodes latus			0			0			0			0			0			0
Mesochra pygmaea			0			0			0			0	1		1			0
Bryocamptus echinatus	1		1			0			0			0			0			0
Heteropsyllus major	1	5	6	5	3	8	5	5	10	3	1	4	5	1	6	3	2	5
Heteropsyllus major sensu bodin	1		1	1		1	2		2			0			0			0
Cletodes limicola			0			0			0			0			0			0
Cletodes longicaudatus			0			0	2	1	3			0	1	1	2	1	9	10
Cletodes tenuipes	1	3	4		3	3	1	2	3			0		5	5		2	2
Cletodes smirnovi			0			0			0			0			0			0
Cletodes spp.			0			0		2	2			0			0			0
Enhydrosoma curvirostre		1	1		1	1			0			0			0			0
Enhydrosoma longifurcatum			0	1	3	4			0		1	1	1		1		3	3
Enhydrosoma sarsi	1	3	4			0			0			0			0			0
Enhydrosoma M sp. A	1		1			0			0			0			0	1		1
Enhydrosoma spp.			0			0		6	6		2	2		7	7			0
Stylicletodes logicaudatus		1	1			0			0			0			0			0
Cletodidae spp.		2	2			0		2	2			0			0			0
Danielssenia typica			0			0			0			0			0	1		1
Stenhelia gibba	83	76	159	58	97	155	72	99	171	27	36	63	34	40	74	58	54	112
Stenhelia giesbrechti	2	1	3	3		3	2	2	4	1	4	5	2	1	3		2	2
Stenhelia hanstromi			0			0			0			0			0			0
Stenhelia mastigochaeta			0			0		2	2			0	1	1	2		1	1
Stenhelia normani			0			0			0			0			0	1		1
Stenhelia reflexa	1	2	3	1	2	3	1	3	4		4	4		1	1		1	1
Stenhelia spp.		1	1		5	5		8	8			0		1	1		1	1
Amphiascus minutus			0			0			0			0			0			0
Amonardia normani			0			0			0			0			0			0
Bulbamphiascus imus			0			0			0			0			0	2		2
Paramphiascella hyperborea	1	1	2		1	1		3	3	1	5	6	1	2	3	1	4	5
Haloschizopera bulbifera	1		1	1		1			0	1		1	2		2	1		1
Haloschizopera lionensis			0			0			0			0			0			0
Haloschizopera sp. *A*	6		6	7		7	8	1	9	3		3	6		6	8		8
Haloschizopera scotti			0			0			0			0			0			0

Haloschizopera tenuipes			0			0			0			0			0			0
Haloschizopera sp. 1			0	1	1	2			0			0			0			0
Haloschizopera sp.		5	5		6	6		12	12		2	2		4	4		3	3
Ectinosoma sp. 2			0			0			0			0	1	2	3			0
Ectinosoma M. sp. A			0			0			0			0			0			0
Ectinosoma aff. *breviarticulatum*			0			0			0	1		1			0			0
Ectinosoma aff. *californicum*			0			0	1		1			0			0			0
Ectinosoma spp.			0			0			0		1	1			0			0
Bradya scotti	1		1	1	1	2	1		1	1		1			0		1	1
Bradya typica	1		1	1		1			0			0			0			0
Bradya spp.		1	1		1	1		2	2		1	1			0		1	1
Halophytophilus similis			0			0			0			0	1		1			0
Halectinosoma cooperatum	1		1	2	1	3	1		1			0			0			0
Halectinosoma mixtum	1		1			0			0			0			0			0
Halectinosoma pygmeum	5		5	8		8	2	5	7		3	3	2	2	4	3	3	6
Halectinosoma angulifrons	5	7	12	4	14	18	8	11	19	1	7	8	5	18	23	12	22	34
Halectinosoma crenulatum			0			0			0			0			0			0
Halectinosoma denticulatum			0			0			0			0			0			0
Halectinosoma distinctum			0	6		6	1	1	2			0			0	2	1	3
Halectinosoma spp.		10	10		6	6		10	10		6	6		2	2		3	3
Ectinosomatida*e* spp.		8	8		5	5			0		4	4		8	8			0
Pontopolites typicus	4	6	10	7	3	10	6	5	11	1	1	2	1	2	3	1	1	2
Laophonte longicaudata			0			0	2	1	3	1		1			0		2	2
Normanella incerta	1	2	3	1	5	6	2	10	12		2	2		14	14		7	7
Normanella mucronata typica	2		2	2		2	1		1	1		1			0			0
Normanella spp.		1	1		4	4			0		2	2			0			0
Rhizothrix curvata		2	2		4	4		2	2		1	1	1	1	2		3	3
Zosime major	1		1			0			0			0			0			0
Zosime spp.			0		1	1		3	3			0		1	1			0
Tachidiella minuta	4	2	6	1		1	6	4	10	4		4	2		2	3		3
Cyclopoida indet.			0		1	1			0	3	1	4	2		2	2	2	4
Copepoda indet.		1	1		4	4		1	1			0			0		2	2

APPENDIX IV continued...

10 Day Samples

	0 m Station									10 m Station								
	Repl. 1			Repl. 2			Repl. 3			Repl. 1			Repl. 2			Repl. 3		
	A	C	T	A	C	T	A	C	T	A	C	T	A	C	T	A	C	T
Longipedia coronata	2		2			0			0			0	1		1			0
Longipedia helgolandica	1	1	2			0			0	4		4	2		2			0
Longipedia minor			0			0			0	1		1			0			0
Longipedia scotti	3		3			0	1		1		1	1	1		1			0
Longipedia spp.		3	3		8	8		7	7		5	5		4	4		9	9
Pseudameira sp. 1	8	1	9	6	1	7	2		20	9	2	11	15		15	7	2	9
Pseudameira sp. 2	1		1	2		2			0	2		2	1		1	2		2
Sarsameira longiremis	1		1			0			0			0			0	3		3
Sarsameira parva			0	1	1	2	2	1	3			0			0	1		1
Sarsameira sp. 1			0			0			0			0	1		1			0
Sarsameira sp.			0			0			0			0			0		2	2
Proameira sp. 1			0			0	2		2			0			0	2		2
Eurycletodes latus			0			0			0			0			0	1		1
Heteropsyllus major	4	1	5	7	2	9	7	2	9	7	3	10	5	1	6	9	4	13
Heteropsyllus major sensu bodin			0	2		2	2		2			0			0			0
Cletodes limicola	2		2		1	1			0	1	1	2	1	1	2			0
Cletodes longicaudatus	1	8	9	2	1	3	2		2		10	10	1	4	5		4	4
Cletodes tenuipes		5	5		2	2		2	2	1		1	1	3	4		4	4
Cletodes smirnovi		2	2	1		1			0	1		1			0			0
Cletodes spp.		1	1		2	2		2	2			0			0		4	4
Enhydrosoma curvirostre		1	1			0			0			0			0			0
Enhydrosoma longifurcatum			0			0	1		1			0			0			0
Enhydrosoma sarsi			0			0			0			0			0	1		1
Enhydrosoma spp.		1	1		1	1		2	2		2	2		5	5		4	4
Cletodidae spp.		2	2		2	2		3	3			0		1	1			0
Danielssenia typica	1		1			0	1		1	2		2			0			0
Stenhelia gibba	3	58	88	45	62	107	39	49	88	29	50	79	35	58	93	29	89	118
Stenhelia giesbrechti		3	3			0	3	3	6	3		3	1	1	2	4	2	6
Stenhelia hanstromi			0			0			0	1		1			0	1		1
Stenhelia mastigochaeta	1		1			0	1		1	1	1	2	1		1			0
Stenhelia normani			0			0			0			0	1		1			0
Stenhelia reflexa		3	3		6	6			0	3	1	4	3	2	5	2	1	3
Amphiascus minutus			0			0			0	1		1			0			0
Amonardia normani			0			0		2	2			0			0			0
Paramphiascella hyperborea	2	3	5	2	2	4	1	3	4		8	8	3	5	8	2	2	4
Haloschizopera bulbifera	3		3	5	1	6	6		6	5		5	3		3	5		5
Haloschizopera lionensis			0	1		1			0	5		5			0			0
Haloschizopera sp. A	4	1	5	9		9	7		7			0	5		5	6		6
Haloschizopera scotti			0	1		1			0			0			0			0
Haloschizopera tenuipes			0			0			0			0	1		1			0
Haloschizopera sp.		7	7		8	8		9	9		5	5		6	6		8	8
Ectinosoma sp. 2			0			0			0	1	1	2	1	1	2	2	2	4
Ectinosoma M. sp. A	1		1			0			0			0			0			0
Ectinosoma aff. *breviarticulatum*	1		1	1		1	1		1			0			0			0
Ectinosoma aff. *californicum*	1		1			0			0			0			0			0
Ectinosoma spp.			0			0			0		1	1			0			0
Bradya scotti	1		1	3		3			0			0	1		1	1		1
Bradya spp.	1		1			0		1	1			0		3	3		1	1
Halophytophilus similis			0			0	1		1	1		1			0			0
Halectinosoma cooperatum	7		7	1		1			0			0	3		3			0

Halectinosoma mixtum			0	1		1			0			0			0	1	1	2
Halectinosoma pygmeum	4	2	6	3	1	4	2	1	3		2	2	1	1	2	5		5
Halectinosoma angulifrons	7	6	13	3	12	15	7	16	23	7	10	17	1	14	24	13	17	30
Halectinosoma crenulatum	3		3			0			0			0			0			0
Halectinosoma denticulatum			0			0			0			0			0	1		1
Halectinosoma distinctum	3		3	2	1	3	2	1	3	1		1			0	2		2
Halectinosoma spp.			0		3	3		2	2		5	5		4	4		10	10
Ectinosomatidae spp.		6	6		2	2		6	6		3	3		4	4		2	2
Pontopolites typicus	8	7	15	11	10	21	8	4	12	3	3	6	3	7	10	11	11	22
Laophonte longicaudata		1	1	2	2	4	4	1	5			0		1	1		1	1
Normanella incerta	3	7	10	5	12	17	3	6	9		4	4		4	4		13	13
Normanella mucronata typica	1		1	2		2	2	1	3	3	3	6			0	1		1
Rhizothrix curvata	1	3	4	5	1	6	2	3	5		9	9		4	4	4	7	11
Zosime major		1	1			0	3		3			0			0			0
Zosime spp.		2	2			0		2	2		2	2		3	3		5	5
Tachidiella minuta	7	1	8	3		3	3	2	5	4	3	7	2	1	3	5		5
Cyclopoida indet.		1	1			0			0	1		1	2	1	3	2	2	4
Copepoda indet.		1	1		4	4		1	1		3	3			0		1	1

APPENDIX IV continued...

30 Day Samples

	0 m Station									10 m Station								
	Repl. 1			Repl. 2			Repl. 3			Repl. 1			Repl. 2			Repl. 3		
	A	C	T	A	C	T	A	C	T	A	C	T	A	C	T	A	C	T
Longipedia coronata			0	2		2			0			0	1		1	1		1
Longipedia helgolandica	2	1	3			0	3	1	4	3		3	2		2	2		2
Longipedia minor			0	2		2			0			0			0			0
Longipedia scotti	1		1	1		1			0			0	1		1			0
Longipedia spp.		3	3		1	1		6	6		3	3		4	4		1	1
Pseudameira sp. 1	9	1	10	13		13	6		6	9		9	5		5	12	1	13
Pseudameira sp. 2			0			0			0			0	2		2	1		1
Pseudameira sp. 3			0			0			0	1		1			0	1		1
Sarsameira longiremis			0			0			0	1		1			0			0
Sarsameira parva	2		2	1		1	3		3			0	3	2	5	1		1
Proameira sp. 1			0	2		2			0	1		1	2		2	1		1
Mesochra pygmaea			0	1		1			0			0			0			0
Heteropsyllus major	11	4	15	5	1	6	7	1	8	7	1	8	9	1	10	7	2	9
Heteropsyllus major sensu bodin	3		3			0	2		2	1		1	1		1	2		2
Cletodes limicola	1		1			0	1	1	2			0			0	1		1
Cletodes longicaudatus		10	10	3	8	11	2	5	7		3	3	2	2	4	1	2	3
Cletodes tenuipes	1	2	3		2	2	2	2	4		2	2	1	9	10	1	5	6
Cletodes smirnovi	1		1	1		1			0	2		2			0			0
Cletodes spp.			0			0		3	3		1	1			0			0
Enhydrosoma curvirostre			0		1	1			0			0			0			0
Enhydrosoma longifurcatum			0			0			0			0			0	2		2
Enhydrosoma sarsi			0			0			0			0	1		1	1		1
Enhydrosoma M sp. A			0			0			0	1		1			0			0
Enhydrosoma spp.			0			0		3	3		2	2			0		3	3
Cletodidae spp.			0		1	1			0			0			0			0
Danielssenia typica	2		2			0			0			0			0			0
Psammis longisetosa			0			0			0	1		1			0			0
Stenhelia gibba	66	10	76	5	9	59	46	25	71	39	16	55	54	23	77	53	25	78
Stenhelia giesbrechti	1		1	1		1	2		2			0			0			0
Stenhelia hanstromi			0	1		1			0			0			0			0
Stenhelia mastigochaeta	2		2	2		2			0			0	2	2	4	1		1
Stenhelia reflexa	1	2	3	1	2	3	3		3	1	2	3			0		2	2
Stenhelia spp.			0			0		1	1			0			0			0
Typhlamphiascus confusus			0			0		1	1			0			0			0
Paramphiascella hyperborea	3	3	6	2	2	4	2	1	3	2	2	4	2	2	4	6	6	12
Haloschizopera bulbifera	3		3	13		13	7		7	6		6	5		5	5		5
Haloschizopera sp. A	12		12	7		7	11		11	2		2	4		4	5		5
Haloschizopera tenuipes			0			0			0			0	1	1	2			0
Haloschizopera sp.		5	5		4	4		6	6		8	8		6	6		6	6
Ectinosoma sp. 2			0			0			0			0	2	2	4			0
Ectinosoma M. sp. A			0		1	1			0	1		1			0			0
Ectinosoma aff. *breviarticulatum*			0			0	1		1			0	3		3	1	1	2
Ectinosoma aff. *californicum*			0		1	1	2		2			0	1		1			0
Ectinosoma spp.		4	4		1	1		3	3		1	1		1	1		1	1
Bradya scotti	2		2	1		1	3		3	1		1	1		1	1		1
Bradya typica			0	1		1			0			0			0			0
Bradya spp.		1	1			0		3	3		1	1		2	2		4	4
Halectinosoma cooperatum			0	2		2	1	3	4		2	2	1		1			0
Halectinosoma mixtum			0			0			0			0			0	2		2
Halectinosoma pygmeum	1		1	3		3			0	5		5	1		1	1	1	2

Halectinosoma argyllensis			0			0	1		1	1		1	1		1			0
Halectinosoma angulifrons	5	2	7	14	1	15	7		7	7	5	12	16	3	19	7	5	12
Halectinosoma denticulatum			0			0	1		1			0			0			0
Halectinosoma distinctum	1		1			0	2		2	2		2	2		2	1		1
Halectinosoma similidistinctum			0			0			0			0			0			0
Halectinosoma spp.		5	5		4	4		3	3	1	5	6		1	1		1	1
Ectinosomatidae spp.		3	3		3	3		1	1		3	3		2	2		3	3
Pontopolites typicus	12	2	14		1	1	5	2	7			0	17	3	20	5	6	11
Laophonte longicaudata	1		1	1		1	2	1	3	1		1	3		3	2		2
Normanella incerta	3	9	12	2	2	4	2	7	9	1	5	6	3	4	7	3	8	11
Normanella mucronata typica	1		1	3		3			0	2		2	1		1			0
Normanella spp.		1	1			0		1	1			0			0			0
Rhizothrix curvata	2	6	8	1	1	2	5	3	8	2	1	3	3	5	8	3	8	11
Zosime major			0			0	3		3			0	3		3			0
Zosime spp.		6	6		3	3		1	1		3	3		2	2		5	5
Tachidiella minuta		1	1	2		2	9	1	10	3	3	6	5	1	6	5	1	6
Cyclopoida indet.			0			0			0		1	1		2	2	2	1	3
Copepoda indet.		4	4		1	1		1	1			0			0		2	2

APPENDIX IV continued...

90 Day Samples

	0 m Station									10 m Station								
	Repl. 1			Repl. 2			Repl. 3			Repl. 1			Repl. 2			Repl. 3		
	A	C	T	A	C	T	A	C	T	A	C	T	A	C	T	A	C	T
Longipedia helgolandica	11		11	7		7	14	1	15	5	1	6	6		6	7		7
Longipedia minor			0			0	1		1			0			0			0
Longipedia scotti	5		5	2		2	6		6			0	1		1			0
Longipedia spp.		21	21		14	14		10	10		5	5		7	7		8	8
Pseudameira sp. 1	9		9	2		2	2		20	2		2	7		7	2	1	3
Pseudameira sp. 2			0			0			0	1		1	1		1		1	1
Sarsameira parva			0	2	1	3	1		1	2	1	3	1		1	3	2	5
Proameira sp. 1			0	2		2			0			0			0	1	1	2
Eurycletodes sp. A			0			0			0			0	2		2			0
Heteropsyllus major	3	1	4	8	1	9	4	1	5	2		2	8	3	11	5	4	9
Heteropsyllus major sensu bodin	2		2			0	4		4	3	1	4			0			0
Cletodes limicola	3	1	4	1	1	2	1		1			0			0			0
Cletodes longicaudatus			0	6	11	17	1	14	15	2	10	12		3	3	2	11	13
Cletodes tenuipes	2	5	7	1	2	3	1		1	1		1	1	2	3		1	1
Cletodes smirnovi	3		3			0	3		3			0			0			0
Enhydrosoma curvirostre			0	1		1	1	4	5			0	3		3			0
Enhydrosoma longifurcatum			0			0			0			0	1	2	3	1		1
Enhydrosoma sarsi	1		1			0	1		1			0			0			0
Enhydrosoma spp.			0			0			0		1	1		1	1			0
Acrenhydrosoma perplexum			0	1		1			0			0			0			0
Cletodidae spp.		4	4		1	1		2	2		1	1		4	4		3	3
Danielssenia typica			0			0			0	1		1			0			0
Stenhelia gibba	52		52	62		62	68		68	57		57	43		43	63		63
Stenhelia giesbrechti	1		1			0		1	1	4		4	1		1	4		4
Stenhelia mastigochaeta			0			0			0	1		1	1		1	1	1	2
Stenhelia reflexa	1		1			0			0	2	3	5			0	4	1	5
Stenhelia spp.		1	1			0			0		1	1			0			0
Bulbamphiascus imus			0	1		1			0			0			0			0
Typhlamphiascus confusus			0			0			0			0		1	1			0
Paramphiascella hyperborea	3		3	3		3	1		1	2		2	2	1	3	1		1
Haloschizopera bulbifera	12		12	5		5	1		10	8		8	13	1	14	2		2
Haloschizopera sp. A	5		5	7		7	12		12	1		1			0	11		11
Haloschizopera sp.			0		3	3		2	2			0			0		2	2
Ectinosoma sp. 1	2		2	2		2			0			0			0			0
Ectinosoma sp. 2	3		3			0	1		1	1		1			0			0
Ectinosoma M. sp. A	1		1	1		1	1		1			0			0			0
Ectinosoma aff. *breviarticulatum*	2		2	1	1	2	1		1			0	1		1	1		1
Ectinosoma aff. *californicum*	2		2			0	2		2			0			0			0
Ectinosoma spp.			0		1	1			0			0	2		2			0
Bradya scotti			0	1		1	2		2			0	2		2	1		1
Bradya typica	3		3			0			0	1		1			0	2		2
Bradya spp.			0		2	2		1	1			0			0		2	2
Pseudobradya similis			0			0			0	1		1	1		1			0
Halectinosoma cooperatum	6		6	4		4	5		5	2		2			0	1		1
Halectinosoma mixtum			0			0			0	1		1	4		4			0
Halectinosoma pygmeum	8		8	3		3	3		3			0	1		1	1		1
Halectinosoma argyllensis			0	1		1			0			0			0			0
Halectinosoma angulifrons			0	4		4	6		6	4		4	14		14	1		10
Halectinosoma crenulatum			0			0			0			0	1		1			0
Halectinosoma denticulatum			0			0			0	1		1	1		1			0

Halectinosoma distinctum	3		3			0	8		8	2		2	3		3	3		3
Halectinosoma similidistinctum	1		1			0			0			0			0			0
Halectinosoma spp.			0		1	1		2	2		1	1		3	3		3	3
Ectinosomatidae spp.		3	3		6	6		6	6		1	1			0			0
Pontopolites typicus	8		8	18		18	14		14	4		4	2		2	7		7
Laophonte longicaudata			0	1	1	2			0	2		2	2	1	3			0
Asellopsis hispida			0			0			0			0			0	1		1
Normanella incerta	1	1	2	3		3	5		5	3	1	4			0	1	3	4
Normanella mucronata typica	1		1			0			0	2		2			0	1		1
Normanella tenuifurca			0			0			0		1	1			0		1	1
Normanella spp.		1	1		1	1		5	5		3	3		1	1			0
Rhizothrix curvata	1	3	4	8		8	2	3	5	1		1	3		3	1	2	3
Zosime major			0			0			0			0	2	1	3	1		1
Zosime spp.		6	6		9	9		5	5		7	7		6	6		6	6
Tachidiella minuta	2	1	3	2		2		2	2	2	2	4	3	2	5	1		1
Cyclopoida indet.			0			0		3	3			0	1		1		1	1
Copepoda indet.		4	4			0		1	1		2	2		1	1			0

APPENDIX V

Copepod species abundance matrix for the enclosure field experiment

Column heading letters are as follows: A; adult, C; copepodite, T; total (all age group) abundance.

Control (30 Day) Samples

	Repl. 1			Repl. 2			Repl. 3			Repl. 4		
	A	C	T	A	C	T	A	C	T	A	C	T
Longipedia coronata			0	1		1			0			0
Longipedia helgolandica	6		6	1		1	6		6	6		6
Longipedia minor	2		2			0			0			0
Longipedia scotti	4	6	10	1	1	2			0	4		4
Longipedia sp.		15	15		11	11		3	3		11	11
Pseudameira sp. 1	1		1			0	2		2	2		2
Pseudameira sp. 2	3	1	4	2		2			0	3		3
Pseudameira sp. 3			0			0			0	1		1
Sarsameira parva	1		1	2		2			0			0
Sarsameira sp. 2			0			0			0	1		1
Proameira sp. 1			0			0	4		4			0
Mesochra pygmaea			0	1		1			0			0
Mesochra M sp. A			0	1		1			0			0
Nanaomesochra sp. 1			0	1		1			0			0
Heteropsyllus major			0	4		4	1		1	1	2	3
Heteropsyllus major sensu bodin			0	1		1			0			0
Cletodes limicola			0			0		1	1			0
Cletodes longicaudatus	1	2	3		1	1		2	2		3	3
Cletodes tenuipes			0		1	1		1	1			0
Enhydrosoma longifurcatum			0			0	5		5			0
Enhydrosoma sp.			0		1	1		6	6			0
Stenhelia mastigochaeta	1	1	2		1	1			0			0
Stenhelia gibba	2		2	3	3	6	1	1	2			0
Stenhelia giesbrechti		3	3		2	2		4	4		1	1
Stenhelia reflexa			0	1	1	2		1	1	1	1	2
Amphiascopsis sp. 1 (?)		1	1			0			0			0
Amonardia normani	1	1	2	4	27	31		2	2	1	14	15
Amonardia sp.		10	10			0			0			0
Paramphiascella hyperborea		2	2			0			0		1	1
Haloschizopera bulbifera			0	1		1			0	2		2
Haloschizopera sp. A	1		1	4		4	11		11	1		1
Haloschizopera scotti			0			0	1		1			0
Haloschizopera sp.		1	1		3	3		3	3			0
Diosacchidae spp.			0			0		1	1			0
Ectinosoma aff. californicum	1		1			0	1	1	2			0
Ectinosoma M sp A.	1	1	2			0			0			0
Ectinosoma spp.			0		3	3		1	1		2	2
Bradya scotti	1		1	1		1	1		1			0
Bradya typica			0			0	1		1			0
Bradya spp.		6	6		14	14			0		3	3
Pseudobradya similis	1		1			0			0			0
Pseudobradya spp.			0		2	2			0		1	1
Halophytophilus similis			0	1		1			0			0
Halectinosoma angulifrons	3	1	4	4	1	5	3	3	6			0
Halectinosoma argyllensis			0			0			0	4		4
Halectinosoma cooperatum	1		1	5		5	25		25			0
Halectinosoma distinctum			0			0	1		1			0

Halectinosoma pygmeum		1	1	1		1	4		4	1		1
Halectinosoma spp.		2	2		6	6		3	3		5	5
Ectinosomatidae spp.		2	2		2	2			0		2	2
Harpactacus obscurus			0			0			0	1	2	3
Harpactacus sp.		1	1		2	2			0			0
Pontopolites typicus	1	1	2			0			0			0
Laophonte longicaudata	1		1	5	3	8			0			0
Normanella incerta	1	3	4	1	9	10			0			0
Normanella mucronata typica			0	1		1	1	1	2			0
Rhizothrix curvata	4	3	7	6	8	14	1		1	1	3	4
Thalestridae sp.			0			0		1	1			0
Dactylopodia vulgaris			0			0			0	1	5	6
Dactylopodia sp.		2	2			0			0			0
Paradactylopodia sp. 1			0			0	1		1	5		5
Tisbe sp.			0		1	1			0	1	1	2
Zosime sp.		5	5		4	4			0		2	2
Tachidiella minuta	11	7	18	2	11	13	19	2	21	7	4	11
Harpacticoidea indet.		3	3		8	8		2	2		3	3
Cyclopoida sp. 1	4		4	2		2	1		1	3		3
Cyclopoida sp. 2			0			0	5		5	2		2
Cyclopoida sp. 3			0			0	3		3			0
Cyclopoida M sp. A	1		1			0			0			0
Cyclopoida spp.		10	10		14	14			0		4	4
Calanoida sp.	2		2	1		1		1	1		2	2

APPENDIX V continued...

Low Copper Concentration (30 Day) Samples

	Repl. 1			Repl. 2			Repl. 3			Repl. 4		
	A	C	T	A	C	T	A	C	T	A	C	T
Longipedia helgolandica	4		4	2		2	2		2	3		3
Longipedia scotti	1		1	1		1			0	1	1	2
Longipedia sp.		12	12		6	6		6	6			0
Ameira sp. 1	3	1	4			0			0	3		3
Pseudameira sp. 1	3		3	1		1	2		2	1		1
Pseudameira sp. 2	2		2	2		2	1	2	3	2		2
Pseudameira sp. 3	1		1			0			0			0
Sarsameira parva	2		2			0			0	2		2
Sarsameira longiremis			0			0	1		1	1		1
Proameira sp. 1			0	1		1		1	1			0
Mesochra pygmaea			0			0			0	3	2	5
Mesochra M sp. A			0	1		1			0	2		2
Heteropsyllus major	2	2	4	2		2	1	1	2	2	3	5
Cletodes longicaudatus	1	2	3	1	1	2			0	1	4	5
Cletodes tenuipes		1	1			0			0			0
Enhydrosoma longifurcatum			0			0			0	1		1
Enhydrosoma sp.			0		1	1		3	3			0
Stenhelia mastigochaeta			0	1		1			0	1	1	2
Stenhelia gibba	2		2	4		4		1	1		1	1
Stenhelia giesbrechti		4	4	1	2	3	2	1	3	3	1	4
Stenhelia reflexa	1		1	1	1	2			0	1		1
Diosaccus tenuicornis			0			0		1	1		1	1
Amphiascoides sp. 1			0			0			0	1		1
Amonardia normani	3	9	12	3	12	15	1	8	9	4	21	25
Bulbamphiascus imus			0			0			0		1	1
Paramphiascella hyperborea			0		1	1			0	1		1
Haloschizopera bulbifera			0			0	1		1	1		1
Haloschizopera sp. A	4		4	3		3	4		4	3		3
Haloschizopera sp. 2			0			0			0	1		1
Haloschizopera sp.		1	1		2	2		7	7		4	4
Diosacchidae		1	1		3	3		2	2			0
Ectinosoma aff. breviarticulatum			0			0			0	1		1
Ectinosoma aff. californicum	1		1	3	1	4	3	1	4		1	1
Ectinosoma sp. 2			0			0			0	1		1
Ectinosoma spp.		6	6			0		2	2		1	1
Bradya scotti			0			0			0	1		1
Bradya typica			0	2		2			0	1		1
Bradya spp.		6	6		5	5		4	4		9	9
Pseudobradya similis		1	1			0	1	4	5	6		6
Pseudobradya spp.		1	1		2	2		2	2			0
Halophytophilus similis	1		1			0			0			0
Halectinosoma angulifrons	3		3	4	3	7	1	1	2	1		1
Halectinosoma argyllensis			0	1		1			0	2		2
Halectinosoma cooperatum	1		1	1		1			0	1		1
Halectinosoma crenulatum	2		2			0			0	1		1
Halectinosoma pygmeum	2		2			0	1		1			0
Halectinosoma similidistinctum			0			0		1	1			0
Halectinosoma spp.		1	1		7	7		12	12		16	16
Ectinosomatidae spp.		3	3		2	2		3	3		3	3
Harpactacus obscurus			0			0		2	2			0
Pontopolites typicus			0			0			0	1	1	2

Laophonte longicaudata	4	3	7	1	2	3		8	8	3	12	15
Normanella incerta		3	3		3	3		14	14		6	6
Normanella tenuifurca			0			0	1		1			0
Rhizothrix curvata	2	5	7	6	5	11	3	3	6	4	5	9
Dactylopodia vulgaris			0			0		2	2			0
Dactylopodia sp.			0		1	1			0			0
Paradactylopodia sp. 1			0	2	3	5		4	4		1	1
Tisbe sp.		2	2			0			0		2	2
Zosime sp.		2	2			0		3	3		4	4
Tachidiella minuta	14	10	24	6	12	18	24	30	54	12	14	26
Harpacticoidea indet.			0			0		1	1		4	4
Cyclopoida sp. 1	2		2	4		4			0	1		1
Cyclopoida sp. 2	2		2	3	1	4		2	2		1	1
Cyclopoida sp. 3			0	1		1			0			0
Cyclopoida spp.		6	6		12	12			0		6	6
Calanoida sp.		1	1	3	6	9	1	9	10		2	2

APPENDIX V continued...

Medium Copper Concentration (30 Day) Samples

	Repl. 1			Repl. 2			Repl. 3			Repl. 4		
	A	C	T	A	C	T	A	C	T	A	C	T
Longipedia coronata			0			0	1		1			0
Longipedia helgolandica	8	1	9	1		1			0	2		2
Longipedia minor	1	1	2			0			0			0
Longipedia scotti	6		6	1		1			0			0
Longipedia sp.		8	8		5	5		2	2			0
Canuella perplexa			0		1	1			0			0
Ameira sp. 1			0	1		1			0			0
Pseudameira sp. 1	4		4	6		6	2		2	2	1	3
Pseudameira sp. 2	5		5			0			0			0
Pseudameira sp. 3			0	1		1			0			0
Sarsameira parva	1		1			0			0			0
Sarsameira spp.		2	2			0			0			0
Mesochra pygmaea	1		1			0			0		2	2
Mesochra M sp. A			0			0	1		1	1		1
Heteropsyllus major		1	1	1	1	2			0			0
Heteropsyllus major sensu bodin	1		1	1		1			0			0
Cletodes limicola			0	2		2			0			0
Cletodes longicaudatus	3	2	5		3	3		3	3	1	2	3
Cletodes tenuipes		2	2			0			0	1		1
Enhydrosoma longifurcatum			0			0			0	1		1
Enhydrosoma sp.		3	3		3	3		1	1			0
Cletodidae			0			0			0		1	1
Stenhelia mastigochaeta	1	1	2			0			0	1		1
Stenhelia gibba	5	10	15	4	11	15	2	3	5	5	6	11
Stenhelia giesbrechti	2	1	3		5	5			0		1	1
Stenhelia reflexa		2	2			0			0			0
Amonardia normani	2	11	13	1	18	19	1	8	9	7	17	24
Paramphiascella hyperborea		3	3		4	4			0		1	1
Haloschizopera bulbifera	2	1	3			0	2	1	3			0
Haloschizopera pygmaea			0			0	1		1			0
Haloschizopera sp. A	1		1	1		1			0			0
Haloschizopera sp.		12	12		1	1			0			0
Ectinosoma aff. californicum	4		4			0			0			0
Ectinosoma spp.		6	6		1	1			0			0
Bradya scotti	1		1			0			0			0
Bradya typica			0			0	1		1			0
Bradya spp.		14	14		7	7		2	2		1	1
Pseudobradya similis	1		1	1		1	2		2	6		6
Pseudobradya spp.		7	7		5	5		7	7		10	10
Halophytophilus sp.1			0			0			0	1		1
Halectinosoma angulifrons	5	1	6			0	2		2			0
Halectinosoma argyllensis			0			0			0	1		1
Halectinosoma cooperatum	1		1	4		4			0			0
Halectinosoma pygmeum	2	1	3	1	1	2			0	1		1
Halectinosoma spp.		4	4		1	1		1	1	4		4
Ectinosomatidae spp.		2	2		2	2			0			0
Harpactacus obscurus			0			0		1	1			0
Harpactacus sp.		1	1		1	1			0			0
Pontopolites typicus			0			0			0	1	1	2
Laophonte longicaudata	2	7	9		3	3		2	2		4	4
Normanella incerta	2	12	14	2	4	6		6	6		7	7

Normanella mucronata typica	1	4	5			0			0		1	1
Rhizothrix curvata	8	12	20	5	13	18	2	6	8	1	9	10
Dactylopodia vulgaris	1	1	2			0	1	4	5		2	2
Dactylopodia sp.		1	1			0			0			0
Tisbe sp.	2	5	7			0			0			0
Zosime major	1		1			0			0			0
Zosime sp.		2	2		2	2			0		1	1
Tachidiella minuta	27	19	46	7	2	9	1		1	3	1	4
Harpacticoidea indet.		9	9		1	1			0		2	2
Cyclopoida sp. 1	5	1	6	2		2	2		2			0
Cyclopoida sp. 2	4		4			0	6	2	8			0
Cyclopoida sp. 3			0			0	2		2			0
Cyclopoida M sp. A	1		1			0			0			0
Cyclopoida spp.		17	17		11	11		13	13		9	9
Calanoida sp.		2	2		2	2		1	1			0

APPENDIX V continued...

High Copper Concentration (30 Day) Samples

	Repl. 1			Repl. 2			Repl. 3			Repl. 4		
	A	C	T	A	C	T	A	C	T	A	C	T
Longipedia sp.			0			0		1	1			0
Pseudameira sp. 1	1		1			0	1		1	1		1
Proameira sp. 1	1		1			0			0			0
Heteropsyllus major	1		1			0			0			0
Cletodes limicola	1		1			0			0	1		1
Cletodes longicaudatus	2	4	6	1	5	6	1	9	10		4	4
Cletodes tenuipes			0		1	1	1	3	4		2	2
Enhydrosoma sp.		1	1			0			0			0
Cletodidae			0			0			0		1	1
Stenhelia gibba	2		2	1	4	5		1	1			0
Stenhelia giesbrechti		1	1			0			0			0
Stenhelia reflexa		1	1			0		3	3	1		1
Amonardia normani			0			0	2	4	6			0
Amonardia sp.		1	1			0			0			0
Robertgurneya sp.A	1		1			0			0			0
Paramphiascella hyperborea	1	1	2			0			0			0
Haloschizopera bulbifera	1		1			0			0			0
Haloschizopera sp. A			0			0			0	1		1
Haloschizopera scotti			0			0	1		1			0
Haloschizopera sp.			0		1	1		1	1		2	2
Ectinosoma aff. californicum	1		1	1		1			0			0
Ectinosoma sp. 2			0			0	1		1			0
Bradya spp.	1	2	3			0			0		2	2
Pseudobradya similis		1	1			0	1		1			0
Pseudobradya spp.			0			0		3	3			0
Halectinosoma angulifrons	1		1			0			0			0
Halectinosoma cooperatum	2		2			0	6		6			0
Halectinosoma crenulatum			0			0			0	1		1
Halectinosoma dendiculatum			0	1		1			0			0
Halectinosoma spp.		1	1			0		4	4			0
Harpactacus obscurus			0			0	3		3			0
Pontopolites typicus		1	1			0			0			0
Laophonte longicaudata		2	2	1	1	2	6	6	12	2	4	6
Normanella incerta	1		1			0	1	2	3		2	2
Normanella mucronata typica			0			0	1		1			0
Rhizothrix curvata	3	1	4	4	2	6	1	7	8	2	2	4
Dactylopodia vulgaris			0			0			0	1	1	2
Dactylopodia sp.		1	1		2	2			0			0
Paradactylopodia sp. 1			0			0		7	7			0
Tisbe sp.			0			0		1	1			0
Zosime sp.			0			0			0		1	1
Tachidiella minuta			0		1	1	1		1		1	1
Harpacticoidea indet.			0		1	1		5	5			0
Cyclopoida sp. 1	2		2	1		1	7	1	8			0
Cyclopoida sp. 2			0			0	2		2	1		1
Cyclopoida sp. 3			0			0	13		13	3		3
Cyclopoida spp.		2	2		7	7		31	31		11	11
Calanoida sp.			0			0			0		4	4

APPENDIX V continued...

Background Core (1 Day) Samples

	Repl. 1			Repl. 2			Repl. 3			Repl. 4		
	A	C	T	A	C	T	A	C	T	A	C	T
Longipedia helgolandica			0	1		1			0			0
Longipedia scotti			0	1		1			0			0
Longipedia sp.		3	3		3	3		2	2			0
Pseudameira sp. 1	6	6	12	8	7	15	2		2	6	2	8
Pseudameira sp. 2			0			0			0	2	1	3
Sarsameira parva	1		1	2		2	1	1	2		1	1
Proameira sp. 1			0			0	1		1			0
Mesochra pygmaea			0			0			0	1		1
Heteropsyllus major	2	3	5	3	3	6	2		2	3	1	4
Heteropsyllus major sensu bodin			0			0	1		1	1		1
Cletodes limicola		1	1			0			0			0
Cletodes longicaudatus		1	1		5	5	1	2	3	2	3	5
Cletodes tenuipes		3	3		5	5			0		2	2
Enhydrosoma curvirostre			0	1		1			0			0
Enhydrosoma sp.			0		5	5			0		2	2
Stenhelia hanstromi		1	1			0			0			0
Stenhelia mastigochaeta	1	1	2	1	1	2			0	3		3
Stenhelia gibba		2	2	1	21	22	1	4	5		11	11
Stenhelia giesbrechti	1	1	2		2	2		1	1		2	2
Stenhelia reflexa			0	1	3	4	2	1	3	1	1	2
Amonardia normani			0			0		1	1			0
Paramphiascella hyperborea		1	1		5	5			0		3	3
Haloschizopera bulbifera	1		1	2		2			0			0
Haloschizopera sp. A			0	7		7	2		2	5		5
Haloschizopera sp.		4	4		11	11		4	4		9	9
Ectinosoma spp.		2	2			0		1	1			0
Bradya scotti			0			0	1		1	1		1
Bradya typica			0			0	1		1			0
Bradya spp.		2	2		2	2		3	3		4	4
Halophytophilus similis			0			0			0	1		1
Halophytophilus sp.1		1	1			0			0			0
Halectinosoma angulifrons	1	8	9	2	7	9	1	1	2	2	5	7
Halectinosoma argyllensis			0	1		1			0			0
Halectinosoma cooperatum			0	1		1			0			0
Halectinosoma paragothiceps			0			0	1		1			0
Halectinosoma pygmeum		1	1	3		3	1		1	4		4
Halectinosoma spp.		4	4		16	16		7	7		9	9
Ectinosomatidae spp.		4	4		3	3		2	2			0
Harpactacus sp.			0			0			0		1	1
Pontopolites typicus	1	1	2	3	4	7			0			0
Laophonte longicaudata	3	7	10	5	23	28	2	4	6	1	11	12
Normanella incerta		3	3		6	6		4	4		2	2
Normanella mucronata typica	2		2	1		1	1		1	1		1
Rhizothrix curvata			0	2	14	16		3	3		1	1
Dactylopodia vulgaris			0			0		1	1			0
Zosime sp.		1	1		3	3			0		2	2
Tachidiella minuta	2	6	8	7	6	13	1		1	4	5	9
Harpacticoidea indet.			0		5	5		1	1		2	2
Cyclopoida sp. 2		1	1			0	1		1			0
Cyclopoida sp. 3	1		1			0			0			0
Cyclopoida spp.		3	3			0		6	6		1	1

APPENDIX V continued...

Background Core (30 Day) Samples

	Repl. 1			Repl. 2			Repl. 3			Repl. 4		
	A	C	T	A	C	T	A	C	T	A	C	T
Longipedia helgolandica	4		4	7		7	6		6	7		7
Longipedia scotti	1		1	1		1	2		2	3		3
Longipedia sp.			0		6	6		5	5		5	5
Pseudameira sp. 1	2		2	8	1	9	7	1	8	6	1	7
Sarsameira longiremis			0	1	1	2	1	2	3			0
Sarsameira spp.			0		1	1			0		2	2
Heteropsyllus major	2	1	3	1		1			0	7		7
Cletodes limicola			0			0		1	1	1		1
Cletodes longicaudatus	1	1	2			0		1	1	1	1	2
Cletodes tenuipes			0		4	4		2	2		3	3
Enhydrosoma longifurcatum			0			0			0	1		1
Enhydrosoma sp.			0		4	4			0			0
Danielessenia typica			0			0	1		1			0
Stenhelia mastigochaeta			0	2	1	3			0			0
Stenhelia gibba	3		3	6	5	11			0	2	3	5
Stenhelia giesbrechti			0		1	1	1	2	3	2	3	5
Stenhelia reflexa	1		1	2		2		1	1	1	1	2
Paramphiascella hyperborea		1	1		2	2		1	1		1	1
Haloschizopera bulbifera	5	2	7	7		7	1		1	2		2
Haloschizopera sp. A		3	3	8		8	2		2	4		4
Haloschizopera sp.		2	2		5	5		4	4		7	7
Ectinosoma aff. breviarticulatum		1	1			0			0			0
Ectinosoma aff. californicum			0	3		3	2		2			0
Ectinosoma spp.		1	1			0		4	4		6	6
Bradya scotti			0			0		1	1	3		3
Bradya typica			0	1		1	2		2			0
Bradya spp.		2	2		6	6		1	1		11	11
Halophytophilus similis			0			0	1		1	1		1
Halectinosoma angulifrons			0		3	3	3		3	8		8
Halectinosoma argyllensis			0	1		1			0			0
Halectinosoma cooperatum	1		1	2	1	3			0	2		2
Halectinosoma dendiculatum			0			0	1		1	1		1
Halectinosoma distinctum			0			0	1		1			0
Halectinosoma mixtum			0			0			0	1		1
Halectinosoma pygmeum	1		1	3		3			0	4		4
Halectinosoma spp.			0		4	4		7	7		7	7
Ectinosomatidae spp.		1	1		2	2			0		2	2
Pontopolites typicus	1		1	2		2			0	6	1	7
Laophonte longicaudata	1	1	2	1	2	3		1	1	3	1	4
Normanella incerta		2	2		4	4		1	1	1	4	5
Normanella mucronata typica		1	1		2	2		1	1		2	2
Rhizothrix curvata	1	7	8	11	23	34	2	4	6	2	4	6
Zosime sp.		2	2		5	5		2	2		5	5
Tachidiella minuta	6	1	7	9	6	15	11	7	18	4	6	10
Harpacticoidea indet.		5	5			0			0		1	1
Cyclopoida sp. 2	2		2	2		2	1		1	1		1
Cyclopoida sp. 3			0			0	2		2			0
Cyclopoida spp.		6	6		3	3			0		5	5
Calanoida sp.	1	2	3			0			0		1	1