EVALUATION OF STREAM MEIOFAUNA AS A MONITOR OF TRACE METAL CONTAMINATION

by

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ABSTRACT

Members of the meiobenthos have been used extensively to determine the effects of anthropogenic perturbation in marine systems (Coull & Chandler, 1992). Despite this, the meiofauna has been virtually excluded from freshwater pollution monitoring. This thesis aimed to address this research caveat, by evaluating the potential of stream meiofauna for monitoring metal-contamination. Meiofaunal communities were sampled from streams in SW England representing a gradient in metal contamination. Environmental variables in these streams were also measured to identify the important forcing agents structuring the stream benthos. Multivariate techniques demonstrated Cu, either alone or in combination with other environmental variables was of most importance in correlations with the composition of meiofaunal communities. Comparison with the macrofaunal data demonstrated that both components of the benthos responded in a similar way to metal contamination, although the meiofauna also highlighted other differences in water chemistry. The combination of meiofauna, macrofauna and temporary meiofauna in a combined metazoan community analysis gave the best discrimination of sites. Detection of metal-contamination was retained in meiofaunal data aggregated to the family level.

The abundances of the harpacticoid copepod *Bryocamptus zschokkei* were consistently important in contributing to between-site differences in community structure. The harpacticoid, therefore was selected as an ecologically-relevant freshwater toxicity test for Cu. Laboratory experiments demonstrated that Cu had toxic effects on the survival and reproduction of *Bryocamptus zschokkei*. Although acute toxicity tests gave more rapid results, these effects on survival occurred at a higher Cu concentration than those in the chronic tests. Sub-lethal concentrations of Cu led to a reduction in the numbers of offspring per brood. Animals with pre-exposure to chronic concentrations of Cu exhibited greater tolerance to this metal.

In conclusion, more information may be gained by including the meiofauna, alongside the macrofauna, when monitoring the impact of contaminants on freshwater systems. To reduce the effort of processing samples it appears family level data could be used to detect metal-contamination. The novel use of *B. zschokkei* in laboratory tests, where it showed lethal and sub-lethal responses to Cu, demonstrated that this species may have much potential as an ecologically-relevant freshwater bioassay organism for this metal. The advantages of using meiofaunal species such as *B. zschokkei* as toxicity test organisms are discussed.

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Author's declaration

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A programme of advanced study was undertaken, which included instruction in freshwater meiofaunal identification, Atomic Absorption Spectrometry, copepod culture techniques, PRIMER usage for multivariate analysis of data and Statgraphics.

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* Poster presentation at the International Meiofauna Conference, Perpignan, France, May, 1995, entitled: The effect of heavy metal contamination on stream meiofaunal communities

* Poster presentation at the British Ecological Society, Sheffield, December 1995, entitled: The Effect of heavy metal contamination on stream meiofaunal communities.

* Poster presentation at SETAC (Society for Environmental Toxicology and Chemistry) UK, Luton, June 1996 entitled: The Effect of heavy metal contamination on stream meiofaunal communities.

* Oral presentation to the Department of Biological Sciences, University of Plymouth November 1996, entitled: The evaluation of stream meiofauna as a monitor of heavy metal contamination

* Poster presentation at S.E.T.A.C. , Washington D.C., November 1996, entitled: The effect of heavy metal contamination on stream meiofaunal communities.

* Oral presentation to the Stream Ecology Group, University of Maryland, Washington D.C., November 1996, entitled: The evaluation of stream meiofauna as a monitor of heavy metal contamination

* Oral presentation at the British Ecological Society Annual Winter Meeting, Durham, December 1996, entitled: The evaluation of stream meiofauna as a monitor of heavy metal contamination.

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Signed..... S Bunton .....

Date..... 27.03.98 .....

CHAPTER 1

General Introduction

The principal objective of this thesis was to evaluate the use of the freshwater meiofauna for assessing the biological impact of metal contamination in streams in south west England. To achieve this objective the meiofaunal communities in metal-contaminated streams were described and correlations between stream meiofaunal and macrofaunal communities and metal contamination in the field were compared. In addition, the potential of the harpacticoid copepod *Bryocamptus zschokkei* as a toxicity test organism for predicting the effects of Cu on the stream benthos was evaluated. As a prelude to these studies this chapter reviews the current knowledge of the effects of metals on stream biota, discusses the different approaches taken to assess water quality and considers the potential advantages of stream meiofauna as a monitor of trace metal contamination.

1.1 The influence of trace metal contamination on stream ecosystems

1.1.1 Biological effects

Trace metals are released continuously into freshwater ecosystems from natural processes such as the weathering of rocks and volcanoes (Kelly, 1988). At low concentrations, some trace metals [e.g. copper (Cu), zinc (Zn) and iron (Fe)] are essential for biological processes. For example, Cu is an important component of many metalloenzymes and respiratory pigments (Hebel *et al.*, 1997). Increased concentration of metals has occurred in many river systems throughout the world in the last century due to industrial processes such as the processing of metal ores. This elevation of metal concentrations in running waters has had a profound and adverse effect on the biota (Kelly, 1988). As metals are conservative pollutants their impact will have long-term consequences on the aquatic environment. Aquatic organisms exposed to elevated trace metal concentrations often accumulate metals directly from the water or via their food (Abel, 1996). In some cases, where carnivores at the top of the food chain obtain their pollutant burden from ingestion, the potential exists for considerable biomagnification of the trace metal (Mason, 1996). As

well as an increase in the concentration of metals measured in stream biota, many studies have demonstrated that large alterations to the structure of stream communities occur at high metal concentrations in the water (Winner *et al.*, 1980; Leland *et al.*, 1989; Clement, 1994; Gower *et al.*, 1994). Metal-contaminated streams often have a lower species richness and a lower abundance of biota compared with streams that are uncontaminated by trace metals. Exposure of many stream species to elevated trace metal concentrations in the laboratory results in mortality, lower fecundity or a decrease in the rate of development of the individual (Abel, 1996); such effects will, ultimately, cause a reduction in population size. In contrast, some species appear to tolerate high metal concentrations [e.g. many species of Plecoptera (Kelly, 1988)] and these species replace the more sensitive species in metal-contaminated streams. This tolerance may occur by a decrease in the uptake of the metal by the organism, by an increase in its excretion, or by an increase in the production of substances, such as metallothionein, which binds with the metal rendering it biologically unavailable (Abel, 1996).

Changes in the abundance and distribution of species, however, may not necessarily result from the direct effect of metal contamination. Rather, these changes may result from indirect trace metal effect (e.g. through the effect of the metal on other species). The effect of the metal on a species may be accentuated, or attenuated, depending upon the nature of interspecific relationships which exist in the environment. A reduction in the population size of a superior competitor may allow the population of a lesser competitor but a species more tolerant to the contaminant to increase. For example, after exposure to Cu of mixed cultures of *Daphnia pulex* and *Daphnia magna*, the population size of the previously dominant, *D. pulex*, was reduced and the more Cu tolerant cladoceran *D. magna* dominated (Leblanc, 1985). In another study, the vulnerability of two species of net-spinning caddisflies (*Chimarra sp.* and *Hydropsyche morosa*) to predation by the stonefly

Paragnetina media was significantly greater in experimental streams dosed with copper than in control streams (Clements *et al.*, 1989).

There can also be a large amount of intraspecific variation of resistance to metal toxicity. Previous exposure of individuals to low metal concentrations can increase their metal tolerance, either through acclimatisation effects (where exposure to low concentrations of metals confer increased resistance to subsequent high-dose exposure) or through genetic adaptation (where the selection of tolerant individuals within a population has occurred through exposure). Physiological acclimation of individuals of *Gammarus pulex* to cadmium after pre-exposure to sub-lethal concentrations of cadmium and zinc in the laboratory was reported by Stuhlbacher and Maltby (1992). An example of genetic adaptation was shown by Brown (1977a) who reported that individuals of the isopod crustacean *Asellus meridanus* from metal-polluted streams were more resistant to Cu and lead than individuals from clean streams. This resistance persisted in to the F2 generation after *Asellus meridanus* was reared in clean water, demonstrating that the resistance to Cu had a genetic basis. Different life stages within ones species can also vary in their susceptibility to the toxic effects of metals; generally, the early life stages are often more vulnerable to toxicity than are the later stages. For example, the toxicity of Cu decreased in the midge larvae *Chironomus tentans* in successive developmental stages (Gauss *et al.*, 1985).

1.1.2 Metal bioavailability

In aquatic systems, trace metals are rarely encountered in isolation, and hence, it is often difficult to determine which metals, if any, are of primary importance in influencing components of the biota. Further complications arise due to the fact that there may be interactive effects amongst metals and the toxicity of any one metal can be influenced by

the presence of others. Three types of interactions amongst trace metals have been observed. Firstly, there may be an additive effect, whereby the combined effect of the metals is equal to the sum of the individual metal toxicities. For example, the avoidance behaviour of the oligochaetes *Tubifex tubifex* and *Limnodrilus hoffmeisteri* in laboratory trials increased in proportion to the combined effects of sub-lethal doses of Cu and Zn (McMurty, 1984). Secondly, a synergistic interaction may occur, whereby the combined effect of the metals is more than the sum of individual metal effects. For example, 'more-than-additional' toxicity was reported by Borgman (1980) for the combined effect of Zn and arsenic on the biomass production rates of natural assemblages of freshwater copepods. Finally, the metals may interfere with each other leading to an antagonistic effect, where by the combined effect of the metals is less than the sum of the individual metal effects. This type of effect was found for the combined lethal effect of Zn and Cd on the freshwater prawn *Paratya tasmaniensis* (Thorp & Lake, 1973).

The bioavailability and, hence, the toxicity of trace metals to aquatic organisms is also affected by their chemical form (speciation). The 96-h LC_{50} values for the fathead minnow, *Pimephales promelus,* for Cu ranged over two to three orders of magnitude depending on the copper species (Pagenhopf *et al.*, 1974). The cupric ion was most toxic, followed by copper (II) hydroxide. Copper carbonate, copper bicarbonate and copper (I) hydroxide contributed little to the toxic action of Cu (Pagenhopf *et al.*, 1974). Speciation is influenced by environmental variables and it is important that those variables which cause speciation are measured alongside the trace metals. The modification of metal toxicity by pH is, perhaps, one of the most well-cited examples of this topic. The toxicity of metals increases generally as the pH decreases (as high acidity brings many metals into solution) (Gerhardt, 1993). For example, at low pH, Al is in a soluble monomeric form (Hall *et al.*, 1987), which is highly toxic to invertebrates and fish (Ormerod *et al.*, 1987). In some cases,

however, a decrease in pH can have a mitigating effect on metal toxicity (due to H+ ions interfering with the uptake of metal ions) (Campbell & Stokes, 1985). This latter occurrence has been cited frequently for copper and zinc toxicity to fish (Howarth & Sprague, 1978; Cusimano *et al*, 1986) and invertebrates (Borgman 1983).

Dissolved organic matter and water hardness have important influences on metal speciation. Dissolved organic matter (humic and fulvic acids) has a high affinity with metals and its presence is important for complexing Cu in freshwaters (Spear & Pierce, 1979). Mantoura *et al.* (1978) found 90% of the Cu in fresh water was complexed by humic ligands. Once metal ions have formed complexes with these organic agents, they are generally unavailable to aquatic organisms. For example, the toxic action of monomeric Al on the stream benthic macroinvertebrate community structure in streams in Sweden was appreciably reduced by chelation with DOM (Kullberg, 1992). This reported modification of the stream community may also have been influenced by pH, as the amount of metals in humic complexes reduce under acidic conditions in streams (Kullberg, 1992). In waters of high hardness, metal ions form inorganic complexes which are of a low toxicity. Copper was four to six times more toxic to *Gammarus pulex* in soft than in hard water (Stephenson, 1983), and Howarth & Sprague (1978) demonstrated that the lethal toxicity of Cu to the rainbow trout, *Salmo gairdnerii*, decreased with increasing hardness.

The physical variables of flow and temperature will also influence the impact of metal contamination on the biota. At present, the exact relationship between metal concentrations and discharge is unclear, although some workers have recorded increased metal concentrations after periods of high flow, due to the greater scouring action resuspending bottom sediments (Williams *et al.*, 1973; Brown, 1977b). Conversely, a decrease in dissolved metal concentrations occurred, due to dilution of the metals, at high flows

(Johnson & Thornton, 1987). With increases in temperature, the time taken for organisms to react to a given metal concentration generally reduces. The properties of the metal itself may be altered, resulting in an increase in its soluble, bioavailable phase (Newman & McIntosh, 1991), whilst an influence on the rate of metabolic processes may increase the uptake of the metal.

1.1.3 Direct effects of environmental variables (other than trace metals) on the stream biota

When assessing the impacts of trace metals on stream communities, it is important to understand that physicochemical variables other than the metals themselves can also have a profound effect on stream biota. The effects of pH on streams have been documented extensively (Steinberg & Wright, 1994). Field and laboratory experiments have established an increase in mortality of stream benthic invertebrates with increasing acidity (Burton *et al.*, 1985; Willoughby & Mappin, 1988). This mortality has implications for the diversity of stream invertebrates (Weatherly & Ormerod, 1987; Rundle & Hildrew, 1990). Water hardness has also been linked to the community structure and composition of stream macroinvertebrate communities, and higher diversities are found in streams of high water hardness (Ormerod & Edwards, 1987).

There is a wealth of literature on the effect of flow on stream biota and its rôle in shaping community structure (Hildrew & Giller, 1995; Allan, 1995). Temperature has a profound influence on stream biota by affecting the rate of development and timing of their life cycles (Allan, 1995). (Refer to Chapters 2 and 3 for more detailed discussion of the effects of environmental variables on stream biota.)

1.2 The use of stream biota for monitoring water quality

1.2.1 Community structure as a monitoring tool

Using the biota as a monitor of water quality is often preferable to using chemical measurements alone, as changes in the abundance and presence of biota will occur as a result of episodic contamination events and low-level contamination. Thereby, the biota reflect the total conditions found in the stream over a period of time. The ideal approach to assessing ecosystem health is to use the biota at the community level (Rosenberg & Resh, 1992). The response of the community integrates the effects of competition, plant-herbivore and predator-prey interactions, and the influence of environmental variables. Ideally, the entire aquatic community should be studied to assess water quality. This approach is, however, impractical as it requires a large degree of expertise and a great deal of time. Therefore, regulators usually focus on one particular component of the ecosystem. Benthic macroinvertebrates are the most commonly-used group of organisms to assess stream water quality and they have many attributes which make them suitable monitoring tools (Rosenberg & Resh, 1992). Firstly, they are ubiquitous and, therefore, can be affected by environmental perturbations in many different types of freshwater systems. Secondly, macroinvertebrates are relatively sedentary and, therefore, respond to the integrated environmental conditions of the local area. Thirdly, they represent a diverse range of trophic levels and feeding types and, hence, offer a spectrum of responses to contamination. The probability that at least some of these organisms will react to a particular change in environmental conditions is, therefore, high. Finally, sampling is also relatively easy and only simple, inexpensive equipment is required for collection.

1.2.2.1 Univariate measures of community response

Different techniques have been used to assess between-site differences in invertebrate community structure. Univariate measures, such as diversity and biotic indices, have been

implemented frequently to monitor freshwater systems (Cairns *et al*, 1968; Wilhm & Dorris, 1968; Norris *et al.*, 1982; Roline, 1988; Camargo, 1993; Schmidtz & Nadel, 1995; Joshi *et al*, 1995). This high use of univariate methods probably stems from the fact that they are expressed in a single mathematical expression, thereby, allowing a value judgement to be placed on the community. With such diversity indices, a low diversity is supposedly indicative of high contamination. Biological information is lost, however, when using diversity indices, as species do not retain their identities in the calculations and the differing levels of tolerance among taxa is ignored. The response of diversity indices to contamination is also not necessarily monotonic and, in some cases, moderate pollution increases the abundance and diversity of biota (Pindar & Farr, 1987). Biotic indices were developed to retain information on relative species tolerances. Most of the biotic indices currently under use were developed to measure organic pollution. In their simplest form, these indices are ratios of tolerant to sensitive species; for example, the '*Asellus* : *Gammarus*' index developed by Watton & Hawkes (1984). Most biotic indices involve assigning each species a value depending on its sensitivity to a particular pollutant, and rely solely on presence and absence data or on weighting the species present according to their abundance. One of the first indices, the Trent Biotic Index (TBI), was developed in the UK, originally for use in the Trent River Authority area (Woodweis, 1964). In the TBI, rankings of sites or systems were made according to the presence or absence of six groups of key organisms. In Scotland, a variation of the TBI (Chandler's Biotic Score) was developed and involved dividing the fauna into key indicator groups, but required quantitative data and a higher degree of taxonomic expertise (Chandler, 1970). In 1979, the Biological Monitoring Working Party (BMWP) score was introduced as a standardised biotic system for assessing biological quality of rivers (ISO, 1979). This index requires family-level identification, which saves time, reduces the variability due to misidentification and allows a wider geographical application. Finally, to avoid the score

being influenced disproportionately by the presence/absence of particular taxa, depending on the sampling effort, the BMWP score can be divided by the number of scoring taxa in the sample to give an average score per taxon (ASPT) (Armitage *et al.*, 1983).

As the TBI, Chandler and BMWP indices were developed specifically to measure responses to organic pollution they may be unsuitable for detecting other forms of pollution. Organisms considered to be intolerant of organic pollution are sometimes very tolerant to specific toxicants and *vice versa*. Using acute toxicity tests, Slooff (1983) compared the relative tolerances (lethal responses) of 12 invertebrates from various taxonomic groups to 15 chemicals (including both metals and organic compounds) and a mixture of organics concentrated from River Rhine water. The results of the bioassays demonstrated that the toxicity of chemicals to invertebrates varies widely with the test species and that the tolerances of macroinvertebrates are species-specific.

At present, there are no biotic indices in common use for forms of pollution other than organic. Some information, however, exists on the tolerance of benthic macroinvertebrates to trace metals, with a decrease in metal tolerance generally occurring from chironomids, through caddis flies and stoneflies, to mayflies (Winner *et al.*, 1980; Clements *et al.*, 1988). This scheme is highly generalised and exceptions to this pattern have been found. For example, tanytarsinid chironomids, were as sensitive to metals as mayflies in experimental streams and field biomonitoring at the Clinch River (USA) (Clements *et al.*, 1988, 1989). An Index of Community Sensitivity (ICS), based on sensitivity and relative abundance of dominant taxa, was, however, developed for the Clinch River system (Clements *et al.*, 1992). The ICS was highly sensitive to metals and distinguished clean and impacted sites within the study area, although, it was advised that the index should be restricted to local use, as the sensitivity values were obtained from a single set of experiments, exposing a

specific community of benthic invertebrates to Cu for ten days. Other regions, containing very different species assemblages, may respond differently and further surveys are required to assess the response of invertebrate communities in other streams (Clements *et al.*, 1989). Another difficulty of using a biotic index to monitor trace metal contamination in streams is that metal effluent is often a mixture of metals that will result in a complex response. Finding a consistent gradient in response to metal contamination is, therefore, difficult. Factors, such as substratum composition, flow regime and nutrient concentrations are also likely to influence invertebrate distribution, and would potentially confound investigations of the effects of metals on stream fauna (LaPoint *et al.*, 1984). One way of identifying the contribution of these variables to faunal distribution is by using multivariate analysis (Resh & Jackson , 1992).

1.2.2.2 Multivariate analyses

With the advancement of information technology, sophisticated multivariate statistical approaches are being used more frequently for the biological assessment of water quality (Norris & Georges, 1993; Rutt *et al.*, 1993; Gower *et al.*, 1994). Multivariate techniques have the advantage that they take into account the identity of each species, not just the distribution of individuals amongst species. So, unlike diversity indices, subtle changes in species composition across sites are not missed by the need to summarise the combined characters of the site as a single value. As multivariate measures contain more biological information, they are often more sensitive and better at discrimination between sites of differing levels of pollution than univariate measures (Norris *et al.*, 1982; Warwick & Clarke, 1991; Perryman, 1996). Cao *et al.* (1996) compared the abilities of biotic indices, and multivariate and diversity measures to detect the change of the macroinvertebrate community along a gradient of pollution caused by sewage effluent in the River Trent system. These authors found that multivariate analyses clearly illustrated differences in

community structure along the pollution gradient, whereas none of the biotic indices were sensitive over the whole range of water quality (Cao *et al.*, 1996); neither the Simpson's, Shannon's or the Evenness Indices demonstrated the effects of pollution on macroinvertebrate communities, whilst the Margalefs index was depressed only at heavily polluted sites (as was species richness) (Cao *et al.*, 1996).

Multivariate statistical techniques have also been used in the development of predictive models for assessing water quality. In Britain, the River InVertebrate Prediction And Classification Scheme (RIVPACS) is an example of one such model. It was developed by the Institute of Freshwater Ecology in collaboration with the water industries (Wright *et al.*, 1984), and is based on a survey of invertebrates and physicochemistry at 438 'pristine' streams. TWINSPAN (Hill, 1979) classification was used to classify the running waters on the basis of their macroinvertebrate fauna. Multiple discriminant analysis was then used to identify a number of key factors which influenced the observed pattern of distribution of the taxa. It was, thereby, possible to predict the probability with which a given species or family will be captured at a particular site using environmental data. The predicted target assemblage of macroinvertebrates can be used to generate expected BMWP or ASPT scores against which to assess the results of field surveys. The RIVPACS is now in use by the Environment Agency to assist in the analysis and interpretation of survey data.

1.3 Single species as monitoring tools

Although the best assessment of the effect of a contaminant on the biota is by direct monitoring of the community, there are many cases where this is not possible. Regulators are often required to predict the effects of metals (and other anthropogenic inputs) on a freshwater system prior to the introduction of the chemical. For example, compliance with a toxicity standard may be a legal requirement to obtain consent to discharge an effluent,

while identification of the more toxic components of complex effluents may be necessary in order to improve treatment processes. As the effects of thousands of compounds and effluents require screening, risk assessment predictions need to be quick, easy and inexpensive. Thus, single species toxicity tests on several, or even just one organism, provide a means of assessing the toxicity of chemicals before they are discharged into freshwater systems. A major assumption with these toxicity tests is that the response of the organism used is indicative of the response of the resident aquatic biota in the receiving water.

1.3.1 Choice of test organism

Many of the early toxicity tests were undertaken on fish (Lloyd, 1972), mainly because fish attracted public and political interest. It soon became apparent, however, that water quality standards set using fish were inappropriate for protecting more sensitive species in the system and, hence the 'ecological integrity' of ecosystems. Thus, interest developed in performing tests on other components of the community, including invertebrates.

Important criteria in the choice of test organism, were recommended by the U.S. Environmental Protection Agency (USEPA, 1979), included that the organism should:

1) represent an ecologically or economically important group,

2) be widely available,

3) be easily maintained,

4) be sensitive to chemicals,

5) have a response that is easily identifiable,

6) have a response that is comparable to those of indigenous species, and

7) have a well known physiology, taxonomy and ecology.

Few, if any species, meet all these criteria and the choice of organisms used in bioassays has been heavily criticised (Cairns & Pratts, 1989; Gray, 1989; Maltby & Calow, 1989). Test organisms are often chosen primarily because of their availability and robustness in laboratory cultures, rather than their ability to indicate subtle ecological consequences of contamination (Gray, 1989). This is, in part, due to the necessity of having validated, standardised test procedures that allow a comparison between laboratories, which has often limited the choice of organisms to groups such as cladocerans (e.g. *Daphnia*) and fathead minnows (*Pimephales promelus*). A comparison of toxicity data from different laboratories may require a fixed procedure, but standardisation is overly restrictive in predicting the effects of a chemical on the community in a specific receiving system. For example, *Daphnia spp.* would not be the appropriate species to predict the effects of contaminants on freshwater streams, as this genus is planktonic and usually does not occur in flowing waters (Fitter & Manuel, 1995). Although bioassays should ideally be rapid, simple and inexpensive, one of the main criterion should, perhaps, be that the species used is ecologically relevant to the system under study (See Chapter 5).

1.3.2 Sub-lethal versus lethal toxicity tests

Another criticism of many bioassays has been the predominance of lethal rather than sub-lethal toxicity tests (Birge & Black, 1985; Richardson & Martin, 1994; Forbes & Forbes, 1994). Maltby & Calow (1989) found that from 1979 to 1987, 80% of toxicity tests used survival as a criterion of toxicity. The response of field populations, however, is likely to first occur at sub-lethal concentrations of contamination. Any toxic effect on the physiology or behaviour of organisms is of significance if it affects growth, reproduction, mortality or dispersal; factors which will influence the abundance and distribution of the species. Thus, sublethal responses to a pollutant, such as physiological responses [for example, changes in respiration rate of oligochaetes (Chapman, 1987)], behavioural

responses [such as grazing rates of the estuarine harpacticoid *Schizopera knabeni* (Lotufo, 1997)], and changes in growth rates [for example, the effect of Cu on the growth of *Tubifex tubifex* (Wiederholm *et al.*, 1987)], have been used to detect the effects of contaminants. If an ultimate aim is to predict the effects of contaminants on the population dynamics of individual species, perhaps the best criteria is to look at the chronic effects on development and reproduction together with survivorship values, as these variables characterise the most ecologically relevant sub lethal endpoints (Transpurger & Drews, 1996).

Even so, the measure of response in sub-lethal toxicity tests may be time consuming and it is important to develop, and evaluate, rapid methods for measuring sub-lethal toxicity. Analysis of the results of many partial and complete life-cycle tests demonstrated that, in the majority of cases, the early life stages are the most sensitive stage (Macek & Sleigh, 1977; McKim, 1977). The 'no observable effect' concentration, based on the embryo-larval stages, generally lies very close to the value obtained when the whole life cycle is considered (Macek & Sleigh, 1977; McKim, 1977). Thus, rapid and cost-effective alternatives to established chronic toxicity tests have focused on the development of test methods using sensitive early life stages (Mount & Norberg, 1984; Norberg & Mount, 1985). Caution using these tests is still required, however, as there are concerns that relatively short-term exposure durations may not reflect accurately biological effects due to prolonged exposure to toxicants (Suter, 1990; Chapter 5).

1.3.3 Linking different levels of biological organisation

In recent years, there has been a growing concern over the lack of laboratory-to-field verification of toxicity tests by regulators (Cairns & Pratt, 1989; Maltby & Calow, 1989; Richardson & Martin, 1994). The results of studies that have tried to verify the predictions

made in the laboratory with effects in the field have shown that, in some cases, extrapolation from bioassays in the laboratory to the field are accurate, whereas in other cases they are misleading (Cairns & Pratt, 1989). An alternative approach suggested by Gray (1989), was to select ecologically sensitive species objectively from a pollution gradient.

1.4 Stream meiofauna: description and potential as biomonitors

Stream meiofauna are defined as those animals which pass through a 500 μm mesh sieve and are retained on a 63 μm sieve (Giere, 1993). Typically, stream meiofaunal communities are dominated by harpacticoid and cyclopoid copepods, nematodes and rotifers; cladocerans, ostracods, hydrachnellid and halacarid mites, tardigrads and gastrotrichs may also be abundant, although the proportions and abundances of these taxa vary (Palmer, 1990; Rundle & Hildrew, 1990; Rundle & Ormerod, 1991; Suren, 1992; Borchardt & Bott, 1995). Many surveys of the stream benthos, however, have failed to acknowledge the significance of the meiofauna due to the mesh of most sampling nets being too coarse to retain animals of their size. Stream meiofauna were also considered, until recently, as interstitial species of the hyporheic region and meiofauna found in the epibenthos were thought to be chance encounters of these interstitial species (Ham, 1982; Shiozowa, 1985). Thus, knowledge of the biology of freshwater meiofauna has been obtained mostly through groundwater and lentic studies. Only of late have quantitative studies of the distribution of the stream meiobenthos taken place. Even so, the meiofauna of streams is diverse and abundant. For example, of the 194 species of fish and benthic invertebrates recorded in surveys of streams in southern England a third were found to be microarthropods and these are only a small component of the meiofauna (Townsend *et al.*, 1983; Rundle & Hildrew, 1990).

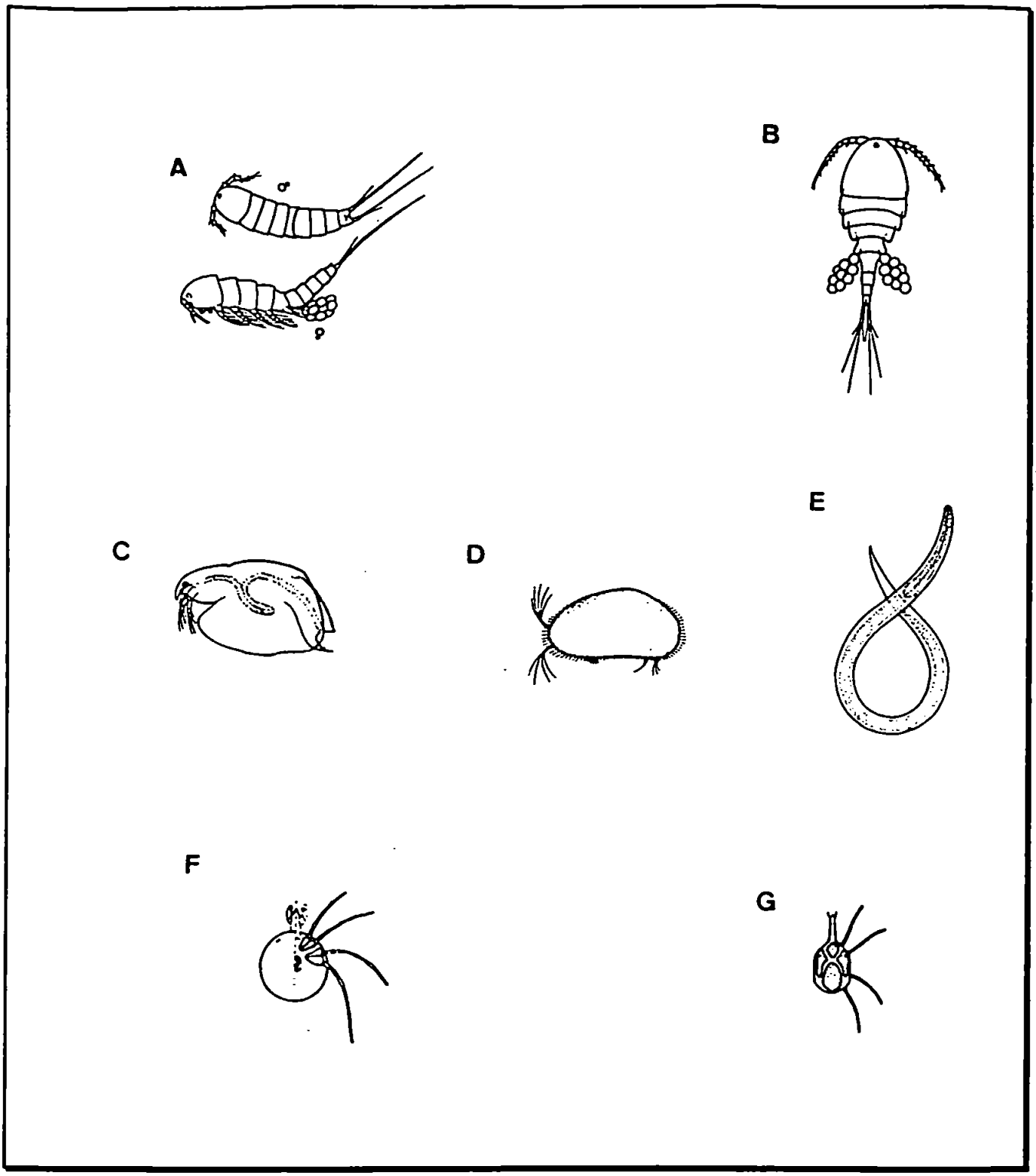


Figure 1.1 Stream meiofauna: **A)** Order Harpacticoidea (Length = 0.2-2.5 mm), **B)** Order Cyclopoidea (Length =0.5-3 mm), **C)** Class Cladocera (Length = 0.3-1.3 mm), **D)** Class Ostracoda (Length = 0.2-7 mm), **E)** Phylum Nematoda (Length = 1-3 mm), **F)** Family Hydrachnellae (Length = 0.5-2 mm), **G)** Family Halacaridae (Length = 0.2-1 mm). (Figure adapted from Fitter & Manuel, 1995)

The microcrustaceans are particularly abundant in running waters. For example, more microcrustaceans, were found within a stream bed than within the plankton of a nearby mountain lake in Colorado (Pennak & Ward, 1986). Of the microcrustaceans, harpacticoid and cyclopoid copepods are often the dominant groups in the stream benthos. Stream harpacticoid copepods are highly adapted for burrowing within the interstitial environment, and have thin, rather linear bodies, minute legs and short, non-protruding appendages, whilst uniform segmentation makes them highly flexible (Fig. 1.1). At first harpacticoids were thought to feed on the detritus within the sediment, however, recent marine studies have shown some harpacticoids selectively graze on single food particles (diatom cells, bacteria and protozoans) (Marcotte 1983) and it appears that this may also to be the case for stream harpacticoids (Perlmutter & Meyer, 1991). Most cyclopoid copepods live epibenthically or among macrophytes (Giere, 1993). Besides being smaller than planktonic cyclopoids, many also have fewer eggs (Fig. 1.1) and some have even lost the typical egg sacs, carrying their eggs on long filaments (e.g. *Speocyclops* and *Graeteriella*) (Giere, 1993). Most stream cyclopoids are predacious carnivores.

Cladoceran and ostracod crustaceans can also be abundant in streams. Most benthic cladocerans never exceed 1 mm body length unlike the planktonic cladocerans which often exceed this meiobenthic size. Meiobenthic cladocerans dig through the sediment using their large, muscular antennae and thoracic appendages (Fig. 1.1) and feed on algae and detrital particles. Meiobenthic ostracods, also adapted for burrowing within the sediment, have shells which are laterally compressed, or ventrally flattened, while they have strong legs armed with claw-like setae used to burrow through the sediment. They also have a complex of spinneret glands on the second antennae that can release adhesive fibres from openings in long setae; these fibres can be used to cling onto particles. Ostracods feed on the bacteria and detritus within the sediment.

Aquatic mites also frequent the stream benthos (Fig 1.1). The larval phase of hydrachnellid mites are parasites of adult insects. Halacarid mites are adapted to life in exposed and agitated coarse substrata, with their roundish bodies armoured with solid plates and their legs pressed to the body in depressions of the cuticle. Both groups of mites are carnivorous with piercing mouthparts. Nematodes (Fig 1.1) can also reach high abundances in streams (Palmer, 1990). They are able to move through the sediment by characteristic dorso-ventral wriggling movements. Most nematode species specialise on one type of food with their mouthparts being adapted to deal with specific food items.

Thus, stream meiofauna species have several features that enable them to cope with high flows found in streams. Meiofaunal communities are disturbed by sudden floods, but faunal reduction is compensated for relatively rapidly (in a few weeks) possibly through transport from regions higher up the river, but also from the refuge areas represented by debris accumulation (Giere, 1993). Recent investigations have demonstrated that meiofaunal distribution is linked to stream physicochemistry. Flow, temperature and pH, amongst other factors are important in determining stream meiofaunal community structure (see Chapter 3 for further discussion).

Although the presence of lotic meiofauna has been recorded only occasionally, their high densities (e.g. Zullini & Ricci, 1980; Shiozawa, 1985) and high production values (O'Doherty, 1985) imply that these animals contribute significantly to energy dynamics in streams. The detritivorous harpacticoid copepod, *Attheyella illinoisensis*, has been shown to effectively remove accumulated organic material, fungi and bacteria from detritus and can enhance production in detritally-associated bacteria (Perlmutter & Meyer, 1991). Thus, harpacticoids may alter substantially the quality of detritus, influencing the rate of its

consumption by larger stream invertebrates. In such a way, harpacticoids may play a significant rôle in the detrital dynamics of streams. Furthermore, meiofaunal taxa such as copepods and nematodes have no emergent stages and, consequently, most carbon assimilated and not respired by these animals will remain in the stream, unlike most macroinvertebrate insects which leave the stream on becoming adults. Thus, the meiofauna is likely to have a significant role in stream ecosystem functioning (Meyer *et al.*, 1988; Rundle, 1988) and may be important in lotic food webs as consumers of detritus and microbes, contributing to the transfer of energy from microbes to macrofauna (Lancaster & Robertson, 1995). Meiofauna may also be important prey of many stream biota, and have frequently been found to be present in the diet of fish such as cyprinids and salmonids (Rundle & Hildrew, 1992) and predatory invertebrates such as caddis flies, alderflies and tanypoid chironomids (Hildrew *et al.*, 1985; Lancaster and Robertson, 1995).

In marine ecosystems, there is growing interest in using the benthic meiofauna as a monitor of pollution (Moore & Bett, 1989; Coull & Chandler, 1992). As with the macrofauna, the meiofauna are ubiquitous, sedentary, relatively easy to sample, and represent a diverse range of trophic levels and feeding types (Section 1.2.1). Marine meiofaunal communities are sensitive to a wide range of contaminants including organic pollution (Keller, 1985; Sanduli & de Nicola, 1991), crude oils (Kontogiannis & Barnett, 1973; Ustach, 1979), trace metals (Hoppeneit & Sperling, 1977; Brand *et al.*, 1986) and pesticides (Bengtsson, 1978; Bengtsson & Bergstrom, 1987). Several advantages of using marine meiofauna, rather than macrofauna, as monitors of contamination has been suggested and these may apply also to the freshwater meiofauna (Heip, 1980; Hicks, 1991; Warwick, 1993). Firstly, meiofaunal species have short generation cycles and rapid growth rates, thus, measurable structural changes in the community will be measurable in a shorter time than macroinvertebrates. Secondly, meiofaunal species remain in the benthos throughout their

entire life cycles and are, therefore, closely linked to the dynamics of this environment. Many stream macrofauna species, on the other hand, have a terrestrial adult stage which results in episodic recruitment to the stream benthos being dependent on factors other than those within the locality of the benthos. Finally, the meiobenthic fauna has an intimate association, and dependency, on the sedimentary environment. As the sediment is the sink for many contaminants, the meiobenthos are impacted directly by the long-term consequences of contamination.

Recently, studies of the effects of contaminants on the marine benthos have included both the macrofauna and meiofauna (Read *et al.*, 1983; Austen *et al.*, 1989; Somerfield *et al.*, 1995). By including the meiofauna, a more comprehensive comparison between sites can be made due to the more diverse assemblage of organisms (Newell *et al.*, 1990a). Meiofauna and macrofauna represent two distinct components of the benthos, with a number of differentiating features beside size (e.g. they have short generation times and usually complete their entire life cycle in the benthos). Therefore, macrofaunal and meiofaunal communities may respond differently to contamination, and studies have found this to be the case (Austen *et al.*, 1989; Somerfield *et al.*, 1994). (Refer to Chapter 4 for a more detailed comparison of the responses of macrofaunal and meiofaunal communities to pollution.)

Evidence suggests that the freshwater meiofaunal community structure has potential as a monitor of anthropogenic inputs. For example, the meiobenthos responded in a comparable way to the macrobenthos when exposed to industrial and municipal effluents in Lake Vanajavesi, southern Finland (Kansanin, 1981). The diversity of both faunal components increased downstream of the point source of industrial discharge (Kansanin, 1981). The hyporheic meiobenthos of a small German mountain stream was influenced by a discharge

of domestic and brewery effluent (Pieper, 1976). In the vicinity of the outfall, the fauna was impoverished, but at downstream stations, the normal community composition was restored (Pieper, 1976). Stream meiofaunal communities responded to the effects of acidification, and very distinct communities were found at sites of different pH in streams in mid-Wales (Rundle & Hildrew, 1990) and in the Ashdown Forest, England (Rundle & Ormerod, 1991). Lower abundances of some meiofaunal groups (e.g. harpacticoid copepods) appeared to be linked to higher levels of Al in the streams in mid-Wales (Rundle & Ormerod, 1991), suggesting that some stream meiofaunal species are sensitive to elevated concentrations of this metal.

Stream meiofauna may also show potential as toxicity test organisms. Freshwater nematodes are increasingly being used as toxicity test organisms using lethal, sub-lethal and genetic endpoints (Samiloff, 1987; Bongers & Van de Haar, 1990; Transpurger *et al.*, 1995). Marine nematodes and copepods have also been used frequently as toxicity test organisms, and are highly sensitive to contaminants (Coull & Chandler, 1992). For example, the marine copepod *Tisbe battagliai,* was more sensitive to Cu and hexavalent chromium than the standard toxicity test organism, the mysid crustacean *Mysidopsis bahia,* (Hutchinson *et al.*, 1994). The high fecundity, fast development rates and short generation times also make meiofauna excellent potential test organisms for assessing contaminant effects on reproduction. Results of these tests are obtained quickly and cost effectively, and sub-lethal endpoints are more sensitive indicators of toxicity than mortality (Lotufo, 1997; Williams, 1997).

1.5 Outline of the thesis

It is apparent that freshwater meiofauna may have potential as a biomonitor of water quality. Within this context this thesis aims to investigate the potential of meiofauna at the community and individual level as a monitor of metal-contamination. Chapter 2 describes the physicochemistry of twelve sites in streams representing a gradient of Cu contamination. The environmental variables in these streams were measured to identify the important forcing agents structuring the meiofaunal community. In Chapter 3 the meiofaunal communities found in these streams are described and multivariate analyses were used to establish whether differences in metal concentrations or other environmental variables explained the inter-site differences in meiofaunal communities. In Chapter 4 correlations between subsets of the benthic invertebrate community and environmental variables are compared across a metal contamination gradient, with a view to using these faunal components as monitors of metal contamination. Univariate measures, the Index of Multivariate dispersion and higher taxonomic levels than species, for both macrofaunal and meiofaunal communities, were also used to examine the pattern of perceived impact (Chapter 4).

The harpacticoid copepod *Bryocamptus zschokkei* was sensitive to the subtle differences in Cu contamination measured at the sites representing a gradient of metal contamination (Chapter 3). It was, thereby, selected as a potential toxicity test organism for Cu. Chapter 5 describes laboratory experiments assessing the toxic effects of Cu on the survival, development and fecundity of *B. zschokkei*. The acute toxic effects of Cu on individuals from contaminated and reference sites were compared to assess whether field populations had developed tolerance to Cu (Chapter 5). Chapter 6 provides a general discussion of the potential of meiofauna at the community and individual level as a monitor of metal contamination. Where appropriate, recommendations are made for future research.

CHAPTER 2

Stream physicochemistry

2.1 Introduction

It is well established that the composition of stream communities is influenced strongly by physical and chemical factors (Allan, 1995). Of the physical variables, water velocity has been identified as one of the most significant influences on meio- and macrobenthic communities in running waters. Previous studies have shown that flow causes a patchy distribution in the abundance of stream macroinvertebrates (Hildrew & Giller, 1994), while Palmer (1990) reported a reduced abundance of meiofaunal oligochaetes, rotifers and copepods after floods in a North American creek. Temperature is another highly influential variable which impacts on stream communities. For example, seasonal variation in temperature is critical to the successful completion of the life cycle of aquatic organisms, affecting the timing of hatching and the rate of growth (Hynes, 1970). Many studies that have shown high temperatures will increase the rate of development of stream biota (Allan, 1995), resulting in increased abundances of the fauna.

Stream chemistry is also an important factor shaping benthic communities. Ormerod & Edwards (1987) recorded higher numbers of macrofaunal species in streams of high rather than low water hardness. Increased concentrations of dissolved organic carbon (DOC) have a direct effect on primary production and, therefore, on the abundance and diversity of the benthic community (Allan, 1995). Stream chemistry, which has been altered due to anthropogenic inputs, often has the most dramatic impact on stream biota. For example, naturally acidic streams seem to be less affected than those acidified by atmospheric acidification (Allan, 1995). The deleterious effect of acidic stream waters is primarily in terms of reducing the number of species and individuals of both the macrofaunal (Weatherly & Ormerod, 1987) and meiofaunal communities (Rundle & Hildrew, 1990). Of the other anthropogenic inputs which have had impacts on stream biota, trace metal contamination is one of the most severe. Field studies have shown that trace metal

contamination often reduces the abundance and species richness of stream macroinvertebrates, and changes the proportional abundance of different groups (Willis, 1985; Clements *et al.*, 1988; Kiffney & Clements, 1994). There is little information available on the effect of trace metal pollution on stream meiofauna (Chapter 3).

In the case of aquatic ecosystems impacted by trace metals, water toxicity will depend not only upon the concentrations and combinations of the trace metals present, but also on the interactions of metals with other environmental variables that may influence metal toxicity (Chapter 1). Therefore, in the present study, it was considered important to measure these environmental variables. As the meiofauna tend to live interstitially, metal concentrations in the interstitial waters were measured at each site as other workers have reported differences in water chemistry between surface and interstitial waters (Pennak, 1988). It was also important to measure temporal changes in environmental factors and metal concentrations (Williams, *et al.*, 1973; Brown, 1977b), as seasonal changes in river flow can, for example, lead to a decrease in dissolved metal concentrations by dilution (Johnson & Thornton, 1988). Conversely, high flows may increase metal concentrations by the greater scouring action of bottom sediments (Williams *et al.*, 1973; Brown, 1977b).

The main aim of this chapter was to document the physicochemistry of the sites selected to investigate relationships between meiofaunal communities and trace metals. Comparisons between interstitial and surface water concentrations were made for metals and DOC concentrations. Overall trends in physicochemistry among sites were also investigated using the ordination technique Principal Component Analysis (PCA).

2.2 Study area and sampling sites

The study area incorporated the streams of the south-east corner of Bodmin Moor, south-west England (Fig. 2.1). All streams were fast flowing, first order tributaries typical of Cornwall's narrow, moorland ridge. As a result of geological processes, pockets of metals, chiefly tin and copper ores, have been deposited in this region (Dines, 1956). In particular, there are several lodes of tin (Sn) and copper (Cu) in close association, and these run parallel in an east-west direction; some lodes of lead (Pb) and zinc (Zn) run in a north-south direction (Fig. 2.1). Intensive mining activity in the 19th and early 20th Centuries (Shambrook, 1986) led to an extensive network of underground workings, and an increase in the surface area of contact between metal ores and ground water. Hence, the drainage water in the study area is vulnerable to contamination from groundwater and surface runoff. As the area has never become a major population centre (NRA, 1994), it has not been subject to other sources of environmental contamination. Hence, the effects of trace metals on stream biota have not been compounded by interaction with other forms of contamination, making it an ideal location for identifying the effects of trace metals on stream benthic communities.

Sampling sites were located on tributary streams of the Rivers Lynher [Darley Brook (D), Upton Cross stream (U), Longridge tributary (L), Berriowbridge tributary (B), Rilla Mill tributary (R) and Trebartha tributary (T)] and Seaton (S) (Fig. 2.2). These sites were chosen as their chemistry and macroinvertebrate communities have been monitored previously (e.g. Gower *et al.*, 1994). The tributaries of the Rivers Lynher and Seaton have a long history of trace metal contamination from mine drainage and, initially, were the subject of several studies on water quality. South West Water (1984) recorded elevated Cu levels in Darley Brook, Upton Cross stream and Longridge tributary, and high Zn concentrations in

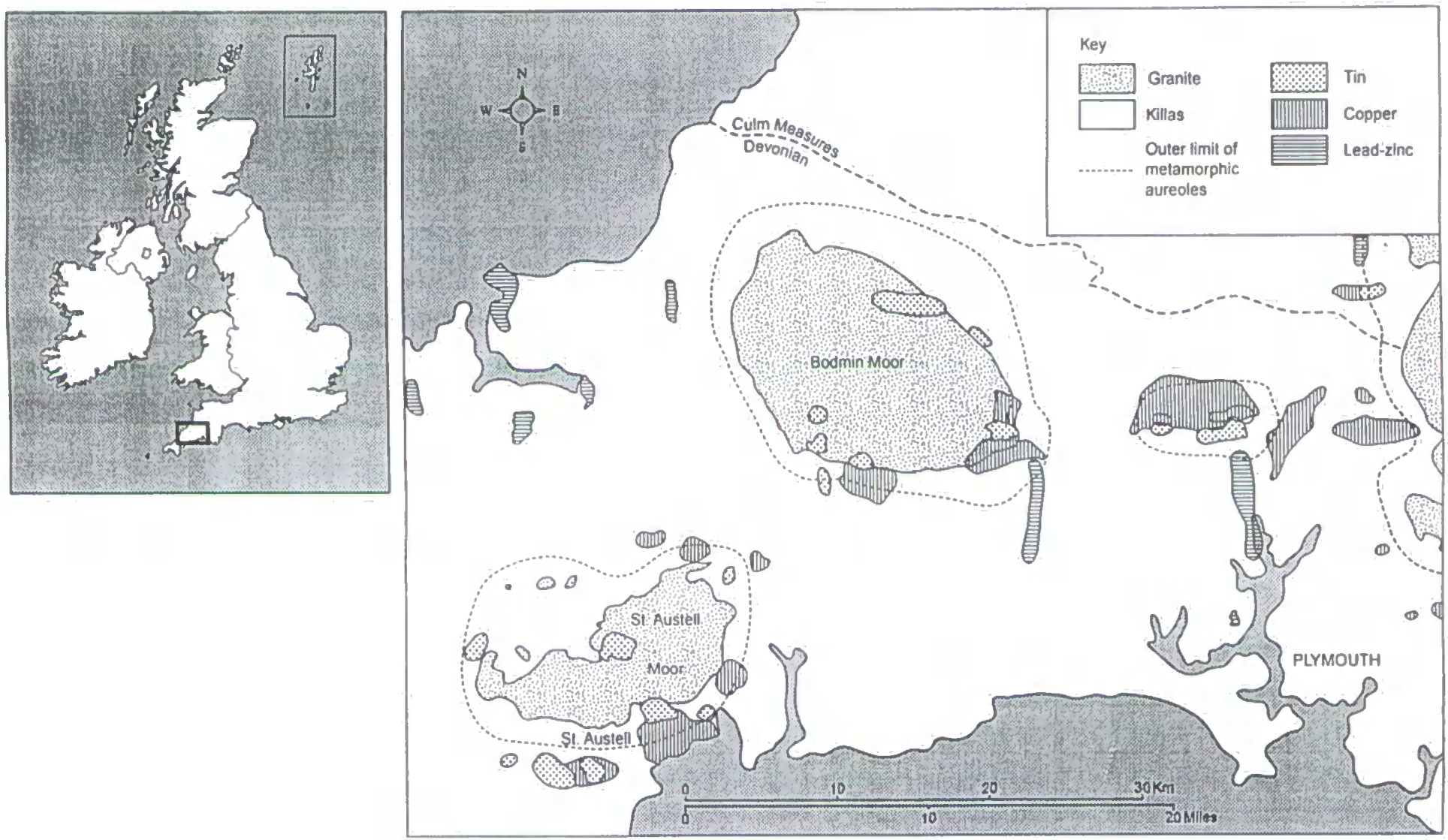


Figure 2.1 Principal lodes in south-west England. Note the lodes of copper, lead and zinc in the south-east corner of Bodmin Moor where streams were sampled (From Dines, 1956).

the Upton Cross stream. Following these initial findings, Darlington (1987) measured high concentrations of Cu in Darley Brook and reported decreasing Cu concentrations further downstream. Finally, Gower *et al.* (1994) reported high concentrations of Cu, Zn, Al and Fe in many of the stream tributaries of the Rivers Lynher and Seaton, and showed that Cu was highly correlated with macroinvertebrate community structure.

As Cu was shown to be an important variable shaping biotic communities (Gower *et al.*, 1994), this trace metal was chosen as the major water chemistry gradient against which to gauge meiofaunal community response. Ten sites, selected along a copper gradient included highly contaminated sites (U2, L4, S8 and D5) below adit portals, sites of intermediate contamination (D1, S9, U3 and L1) some distance from the mine adits, and sites with no obvious source of contamination (R2 and B2) (Fig. 2.2). These sites were sampled during the spring and autumn. In the summer, two additional sites were included to increase the resolution of the gradient in Cu contamination. These were a "high quality" RIVPAC site (T1), classified as a biological class A site in 1990 (NRA, 1994), and a site (D3) with intermediate Cu concentrations (Fig. 2.2).

2.3 Materials and methods

2.3.1 Collection of water samples

Surface water samples were taken in November 1994, May and August 1995, and January 1996, and interstitial samples in August and November 1995, and January and May 1996. All samples were taken within one week of sampling the stream biota. At each site, on each sampling occasion, two 50 ml samples each of surface and interstitial water were collected in acid-washed propylene bottles; one sample was for metal analysis, the other was for DOC analysis.

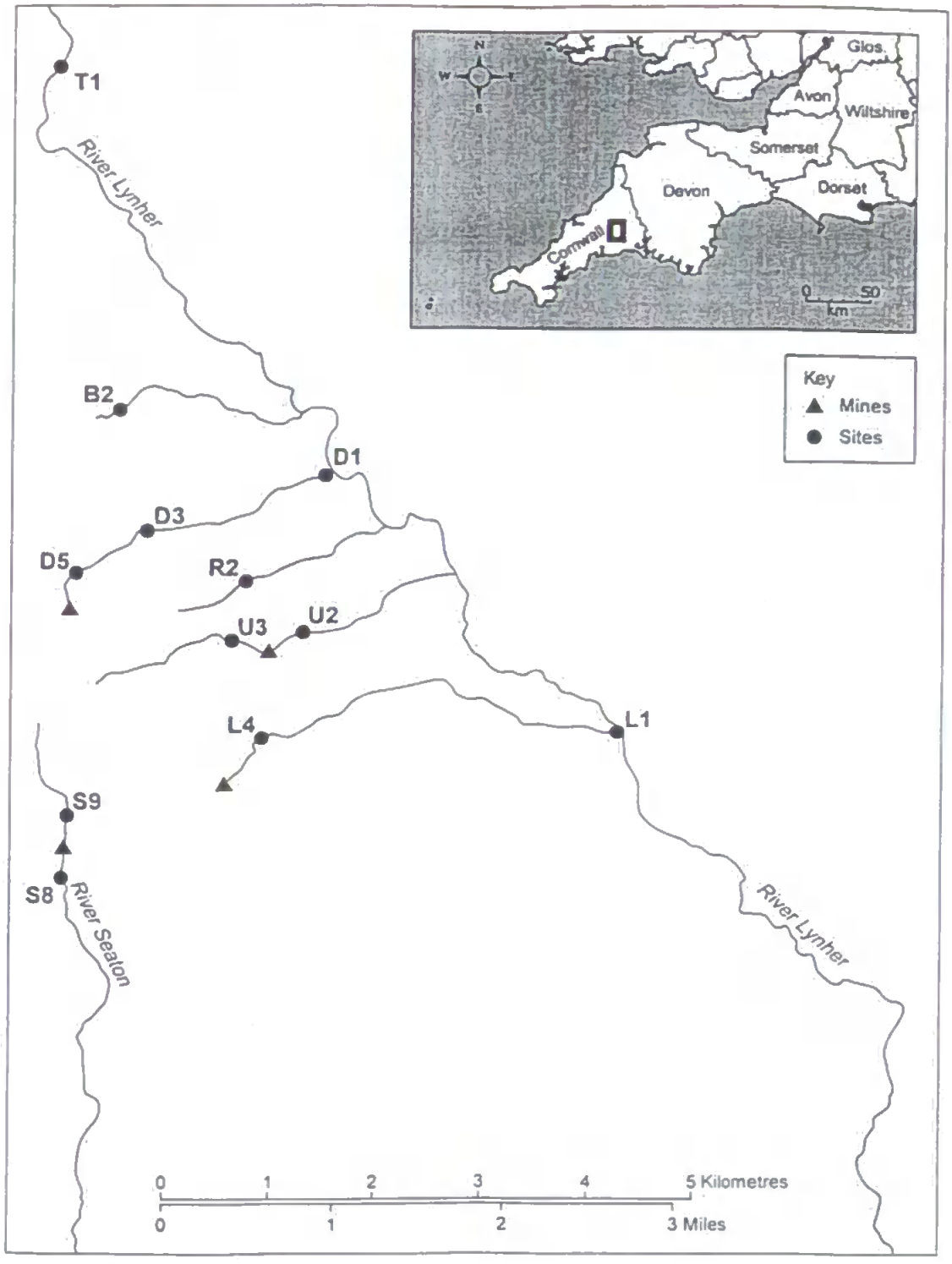


Figure 2.2 Map of the tributaries of the Rivers Lynher and Seaton, south-west England, showing the location of the twelve sites used in the surveys.

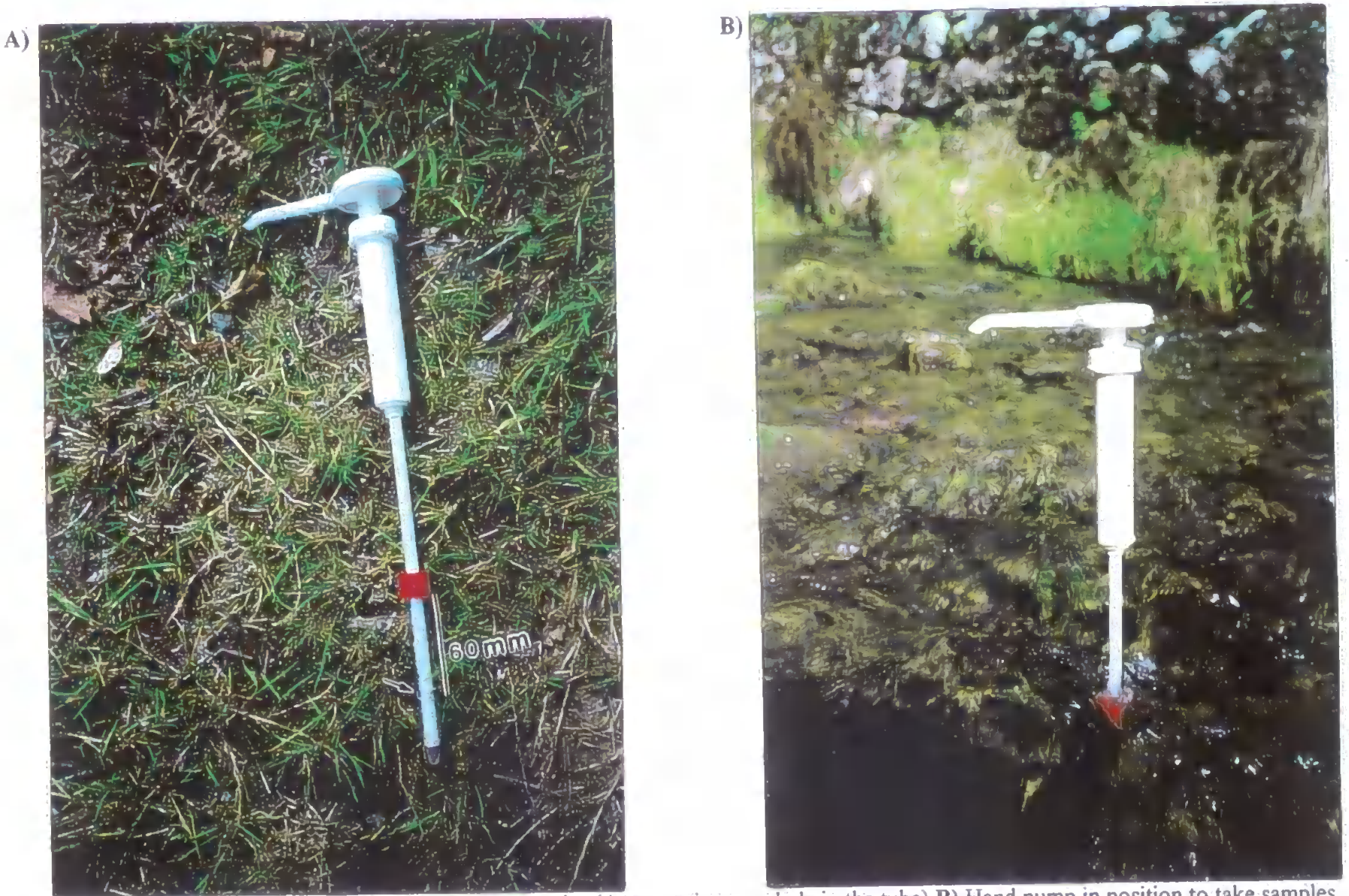


Plate 2.1. A) Hand pump and tube used to obtain interstitial water samples (Arrow points to a hole in the tube) **B)** Hand pump in position to take samples.

Interstitial water was obtained using a centrifuge tube (length=100 mm; depth=15 mm) with a lid inserted into the sediment two weeks prior to sampling (Plate 2.1). In winter (January, 1996), two tubes were placed at each site as a precaution against their possible loss due to high flow rates. The two test tubes used in winter provided the additional advantage of giving some idea of the variation in the method of sampling interstitial metal concentrations. Each tube contained six holes (each of 5 mm diameter), three holes on each side; the holes were positioned so that water was sampled at 20, 40 and 60 mm depth in the sediment. Water was drawn from the tube using a hand pump (Plate 2.1A). In the field, interstitial and surface water samples were filtered through a 0.45 μm Millipore membrane filter. Any metals passing through this filter are considered available for uptake by the biota (Wilson, 1976). Water samples for metal analysis were acidified in 5% spectrosol grade nitric acid to ensure the metal ions remained in solution (Smith, 1973). All equipment used for sample collection and storage was washed in 5% nitric acid and distilled deionized water.

2.3.2 Metal analysis

Concentrations of Cu, Zn, Fe, Al, Ca and Mg were measured using a GBC 902 flame atomic absorption spectrophotometer. For each metal, a fine mist of sample is sprayed into a flame where atomization occurs. The resulting metal atoms are excited by a hollow cathode lamp (specific to each metal) and by absorbing energy allow a transition from the ground state to a higher energy level. The amount of energy absorbed is related to the concentration of the metal. The limits of detection for Cu, Zn, Al, Fe, Ca and Mg found when using the flame atomic absorption spectrophotometer were 3 $\mu g\ l^{-1}$, 0.8 $\mu g\ l^{-1}$, 30 $\mu g\ l^{-1}$, 6 $\mu g\ l^{-1}$, 1 $\mu g\ l^{-1}$ and 3 $\mu g\ l^{-1}$, respectively. The detection limit being defined as the value that can be detected with a 95 % confidence level. Ca and Mg form stable compounds with anions such as phosphate, which hinder the formation of atoms. This was overcome by adding an excess of potassium chloride (10,000 mg l^{-1}), which causes the interfering anion

to form a compound with the potassium, thereby, releasing Ca and Mg. Concentrations of Ca and Mg in surface water samples were used to calculate total water hardness for each site using the equation:

$$\text{Hardness} = (\text{Ca} * 2.50) + (\text{Mg} * 4.12)$$

where all concentrations are in mg l^{-1} (Gower *et al.*, 1994).

2.3.3 DOC Analysis

Non-acidified water samples were stored at 4°C for up to one week in acid-washed propylene bottles prior to measurement. DOC concentrations were measured using a SHIMADZU Total Organic Carbon Analyser 5000.

2.3.4 Measurement of other physicochemical variables

Field measurements of other environmental variables, made at the same times as the surface water samples included one measurement of each variable taken per visit. A Phox 2E meter was used to measure pH, and a HANNA HI 8633 meter to measure conductivity and temperature. Measurements were taken by placing the probes 2 cm under the surface in a slow flowing region of the stream.

A single measurement of stream discharge was made in November 1995, and January, May and August 1996 using a modified version of the area-velocity method developed by Lancaster & Hildrew (1993). A random point was chosen along a line bisecting the stream channel longitudinally, and the water depth (d), stream width (w) and near bed current velocity (m s^{-1}) were measured at this point and at two equidistant points either side. Measurements were taken 3 cm above the stream bed using a Valeport current meter. This procedure was repeated at two other random points along the transect and discharge was calculated ($m^3 s^{-1}$) by multiplying the mean current velocity by stream area (d * w).

2.3.5. Statistical analyses

Due to the right skewed nature of all the environmental data (apart from pH), a log (n+1) transformation was applied. Principal Components Analysis (PCA) was used to display (as two dimensional ordinations) inter-site differences in environmental variables for individual seasons and all seasons combined (Clarke & Warwick, 1994a). Principal components are linear combinations of the original variables, with there being as many principal components as there are variables. The degree to which the two-dimensional PCA succeeded in representing the data was shown by the percentage of total variance explained by the first two principal components. The biggest differences between sites takes place along the first principal component axis (PC1) (the axis which maximises the variance of points projected perpendicular onto it). The second principal axis, perpendicular to PC1, was chosen to maximise the variance of points. The contribution of an environmental variable to PC1 and PC2 is indicated by its coefficient in the linear combination of variables making up the PC. It was important to normalise all the PC axes due to the mix of measurement scales used for the environmental variables (e.g. $\mu g\ l^{-1}$, $m^3\ s^{-1}$, $\mu S\ cm^{-1}$), otherwise, points on the ordination could be made to appear closer, simply by a change of scale on one of the axes. Data were normalised by subtracting the environmental variable for each site by the mean across sites and then dividing by the Standard Deviation across sites to give a correlation-based PCA (Clarke & Warwick, 1994a). This procedure equalised the variance of samples along all the environmental axes, so that all the environmental variables were, potentially, of equal importance in determining the principal components.

Lower triangular Euclidean distance matrices relating to the ordination for environmental variables for individual seasons and all seasons combined were constructed from the original data using the PRIMER program CLUSTER. The Euclidean distance is the natural distance

between any two points in space. The Euclidean distance between the *j*th and the *k*th sample (d*jk*) is calculated using the equation:

$$djk = \sqrt{[\sum_{i=1}^{p} (yij - yik)^2]}$$

where y*ij* is the *i*th variable in the *j*th sample. Similarly y*ik* represents the *i*th variable in the *k*th sample. $\sum$ is the sum of all variables. To verify whether sites were separated in a comparable way in each season, Spearman Rank correlations (ρ) between the corresponding elements of each pair of the Euclidean distance matrices for environmental variables were computed using the PRIMER program RELATE. The significance of the correlation determined by using a Monte Carlo permutation procedure (Clarke & Warwick, 1994b).

Relationships between site differences in the environmental variables in all seasons and between surface and interstitial water samples in August 1995 and January 1996 , were assessed using product moment correlation coefficients derived from linear regression analyses (Devore & Peck, 1990).

2.4 Results

2.4.1 Trace Metals

The twelve sites represented a clear gradient in surface and interstitial Cu concentrations, with mean surface water and interstitial concentrations ranging from 10-766 μg l^{-1} and 7-1820 μg l^{-1}, respectively, across the sites (Table 2.1; Fig. 2.3). The highest concentrations of Cu were found at sites downstream of adit portals (U2, L4, S8 and D5) and lowest Cu concentrations were measured at sites where there was no obvious input source of metals (T1, R2, B2). There was a wide range in the concentrations of the other metals in both the surface and interstitial waters (Table 2.1 & 2.2). Highest Zn concentrations were found at sites located downstream of the adit portals, with exceptionally high maximum surface and interstitial Zn concentrations at U2 (Table 2.1). At other sites, mean Zn concentrations ranged from 52-374 and 81-377 μg l^{-1} in the surface and interstitial waters, respectively. Concentrations of surface Fe were highest at U2 and L4, and mean surface concentration of Fe found at the other sites ranged from 51-140 μg l^{-1} (Table 2.2). The concentration of interstitial Fe was highest at R2, U2 and L4 and the mean interstitial concentration of Fe found at the other sites ranged from 113-264μg l^{-1}. Concentrations of surface and interstitial Al were highest at L4 and S8 (Table 2.2). The mean surface and interstitial concentrations of Al found at other sites ranged from 118-474 and 152-821 μg l^{-1} Al, respectively.

Interstitial Cu and Zn concentrations in August 1995 and January 1996 showed strong correlations ($p<0.05$) with those measured in surface waters samples taken at these times (Fig. 2.4; Table 2.4). Iron interstitial and surface water concentrations correlated significantly in January 1996 and aluminium interstitial and surface water concentrations correlated significantly in August 1995 (Table 2.4). There was, however, no significant correlation between summer interstitial and surface water Fe concentrations and winter interstitial and surface water Al concentrations (Table 2.4).

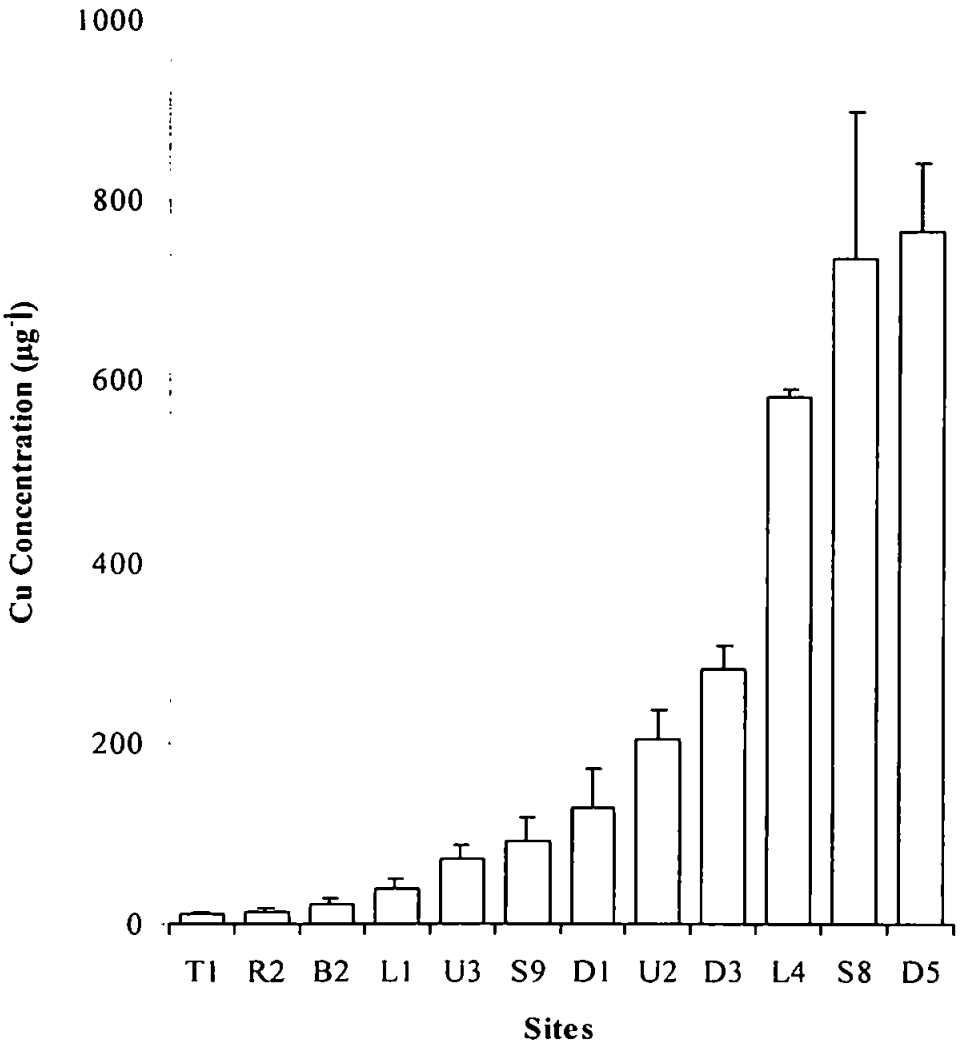


Figure 2.3. Mean surface water Cu concentrations (µg l^{-1}) at the twelve sites located on the tributaries of the Rivers Lynher and Seaton. Sites are arranged in order of Cu concentration to demonstrate the trend (n = 4 for each site, vertical bars = + 1 SD). See Figure 2.2 for location of each site.

Table 2.1 Mean and maximum concentrations (μg l^{-1}) of Cu and Zn at the twelve sites used in the surveys (sites T1 and D3 were only used in the summer survey). See Figure 2.2 for location of each site. (Interstitial and surface water measurements not directly comparable).

Site	Copper (μg l^{-1})				Zinc (μg l^{-1})			
	Surface Water		Interstitial Water		Surface Water		Interstitial Water	
	Mean	Maximum	Mean	Maximum	Mean	Maximum	Mean	Maximum
T1	10	17	9	20	101	156	160	271
R2	13	20	40	62	119	175	160	199
B2	22	37	24	47	115	246	112	151
L1	40	62	290	649	89	163	229	317
U3	71	116	117	167	124	272	98	129
S9	92	144	362	479	52	80	81	92
D1	128	247	185	252	63	106	101	239
U2	203	302	423	490	874	1121	955	1083
D3	283	352	651	1183	343	458	190	296
L4	582	602	345	618	273	420	216	444
S8	736	1205	1820	1890	374	424	377	446
D5	766	928	1286	1763	217	313	226	329

Table 2.2 Mean and maximum concentrations (μg l^{-1}) of Al and Fe at the twelve sites used in the surveys (sites T1 and D3 were only used in the summer survey). See Figure 2.2 for location of each site (Interstitial and surface water measurements not directly comparable).

Site	Aluminium (μg l^{-1})				Iron (μg l^{-1})			
	Surface		Interstitial		Surface		Interstitial	
	Mean	Maximum	Mean	Maximum	Mean	Maximum	Mean	Maximum
T1	402	713	152	354	157	193	131	140
R2	118	187	821	1693	139	186	757	1705
B2	243	413	578	677	94	109	264	581
L1	457	520	449	602	132	184	200	252
U3	404	440	380	630	140	337	211	492
S9	330	347	533	538	79	101	132	136
D1	305	386	438	890	51	98	193	364
U2	474	938	469	1229	396	780	487	1146
D3	402	534	388	549	108	136	113	201
L4	1084	1615	1109	1559	212	599	709	1492
S8	893	1280	1084	1740	76	86	142	180
D5	245	346	571	917	66	85	118	296

Table 2.3 Matrix of product moment correlation coefficients between mean surface metal concentrations from the twelve sites (** P<0.001, * P<0.05).

	Cu	Max Cu	Zn	Max Zn	Fe	Max Fe	Al	Max Al
Max Cu	0.995**							
Zinc	0.577	0.551						
Max Zn	0.376	0.484	0.820**					
Fe	-0.127	-0.174	0.554	0.603*				
Max Fe	-0.109	0.475	0.475	0.547	0.928**			
Al	0.524	0.522	0.415	0.394	0.281	0.370		
Max Al	0.332	0.590	0.590	0.590	0.418	0.455	0.953**	

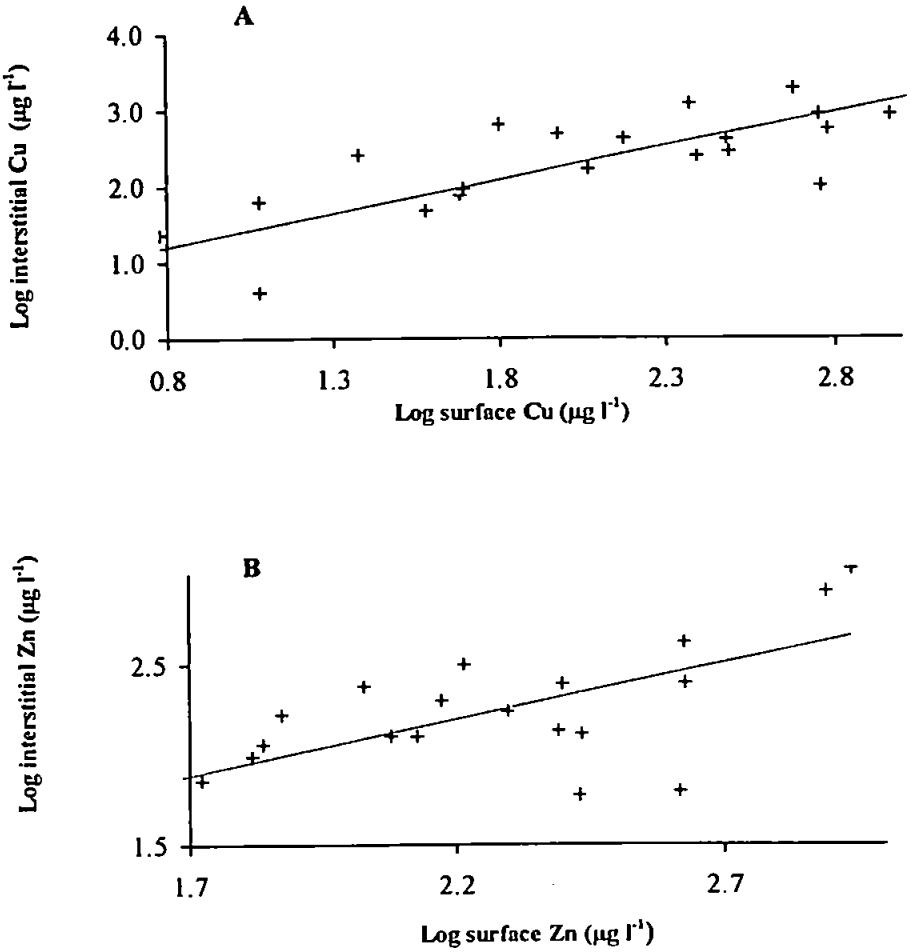


Figure 2.4 Relationship between metal concentrations in surface (*s*) and interstitial (*i*) water samples taken in August 1995 and Winter 1996 (regression equations fitted by least squares regression technique shown in brackets). **A)** Cu [log*Cui* = 0.886(log*Cus*) + 0.488, R^2=0.726]; **B)** Zn [log *Zni* = 0.629(log*Zns*) + 0.814, R^2=0.509];

Using the regression equation derived from summer and winter interstitial and surface water data (Fig 2.4), higher Cu concentrations would be expected in the interstitial than the surface water. For example if surface Cu concentrations were 100 μg l^{-1}Cu the expected interstitial Cu concentration would be 182 μg l^{-1}. However, there appears to be little difference in the concentration of Zn found in the interstitial and surface water. For example, if Zn surface water concentrations were 100 μg l^{-1} the expected concentration of Zn in the interstitial water would be 117μg l^{-1}.

Table 2.4 Product moment correlation values between interstitial and surface water concentrations of **A)** Cu and Zn and **B)** Fe and Al in summer and winter (** $p<0.05$).

A)

Copper		Zinc	
Summer	Winter	Summer	Winter
0.784**	0.777**	0.659**	0.779**

B)

Iron		Aluminium	
Summer	Winter	Summer	Winter
0.597	0.669**	0.694**	-0.080

2.3.2 Other Environmental Variables

Annual mean values of other environmental variables are shown in Table 2.5. Temperature ranged from 10.2-11.2 °C across sites, and the mean pH ranged from 6.0-6.8, except L4 which was highly acidic and had a mean pH of 4.8 (Table 2.5). Total water hardness was highest at sites R2 and B2 (Table 2.5) and ranged from 24.1-35.8 $CaCO_3$ l^{-1} at other sites. Conductivity was relatively low at all sites, ranging from 80 μS cm^{-1} at B2 to 203 μS cm^{-1} at R2, and discharge was highest at the downstream sites (T1, D1 and L1) (Table 2.5).

Table 2.5 Mean values of environmental variables at the twelve sites used in the surveys (see Appendix I for range of values). Sites T1 and D3 were only used in the summer survey. See Figure 2.2 for site locations (Interstitial and sutface DOC measurements are not directly comparable)

Site	pH	Conductivity ($\mu S\ cm^{-1}$, 25°C)	Hardness (mg $CaCO_3\ l^{-1}$)	Discharge ($m^3\ s^1$)	Maximum Discharge ($m^3\ s^{-1}$)	Temperature (°C)	Surface DOC (mg l^{-1})	Interstitial DOC (mg l^{-1})
T1	6.8	159	24.1	0.097	0.236	10.2	11.5	8.5
R2	6.6	203	105.6	0.009	0.017	10.8	7.9	5.9
B2	6.0	80	71.6	0.040	0.060	10.5	12.0	11.3
L1	6.7	180	35.8	0.146	0.200	11.0	8.5	8.6
U3	6.0	108	24.4	0.057	0.0 75	10.7	5.9	3.8
D1	6.6	151	31.9	0.189	0.261	11.0	6.1	5.1
S9	6.4	134	29.4	0.008	0.021	10.4	5.9	5.0
U2	6.3	122	27.2	0.065	0.094	11.2	5.2	4.4
D3	6.6	110	26.0	0.009	0.011	11.1	4.3	3.7
L4	4.8	127	29.6	0.008	0.015	10.8	5.3	3.9
S8	6.0	141	30.3	0.082	0.125	11.1	5.3	3.5
D5	6.2	116	24.1	0.025	0.039	11.2	5.2	3.2

The mean discharge at the other sites ranged from 0.008 to 0.082 $m^3 s^{-1}$. The pattern of DOC concentrations across sites was opposite to that for Cu. Sites of low metal concentrations (R2, B2, T1 and L1) had DOC concentrations ranging from 7.9-12 mg l^{-1} in the surface waters and from 5.9-11.3 mg l^{-1} in the interstitial waters; DOC concentrations in highly contaminated sites ranged from 4.3-6.1 mg l^{-1} in the surface water and from 3.2-5.1 mg l^{-1} in the interstitial water. A positive correlation was evident between interstitial and surface DOC concentrations in summer ($r=0.926$, $p=0.001$) (Fig. 2.5), but not winter ($r=0.162$, $p=0.633$). Surface water Cu and DOC concentrations were also correlated with temperature and negative correlations were found between mean surface water Al concentrations and water hardness (Table 2.6).

2.3.3 Temporal Variation

Of the environmental variables, only temperature showed a clear seasonal pattern (Fig. 2.6), with the water temperature being lowest in winter and highest in summer at all sites. Even so, seasonal temperature differences were small and ranged from 7.9-9.4°C (winter) and 11.1-12.9°C (summer). Lowest discharge values were consistently measured in the summer (Appendix I), although, no seasonal pattern in discharge was found for spring, autumn and winter. There was no seasonal pattern in metal variability, but the variation in metal concentrations measured in the four seasons was high at some sites (Appendix I, Fig. 2.7).

Table 2.6 Matrix of product moment correlation coefficients between mean values of physicochemical variables from twelve sites (** $p<0.01$, * $p<0.05$).

	Conductivity	Hardness	PH	DOC	Temperature	Discharge	Maximum Discharge
Cu	-0.278	-0.536	-0.504	-0.850**	0.692*	-0.175	-0.381
Maximum Cu	-0.277	-0.549	-0.467	-0.841**	0.692*	-0.102	-0.341
Zn	-0.264	-0.239	-0.337	-0.517	0.464	-0.285	-0.384
Maximum Zn	-0.379	-0.210	-0.401	-0.442	0.432	-0.277	-0.425
Fe	0.045	-0.025	-0.245	0.015	-0.170	-0.211	0.006
Maximum Fe	0.012	-0.131	-0.429	-0.133	-0.090	-0.266	-0.124
Al	-0.114	-0.616*	-0.552	-0.307	0.014	0.136	0.213
Maximum Al	-0.580	-0.161	-0.500	-0.245	0.005	0.111	0.084
Conductivity		0.188	0.409	0.107	-0.067	0.282	0.316
Hardness			0.083	0.488	-0.158	-0.179	-0.278
PH				0.266	-0.044	0.378	0.404
DOC					-0.736**	0.254	0.433
Temperature						0.021	-0.542
Discharge							0.411

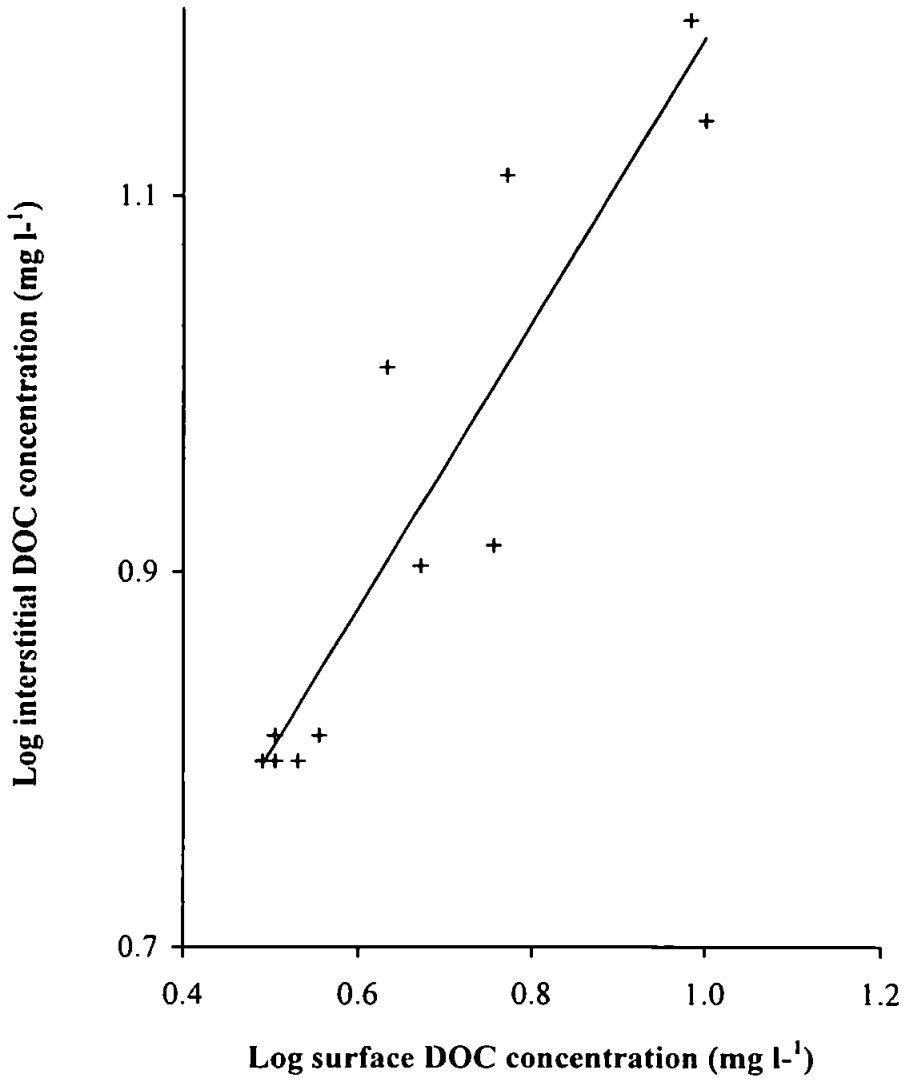


Figure 2.5 Relationship between DOC concentrations in surface (*s*) and interstitial (*i*) water in summer (logDOC*i* = 0.759(logDOC*s*) + 0.425, R2=0.855).

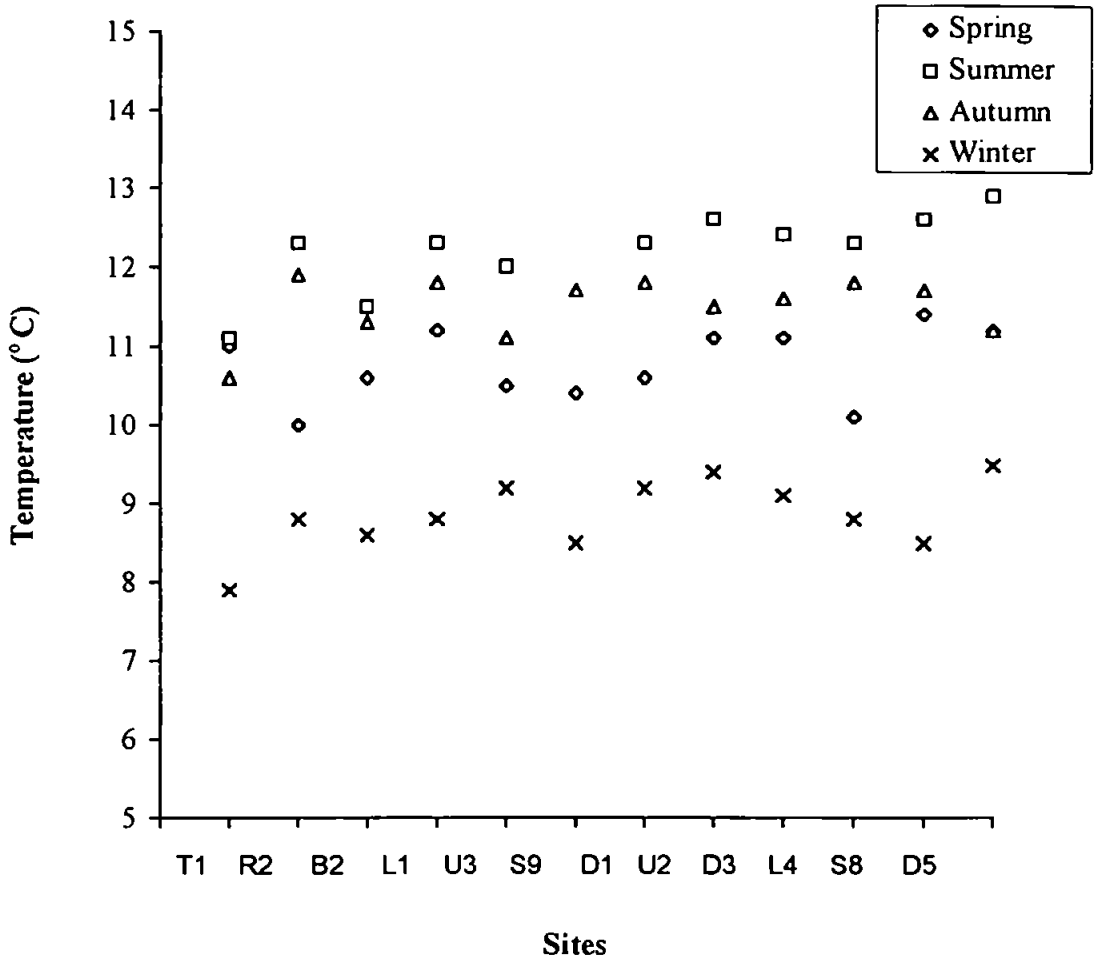


Figure 2.6 Seasonal variation in temperature. Each point represents a single measurement taken that season. Sites are arranged in order of Cu concentration. See Figure 2.2 for the location of the sites.

The PCA of the mean values of the four seasonal measurements combined, demonstrated a general pattern of increasing metal contamination from left to right on the first PC axis with the five most contaminated sites separated distinctly from the rest (Fig. 2.8A). The variables contributing most to the separation of the sites on axis one (PC1) were Cu and Al (increasing from left to right) and DOC (increasing from right to left) (Table 2.7). The second axis represented increasing conductivity, pH and discharge (increasing from top to bottom). These first two principal components accounting for 59.1 % of the variation.

The PCA ordinations for spring, summer, autumn and winter also separated sites of high metal contamination from other sites (Fig. 2.8 B-E). Again, Cu was consistently important in contributing to the separation of sites in each of the four seasons. Zinc, Al and DOC were also important in discriminating between sites along axis one in autumn and spring; winter and autumn, and summer, respectively. For the second axis the environmental variables most important in contributing to the separation of sites were hardness and conductivity in autumn, temperature and discharge in spring, hardness in summer, and hardness and discharge in winter.

Thus, despite the temporal variation found for some environmental variables, principal components analysis demonstrated that physicochemical data separated sites in a consistent seasonal pattern (Fig. 2.8). This consistent pattern was confirmed by significant ($p<0.05$) correlations between the Euclidean distance matrices of environmental variables for each season, and between the Euclidean distance matrices of environmental variables for each season and the matrix of mean environmental variables (Table 2.8).

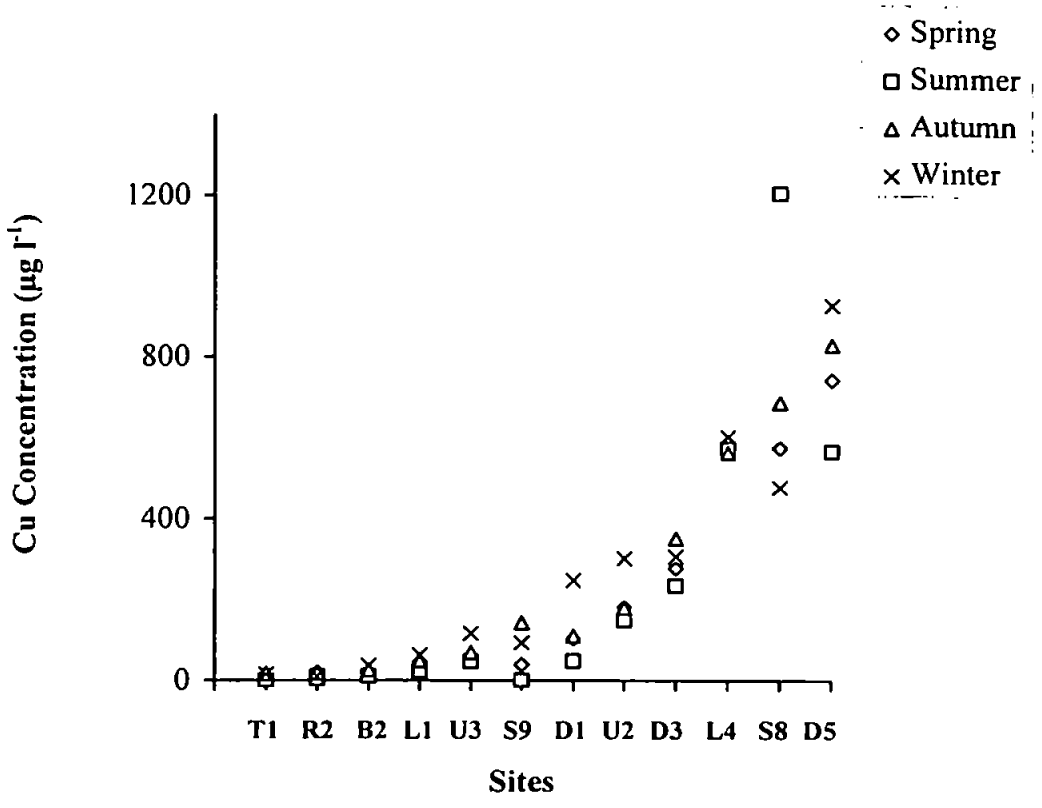


Figure 2.7 Seasonal Cu concentrations in surface waters. Each point represents a single measurement taken that season. Sites are arranged in order of Cu concentration. See Figure 2.2 for the location of sites.

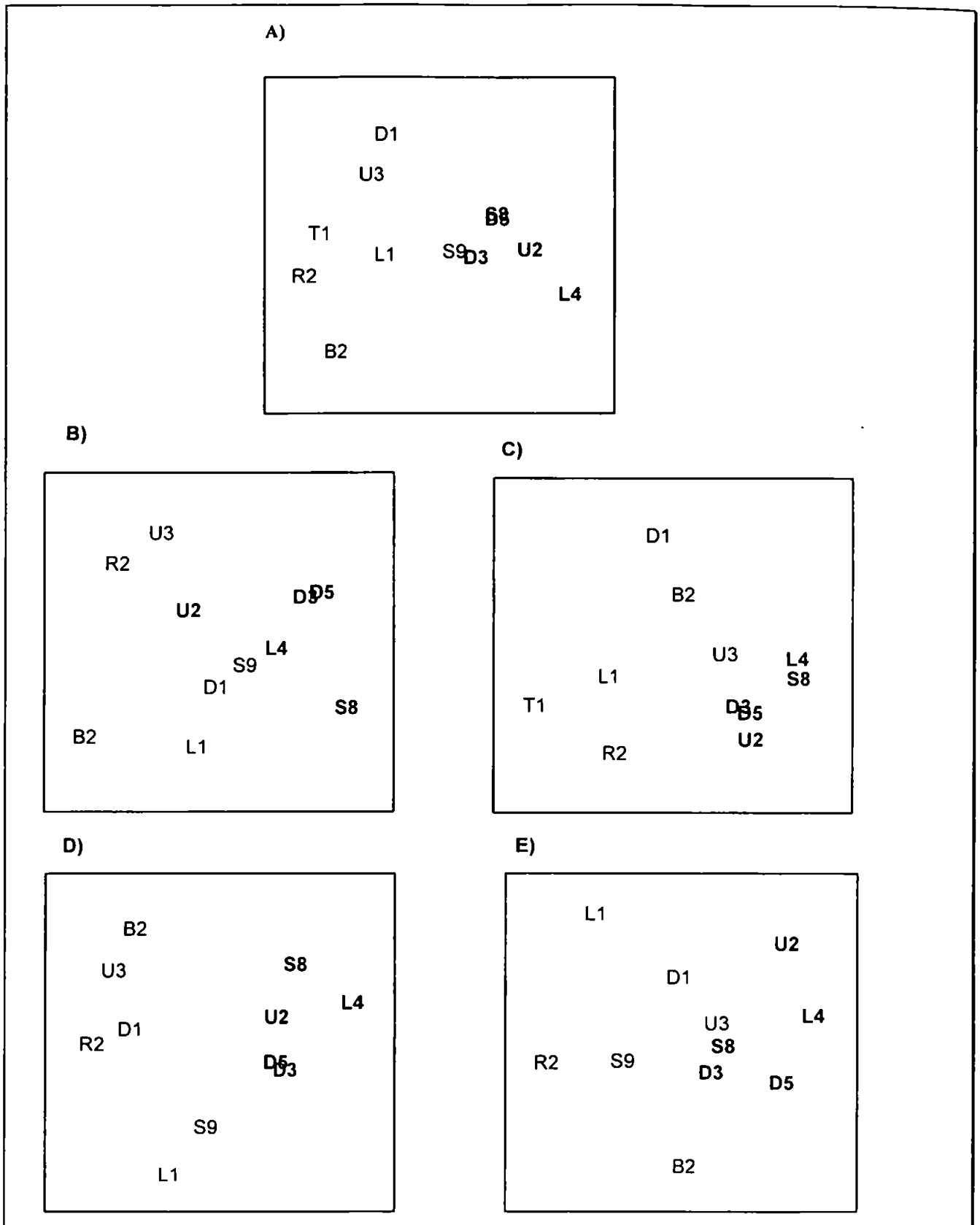


Figure 2.8 Correlation based PCA of twelve sites on the tributaries of the Rivers Lynher and Seaton in: **A)** combined seasons, **B)** spring, **C)** summer, **D)** autumn and **E)** winter. The five most heavily contaminated sites are in bold. Site S9 was omitted from summer and autumn environmental data analysis, and T1 from winter data analysis as some measurements could not be taken at these sites due to no flow or high flow.

Table. 2.7 Eigen vectors for PC1 and PC2 (coefficients in the linear combinations of variables making up PCs) for the autumn, spring, summer and winter environmental data (excluding interstitial measurements). Variables that contribute highest to the PCs are shown in bold. Data set excludes T1 data in the winter, and S9 data in the summer and autumn as some measurements could not be taken at these sites either due to high flow or no flow (Appendix I).

Variable	Combined		Autumn		Spring		Summer		Winter	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
% Variation Explained by PCs	*39.8*	*19.3*	*31.6*	*22.0*	*31.1*	*22.1*	*36.7*	*22.4*	*29.5*	*23.0*
Copper	**0.448**	0.109	**0.492**	0.073	**0.495**	0.032	**0.487**	-0.009	**0.496**	0.105
Zinc	0.362	-0.127	**0.411**	0.317	**0.414**	-0.034	0.352	-0.390	0.275	0.086
Aluminium	**0.400**	-0.041	0.162	0.052	0.397	0.300	0.251	0.150	**0.400**	0.369
Iron	0.173	-0.294	0.032	-0.152	0.130	-0.174	0.060	**-0.562**	0.212	-0.319
Hardness	-0.377	0.198	0.074	**0.604**	-0.363	0.344	-0.173	**0.480**	-0.113	**0.590**
Conductivity	-0.087	**0.446**	-0.267	**0.563**	-0.195	0.268	-0.321	0.205	-0.306	0.359
DOC	**-0.402**	-0.213	**-0.476**	0.182	-0.238	-0.254	**-0.447**	0.187	-0.378	0.215
PH	-0.311	**0.454**	-0.367	0.037	-0.389	0.378	-0.351	-0.201	-0.381	0.144
Temperature	0.265	0.318	0.135	0.382	0.154	**0.511**	0.339	-0.158	0.269	0.195
Discharge	0.000	**0.542**	-0.325	0.078	-0.086	**0.467**	-0.032	0.368	-0.077	**0.402**

Table 2.8 Matrix of pairwise Spearman's Rank Correlation coefficients between Euclidean distance matrices of environmental data. Data from S9 and T1 excluded as some measurements could not be taken at these sites due to no flow in the summer and high flow in the winter respectively (Appendix I) (*p<0.05).

	Combined	Spring	Summer	Autumn
Spring	0.917*			
Summer	0.637*	0.642*		
Autumn	0.713*	0.681*	0.571*	
Winter	0.700*	0.687*	0.380*	0.609*

2.6 Discussion

Physicochemical measurements confirmed that the twelve sites chosen for this study represented a wide range of trace metal concentrations. The concentration of metals found at the sites was related to the types of ores drained (Shambrook, 1986), and to the amount of dilution and chemistry of the streams (Darlington, 1987). High concentrations of Cu and Zn were measured at all sites located downstream of the adit portals (U2, L4, S8, D5). Of these sites, L4 and U2 had higher Fe concentrations than S8 and D5. Upton Cross stream drains a lode that is rich in chalcopyrite (a mineral ore of high Fe content) (Shambrook, 1986), which explains the high Fe concentration at U2. The higher pH conditions at U2 compared with L4 were also conducive to the formation of iron hydroxide precipitate (Smith, 1973) (Plate 2.2). Iron hydroxide precipitate is a strong adsorption agent of copper (Johnson & Thornton, 1987), explaining the low concentration of dissolved Cu at U2 compared with L4, S8 and D5. The surface and interstitial waters of sites S8 and L4 contained very high concentrations of Al, and the acidic waters at L4 are likely to release monomeric Al (Hall *et al.*, 1987). Concentrations of Cu and Al decreased rapidly downstream of the adit portals in Darley Brook (D3, D1) and in the Longridge tributary (L1), with the two tributaries joining these streams increasing the dilution. Downstream of the adit, the pH increased and may have led to the formation of a Cu-Al precipitate (Darlington, 1987). At D5, this Cu-Al precipitate (Plate 2.3) may make this site unfavourable physically, as well as chemically, for benthic fauna. Gower *et al.* (1994) recorded similar patterns of contamination for Cu, Zn and Al at these sites. Therefore, it appears that the difference in water quality of the sites is relatively constant over time and the input of trace metals into the system is causing a persistent chemical change.

At all sites, the Cu and Zn concentrations (range 10-766 and 52-874 $\mu g\ l^{-1}$, respectively) were higher than those considered to be 'natural' concentrations for running water.

Plate 2.2. Site U2: Note the red / brown iron hydroxide precipitate on the surface of stones / cobbles.

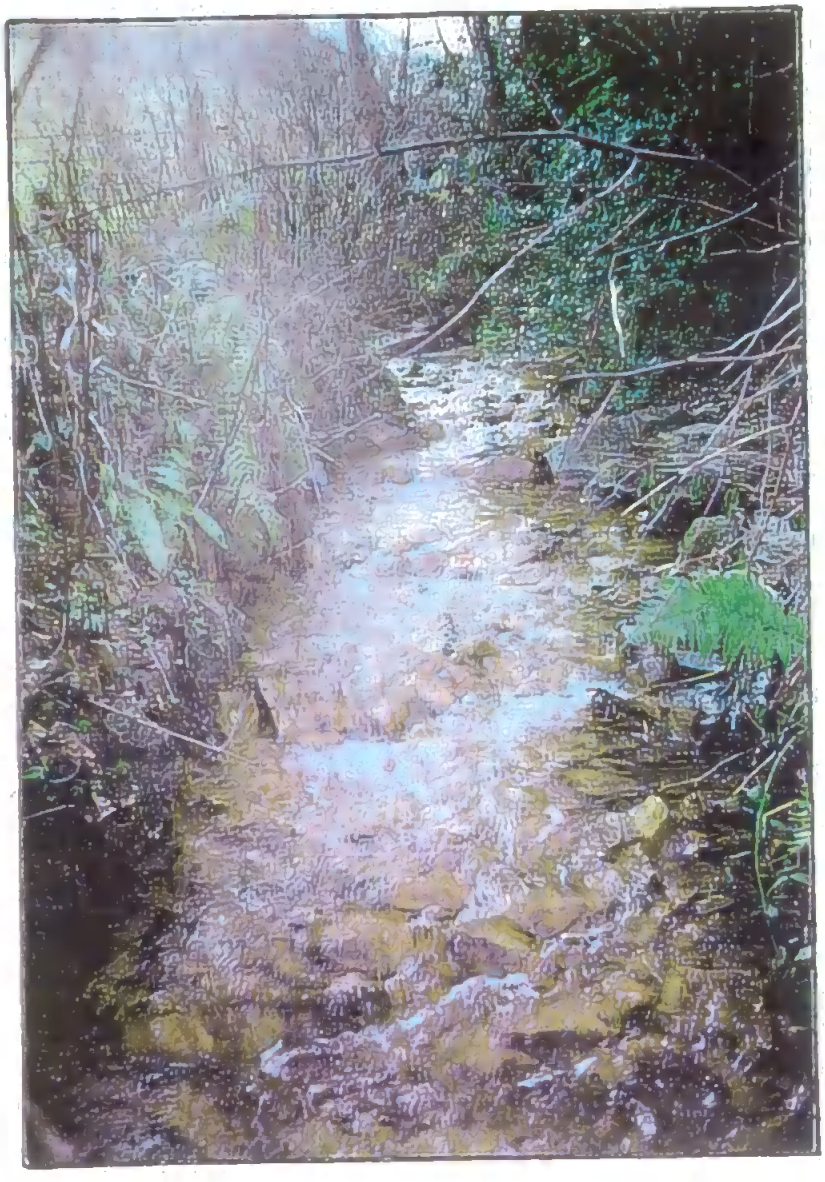


Plate 2.3 Site D5: Note grey / green copper-aluminium precipitate covering stones / cobbles

Turekian (1971) quoted 'typical' stream values of 7 μg l^{-1} (Cu) and 20 μg l^{-1} (Zn). In uncontaminated streams. Moore & Ramamoorthy (1984) cited 0.5 - 1.0 μg Cu l^{-1} and 0.5 - 15 Zn μg l^{-1} as being the typical values for Cu in uncontaminated sites. At all sites, Cu and Zn concentrations exceeded the levels regarded as acceptable by the EC Freshwater Fish Directive and the UK Environmental Quality Standards (EQS) (NRA, 1994). Due to such high Cu and Zn concentrations, all sites (except R2 and T1) were classified as being of poor water quality using the standards set by the National Rivers Authority in their River Ecosystem Use Classification scheme (NRA, 1994). Thus, toxic effects on the benthos may be occurring at all sites demonstrating the difficulty of finding 'true control' sites in this region of extensive mining activity.

Higher interstitial concentrations of Cu, compared with surface waters, were recorded at some sites. Adsorption of metals onto particulates (Nienke & Leek, 1982) and increases in pH leading to precipitation (Jennet & Foil, 1979) are known to result in metals accumulating in the sediment of running waters. Darlington (1987) reported that Cu concentrations in the sediment of Darley brook were more than twice that found in the surface water. Chemical changes in the sediment may cause increased concentration of bioavailable metals. For example, Whitman & Clark (1984) found that the mean pH of surface water of 6.0 decreased to 5.5 at a depth of 10 cm in a small stream in Texas. Hence, the invertebrates inhabiting interstitial waters (e.g. many meiofaunal species) may be exposed to very different water chemistry conditions, including higher concentrations of trace metals, than surface water measurements indicate. Further investigations are required into the spatial variation of chemical variables, including metal concentrations, in the interstitial environment before any definite conclusions can be made of the relationship between surface and interstitial waters. The large difference in the concentration of metals found in the two interstitial samples taken at some sites in winter, suggests metal

contamination is patchy and this may influence the distribution of invertebrates on a finer scale. The large variation in the four interstitial metal concentrations between seasons may be due to the high spatial variation of interstitial metal concentrations.

There are suggestions in the literature (Howarth & Sprague, 1978; Mantoura *et al.*, 1978; Borgman, 1983) of a link between trace metal ions and other environmental variables in aquatic systems. The strong negative correlation between Cu and DOC concentrations in surface and interstitial stream water reported in this study supports the findings of Gower *et al.* (1994). Gower *et al.* (1994) explained this relationship by suggesting that Cu complexed with fulvic and humic acids, resulting in the ultimate removal of DOM from the water. Humic and fulvic acids are known to be the most important complexing agents in fresh water (Mantoura *et al.*, 1978). Therefore, there is likely to be an increase in the concentration of organic Cu complexes at sites with high DOC concentrations and these complexes are thought to be less toxic to organisms than the free ion form (Spear & Pierce, 1979). Temperature was also correlated positively with copper concentrations and negatively with DOC. The small variation in temperatures (10.2-11.2 °C) between sites, however, suggests that these correlations were probably coincidental. As the streams originate from groundwater running through hard granite rock, low conductivities and low water hardness were measured at all sites. Thus, in these streams, toxicity of the metals may be considered as particularly pronounced due to the poor buffering capacity of the water.

Water temperature was the only environmental variable that showed a consistent seasonal pattern, with highest temperatures in the summer and lowest in the winter. Even though these temperature differences were small they may be of significance to the benthos, as previous studies have recorded that slight temperature changes have a significant impact on the timing of hatching and the rate of development of many species resulting in temporal

changes in their abundance (Hynes, 1970). Some workers have recorded seasonal trends in stream discharge (Brown, 1977b; Palmer, 1990). No seasonal trend was recorded in this study apart from discharge values which were consistently lowest in the summer at each site. Changes in flow are unpredictable, making their effects on the stream system difficult to measure. At the three sites located on Darley Brook, low summer flows corresponded with low Cu concentrations. This is in agreement with Darlington (1987) (in Darley Brook) who demonstrated that Cu concentrations were correlated positively with discharge. It was suggested that higher concentrations of Cu in the other seasons was due to underground chambers filling up with water and siphoning of contaminated water into the stream (Darlington, 1987). This correlation did not occur with the other streams (Appendix I), highlighting the complexity of the effect of flow on metal levels, the converse effect being high flows diluting the metal concentrations in the water.

To establish differences in physicochemistry between sites, many workers have analysed environmental data using principal components analysis (PCA) (Somerfield *et al.*, 1994; Zitko, 1994; Rundle & Attrill, 1995). PCA has been found to be particularly useful in studies on systems contaminated by trace metals. For example, Somerfield *et al.* (1994) clearly separated creeks of the Fal Estuary by their sediment metal concentrations using PCA. As with many other studies (Somerfield *et al.*, 1994; Olsgard & Gray, 1995; Rundle & Attrill, 1995; Antonietti & Sartore, 1996) principal component analysis in the present study aided the visualisation of the separation of sites according to their physicochemistry. The main variables separating sites consistently being Cu, Zn and DOC in all four seasons. Thus, to find environmental variables which best explain differences in faunal community structure across sites, the PCA of averaged values of environmental data can be used, with confidence, as a basis against which groupings of biological data can be compared.

CHAPTER 3

The meiofaunal communities of metal-contaminated streams in south-west England

3.1 Introduction

A key issue in ecology is an understanding of how environmental variables influence the distribution and abundance of organisms. In freshwater ecology, the early emphasis on research on the effects of physicochemistry on individual species (Hynes, 1970) has been superseded by studies of the effects on whole communities (Hildrew & Giller, 1995). Investigations into how various environmental factors influence the structure of species assemblages have been aided by the development of powerful multivariate statistical techniques. Using these techniques, several workers have isolated the environmental variables important in determining the macroinvertebrate community structure in British rivers (Townsend *et al.*, 1983; Wright *et al.*, 1994). Recently, environmental factors influential in determining meiofaunal community structure have also been identified (Palmer, 1990; Rundle & Hildrew, 1990; Suren, 1992). Of these factors, flow was shown to be a particularly important variable, altering the composition of lotic meiofaunal communities (Palmer, 1990; Rundle & Hildrew, 1990). Robertson *et al.* (1995) demonstrated that epibenthic meiofaunal taxa such as cyclopoid copepods, cladocerans and ostracods were more abundant in slow-flowing reaches compared with fast-flowing reaches of a small stream in southern England. In contrast, interstitial harpacticoid copepods showed no significant differences in their abundances between reaches with different flow regimes (Robertson *et al.*, 1995). Studies of meiofaunal communities in North America by Palmer (1990) and Shiozowa (1985) also demonstrated clear differences in flow tolerances among cyclopoid copepod species. In both of these studies, the cyclopoid *Paracyclops fimbriatus* was associated with regions of fast current, whereas the cyclopoids *Eucyclops serrulatus* and *Acanthocyclops vernalis* were associated with areas of slow flow (Palmer, 1990; Shiozowa, 1985).

Temperature is another major physical variable important in influencing meiofaunal community structure. In a survey of the meiofaunal communities of stony streams in the Ashdown Forest (southern England), the distribution and abundances of the three most common harpacticoid species, *Atheyella crassa, Bryocamptus zschokkei* and *Bryocamptus echinatus*, were correlated positively with temperature (Rundle & Hildrew, 1990). A small rise of a few degrees of temperature leads to these three species developing more rapidly (Sarvala, 1979; O'Doherty, 1985). Increased development will, in turn, result in a higher number of generations produced within a set time, thereby, influencing the productivity of these populations.

As well as clear links between meiofaunal communities and physical variables, meiofaunal community composition is influenced by water chemistry. The effect of water chemistry on community structure is usually most pronounced where extreme gradients in the former occur, due either to variation in the geology within a catchment area or as a result of anthropogenic inputs (Allan, 1995). Fryer (1980) was one of the first workers to demonstrate that freshwater meiofaunal communities were influenced by water chemistry. His survey of microcrustaceans from seventy water bodies in three different areas (the Island of Rhum, north-east Yorkshire and the Yorkshire Pennines) demonstrated low species diversity at sites of high acidity (Fryer, 1980). More recently, studies have confirmed the correlation between pH and the distribution and abundance of stream meiofauna in streams of southern England and mid-Wales (Rundle & Hildrew, 1990; Rundle & Ormerod, 1991). Inter-site patterns in species composition in these two regions, and the environmental variables best explaining these patterns, were investigated using multivariate statistical techniques. In both regions, acidic sites had distinctive, species-poor meiofaunal communities, although some species of cyclopoid copepods were tolerant of low pH (Rundle & Ormerod, 1991).

Although no previous study has focused on lotic meiofaunal communities in metal-contaminated streams, there is some evidence to suggest that certain trace metals may influence stream meiofauna. Rundle & Ormerod (1991) demonstrated a negative correlation between Al and the abundance of harpacticoid copepods in streams in mid-Wales. Hummon *et al.* (1989) recorded reduced abundances of meiofaunal taxa and altered community composition in streams in south-eastern Ohio polluted by acid mine drainage and trace metals. Unfortunately, a non-carbonate conductivity value, rather than metal concentrations, was used to indicate the level of contamination in the latter study and taxa were recorded to phyla only. Further involvement of the influence of trace metals on the microcrustacea was proposed by Fryer (1993). He observed that several species of microcrustaceans experienced physiological difficulties in standing waters in Yorkshire compared with microcrustacean species in water bodies with comparable acidities on the island of Rhum. He suggested that the difference was due to the extra stress related to the presence of trace metals at the Yorkshire sites; this hypothesis, however was not tested (Fryer, 1993). Thus, the relationship between trace metal concentrations and stream meiofaunal species assemblages has yet to be investigated rigorously. This chapter addresses this research caveat by investigating the meiofaunal communities of streams known to be contaminated by trace metals (Chapter 2). The main aims were to describe the stream meiofaunal communities (in three seasons) at sites representing a wide range of metal concentrations, and to apply multivariate analyses to establish whether differences in metal concentrations, or other environmental variables, (Chapter 2) correlated with inter-site differences in meiofaunal communities.

3.2 Materials and methods

3.2.1 Meiofauna sampling

Meiofaunal samples were collected from sites R2, B2, L1, U3, S9, D1, U2, L4, S8 and D5 in autumn (21st-25th November 1994) and spring (17th-24th April 1995), and from sites T1, R2, B2, L1, U3, D1, U2, D3, S8 and D5 in summer (8th-17th August 1995) (refer to Figure 2.2 for the location of the sites). Samples could not be taken at S9 and L4 in summer due to these sites drying out. Despite this, the use of additional sites (T1 and D3) allowed the comparison of meiofaunal communities over a wide range of Cu concentrations in this season. No winter samples were taken as previous work had demonstrated that meiofaunal abundances were too low to provide adequate discrimination among sites in temperate streams during this season (Rundle & Hildrew, 1990; Rundle & Ormerod, 1991). Five Surber samples (Area = 0.0225 m^{-2}, Mesh size = 100 μm) were taken from a stony riffle region at each site. Previous studies had shown that the meiofauna was more abundant amongst macrophytes than within the bare substratum of streams (Rundle & Ormerod, 1991; Suren, 1992). The density of macrophyte cover, however, was variable at sites used in the present study. Therefore, to standardise samples across sites, areas with an absence of macrophytes and with a substratum of a mixture of pebbles (2-6 cm) and gravel (< 2 cm), were targeted. The sampling procedure was based on the method used by Surber (1970). The base of the sampler was wedged into the substratum, and pebbles and gravel to a depth of about 5 cm were disturbed for 1-2 mins, washing all animals into the net.

In the field, samples were transferred to pots (volume = 500 ml) containing approximately 100 ml of stream water. Samples were returned to the laboratory, preserved in 4% neutral buffered formalin, and passed through 500 μm and 63 μm sieves to separate the macrofauna and large debris from the meiofauna. Meiofauna retained on the 63 μm sieve were extracted from the mineral debris by floatation, using colloidal silica solution (Ludox TM) with a

specific gravity of 1.15. Samples were poured into 125 ml beakers which were filled to the 125 ml level with Ludox TM solution to allow floatation of organisms. Samples were stirred vigorously and allowed to settle for 1 h. The top fraction, containing the meiofauna, was then poured over a 63 μm sieve and stored in alcohol. This process was repeated three times for each sample using the same Ludox solution.

Copepods, cladocerans, ostracods and mites were mounted on microscope slides using Aquamount (Gurr BDH), and identified under a compound microscope (200-400 times magnification) with conventional bright field illumination. Cyclopoid copepods were dissected initially by separating the anterior part of the animal (i.e. from the third thoracic segment) under a binocular microscope to reveal the fifth leg, a key identification feature (Plate 3.1). Other groups, except for nematodes, were identified without dissection, and were identified to species (using the keys listed in Appendix II) and counted.

3.2.3 Statistical analyses

3.2.3.1 Environmental variables

Annual mean values of the environmental variables (Tables 2.1 and 2.5) were used in the analyses. Mean values from replicate seasonal data provide a robust comparison between sites by accounting for seasonal variability (Rundle & Hildrew, 1990).

3.2.3.2 'PRIMER'

Multivariate statistical techniques in the computer software package PRIMER (Plymouth Routines in Multivariate Ecological Research) were used to investigate changes in meiofaunal community structure across sites. The PRIMER package contains programs which allow community data to be displayed visually (CLUSTER and MDS), enable identification of the environmental variables that best explain community patterns (BIOENV) and identify individual species that contribute most to differences in community


50µm

structure (SIMPER). Prior to analysis, data were double square root transformed to reduce the influence of dominant species. The procedures used are detailed below.

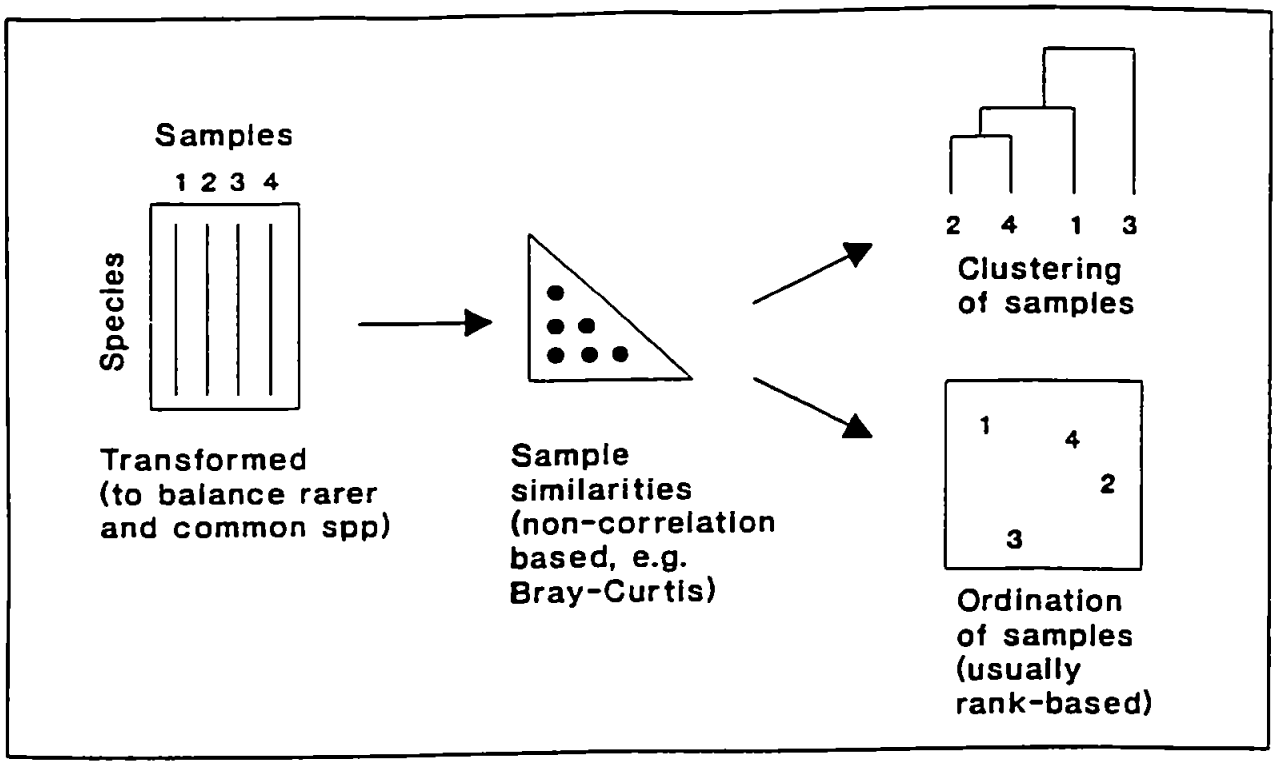
Bray curtis similarity matrix - The starting point for many clustering and ordination techniques is the concept of similarity between the abundances of species found at all pairs of sites. In the present study, the similarity (S) in community structure between all pairs of sites was measured using absolute numbers of mean meiofaunal abundances (i.e. data not standardised) to give a Bray-Curtis similarity coefficient between the range S=0 (for total dissimilarity) to S=100 (for total similarity). The Bray-Curtis similarity coefficient (Bray & Curtis, 1957) is a commonly-used similarity coefficient in ecological studies (Clarke & Warwick, 1994a).

Similarity coefficients between the jth and kth samples (S_{jk}) are calculated using the equation:

$$S_{jk} = 100(1 - \frac{\sum_{i=1}^{p} |y_{ij} - y_{ik}|}{\sum_{i=1}^{p} (y_{ij} + y_{ik})})$$

where y_{ij} represents the ith species in the jth sample (i=1,2,...,p; j=1,2,..., n). Similarly y_{ik} represents the ith species in the kth sample. | ... | represents the absolute value of the difference (the sign is ignored) and $\sum$ is the sum of all species. Computation is simple. Similarity is not affected by species which are absent from both site-pairs, whilst all species contribute to the similarity of sites, though the commoner species are generally given greater weighting than rarer ones. These similarity coefficients, calculated between every pair of samples, were set out in a conventional lower triangular similarity matrix (Clarke & Warwick, 1994a) (Fig. 3.1A).

A



B

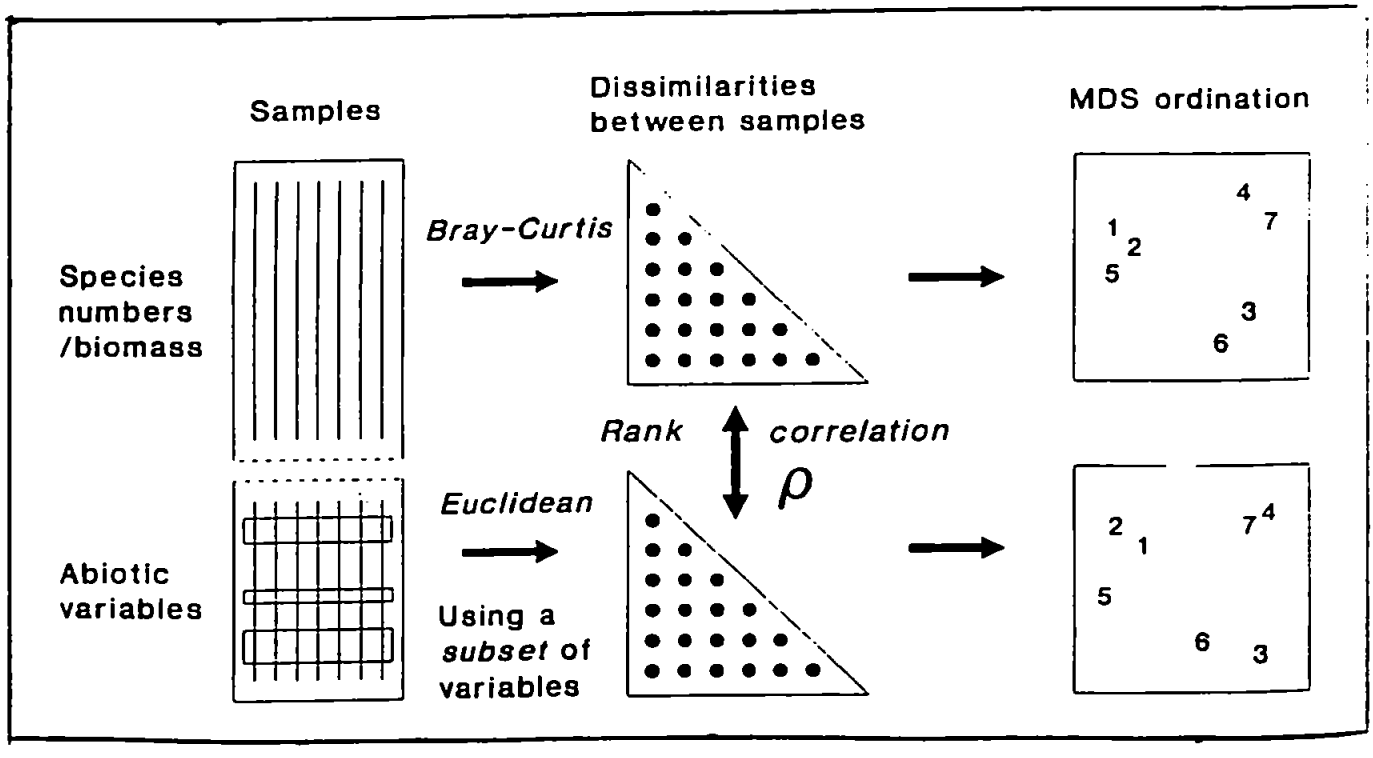


Figure 3.1. A) Stages in a multivariate analysis based on similarity coeffiecients. **B)** The BIOENV procedure - selection of an abiotic variable subset maximizing rank correlation (ρ) between biotic and abiotic (dis)similarity matrices (From Clarke & Warwick, 1994a).

'ANOSIM' - Significant differences in species composition between sites were assessed using ANOSIM (Clarke & Green, 1988; Clarke, 1993). Firstly, to test whether site differences were worthy of further examination, a global test statistic (R) was calculated based on the differences in average rank dissimilarity among replicate samples within and between groups using the equation:

$$R = (r_B - r_W) / (M/2)$$

where r_W is defined as the average of all rank similarities among replicates within sites, r_B is the average of rank similarities arising from all pairs of replicates between sites and $M = n(n-1)/2$ where n is the total number of sites.

Thus, if R=1, all replicates within sites were more similar to each other than any replicates from different sites. Whereas, if R=0, there was on average no difference in the similarities between and within sites. In order to test whether this value is significant, site replicates were repeatedly and randomly reallocated to different groups, and the test statistic 'R' calculated for each of these random combinations. ANOSIM then determined whether the original test statistic was significantly different from the test statistic derived from the repeated random groupings. If the global R value indicates there are site differences worth examining 'somewhere', specific pairs of sites can be compared. Pairwise tests were performed using the same test procedure as described above.

'CLUSTER' - The CLUSTER program determines 'groupings' of samples such that samples within a group are more similar than samples in different groups (Fig. 3.1A). The present study used a hierarchical agglomerate clustering technique (Clarke & Warwick, 1994a). The matrix of pairwise similarity, created from Bray-Curtis similarity coefficients (S), was used to successively 'fuse' the averaged meiofaunal abundances at sites into

groups, starting with communities with the highest mutual similarity, then gradually lowering the similarities at which groups are fused until there was only one single cluster. Similarities between fused groups were determined using group-average linking, that is by using the average of the similarities of pairs of sites from the two groups. This average similarity was weighted by the number of sites in each group. For example, the similarity between a group of one site (S1) and a group of three sites (S2, S3 and S4) would be [S(1,2&3&4) = [[S(1,2)+S(1,3)+S(1,4)]/3].

Multi-dimensional scaling (MDS) - Cluster analysis is not the optimal way to display data if there is a steady gradient in community structure across sites in response to strong environmental forcing. Even for data which are strongly grouped, an ordination plot will allow the relationship between points to be displayed more informatively, as the percentage similarity of samples can only be viewed in one dimension with a dendogram (Fig. 3.1A). The ordination technique used was non-metric multidimensional scaling (MDS) (Kruskal & Wish, 1978) as it is conceptually simple and flexible, making few assumptions about the form of the data or the inter-relationship of the sites unlike ordination techniques such as DECORANA (Hill, 1979) and PCA.

Initially, MDS reduces the similarity matrix to rank form, as absolute similarity values are essentially arbitrary. For example, it cannot be said that the similarity of the community at R2 to B2 is 1.5 times that of R2 to L1. It is just that R2 is relatively more similar to B2 than L1. The regression relationship between distance of ordination points and dissimilarity between site pairs was used to evaluate stress (the scatter around the regression line) for changes in the position of points on the ordination plot. Points were moved to new positions which looked like they would decrease the stress most rapidly. Thus, this complex iterative procedure refined successively the position of the points until the

species composition. The MDS program produced a stress value which indicated how well the two dimensional ordination preserved the site relationships. A stress of <0.05 indicates ordinations were excellent representations with no misinterpretation of site relationships, a stress of <0.1 represented a good ordination with no real risk of misinterpretation, a stress of <0.2 indicated a still useable picture although there is a potential for misinterpretation, and a stress of >0.2 indicated plots are likely to be misinterpreted (Clarke & Warwick, 1994a).

'BIOENV' - The BIOENV program was used to identify the environmental ordinations that best matched faunistic ordinations (Clarke & Ainsworth, 1993). Rank correlations (weighted Spearman rank correlation coefficients) between similarity matrices derived from all the possible subsets of averaged transformed environmental variables, and the similarity matrices derived from the averaged transformed biotic data, were calculated until the combination of variables which gave the highest rank correlation was found (Fig. 3.1 B).

'SIMPER' - The 'exploratory analysis' SIMPER (Clarke, 1993) was used to gain insight into the species most responsible for site groupings (chosen subjectively). In this case, groups were chosen using the site groupings observed using the CLUSTER analysis. Initially, the average dissimilarity between all inter-group sites was examined (i.e. every site in group A was paired with every site in group B). The average dissimilarity was then broken down to contributions of individual species and each species contribution examined. Species which were good at discriminating pairs of group clusters were identified as those which consistently contributed to inter-comparisons between all sites in the two groups.

3.2.3.3 Multiple regression

A stepwise multiple regression analysis was carried out to investigate relationships between physicochemical variables and the abundance of key species found to be important in

explaining community patterns (i.e. those identified by SIMPER to be important discriminatory species).

3.2.3.4 Univariate measures

Correlation between Cu and meiofaunal species richness, and the abundance of major groups, was examined using the product moment correlation coefficient (Devore & Peck, 1990).

3.3 Results

3.3.1 General patterns in species occurrence and abundance

Twenty-four meiofaunal species were identified from the sites sampled on the tributaries of the Rivers Lynher and Seaton (Table 3.1). Hydrachnellid mites and cyclopoid copepods were the most species rich groups (each with seven species), whereas harpacticoid and cyclopoid copepods and nematodes were the numerically dominant groups (Table 3.2). Total meiofaunal abundances, and those of individual groups were highest at most sites in summer. The only exception was the nematodes, whose abundances were lowest in the summer and generally highest in the autumn (Table 3.1).

The number of sites where each species occurred, and their distribution with respect to metal contamination (indicated by the range of Cu concentrations they inhabited) are shown in Table 3.1. Of the three harpacticoid species (family Canthocamptidae), the two most abundant, *Bryocamptus zschokkei* and *Bryocamptus praegeri* (Plate 3.2), were restricted to sites with Cu concentrations of 5 - 182 $\mu g\ l^{-1}$ (Table 3.1). Of the cyclopoid species, only *Paracyclops fimbriatus*, *Diacyclops languidoides* and *Eucyclops serrulatus* were widespread. *Graeteriella unisetiger* (Plate 3.2), only recorded twice before in the UK [North Wales and Oxford (Gurney, 1936)], was limited to U3 and D1, and was found only in autumn. Most of the seven hydrachnellid mites were found in the summer; only one species, *Feltria minuta* (Plate 3.3), was sampled in autumn and spring. Four species of halacarid mite were found, with *Porohalacarus alpinus* restricted to sites of high Cu concentrations. The other halacarid species, *Lobohalacrans weberi* (Plate 3.3), *Soldanellonyx monardi* and *Limnohalacrans wakeri* were found mainly at sites of low Cu concentration. Only one species of ostracod [*Candona candida* (Plate 3.3)] and two species of cladoceran, [*Alona quadrangularis* (Plate 3.3) and *Chydoris sphaericus*] were found, and these were restricted to sites of low Cu concentration (Table 3.1).

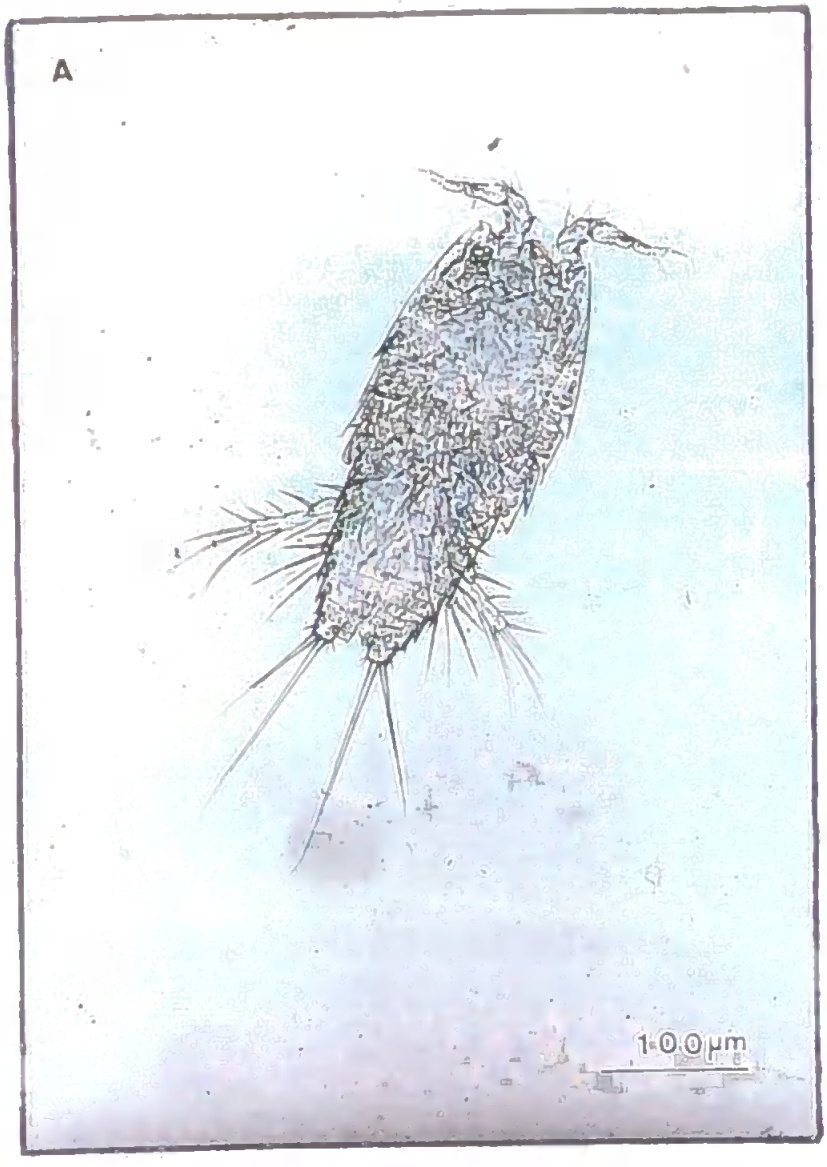
Table 3.1 List of meiofaunal species identified from ten sites on the tributaries of the Rivers Lynher and Seaton sampled in spring, summer and autumn. Frequency of occurrence in each season and the range of surface Cu concentrations in which animals were present are also given.

Species	Occurrence (No. of sites)			Cu range ($\mu g\ l^{-1}$)
	Spring	Summer	Autumn	
PHYLUM CRUSTACEA				
CLASS Copepoda				
ORDER Harpacticoida				
Bryocamptus zschokkei	6	7	7	5-182
Bryocamptus praegeri	3	4	7	5-182
Bryocamptus pygmaeus	4	1	7	13-1205
ORDER Cyclopoida				
Paracyclops fimbriatus	2	6	9	5-1205
Eucyclops serrulatus	2	4	6	52-829
Diacyclops languidoides	8	8	10	5-928
Diacyclops bisetosus	4	5	3	144-766
Speocyclops demetiensis	1	3	3	23-203
Graeteriella unisetiger	0	0	2	49-112
Acanthocyclops vernalis	0	2	0	23-92
CLASS Branchiopoda				
ORDER Cladocera				
Alona quadrangularis	1	1	2	23-112
Chydoris sphaericus	0	0	1	92
CLASS Ostracoda				
Candona candida	4	2	2	23-112
PHYLUM ARTHROPODA				
CLASS Arachnida				
ORDER Acarina				
FAMILY Hydrachnellae				
Feltria minuta	4	4	3	11-182
Hygrobates sp.	5	0	0	5-47
Torrenticola sp.	2	0	0	5-23
Atractides sp.	1	0	0	235
Sperchon sp.	1	0	0	5
Hydrachnellae sp.a	3	0	0	5-235
Hydrachnellae sp.b	3	0	0	5-23
FAMILY Halacaridae				
Lobohalacarus weberi	2	1	5	13-588
Soldanellonyx monardi	1	1	2	9-588
Limnohalacarus wakerii	1	0	2	11-49
Porohalacarus alpinus	0	3	2	566-1205
PHYLUM NEMATODA	10	10	10	5-1205

Table 3.2 Mean abundance ($\bar{x}$) (± 1SE; n=10) of meiofaunal groups found at ten sites on the tributaries of the Rivers Lynher and Seaton in spring, summer and autumn.

Taxa	Spring		Summer		Autumn	
	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*
Harpacticoida	257	*(394)*	3726	*(6118)*	1066	*(2019)*
Cyclopoida	91	*(99)*	442	*(529)*	221	*(138)*
Cladocera	2	*(5)*	36	*(115)*	5	*(8)*
Ostracoda	7	*(9)*	59	*(64)*	4	*(11)*
Hydrachnellae	18	*(32)*	180	*(284)*	8	*(19)*
Halacaridae	5	*(25)*	39	*(55)*	24	*(13)*
Nematoda	539	*(691)*	207	*(193)*	868	*(1253)*

Plate 3.2 A Male *Bryocamptus praegeri*, (Copepoda: Harpacticoida). Note prehensile antennules modified for holding on to the female during copulation. Female *Graeteriella unisetiger* (Copepoda: Cyclopoida) **B** thorax; **C** abdoman. Arrow points to large triangular operculum- a key identification feature of this species.


A
100 µm


B
50 µm

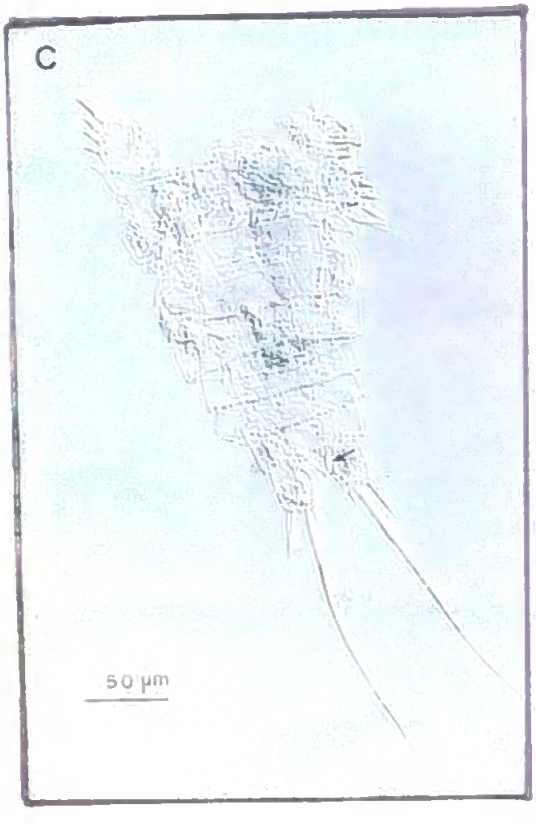
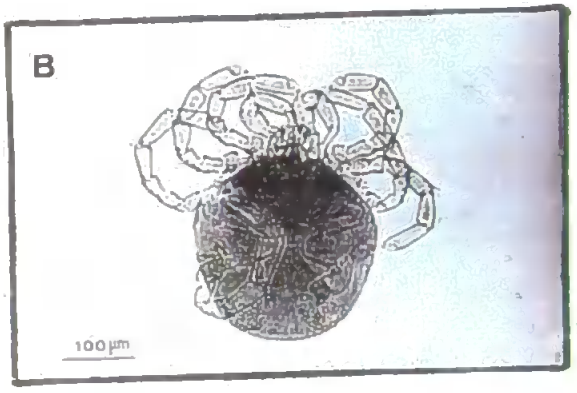
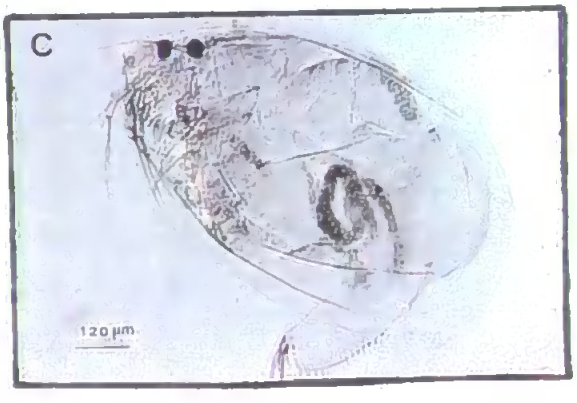
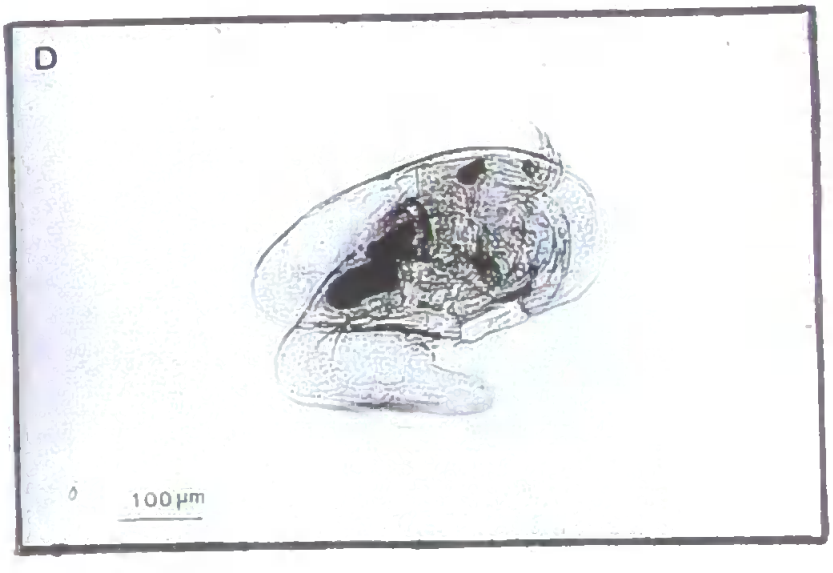

C
50 µm

Plate 3.3 **A** Adult halacarid mite *Lobohalacarus weberi*, a species important in distinguishing L4 (a site of low pH and high Al) from other sites of high metal concnetrations; **B** Adult hydrachnellid mite *Feltria minuta*; **C** Cladoceran *Alona quadrangularis*; **D** Ostracod *Candona candida*. **B, C** and **D** were only found at sites of low metal concentrations.


A
100 µm


B
100 µm


C
120 µm


D
100 µm

3.3.2 General patterns in community composition and seasonality

ANOSIM demonstrated that the composition of meiofaunal communities was significantly different at a high proportion of sites throughout the year (Table 3.3). In other words, distinctive meiofaunal communities were found at sites of differing metal concentrations.

Table 3.3 Results of pairwise tests from 1-way ANOSIM, showing significance (p) of differences in community structure between sites for fourth root, transformed meiofaunal data in spring, summer and autumn at ten sites on tributaries of the Rivers Lynher and Seaton. Communities not significantly different from each other are shown in bold.

	R2	B2	L1	U3	S9	D1	U2	L4	S8
Spring (Global R = 0.521)									
B2	0.01								
L1	0.03	0.01							
U3	0.01	0.01	**0.11**						
S9	0.01	0.01	0.01	0.01					
D1	0.01	0.01	0.01	0.01	0.01				
U2	0.01	0.01	0.01	0.01	0.01	**0.06**			
L4	0.01	0.01	0.02	0.03	0.01	0.01	**0.08**		
S8	0.01	0.01	**0.09**	0.01	0.01	**0.09**	**0.10**	**0.19**	
D5	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Autumn (Global R = 0.657)									
B2	0.01								
L1	0.01	0.01							
U3	0.01	0.01	0.01						
S9	0.01	0.01	0.05	0.01					
D1	0.01	0.01	0.02	0.01	0.01				
U2	0.01	0.01	0.03	**0.56**	0.01	0.01			
L4	0.01	0.01	0.01	0.02	0.01	0.01	**0.07**		
S8	0.01	0.01	0.01	0.01	0.01	0.01	**0.42**	0.01	
D5	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

	T1	R2	B2	L1	U3	D1	U2	D3	S8
Summer(Global R = 0.825)									
R2	0.02								
B2	0.01	0.01							
L1	0.01	0.01	0.01						
U3	0.01	0.01	0.01	0.01					
D1	0.01	0.03	0.01	0.01	0.01				
U2	0.01	0.01	0.01	0.01	0.01	0.02			
D3	0.01	0.01	0.01	0.01	0.01	0.01	**0.05**		
S8	0.01	0.01	0.01	0.01	0.01	0.01	**0.29**	0.01	
D5	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01

In spring, summer and autumn, multidimensional scaling ordinations of averaged meiofaunal abundances showed a clear separation of sites by their meiofaunal community structure. Communities at sites of low metal contamination (i.e. T1, R2, B2, L1) were very different from those found at contaminated sites (i.e. L4, S8, D5) (Figs 3.2-3.4). (Refer to Table 2.1 for the concentrations of metals found at these sites).

Although sites with high and low metal concentrations were clearly separated in every season, there were still seasonal differences in community patterns, as demonstrated by weak or insignificant correlations between seasonal meiofaunal patterns (Table 3.4).

Table 3.4 Matrix of pairwise Spearman's Rank Correlations coefficient (ρ) between similarity matrices derived from averaged fourth root transformed meiofaunal abundance data in spring, summer and autumn (* $p<0.05$).

	Spring	**Summer**
Summer	0.381 *	
Autumn	0.140	0.381

These seasonal differences in community patterns were due to some sites showing large variation in their seasonal composition. The MDS ordinations visually display these seasonal differences in meiofaunal community patterns. In spring, the community at U2 was clearly separated from the other sites at the 50% similarity level (Fig. 3.2). In autumn, the community at L4 was delineated from all others at the 55% similarity level (Fig. 3.4), whilst in summer, communities clustered into two distinct groups at the 30% similarity level (Fig. 3.3). Groupings of sites from the three seasons were later used in SIMPER analysis to

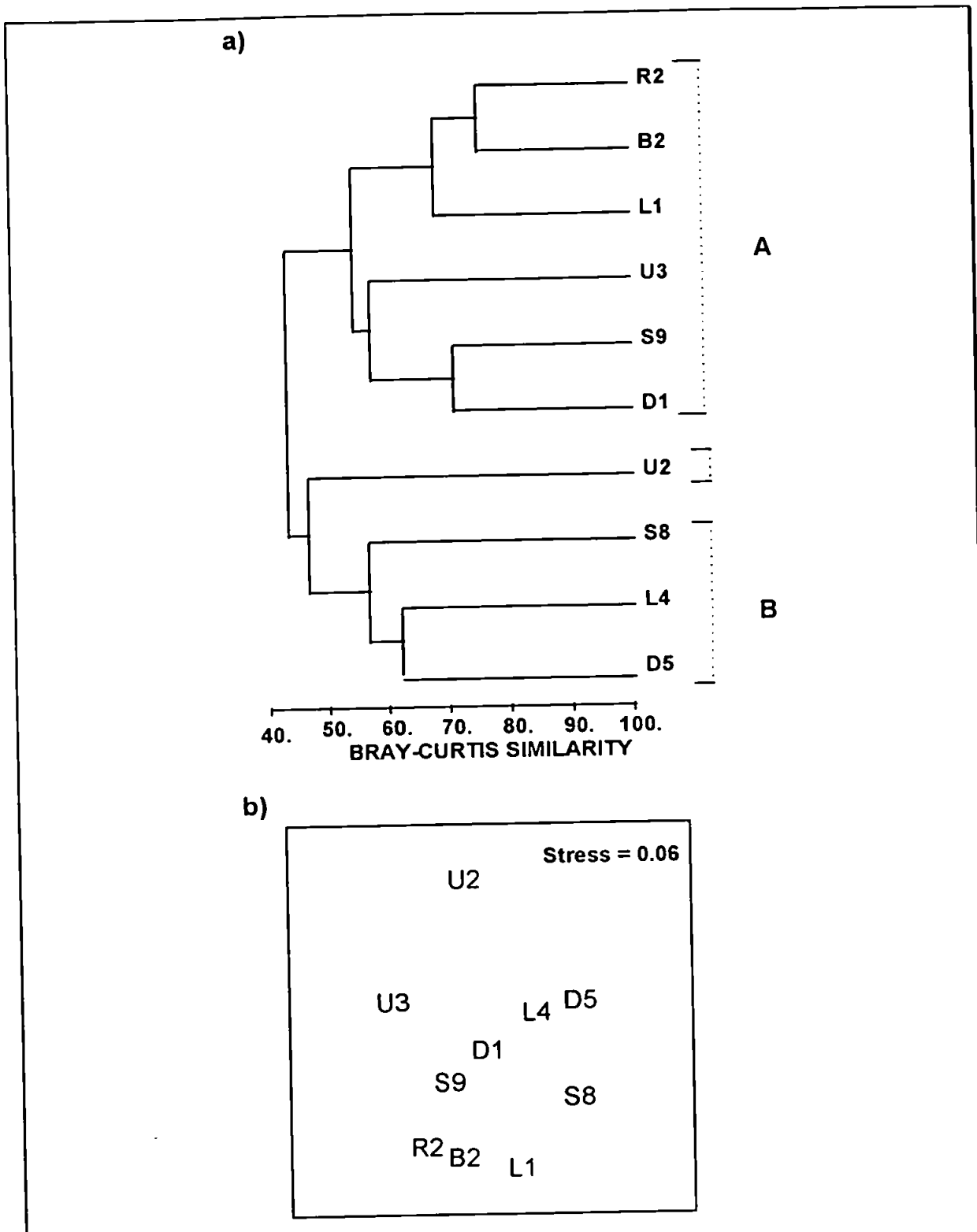


Figure 3.2. a) Dendogram and b) Multidimensional ordination of double square root transformed mean meiofaunal abundances at ten sites on Rivers Lynher and Seaton in spring. See Figure 2.2. for site locations. Sites grouped at the 50 % similarity level (i.e. groups A and B) for SIMPER analysis. Stress value <0.1 indicates the 2-dimensional ordination is a good representation of site relationships.

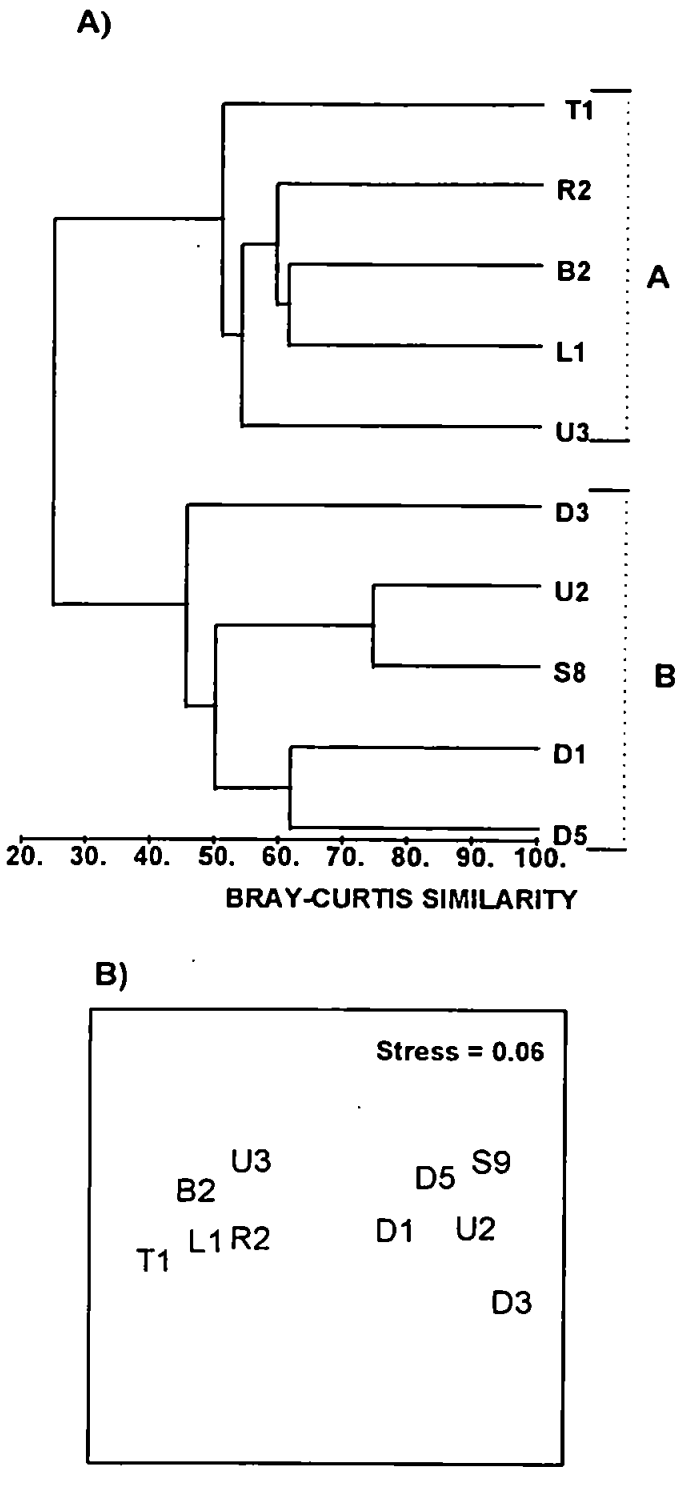


Figure 3.3. A) Dendogram and **B)** Multidimensional ordination of double sqaure root transformed meiofaunal abundances found at ten sites on the Rivers Lynher and Seaton in summer. See Figure 2.2. for site locations. Sites grouped at the 30% similarity level (i.e. groups A and B) for SIMPER analysis. Stress value<0.1 indicates the 2-dimensional ordination is a good representation of site relationships.

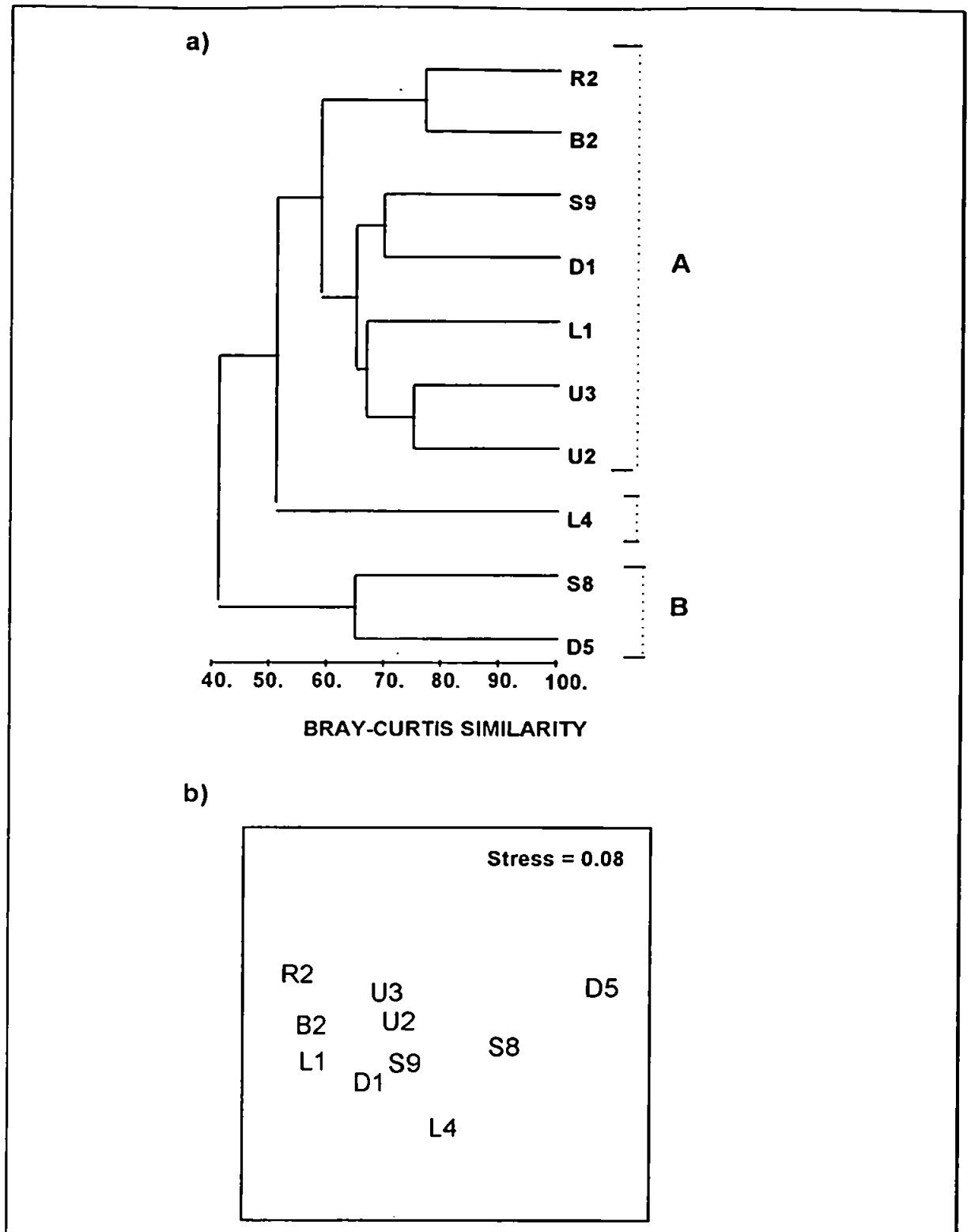


Figure 3.4. a) Dendogram and b) Multidimensional ordination of double square root transformed mean meiofaunal abundances at ten sites on Rivers Lynher and Seaton in autumn. See Figure 2.2 for site locations. Sites grouped at the 55% similarity level (i.e. groups A, B and C) for SIMPER analysis. Stress values <0.1 indicates the 2-dimensional ordination is a good representation of the site relationships.

investigate the species which were important in contributing to the separation of sites by their community structure.

3.3.3 Relationships between community composition and environmental variables

BIOENV analysis demonstrated that Cu was the best single variable explaining inter-site differences in meiofaunal community structure in all seasons (spring r=0.38, summer r=0.64, autumn r=0.58) (Table 3.5). Even so, highest correlations in each season involved Cu in combination with other variables. Autumn meiofaunal community patterns correlated best with Cu and Al , spring communities with Cu and Zn and summer communities with Cu, Zn, DOC and pH (Table 3.5).

Figures 3.5-3.7 show the MDS ordinations of meiofaunal community patterns where site names had been overlaid with symbols proportional in size to the value of key variables, to allow the relationship between important variables and community structure to be visualised. Inter-site differences in meiofaunal community structure can be distinguished clearly in terms of Cu levels in both summer and autumn. The relationship between Cu concentrations and the meiofaunal community structure was less clear in spring compared with the other seasons (Fig. 3.5). The two distinct groupings of communities in summer were also clearly separated by differences in their DOC and Zn concentrations. In autumn, the separation of the community at L4 (see Section 3.3.2) appeared to be linked with the low pH and high Al concentrations at this site, whilst, in spring, the separation of the community at U2 (see Section 3.3.2) appeared to be related to high Zn concentrations.

Table 3.5 Combinations of variables giving the highest rank correlations between biotic and abiotic similarity matrices using BIOENV analyses (lower correlations omitted). Highest correlations are shown in bold.

	Best Variable Combinations		
MEIOFAUNA			
Spring			
1	Cu 0.383	Zn 0.248	
2	**Cu, Zn** **0.501**	Cu, Fe 0.475	Zn, DOC 0.328
3	Cu, Zn, Fe 0.456	Cu, Zn, DOC 0.445	Cu, Fe, DOC 0.444
Summer			
1	Cu 0.640		
2	Cu, Zn 0.622	Cu, DOC 0.618	
3	Cu, Zn, DOC 0.660		
4	**Cu, Zn, DOC, pH** **0.661**	Cu, Zn, DOC, Temperature 0.617	
Autumn			
1	Cu 0.529		
2	**Cu, Al** **0.653**	Cu, pH 0.566	
3	Cu, Al, pH 0.589	Cu, Al, Discharge 0.563	Cu, Al, Hardness 0.535

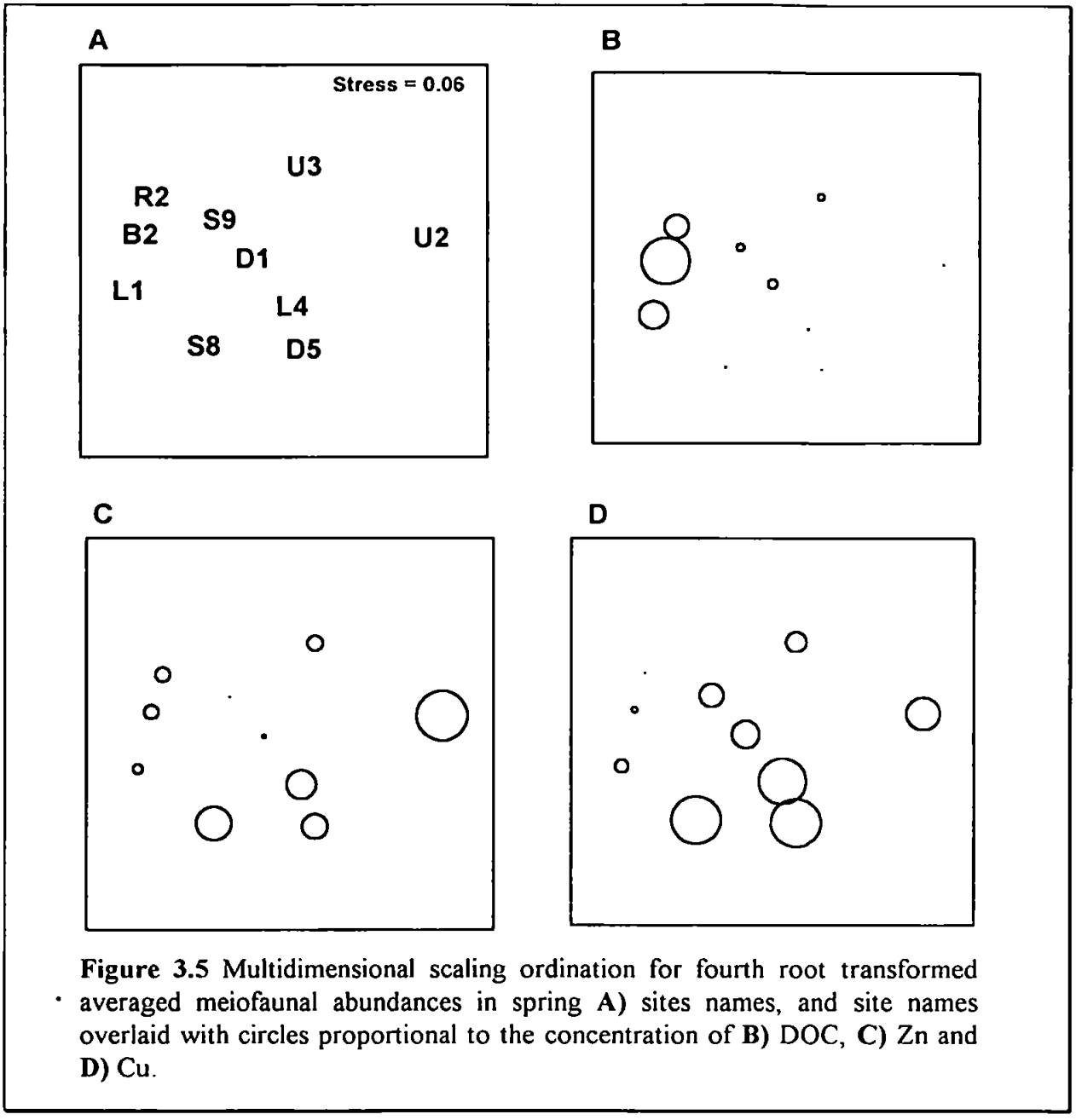


Figure 3.5 Multidimensional scaling ordination for fourth root transformed averaged meiofaunal abundances in spring **A)** sites names, and site names overlaid with circles proportional to the concentration of **B)** DOC, **C)** Zn and **D)** Cu.

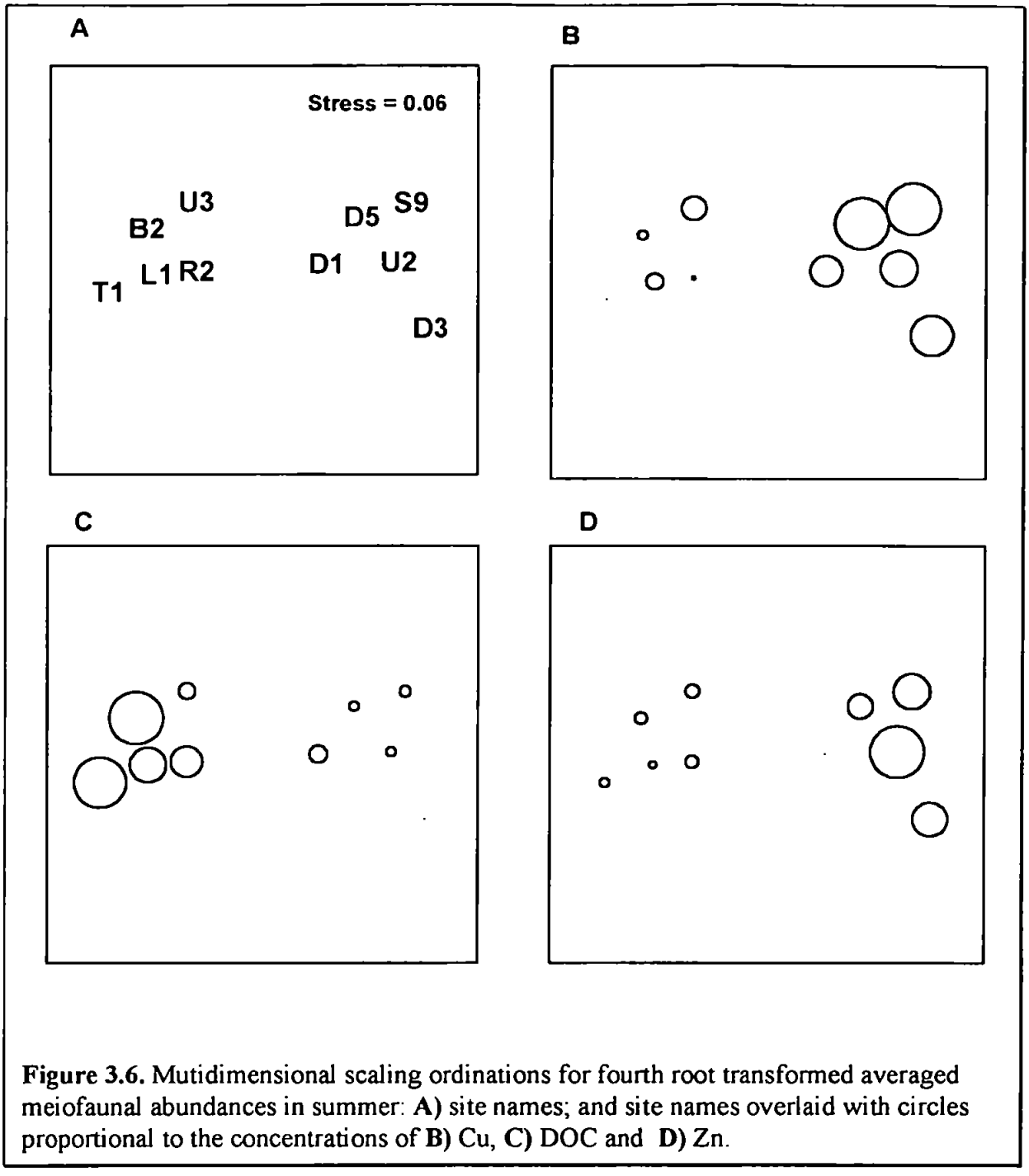


Figure 3.6. Mutidimensional scaling ordinations for fourth root transformed averaged meiofaunal abundances in summer: **A)** site names; and site names overlaid with circles proportional to the concentrations of **B)** Cu, **C)** DOC and **D)** Zn.

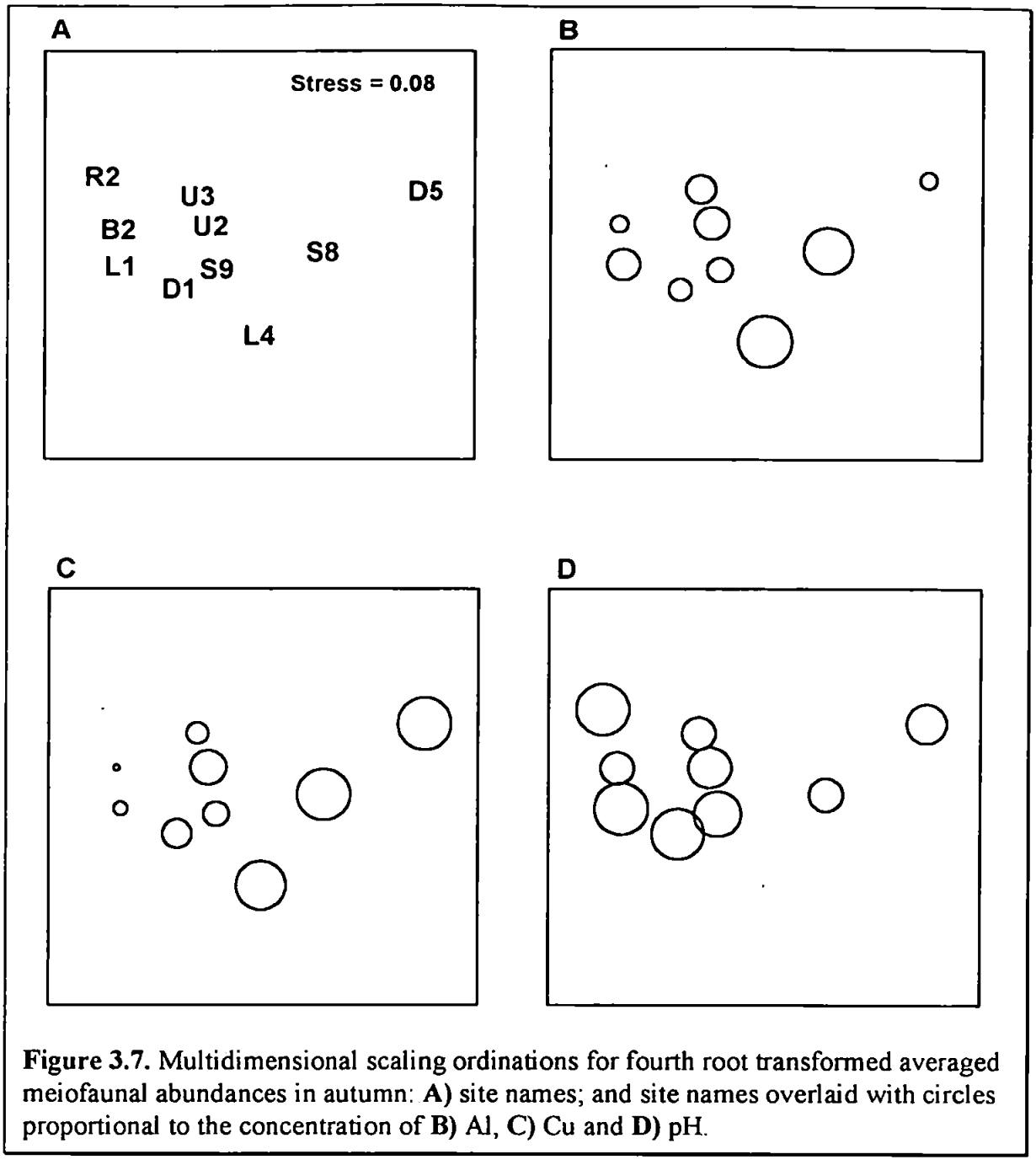


Figure 3.7. Multidimensional scaling ordinations for fourth root transformed averaged meiofaunal abundances in autumn: **A)** site names; and site names overlaid with circles proportional to the concentration of **B)** Al, **C)** Cu and **D)** pH.

3.3.4 Contribution of species in community analyses

SIMPER analyses of meiofaunal abundance data allowed the examination of species which were important in contributing to the dissimilarity between communities at sites of high metal concentrations [CLUSTER group B (Figs 3.2-3.4)], and communities at sites of low metal concentrations [CLUSTER Group A (Figs 3.2-3.4)]. Multiple regression analysis identified the environmental variables that best explained the distribution of these key species (Table 3.6).

In both spring, summer and autumn *Bryocamptus zschokkei* was the species that contributed most to the dissimilarity between communities of high and low metal contamination (Tables 3.6 and 3.7). In all three seasons, sites with high metal concentrations (Group B) were characterised by lower abundances of the harpacticoids *Bryocamptus zschokkei* and *Bryocamptus praegeri* compared with sites of low concentrations (Group A) (Tables 3.6 and 3.7). In spring and summer, metal-contaminated sites were characterised by an absence of the hydrachnellid mite *Feltria minuta* and high abundances of the cyclopoid *D. languidoides*. Low abundances of nematodes were consistent in distinguishing sites of high from low Cu concentrations in spring and autumn, while low abundances of the cyclopoid *Paracyclops fimbriatus* were important in separating sites of high contamination from those of low contamination in summer and autumn. In summer, low abundances of the hydrachnellid mites, and low abundances of the cyclopoid *Eucyclops serrulatus*, also distinguished sites of high metal concentrations. Finally in autumn, the presence of the halacarid mite *Porohalacarus alpinus*, and low abundances of the harpacticoid *Bryocamptus pygmaeus*, characterised sites of low from high Cu concentrations.

Table 3.6 Summary of similarity terms (SIMPER) analysis. Differences (< and >) in average abundances (m^{-2}) of species contributing to dissimilarities between sites of low Cu concentrations (**A**) and sites of high Cu concentrations (**B**) and the separation of U2 from uncontaminated and contaminated site- groups in spring defined from CLUSTER analysis for **a)** spring and **b)** summer. The % contribution of each species to the dissimilarity between groups by their community structure is shown in italics. A cut-off at a cumulative % dissimilarity of 70% was applied (i.e. species listed contributed to 70% of the dissimilarity between groups in their site community structure).

a)

Species	A		B	A		U2		B
Bryocamptus zschokkei	375	> *21.9%*	0	375	> *25.1%*	0		
Diacyclops languidoides	36	< *10.5%*	142	36	> *13.3%*	0	< *41.8%*	142
Bryocamptus praegeri	47	< *7.3%*	0					
Diacyclops bisetosus	13	< *6.8%*	25			0	< 13.5%	9
Feltria minuta	30	> *9.9%*	0	30	> *11.6%*	0		
Nematodes	779	> *10.7%*	23	779	> *18.3%*	18	< *14.7%*	231

Group A=R2,B2,L1,S9,D1,U3 (% similarity=54.5%); U2; Group B=L4,S8,D5 (% similarity=58.7%)

b)

Species	A		B
Bryocamptus zschokkei	6118	> *20.0%*	4
Bryocamptus praegeri	1325	> *9.2%*	0
Paracyclops fimbriatus	195	> *6.3%*	31
Diacyclops bisetosus	0	< *6.3%*	48
Eucyclops serrulatus	220	> *6.1%*	137
Diacyclops languidoides	50	< *5.6%*	126
Hygrobates sp.	97	> *15.2%*	2
Hydrachnellae sp.a	126	> *14.9%*	0
Feltria minuta	50	> *14.8%*	0

Group A=R2,B2,L1,U3,T1 (% similarity=54.6%); Group B=D1,U2,S8,D5,D3 (% similarity=52.0%)

Table 3.7 Summary of similarity terms (SIMPER) analysis. Differences (< and >) in average abundances (m^{-2}) of autumn species contributing to dissimilarities between sites of low Cu concentrations (**A**) and high Cu concentrations (**B**) groups of sites and the separation of L4 from contaminated and uncontaminated sites-groups defined from CLUSTER analysis. The % contribution of each species to the dissimilarity between groups by their community structure is shown in italics. A cut-off at a cumulative % dissimilarity of 70% was applied (i.e. species listed contributed to 70% of the dissimilarity between groups in terms of site community structure).

Species	A		B	A		L4		B
Bryocamptus zschokkei	839	> *18.3%*	0	839	> *19.7%*	0		
Bryocamptus praegeri	648	> *14.2%*	0	647	> *15.3%*	0		
Eucyclops serrulatus	32	< *8.6%*	209	32	> *6.6%*	0	< *19.5%*	209
Diacyclops languidoides				39	< *7.4%*	267		
Diacyclops bisetosus				10	< *8.2%*	36	> *13.8%*	0
Soldanellonyx monardi				3	< *6.6%*	9		
Lobohalacarus weberi						27	> *12.8%*	0
Porohalacarus alpinus	0	< *8.9%*	35			0	< *12.6%*	36
Nematodes	1154	> *8.0%*	213	1154	> *6.2%*	178		
Paracyclops fimbriatus	57	> *7.5%*	4			62	> *11.4%*	4

Group A = R2, B2, S9,L1,U3,D1,U2 (% similarity = 63.5%); Group B=S8,D5 (% similarity = 64.9 %).

The importance of metal concentrations in relation to species abundances was confirmed by multiple regression analyses (Table 3.8). Copper was the most important variable explaining inter-site variation in the abundance of most species. There were negative correlations between Cu and the abundance of the copepods *B. zschokkei*, *B. praegeri* and hydrachnellid mites. Positive correlations occurred between Cu and *Diacyclops languidoides*, and *Porohalacarus alpinus* abundances (Fig. 3.8). However, variables other than Cu were also important in explaining species distributions. The spring abundance of *B. praegeri* was positively correlated with DOC concentrations. Temperature and Al best explained the spring distribution of *Feltria minuta*. The abundance of this hydrachnellid mite correlated negatively with these variables. A negative relationship existed between high Zn concentrations and autumn and spring nematode abundances. Autumn and summer *Eucyclops serrulatus* abundances were negatively related to water hardness and pH, respectively. Autumn *Bryocamptus pygmaeus* abundances correlated positively with Al concentrations (Table 3.8). Summer *Paracyclops fimbriatus* correlated negatively with Al and Zn concentrations.

SIMPER analyses were used also to reveal the species important in distinguishing the community at L4 and U2 from the communities of other sites in autumn and spring, respectively (Tables 3.6 and 3.7). In spring, U2 was separated from the other groupings by an absence of all meiofauna groups apart from nematodes. As with other sites of high metal contamination, L4 differed from uncontaminated sites due to an absence of harpacticoids and a low abundance of nematodes. Site L4 also differed from uncontaminated sites due to a higher abundance of *D. languidoides*, *D. bisetosus* and the halacarid mite, *Soldanellonyx monardi*. The absence of *E. serrulatus*, and the presence of *Lobohalacarus weberi* and *D. bisetosus*, were important in isolating L4 from other sites of high metal concentrations.

Table 3.8 Results of stepwise mutiple regression analysis on species found by SIMPER to be important in contributing to inter-site differences in community structure in spring, summer and autumn. Only species that demonstrated significant correlations with an environmental variable are shown.

	Autumn			Spring			Summer		
Species	**Variable**	**Cumulative %**	**Relationship**	**Variable**	**Cumulative %**	**Relationship**	**Variable**	**Cumulative %**	**Relationship**
HARPACTICOIDA									
Bryocamptus zschokkei	Cu	93.5	Negative	Cu	89.0	Negative	Cu	94.6	Negative
Bryocamptus praegeri	Cu	89.6	Negative	DOC	89.4	Positive	Cu	91.7	Negative
Bryocamptus pygmaeus	Al	34.1	Positive						
CYCLOPOIDA									
Paracyclops fimbriatus							Al	24.9	Negative
							Zn (Interstitial)	53.0	Negative
Diacyclops languidoides				Fe	48.2	Negative	pH	55.2	Negative
				Cu	76.1	Positive	Conductivity	75.7	Negative
Diacyclops bisetosus				Fe	29.	Negative	Cu	72.8	Positive
				Conductivity	46.3	Negative			
Eucylops serrulatus	Hardness	47.4	Negative				PH	49.9	Negative
HYDRACHNELLAE									
Feltria minuta				Temperature	45	Negative	Cu	48.6	Negative
				Al	85.3	Negative	Hardness	69.8	Positive
Hygrobates							Conductivity	35.6	Positive
							Cu	55.4	Negative
Hydrachnellae sp. A							PH	27.3	Positive
							Cu	45.9	Negative
HALACARIDAE									
Porohalacarus alpinus	Cu	40.4	Positive						
NEMATODA	Zn	35.7	Negative	Zn	24.9	Negative			

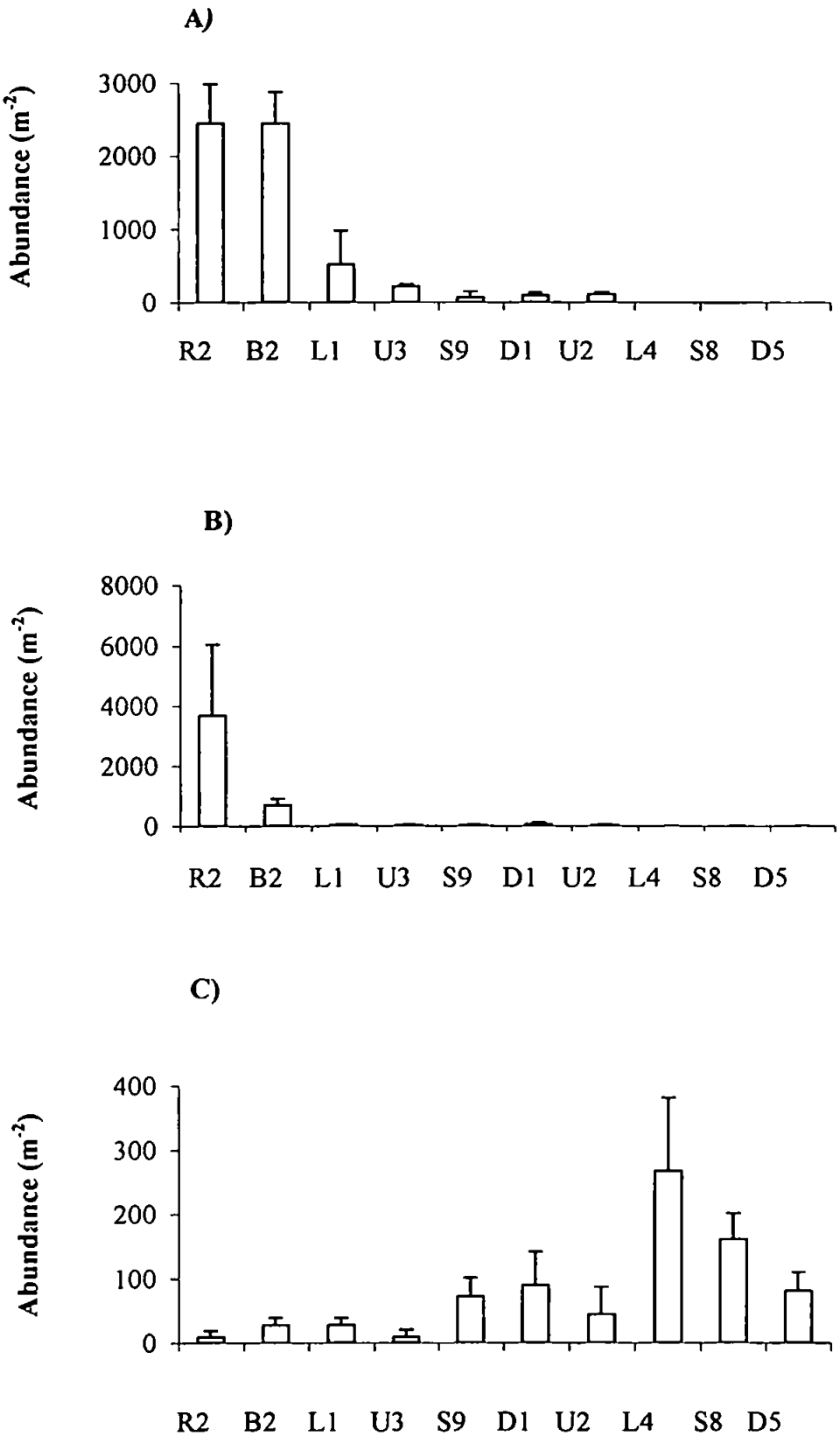


Figure 3.8 Relationship between abundances of **A)** *Bryocamptus zschokkei*, **B)** *Bryocamptus praegeri* and **C)** *Diacyclops languidoides* and the environmental variable shown to explain most of their distribution using multiple regression (i.e. copper) in autumn (n=5 for each site; vertical bars = +1SD). Sites are arranged in order of increasing value of Cu.

3.3.5 Univariate measures

Despite inter-site differences in community structure correlating with Cu in all seasons, the relationship between Cu and the univariate measure of species richness was less clear. In summer, there was a significant negative correlation between total meiofaunal species richness and Cu ($r = -0.822$, $F=12.46$, $p<0.05$), although no such trend was evident for other seasons ($r=-0.376$, $F=2.76$, $p>0.1$ for spring; $r=-0.319$, $F=0.69$, $p>0.1$ for autumn) (Fig. 3.9). Indeed, autumnal meiofaunal diversities were highest at sites of intermediate Cu concentrations.

Differences in total meiofaunal abundances amongst sites were largest in autumn and summer, and there were highly significant negative correlations between total abundance and Cu concentration in autumn ($r=-0.834$, $F=7.32$, $p<0.05$) and summer ($r=-0.880$, $F=28.02$, $p<0.05$). Spring meiofaunal abundances did not correlate significantly with Cu ($r=-0.413$, $F=2.94$, $p>0.1$). The correlation between meiofaunal abundance and Cu was due mainly to sites of high Cu concentrations having a low abundance of harpacticoids (a dominant group at sites of low Cu concentrations) (Fig. 3.10). The other two dominant groups (cyclopoids and nematodes) demonstrated no clear trend of abundances in relation to increases in Cu concentrations.

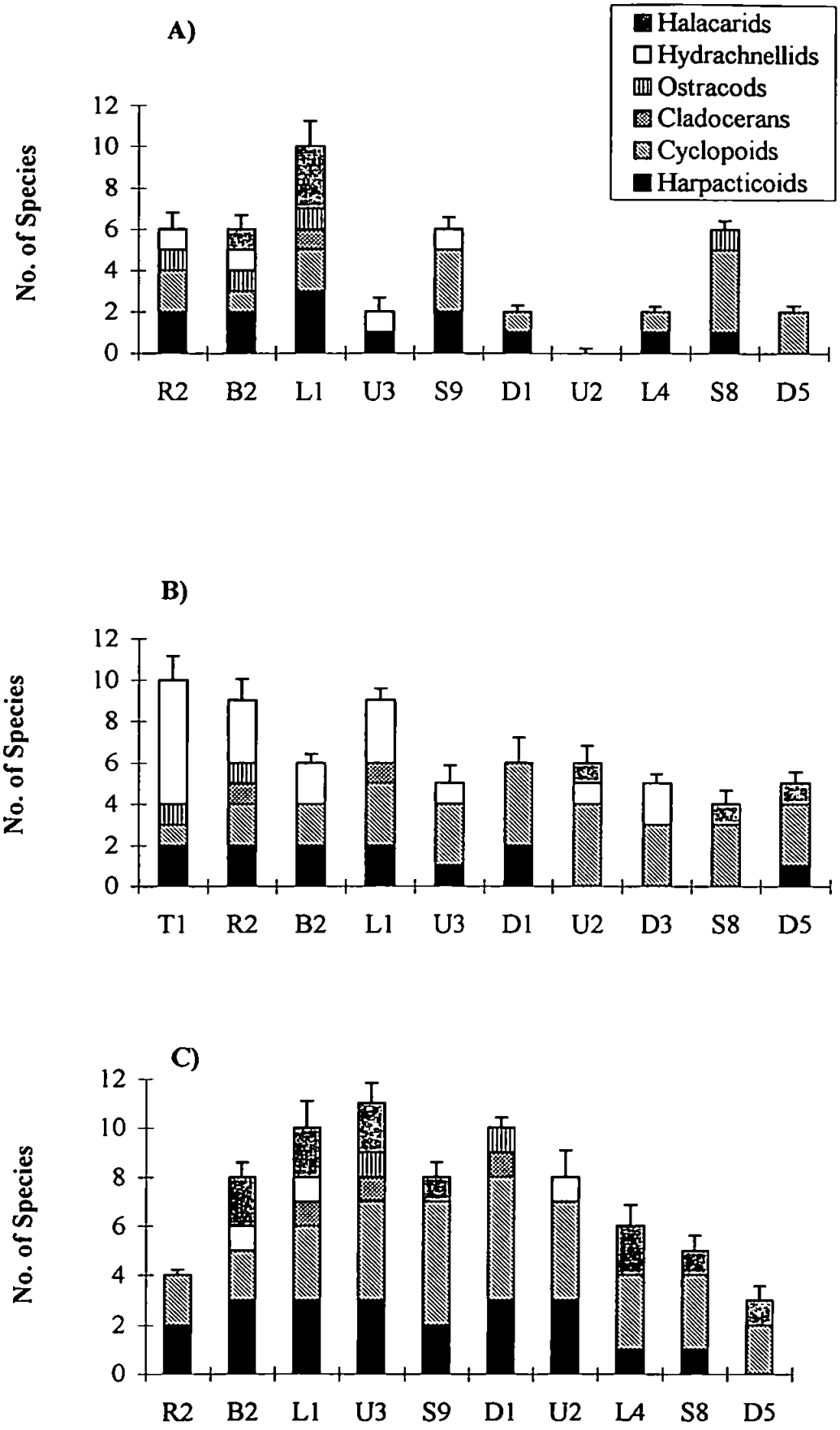


Figure 3.9. Mean species richness of major meiofaunal groups at ten sites on the tributaries of the Rivers Lynher and Seaton (n=5 for each site; vertical bars = +1 SD) A) spring, B) summer and C) autumn. See Figure 2.2 for site locations. Sites are arranged in order of increasing Cu concentration.

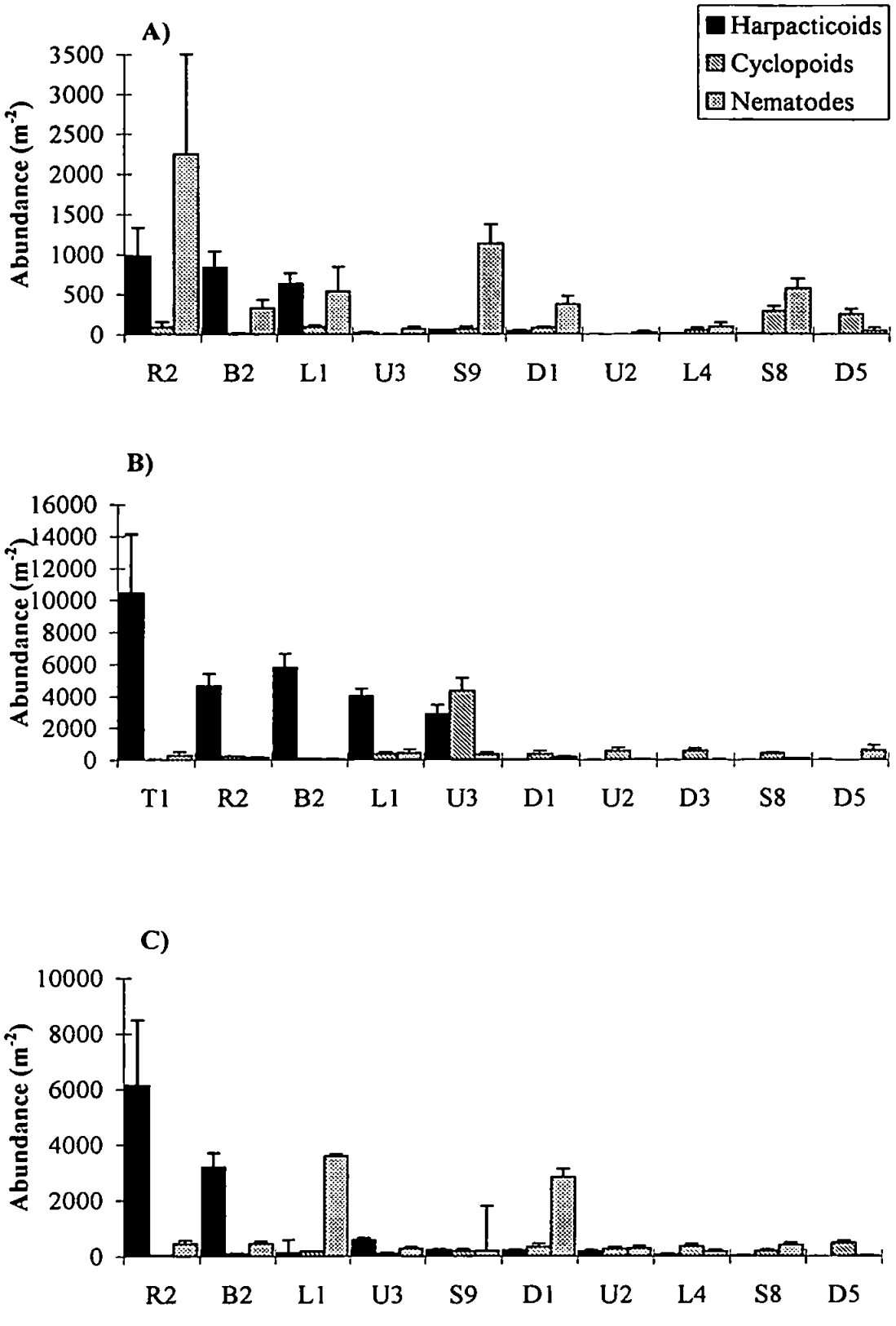


Figure 3.10 Mean abundances of harpacticoids, cyclopoids and nematodes in **A)** spring, **B)** summer and **C)** autumn at ten sites on tributaries of the Rivers Lynher and Seaton (n=5 for each site; vertical bars = + 1 SD). See Figure 2.2 for site locations. Sites arranged in order of increasing Cu concentration.

3.4 Discussion

3.4.1 Links between meiofaunal community structure and environmental variables in metal-contaminated streams

This chapter has demonstrated, for the first time, clear relationships between the concentrations of trace metals and the composition of lotic meiofaunal communities using multivariate analyses. In all seasons, BIOENV analyses implicated Cu as an important variable explaining inter-site differences in meiofaunal community structure, with communities at sites of low Cu concentrations being very different from those of high Cu concentrations. Although there is a lack of literature on the effect of Cu on the freshwater meiobenthos, the high toxicity of Cu to other aquatic biota is well established. Many studies have shown Cu to be a major determinant of stream macroinvertebrate structure (Leland *et al.*, 1989; Kiffney & Clements, 1994). Winner *et al.* (1980) demonstrated that the macroinvertebrate community in a stream contaminated with relatively high, and variable, concentrations of Cu, Cr and Zn was comparable with that in a experimental stream receiving a low, but constant, concentration of Cu, suggesting Cu was the metal most influential in structuring the natural community. Evidence that meiofaunal communities are influenced by Cu has been reported also in the marine environment. Somerfield *et al.* (1994) recorded nematode diversity and changes in nematode community structure in a metal-contaminated estuarine system to be closely correlated with metal levels. Austen *et al.* (1994) also demonstrated an effect of elevated levels of Cu on an estuarine meiofaunal community structure by dosing communities with various concentrations of Cu in microcosm experiments. The meiobenthic community in the mesocosms treated with Cu was significantly different from the controls (Austen *et al.*, 1994).

In their survey of metal contaminated streams (including sites used in the present survey), Gower *et al.* (1994) found that Al, dissolved organic matter and pH appeared to play a major role in determining macrofaunal community structure. The present study demonstrated that the meiofauna apeared to be influenced by these variables. Inter-site variation in Cu in combination with the variables Zn (spring), Zn, DOC, pH (summer), and Al (autumn) best explained the differences in community structure across sites.

Previous studies have demonstrated that aluminium was important in determining stream invertebrate community structure (Ormerod *et al.*, 1987; Wade *et al.*, 1989; Rutt *et al.*, 1990; Gower *et al.*, 1994), including the stream meiofaunal community (Rundle & Ormerod, 1991). Rundle & Ormerod (1991) showed that the total numbers of microcrustaceans and harpacticoid copepods were less abundant at sites of high compared with low Al concentrations. The influence of Zn on stream macroinvertebrate communities is to reduce species richness, reduce abundance and to shift community composition from sensitive to tolerant species (Clements, 1988; Willis, 1988). Laboratory studies have also shown that high Zn concentrations have a toxic effect on marine copepods and nematodes (Coull & Chandler, 1992). From the present study, it appears that high concentrations of Zn may also have a deleterious effect on freshwater copepods and nematodes. BIOENV analyses implicated Zn as an important variable explaining spring and summer meiofaunal community patterns, while Zn was the variable that best explained inter-site variation in nematode abundances.

Other environmental variables have an ameliorating effect on metal toxicity (i.e. pH and DOC) (Campbell & Stokes, 1985; Kullberg, 1992). The importance of the effect of pH on stream meiofaunal community structure has already been reported (Rundle & Hildrew,

1991; Rundle & Ormerod, 1992), with species richness and density of the meiofauna being reduced at acidic sites, whilst acid-sensitive species were replaced by tolerant ones. Under acidic conditions, the toxicity of metals is enhanced by their release from particles into soluble more toxic forms (Campbell & Stokes, 1985). This effect may explain the high Al concentrations found at the most acidic site in the present study.

At some sites, high concentrations of DOC may have had an ameliorating effect on metal toxicity. DOC concentration was highly negatively correlated with Cu concentrations (See Chapter 2), and it is likely that at sites of high DOC, Cu had formed complexes resulting in a reduction of the toxic free ion form of the Cu at these sites (Spear & Pierce, 1979). For example, Austen *et al.* (1994) found the meiofaunal community of estuarine mud to be less affected by Cu and Zn than a community from estuarine sand, and attributed this to Cu binding to the high levels of organic chelaters in the former sample.

3.4.2 Seasonal differences in meiofaunal communities in metal-contaminated streams

Although inter-site differences in meiofaunal community structure were clearly linked to Cu in all three seasons, the importance of the other variables were only highlighted at certain times of the year. In autumn, Al was implicated as an important variable determining community structure, mainly at L4 (a site of high Al concentrations and low pH). Autumnal Al concentrations at L4, however, were no different than those measured in spring (Chapter 2). In spring, the community at U2 was separated from the others due to an absence of all meiofauna except for nematodes. High flows, or high Zn concentrations, occurred at U2 at the time of sampling (Appendix I) and either Zn or flow may be responsible for the absence of microcrustaceans.

The seasonal pattern of abundance of the major meiofaunal groups measured in these metal-contaminated streams was similar to that demonstrated by previous studies of meiofauna of streams in the UK (Robertson, 1990; Rundle, 1990; Rundle, 1993) and in North America (Shiozowa, 1985; Palmer, 1990). Highest abundances of copepods, cladocerans, ostracods and hydrachnellid mites were recorded in summer, whilst lowest abundances were found in spring. This pattern can be explained by several hypotheses. Firstly, low flow occurring during the summer months may provide favourable conditions for population growth, whereas in spring, animals are frequently washed away during spates. For example, Palmer (1990) found a significant decrease in the abundance of benthic copepods, and an increased number of drifting copepods, after spring rainstorms. Secondly, elevated temperatures during the summer may lead to higher abundances in some meiofaunal groups such as harpacticoids and cyclopoids due to the shortening of their developmental times (Sarvala, 1979; O'Doherty, 1985). In contrast to other meiofauna, nematode abundances were lower in summer compared with spring and autumn. Nematodes may not be so vulnerable to erosion as other groups due to their interstitial habitats and this would allow them to increase in numbers when the other groups are in low densities. Low abundances in summer may reflect some influence other than physicochemistry; for example, increased predation pressure at this time. A seasonal survey of meiofaunal abundances in Goose Creek (Virginia) also demonstrated stream nematode abundances to be highest in spring (Palmer, 1990), although nematode abundances in an Italian stream (Zullini & Ricci, 1980) and in a creek in Pennsylvania (USA) (Bott & Kaplan, 1989) showed no seasonal pattern. Further studies are, therefore, required to explain and understand the different seasonal patterns in the abundance of nematodes.

3.4.3 A comparison with meiofaunal communities in other regions

This study focused on streams in an area where trace metal contamination of freshwater is widespread and metal concentrations exceed the recommended UK Environmental Quality Standards for Cu (NRA, 1994). These universally elevated Cu concentrations may explain the low diversities of meiofauna recorded here compared with previous studies of stony streams in the UK. Only 24 species of microarthropods were found in the twelve sites used in this study, whilst Rundle & Hildrew (1990) identified seventy-two species of microarthropods (excluding ostracods) in their seasonal survey of ten stony streams in the Ashdown forest, (southern England). In a survey of thirteen sites in upland streams in mid-Wales, Rundle & Ormerod (1991) recorded twenty-seven microcrustacean species. The lower diversities of meiofauna recorded in the tributaries of the Rivers Lynher and Seaton relative to the two previously published extensive surveys was attributable mainly to a lower number of species of harpacticoid copepods, hydrachnellid mites, ostracods and cladocerans. In south-west England, streams drain systems of base poor geology, resulting in relatively low water hardness, such systems are usually associated with species-poor benthic macroinvertebrate communities (Egglishaw & Morgan, 1965; Ormerod & Edwards, 1987). Therefore, the low diversity in the streams of the tributaries of the Rivers Lynher and Seaton may simply be a reflection of the low stream invertebrate diversity in the South-West.

Despite the diversities of microarthropods in the stream tributaries of the Rivers Lynher and Seaton being lower than those reported for other regions in the UK (Rundle & Hildrew, 1990; Rundle & Ormerod, 1991), similar meiofaunal groups were numerically dominant in both cases (i.e. harpacticoid and cyclopoid copepods). In the present study, nematodes were dominant at sites, irrespective of levels of metal concentrations. Suren (1992) also found nematodes and copepods to be the most abundant permanent meiofauna

in two gravel streams in New Zealand, with ostracods, and aquatic mites contributing a small proportion to the total invertebrate assemblage. In the USA, nematodes and copepods were also found to be abundant in studies of lotic meiofauna in Virginia (Palmer, 1990), Pennsylvania (Borchardt & Bott, 1995) and Ohio (Hummon *et al.*, 1989). The latter study (Ohio) included streams polluted by acid mine drainage, which were dominated by either nematodes or rotifers. The average total meiofaunal densities recorded in the lotic systems sampled in North America were between ten and 100 times higher than those recorded in streams in the UK. This difference may be explained by differences in substratum. In the American streams, the substratum was predominantly fine sediment, whereas other studies were from streams with coarser (stony) substrata. Sediment structure attains a dominant role in meiobenthic ecology. As many meiobenthic animals can exploit the interstitial environment of sandy substrates the proportion and distribution of finer sediment particles will influence the degree of accessibility (Giere *et al.* , 1988).

3.4.4 Indirect effects of trace metals on meiofaunal community structure

Differences in meiofaunal community structure observed across the metal gradient may not be the result of just a direct toxic effect. A decline in the quality of food resources may be responsible for population declines of some taxa. For example, Leland & Carter (1985) reported declines of primary production in creeks exposed to elevated levels of Cu, and heterotropic microbes (bacteria and fungi) have also been shown to be influenced by mine effluent discharge, resulting in reduced decomposition rates (Carpenter *et al.*, 1983; Maltby & Booth, 1983). Trace metals inhibit both the growth and sporulation of aquatic fungi (Duddridge & Wainwright, 1980; Abel & Barlocher, 1984). The resultant reduced decomposition rate of leaf litter due to the lower heteroptrophic activity may also explain the lower concentrations of DOC found at sites of high Cu concentration in the present study. As many species of meiofauna are bacteriophores and algivores (Perlmutter &

Meyer, 1991; Borchadt & Bott, 1995), lower supplies of these food sources are likely to have a significant impact on their community structure.

Sites of high metal concentrations were not significantly less diverse than sites of low metal concentrations. Instead, community structure differed due to metal-tolerant species replacing sensitive species at sites of high metal concentrations. Metals are likely to be affecting predator-prey and competitive interactions occurring within the benthos, allowing tolerant species to increase in abundance due to the removal of a predator or a competitor. In autumn, species richness was highest at sites of intermediate contamination, which corroborates many marine studies demonstrating highest meiofaunal diversities at sites of moderate pollution (Hockin, 1983; Platt & Lambshead, 1985; Hodda & Nicholas, 1986; Moore & Pearson, 1987). Huston (1979) hypothesised that diversities are highest where some limited environmental disturbance prevented dominance by one, or a few, species. Thus, further work is required on the effect of metals on predator-prey and competitive interactions to explain the response of the meiofaunal community to metals.

3.4.5 Links between environmental variables and specific meiofaunal groups in metal-contaminated streams

In the present study, the abundances of the two harpacticoids *Bryocamptus zschokkei* and *B. praegeri* (the dominant species at sites of low contamination) showed strong negative correlations with Cu concentration. Harpacticoids are burrowing interstitial species and, as Cu levels were found to be higher in the interstitial environment then in surface waters (Chapter 2), harpacticoids may be more sensitive to high metal concentrations than epibenthic meiofauna. In the marine environment, Van Damme *et al.* (1984) found a lower abundance of benthic harpacticoids in a metal-contaminated estuary compared with one of low contamination, although no other macrofaunal or meiofaunal element was affected. It

was concluded that this effect was due to Cu, as Cu was persistently present at levels which affected egg production and development of harpacticoids in bioassays (Van Damme *et al.*, 1984). It was also suggested that harpacticoids might be good indicators of Cu contamination due to their apparent high sensitivity (Van Damme *et al.*, 1984). This present study also suggests that harpacticoids, particularly *Bryocamptus zschokkei* and *Bryocamptus praegrei,* may have a role as indicators of Cu contamination.

Unlike harpacticoids, some species of cyclopoid copepod appeared to be highly tolerant of high metal concentration. For example, there were strong positive correlations between the abundances of *Diacyclops languidoides* and *Diacyclops bisetosus* with interstitial Cu concentrations in spring. Many species of cyclopoids (including *D. languidoides*) have also been found to be abundant at acidic sites (Rundle & Hildrew, 1990). This tolerance of cyclopoids to extreme chemical conditions may lead to higher abundances at sites where competition is low and predators are scarce. Caution is required, however, in assuming that this tolerance applies to all cyclopoid species, as the cyclopoid *Paracyclops fimbriatus* appeared to be sensitive to Cu contamination.

Hydrachnellid mites were restricted to sites with low metal concentrations, and abundances of *Feltria minuta* were important in separating sites of low and high Cu concentrations. In spring, however, the abundance of *F. minuta* was negatively correlated with temperature and Al concentrations, whilst in autumn, Cu and hardness best explained its distribution. Despite these correlations with physicochemistry, biological factors are also likely to affect hydrachnellid mite distribution. The majority of hydrachnellid mites are predatory, and most of their larval stages are known to be parasitic on aquatic insects and insect larvae (Gledhill, 1985). It has been suggested that the distribution of these mites is limited by the distribution of their prey (Rundle & Hildrew, 1990). In the present study, the nymphs of

hydrachnellid mites, other than *Feltria minuta* (e.g. *Hygrobates* sp. and *Torrenticola* sp.), were most abundant at sites L1 and T1 (Appendix II), sites of high discharge. Previous studies have shown the hydrachnellid mites *Hygrobates* sp. and *Torrenticola* sp. to be associated with fast-flowing waters (Viets, 1936; Rundle & Hildrew, 1990). The association of hydrachnellid mites with their hosts may also explain the large abundance of nymphs present in the summer in the present study. In most cases, the parasitic larval stage will be feeding on airborne insects and, therefore, their return to the water may be when the host lays its eggs, or after the death of the host, and this is most likely to occur in spring or early summer (Rundle, 1990).

Halacarid mites were restricted mainly to sites of low Cu concentration, however, *Porohalacarus alpinus* was notable for being found only at sites of high Cu concentrations. It is important to note that halacarid mites are generally carnivorous (Green & Macquety, 1987), and *Lobohalacarus weberi* preys on nematodes and oligochaetes (Teschner, 1963). Thus, as with the hydrachnellid mites, halacarid species may be restricted by the occurrence of their prey.

Alona quadrangularis and *Chydoris sphaericus* were the only two cladocerans found in the stream tributaries of the Rivers Lynher and Seaton, and both species were restricted to sites of low Cu contamination. The sensitivity of *C. sphaericus* to Cu is in agreement with Kovisto *et al.* (1992) who demonstrated that long-term exposure of this species to Cu resulted in a reduction in the rate of population growth. *Alona quadrangularis* was found only at sites of high discharge (Appendix II) and this corroborates a study by Rundle and Hildrew (1990) which found maximum discharge best explained the distribution of *A. quadrangularis* in streams in the Ashdown Forest.

Variation in nematode densities were best explained by Zn concentrations; with nematodes being more abundant at low Zn concentration in spring and summer. Marine nematodes are also sensitive to Zn in toxicity tests (Coull & Chandler, 1992). In spring, nematodes were the only taxon found at U2, a site of high Zn contamination. The tolerance of some nematode species to high levels of Zn is in agreement with studies by Somerfield *et al.* (1994) and Austen *et al.* (1994), who found nematodes at very high concentration of Zn and Cu. There may be differences in the tolerance of individual species of nematode. Newell *et al.* (1990a) reported that freshwater nematodes from the same genus had different degrees of tolerance to TiO_2 waste. Thus, future identification of nematodes is likely to reveal a difference in the resistance of nematodes to Zn toxicity.

Conclusion

In summary, metals (particularly Cu) were of overriding importance in determining the inter-site differences in the stream meiofaunal community structure in the present study. Differences in site community structure did not reflect lower species richness at contaminated sites, but were due, primarily, to the replacement of sensitive species by Cu tolerant species at sites of high Cu concentrations. Despite the apparent influence of other variables on stream meiofauna, the structure of the meiofaunal community clearly reflected the gradient in Cu concentrations at the sites surveyed. These results suggest that meiofaunal community structure has potential as a monitor of changes in Cu contamination in streams.

CHAPTER 4

Comparison of stream meiofaunal and macrofaunal community structure across a metal contamination gradient

4.1 Introduction

Whereas macroinvertebrates have been used extensively to monitor the quality of freshwater systems (Rosenberg & Resh, 1992; Mason, 1996), meiofaunal communities have been virtually excluded from freshwater pollution monitoring. The exclusion of the meiofauna from traditional monitoring programmes stems from the fact that this faunal component has only very recently been shown to constitute a significant component of the stream benthos (Chapter 1). The limited information available, however, clearly indicates that freshwater meiofaunal communities are influenced by anthropogenic inputs. For example, the meiofaunal assemblages of acidic streams surveyed in mid-Wales and southern England differed from circum-neutral streams (Rundle & Hildrew, 1990; Rundle & Ormerod, 1991). The distinct species composition in streams of different chemistry suggests that the stream meiofauna has the potential to be a biomonitor of contamination. Further support for this suggestion comes from the marine environment, where meiofaunal communities have been used successfully to detect anthropogenic disturbances (Moore & Bett, 1989). Somerfield *et al.* (1994) examined the meiofaunal community structure in a highly-polluted estuary, and demonstrated that the diversity and community structure of the nematodes was closely correlated with trace metal concentrations. In an estuary contaminated with Zn, Cu and Pb, reduced diversity of meiofauna was also recorded even though there was no alteration of the macrofaunal community (Van Damme *et al.*, 1984). The latter study demonstrates clearly that the extent of contamination may be underestimated when only using one faunal component.

As yet, no comparison of the effect of metal contamination on the meiofaunal and macrofaunal communities of freshwater ecosystems has been made. If there is a comparable response of different faunal components to contaminants, this gives confidence to the

generality of the effect of a pollution event on ecosystems (Warwick, 1993). Similar meiofaunal and macrofaunal community responses were recorded across sites of different pH in streams in southern England (Townsend *et al,* 1983; Rundle & Hildrew, 1990), while marine meiofaunal and macrofaunal communities appeared to change, in a comparable way, across a gradient of trace metal contamination in an estuary in south-west England (Bryan & Gibbs, 1983; Somerfield *et al.*, 1994).

As the meiofauna and macrofauna are ecologically distinct components of the benthos (Warwick, 1984), they may also respond to metal contamination in a different way. Studies that have compared directly the effect of disturbance on marine meiofauna and macrofauna have shown that including both components provides a more comprehensive understanding of the impact of pollution. Somerfield *et al.* (1994) compared community structure of macrofauna and nematodes along a marine transect through a dredging disposal site and related the community structure to a range of environmental variables. The nematodes were more sensitive to the sediment structure and the ongoing disposal of dredging at the site than the macrofauna, whereas the macrofauna was more sensitive to concentrations of trace metals and longer-term events at the site than the nematodes (Somerfield *et al.*, 1994). In another marine study, which examined the impact of sewage enrichment on an intertidal sandy shore in summer and autumn, the macrofauna and meiofauna responded to the sewage enrichment in the vicinity of the sewage outfall, however, the summer macrofaunal community appeared disturbed throughout the area (Austen *et al.*, 1989). It was concluded that the macrofaunal community may have been disturbed by human digging for shellfish occurring in summer. Previous work has shown that the marine meiofauna, unlike the macrofauna, is hardly affected by physical sediment reworking (Thistle, 1980; Sherman & Coull, 1980; Sherman *et al.*, 1983). Some caution must be taken, therefore, if only one

component of the fauna is used to assess the impact of disturbance on the rest of the community.

Another possible caveat using only the macrofauna in freshwater pollution monitoring is the exclusion of their smaller life-history stages during sampling. Current methods of monitoring stream macroinvertebrates use a standard net mesh size of 0.8-1.0 mm. A large proportion of macrofaunal individuals pass through this mesh size range (a large fraction also passes through a 0.5 mm sieve and are classed as temporary meiofauna). To date, no study has included these smaller individuals when measuring the community response of freshwater systems to contamination. By excluding this component of the fauna, it may be argued that the whole community response has not been assessed. Moreover, the inclusion of these smaller life stages may allow contamination to be detected at lower concentrations of chemical input as the former often have greater sensitivity to pollution compared with the adult stages (Diamond *et al.*, 1992; Kiffney & Clements, 1994; Leland *et al.*, 1989).

To increase cost effectiveness, monitoring of fresh water using stream macroinvertebrates is often undertaken using identification to a taxonomic level higher than species (i.e. family) (e.g. Furse *et al.*, 1981; Armitage *et al.*, 1983; Rutt *et al.*, 1990; Rutt *et al.*, 1993). Moreover, measures of response used are often simplistic, and univariate diversity measures, or biotic indices, are preferred to multivariate approaches (Rosenberg & Resh, 1992), under the premise that the former are easier to interpret (see Chapter 1). Previous studies of macroinvertebrate communities, however, have shown that multivariate techniques are the most sensitive analytical method for the detection of contamination in freshwater and marine systems (Norris *et al.*, 1982; Warwick & Clarke, 1991; Cao *et al.*, 1996; Perryman, 1996). Recent studies of the impact of pollutants on marine systems has

led to the development of a multivariate index for the detection of anthropogenic inputs (Warwick & Clarke, 1993a).

The aim of this chapter was to examine which components of the stream benthic community responded most directly to trace metal contamination. To achieve this objective, various faunal components [i.e. macrofauna, meiofauna, total macrofauna (including temporary meiofauna) and total metazoa (macrofauna, meiofauna and temporary meiofauna)], sampled across sites representing a metal gradient, were correlated with environmental variables measured at these sites using multivariate analyses. Higher macrofaunal and meiofaunal taxonomic levels than species, univariate measures and the Index of Multivariate dispersion were also used to examine the pattern of perceived impact.

4.2 Materials and methods

4.2.1 Meiofaunal an macrofaunal sampling

Meiofauna was obtained from samples collected at sites R2, B2, L1, U3, S9, D1, U2, L4, S8 and D5 in autumn (21st-25th November 1994) and spring (17th-24th April 1995) as described in Chapter 3. Macrofauna, and temporary meiofauna, were also obtained from these samples. Macrofauna was removed and identified from material retained on a 500 μm sieve, whilst the temporary meiofauna comprised the macroinvertebrates that passed through a 500 μm sieve. All individuals, apart from chironomids and oligochaetes, were identified to species (using the keys listed in Appendix II), enumerated and a data matrix constructed for the entire metazoan community across sites.

4.2.2 Environmental variables

Annual mean values of the environmental variables (Chapter 2) were used in the analyses. Mean values from replicate seasonal data have been used previously to provide a robust comparison between sites (Rundle & Hildrew, 1990).

4.2.3 Statistical analyses

4.2.3.1 Comparison of patterns between different community subsets

Ranked lower triangular similarity matrices (Bray Curtis Similarity matrices) were constructed for meiofaunal, macrofaunal, total macrofaunal (i.e. macrofauna and temporary meiofauna) and total metazoan data for spring and autumn (Chapter 3). As the fauna varied in abundance within samples from single to thousands of individuals, a fourth root transformation was applied to reduce the influence of dominant species on the analyses. The PRIMER program CLUSTER was used to determine 'groupings' of averaged site communities in such a way that communities within a group were more similar than communities in different groups (Chapter 3). For each subset, multidimensional scaling

ordinations were used to display, visually, inter-site differences in averaged faunal abundances [Kruskal & Wish, 1978; Clarke & Green, 1988 (Chapter 3)].

To compare the discrimination between sites based upon macrofaunal and meiofaunal communities, similarity matrices of averaged macrofaunal (i.e. not including temporary meiofauna) and averaged meiofaunal (i.e. permanent meiofauna) data were compared using the PRIMER program RELATE. Spearman's Rank correlation coefficients (ρ) between the corresponding elements of each pair of matrices were computed and the significance of the correlation was determined using a Monte Carlo permutation procedure (Clarke & Warwick, 1994). The similarities percentage procedure (SIMPER) for each season (Clarke, 1993; Chapter 3) was used to identify the macrofaunal and meiofaunal species important in contributing to the dissimilarities in site groupings derived from the CLUSTER analysis.

To test for significant differences in the community structure between sites, one-way ANOSIM permutation tests (Clarke & Green, 1988; Clarke, 1993, Chapter 3), were used. These tests investigated the discriminatory power of separate community subsets (macrofauna, meiofauna, total macrofauna and total metazoa) in distinguishing between site differences in metal concentrations .

4.2.3.2 Relating faunistic and environmental data

The separation of sites, based upon their community structure, was related to inter-site differences in trace metal concentrations in two ways. Firstly, similarity matrices for averaged faunistic data were compared with the lower triangular Euclidean matrix from a correlation-based PCA of metal data using RELATE (Clarke & Warwick, 1994; Chapter 3). The PCA of metal data was different from the PCA used in Chapter 2 as it included only those sites used to sample meio- and macro-benthic communities in spring and autumn.

Secondly, the relationships between community data and different combinations of environmental variables were investigated using the BIOENV procedure (Clarke & Ainsworth, 1993; Chapter 3).

4.2.3.3 Analysis of varying levels of taxonomic organisation

To investigate whether the discriminatory powers of the data were retained at higher taxonomic levels, macrofaunal and meiofaunal (species) data were aggregated to the level of genus, family and phylum using the PRIMER program AGGREG. The RELATE program (PRIMER) was then used to compute Spearman Rank correlations (ρ) between similarity matrices generated for each taxonomic level. The significance of the correlation was determined using the Monte Carlo permutation procedure (Clarke & Warwick, 1994). Spearman Rank correlations (ρ) were calculated also for inter-faunal matrices at the different taxonomic levels and the dissimilarity matrix for metal data.

4.2.3.4 Univariate measures

Two diversity indices, Shannon-Weiner (H') ($\log_{10}$) and eveness (Pielou's J) ($\log_{10}$), were calculated for the macrofauna and meiofauna in spring and autumn using the PRIMER program DIVERSE. Significant differences between sites were tested using 1-way ANOVA, followed by a multiple range test (Fisher's least significant different process).

4.2.3.5 Index of Multivariate Dispersion

An Index of Multiple Dispersion (using the PRIMER program MVDISP) (Warwick & Clarke, 1993) was calculated to determine the variability of replicate faunal samples at each site. Increased variability amongst replicates, in terms of their species abundances and identities with increased levels of perturbation, were measured previously in four marine communities (Warwick & Clarke, 1993). The Index of Multiple Dispersion may serve,

therefore, as a method of placing a value on community changes in response to pollution, displayed by multivariate analyses.

4.3 Results

4.3.1 Comparison of meio- and macrofaunal community patterns across a gradient of metal contamination

In each season, the MDS ordinations of macrofaunal and meiofaunal communities for the ten sites showed similar patterns (Fig. 4.1). At sites of low Cu concentrations (e.g. R2, B2, L1), communities were separated clearly from those at sites of high Cu concentrations (e.g. L4, S8, D5). These similarities were confirmed by the significant correlations between the similarity matrices underlying spring and autumn macro- and meiofaunal community patterns (Table 4.1). Hence, both faunal communities distinguished sites of differing metal concentrations in a similar way. Spring and autumn similarity matrices correlated significantly for the macrofaunal communities, but there was no significant correlation between meiofaunal communities in the two seasons. This latter result was probably explained by the highly distinctive meiofaunal communities at U2 and L4 in spring and autumn, respectively (Section 3.3.4; Fig. 4.1).

Table 4.1 Matrix of pairwise Spearman's Rank Correlation Coefficients (ρ) between similarity matrices derived from averaged transformed macrofaunal and meiofaunal abundance data (* $p<0.05$) for ten sites on the Rivers Lynher and Seaton.

	Macrofauna (Spring)	Macrofauna (Autumn)	Meiofauna (Autumn)
Meiofauna (Spring)	0.425*		0.140
Meiofauna (Autumn)		0.540*	
Macrofauna (Spring)		0.500*	

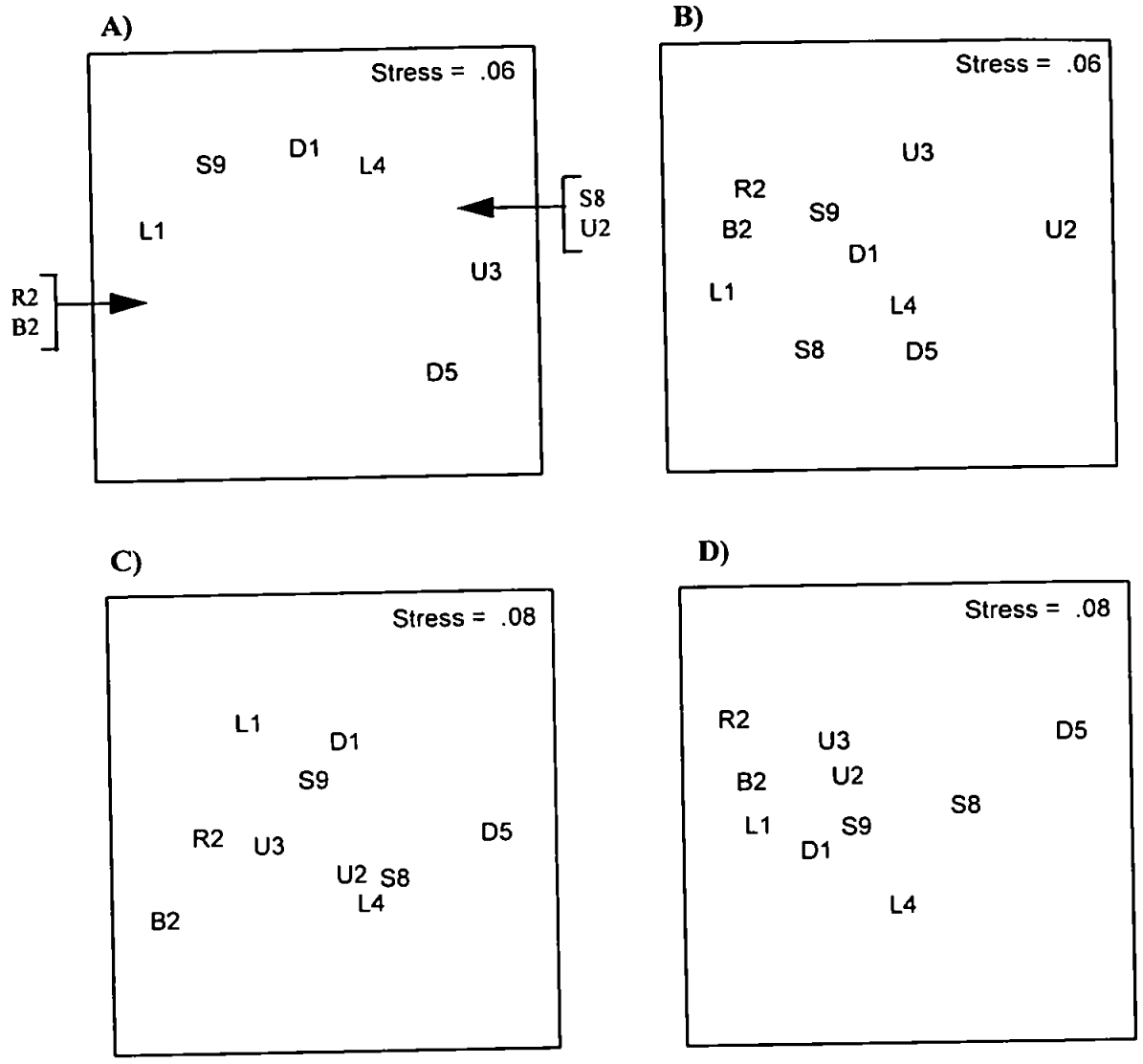


Figure 4.1 MDS ordinations of mean abundances of **A)** spring macrofauna, **B)** spring meiofauna, **C)** autumn macrofauna and **D)** autumn meiofauna at ten sites on the Rivers Lynher and Seaton. Stress values indicate how well the 2-dimensional picture summarises the relationship between site communities. Arrows indicate location of sites listed.

In each season, SIMPER analyses demonstrated that the macrofaunal communities at sites of low Cu concentrations (i.e. R2, B2, L1, U3, S9, D1) were characterised by high abundances of oligochaetes, dipterans (e.g. chironomids), several species of Plecoptera (e.g. *Chloroperla torrentium*) and Trichoptera (e.g. *Hydropsyche siltalai*), the flatworm *Polycelis felina* and the mayfly *Baetis rhodani* (Table 4.2). At uncontaminated sites, spring and autumn meiofaunal communities were characterised by high abundances of the harpacticoids *Bryocamptus zschokkei* and *Bryocamptus praegeri*, and the presence of the mite *Feltria minuta*; these species were absent from contaminated sites (Chapter 3). In contrast, the cyclopoid *Diacyclops languidoides* was abundant at sites with high metal concentrations.

ANOSIM demonstrated that the meiofaunal data discriminated between sites better than the macrofaunal data in autumn (Table 4.3). In spring, however, the macrofauna discriminated better between sites than the meiofauna (Table 4.3). For spring and autumn samples, discrimination amongst sites for total macrofaunal data was better than for the macrofauna (i.e. the discriminatory power of macrofaunal data was improved by the addition of temporary meiofauna). The best discriminatory power was found for the total metazoan data (Table 4.3).

Table 4.2 Summary of similarity terms from SIMPER analysis for a) spring and b) autumn macrofaunal communities. Differences (< and >) in average abundances (m^{-2}) of species contributing to dissimilarities between sites of low Cu concentrations (A) and high Cu concentrations (B) groups defined by CLUSTER analysis are shown. The % contribution of each species to the dissimilarity between groups by their community structure is shown in italics. A cut-off at a cumulative % dissimilarity of 70% was applied (i.e species listed contributed to 70% of the dissimilarity between groups in terms of site community structure).

a)

	A		B
Oligochaeta	124	> *7.6%*	5
Baetis rhodani	270	> *14.9%*	0
Amphinemura sulcicollis	85	> *9.9%*	0
Ceratopognidae	25	> *8.5%*	0
Chloroperla torrentium	53	> *8.5*	2
Chironomidae	404	> *7.8%*	66
Hydropsyche siltalai	23	> *5.2%*	0
Polycelis felina	44	> *5.1%*	4

Group A = R2, B2, L1, U3, S9, D1, L4 (% similarity = 33.7); Group B = U2, S8, D5 (% similarity = 43.0)

b)

	A		B
Oligochaeta	661	> *9.6%*	67
Amphinemura sulcicollis	196	> *8.2%*	4
Baetis rhodani	43	> *7 5%*	0
Pedicia rivosa	16	> *7.0%*	0
Phagocata vitta	13	< *6.2%*	62
Plectronemia conspersa	156	> *5.9%*	0
Polycelis felina	50	> *5.6%*	0
Dicranata sp.	4	> *4.8%*	9
Chironomidae	49	> *4.6%*	29
Leuctra inermis	39	> *3.9%*	0
Hydropsyche siltalai	3	> *3.3%*	0
Rhyacophilia dorsalis	4	> *3.1%*	0

Group A = R2, B2, L1, S9, U3, D1 (% similarity = 54.5); Group B = U2, L4, S8, D5 (% similarity = 58.7)

Table 4.3. Results of pairwise tests from 1-way ANOSIM for fourth root transformed macrofaunal, meiofaunal, total macrofaunal and total metazoan data. Pairwise tests in which differences between sites that were not significant are listed; the fewer of these non- significant differences the better the discrimination between sites.

Macrofauna			Meiofauna			Total Macrofauna			Total Metazoa		
Sites	R	p	Sites	R	p	Sites	R	p	Sites	R	p
Autumn (global R = 0.616)			**Autumn (global R = 0.657)**			**Autumn (global R = 0.737)**			**Autumn (global R = 0.824)**		
L1,U3	0.180	0.10	U3,U2	-0.210	0.056	L4, S8	0.072	0.24			
L1,D1	-0.092	0.67	U2,L4	0.180	0.071						
U3,S9	0.032	0.36	U2,S8	0.024	0.421						
U3,D1	0.180	0.12									
U3,U2	-0.028	0.59									
S9,D1	0.272	0.10									
S9,U2	0.004	0.41									
Spring (global R = 0.604)			**Spring (global R = 0.516)**			**Spring (global R = 0.650)**			**Spring (global R = 0.719)**		
R2,B2	0.068	0.310	R2,B2	0.232	0.087	R2,B2	0.248	0.087	S9,U2	-0.152	0.857
S9,U2	0.056	0.286	R2,U3	0.152	0.087						
S9,L4	0.110	0.159	S9,U2	0.080	0.690						
L4,S8	0.192	0.111	S9,L4	0.094	0.230						
L4,D5	0.294	0.079	S9,S8	-0.020	0.508						
			S9,D5	0.180	0.079						
			U2,L4	0.028	0.310						
			U2,S8	-0.120	0.786						
			U2,D5	-0.016	0.286						
			L4,S8	0.052	0.325						
			L4,D5	0.196	0.079						
			S8,D5	0.048	0.466						

PCA ordinations of the trace metal data demonstrated three main site clusters (R2 and B2; L1, S9, D1 and U3; and S8 and D5) from left to right along axis one (PC1) (Fig. 4.2A). These clusters reflected increasing Cu, Zn and Al concentrations (Table 4.4). Sites L4 and U2 were separated from the other sites, on axis two, due to their high concentrations of Fe (Table 4.4).

Table 4.4 Eigen vectors for PC1 and PC2 for PCA ordination of metal data (excluding interstitial values). (Coefficients in the linear combination of variables making up PCs.)

	Eigen Vectors	
	PC1	**PC2**
% Variation Explained by PC	**55.0%**	**87.7%**
Cu	0.529	-0.490
Zn	0.569	0.332
Al	0.551	-0.292
Fe	0.306	0.752

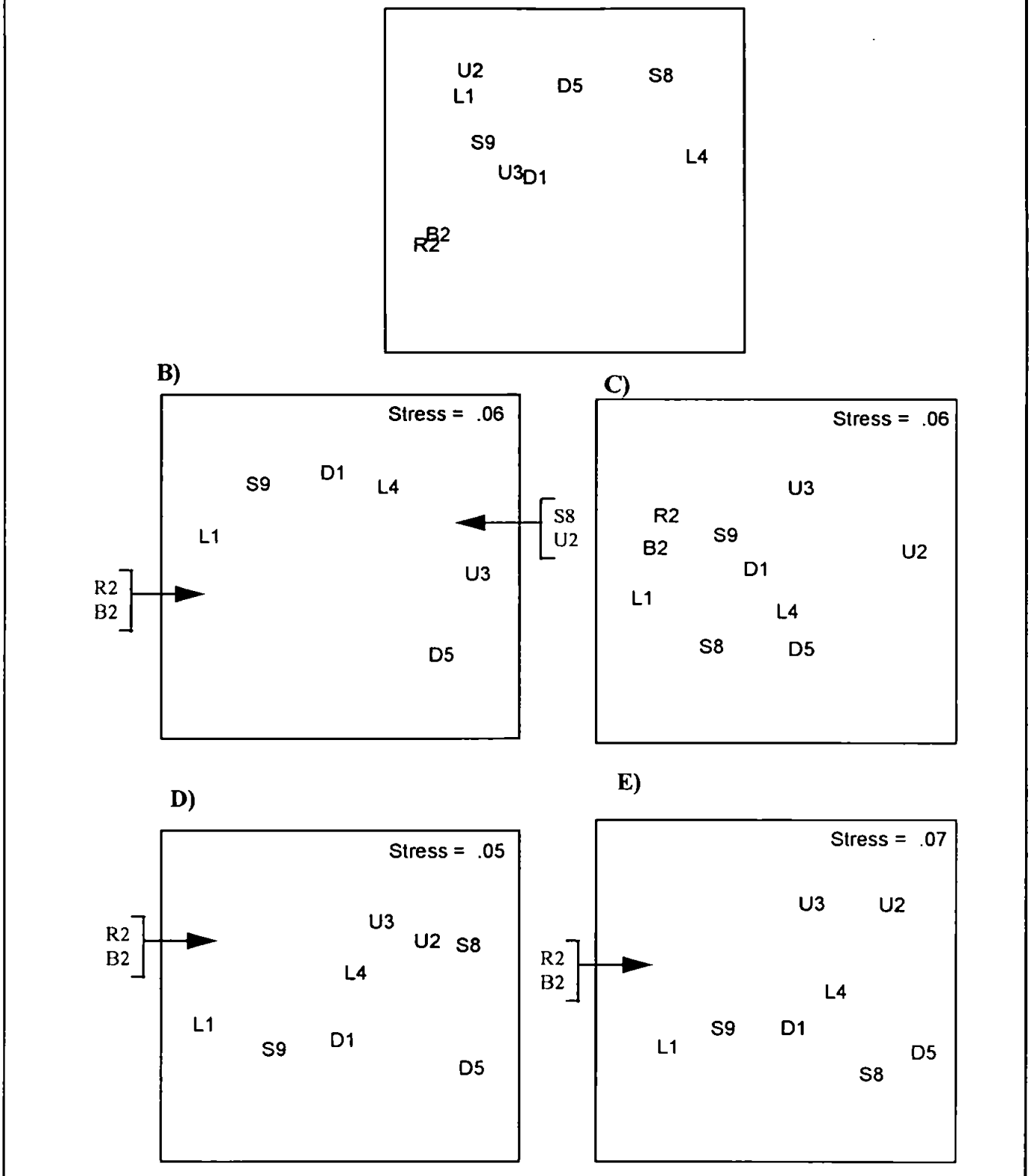


Figure 4.2 Ordination by **A)** PCA of trace metal variables, and by MDS of mean abundances of **B)** macrofaunal, **C)** meiofaunal, **D)** total macrofaunal and **E)** total metazoan data at ten sites on the tributaries of the rivers Lynher and Seaton in spring. Stress values indicate how well the 2-dimensional picture summarises the relationship between communities. Arrows indicate location of sites listed.

For spring and autumn, the MDS configurations of the different faunal components conformed very closely with the PCA configuration for trace metal data (Figs 4.2 & 4.3). At sites of low Cu concentrations (e.g. R2 and B2), communities were very different from those at sites of high Cu concentrations (S8 and D5) for all faunal subsets. This conformity was supported by correlations (Spearman rank) between similarity matrices for community data and the Euclidean distance matrix derived from the metal variables (Table 4.5). There were strong correlations between the trace metal data and similarity matrices for spring meiofaunal and spring total metazoan data ($p<0.01$) (Table 4.5). The similarity matrix for spring and autumn total macrofaunal and macrofaunal data, also correlated with the matrix for metal data ($p<0.05$). Other correlations between the faunal and metal data were close to being significant at $P<0.05$ (Table 4.5).

Table 4.5 Spearman rank correlation coefficients (ρ) between the Euclidean distance matrix derived from the metal variables and similarity matrices derived from the averaged transformed biotic data in spring and autumn.

	Spring		Autumn	
	ρ	p	ρ	p
Macrofauna	0.359	0.019	0.452	0.007
Meiofauna	0.484	0.004	0.305	0.058
Total Macrofauna	0.301	0.028	0.346	0.046
Total Metazoa	0.541	0.001	0.328	0.053

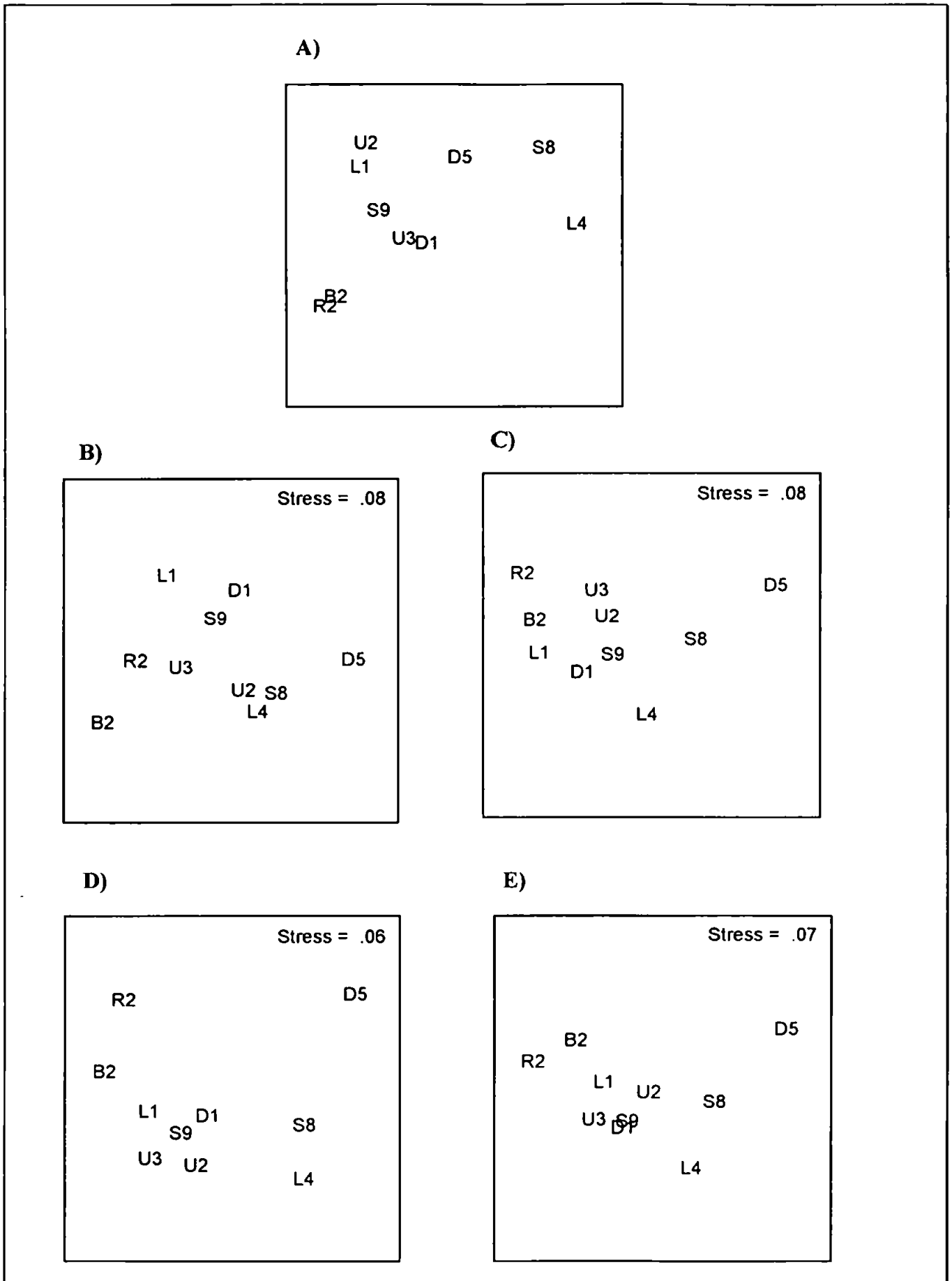


Figure 4.3 Ordination by **A)** PCA of trace metal variables, and by MDS of mean abundances of **B)** macrofaunal, **C)** meiofaunal, **D)** total macrofaunal and **E)** total metazoan data at ten sites on the tributaries of the rivers |Lynher and Seaton in autumn. Stress values indicate how well the 2-dimensional picture summarises the relationship between communities. Arrows indicate location of sites listed.

In both seasons, BIOENV demonstrated that Cu was the best single variable explaining inter-site differences for all the community subsets, except for the autumn macrofauna which correlated best with DOC concentration (Tables 4.6 and 4.7). In spring, the correlation with Cu was higher for macrofauna than meiofauna, whereas in autumn, the best correlation with Cu was with meiofauna rather than macrofauna. In each season, however, the best correlations with Cu were with the total metazoa in spring, and total macrofauna in autumn. For each community subset, the highest correlations involved Cu in combination with other variables. In autumn, the meiofaunal, total macrofaunal and total metazoan community structures correlated best with Cu and Al, while the macrofaunal data correlated best with a combination of the four variables, Cu, Zn, DOC and discharge. In spring, Cu and Zn, either together or in combination with other variables, were the most important variables explaining patterns in the community structure of all data sets.

Table 4.6 Summary of results from BIOENV. Combinations of variables giving the highest rank correlations between biotic and abiotic similarity matrices in spring. Faunal data fourth root transformed, abiotic variables log (1+N) transformed. Best correlations are shown in bold. Lower correlations omitted from the table.

Best Variable Combinations (Spring)		
Macrofauna		
1	Cu 0.453	
2	Cu, Zn 0.483	
3	Cu, Zn, Al 0.532	
4	**Cu, Zn,Al, DOC** **0.549**	
Meiofauna		
1	Cu 0.383	Zn 0.248
2	**Cu, Zn** **0.501**	Cu, Fe 0.475
3	Cu, Zn, Fe 0.486	Cu, Zn, DOC 0.445
4	Cu, Zn, Fe, DOC 0.472	
Total Macrofauna		
1	Cu 0.453	
3	Cu, Zn, DOC 0.532	Cu, Zn, Hardness 0.515
4	**Cu, Zn, Al,** **Conductivity** **0.549**	
Total Metazoa		
1	Cu 0.545	
2	Cu, Zn 0.588	
3	**Cu, Zn, DOC** **0.604**	Cu, Fe, DOC 0.567

Table 4.7 Summary of results from BIOENV. Combinations of variables giving the highest rank correlations between biotic and abiotic similarity matrices in autumn. Faunal data fourth root transformed, abiotic variables log (1+N) transformed. Best correlations are shown in bold. Lower correlations omitted from the table.

Best Variable Combinations (Autumn)		
Macrofauna		
1	DOC 0.532	Cu 0.512
2	Cu, DOC 0.515	
3	Cu, Al, DOC 0.527	Cu, DOC, Discharge 0.521
4	**Cu, Zn, DOC, Discharge** **0.565**	
Meiofauna		
1	Cu 0.592	
2	**Cu, Al** **0.653**	Cu, pH 0.575
3	Cu, Al, pH 0.653	
Total Macrofauna		
1	Cu 0.679	
2	**Cu, Al** **0.788**	
3	Cu, Al, Hardness 0.692	Cu, Al, pH 0.661
Total Metazoa		
1	Cu 0.656	
2	**Cu, Al** **0.730**	
3	Cu, Al, Hardness 0.697	Cu, Al, pH 0.648

4.3.3 Community analyses using different taxonomic levels

Macro- and meiofaunal data sets aggregated into groupings of genus, family and phyla gave a very similar separation of sites as those when species data were used (Figs 4.4 and 4.5). The similarity matrices were correlated significantly for all taxonomic levels apart for the meiofaunal phyla in autumn (Table 4.8). The separation of site U2 from other sites, based upon the spring meiofaunal community, was particularly marked at the phylum level, due to the fact that the Nematoda was the only phylum present at U2 in spring (Fig. 4.4).

Table 4.8 Pairwise Spearman's Rank Correlation coefficients between similarity matrices derived from average transformed meiofaunal and macrofaunal species abundance data aggregated to a range of taxonomic levels. * $p < 0.001$, by a permutation test.

Autumn Meiofauna	Species	Genus	Family
Genus	0.835*		
Family	0.526*	0.687*	
Phylum	0.254	0.254	0.713*

Spring Meiofauna	Species	Genus	Family
Genus	0.909*		
Family	0.884*	0.933*	
Phylum	0.799*	0.834*	0.879*

Autumn Macrofauna	Species	Genus	Family
Genus	0.990*		
Family	0.895*	0.883*	
Phylum	0.656*	0.623*	0.786*

Spring Macrofauna	Species	Genus	Family
Genus	1.0*		
Family	0.995*	0.994*	
Phylum	0.725*	0.723*	0.730*

As faunal data were aggregated to higher taxonomic levels, the correlations between the matrix for the trace metal data, and the matrices for autumn and spring macrofauna and autumn meiofauna decreased (Table 4.9). Despite this decrease, the spring macrofaunal data still correlated with the metal data to the family level, and the autumn macrofaunal data correlated with the metal data to the generic level. The high correlation between spring meiofauna and the metal data also decreased when aggregated to the family level

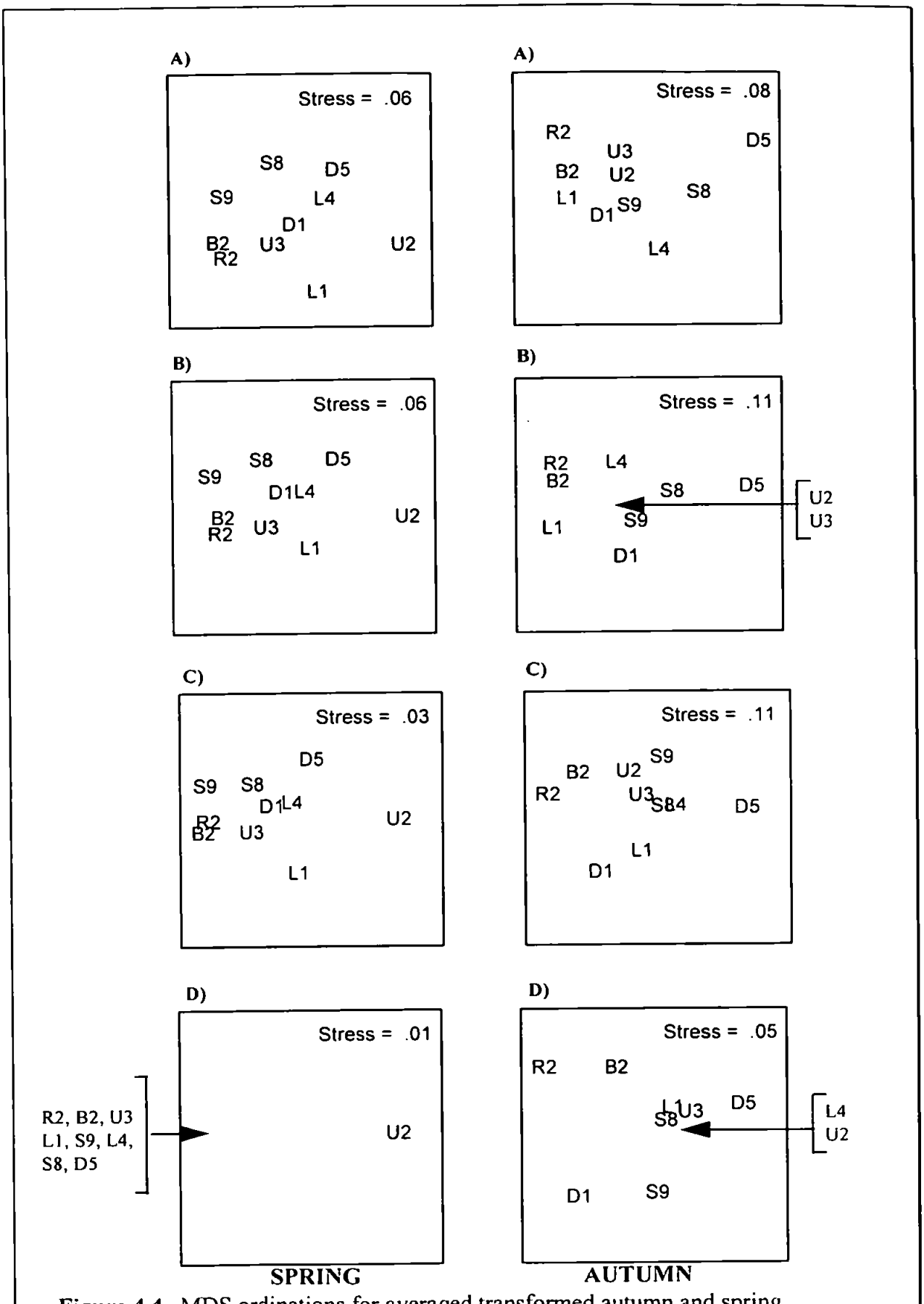


Figure 4.4. MDS ordinations for averaged transformed autumn and spring meiofauna aggregated to **A)** species, **B)** genera, **C)** families and **D)** phyla. Stress value indicates how well the 2-dimensional picture summarises the relationship between site communties. Arrows indicate locations of sites listed.

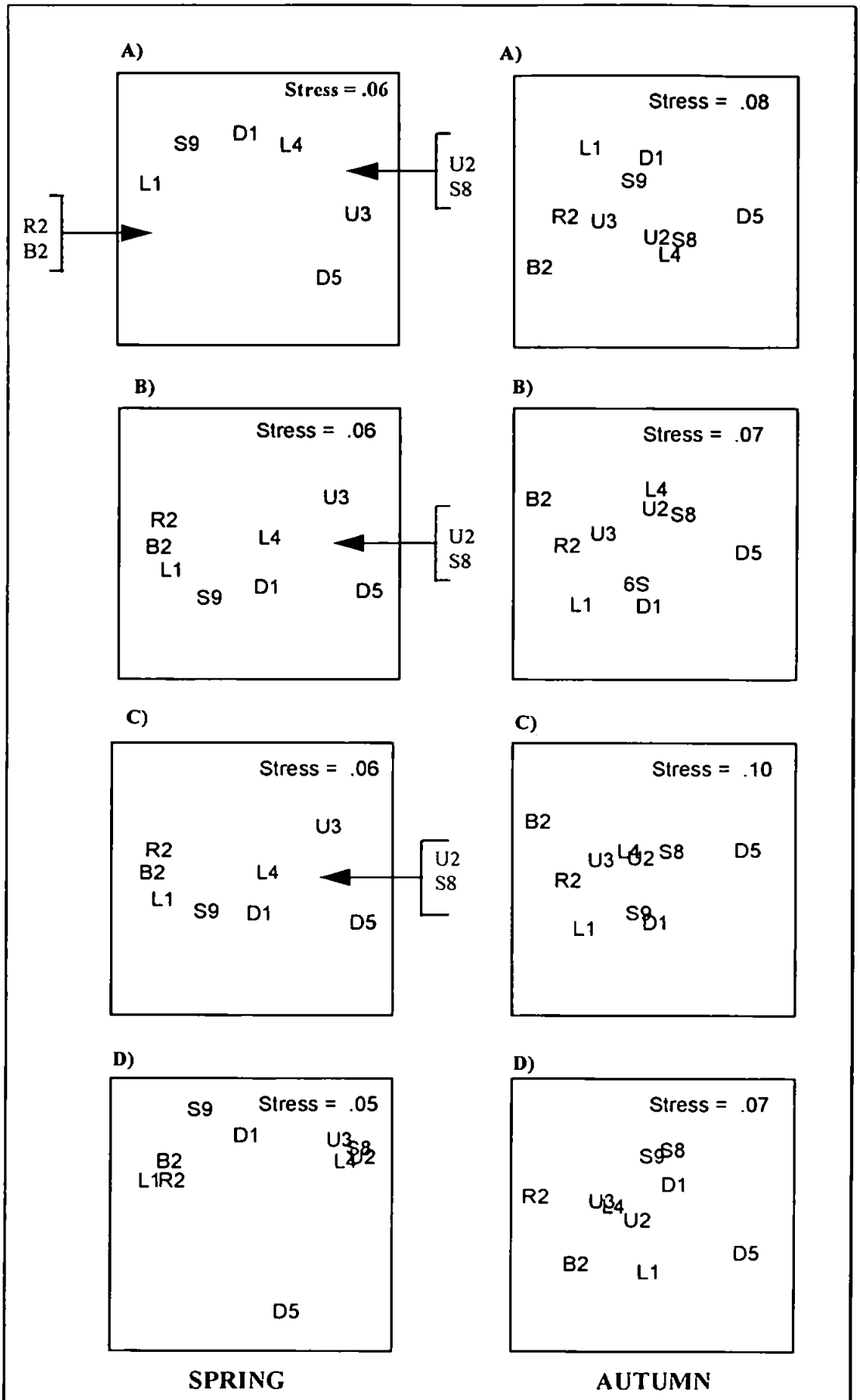


Figure 4.5 MDS ordinations for averaged transformed autumn and spring macrofaunal data aggregated to **A)** species, **B)** genera, **C)** family, **D)** phyla. Stress values indicate how well the 2-dimensional picture summarises the relationship between site communities. Arrows indicate location of sites listed.

(Table 4.9). The highest correlation between the matrices for spring meiofauna and metal data, however, was found at the phylum level, due to these two data sets both clearly separating U2 from the other sites. At the phylum level, the community at U2 was clearly separated from the others due to an absence of all meiofaunal groups, except nematodes, at the site in spring (Fig. 4.4). The trace metal data distinguished U2 from the other data sets due to high Zn and Fe levels at this site.

Table 4.9 Spearman rank correlation coefficients (ρ) between the Euclidean distance matrix derived from the metal variables and similarity matrices derived from spring and autumn transformed biotic data aggregated to a range of taxonomic levels.

	Autumn				Spring			
	Macrofauna		Meiofauna		Macrofauna		Meiofauna	
	ρ	p	ρ	p	ρ	p	ρ	p
Species	0.452	0.060	0.305	0.063	0.305	0.014	0.484	0.004
Genus	0.467	0.04	0.247	0.10	0.247	0.025	0.348	0.027
Family	0.235	0.123	0.250	0.108	0.250	0.02	0.338	0.04
Phylum	0.093	0.30	0.127	0.24	0.127	0.11	0.528	0.004

When the spring and autumn meio- and macrofaunal data were aggregated to higher taxonomic levels, more pairs of sites were not significantly different and values of the ANOSIM statistic (R) in the global tests for differences between sites decreased (Tables 4.10 & 4.11). Thus, data aggregated to higher taxa above the species levels discriminated fewer sites.

Table 4.10 Results of pairwise tests from one-way ANOSIM in which p>0.05 for spring and autumn meiofauna data aggregated to species, genera, family and phyla. Pairwise tests in which differences between sites that were not significant are listed; the fewer of these non-significant differences the better the discrimination between sites.

MEIOFAUNA

Autumn			Spring		
sites	R	p	sites	R	p
Species global R = 0.657			*Species (global R = 0.582)*		
L1, U3	0.180	0.103	R2, B2	0.068	0.310
L1, D1	-0.092	0.667	S9, U2	0.072	0.286
U3, S9	0.032	0.365	S9, L4	0.082	0.206
U3, D1	0.180	0.119	L4, S8	0.236	0.087
U3, U2	-0.028	0.587	L4, D5	0.304	0.071
S9, D1	0.272	0.095			
S9, U2	0.004	0.413	*Genus (global R = 0.518)*		
			R2, B2	-0.008	0.437
Genus (global R =0.604)			B2, U3	0.188	0.103
R2, B2	0.300	0.071	S9, U2	0.000	0.524
L1, S9	0.280	0.071	S9, L4	0.086	0.175
L1, D1	0.000	0.429	D1, S8	0.248	0.079
U3, S9	0.016	0.476	U2, L4	0.324	0.071
U3, U2	0.072	0.246	L4, S8	0.192	0.111
U3, L4	0.200	0.095	L4, D5	0.332	0.063
S9, U2	0.040	0.341	R2, B2	-0.172	0.889
Family (global R = 0.565)			*Family (global R = 0.527)*		
L1, U3	0.208	0.079	R2, U3	0.228	0.063
L1, U2	0.184	0.087	B2, U3	0.212	0.111
U3, S9	0.072	0.246	S9, U2	0.000	0.524
U3, U2	0.068	0.230	S9, L4	0.086	0.175
U3, L4	0.252	0.103	D1, S8	0.248	0.063
S9, U2	-0.008	0.508	U2, L4	0.324	0.071
S9, L4	0.240	0.119	L4, S8	0.332	0.063
U2, L4	0.136	0.111			
L4, S8	0.236	0.111			
Phylum (global R = 0.505)			Phylum (global R = 0.434)		
L1, U3	0.348	0.071	R2, B2	-0.032	0.532
L1, L4	0.428	0.056	R2, U3	0.060	0.310
U3, S9	-0.154	0.889	B2, U3	-0.052	0.587
U3, U2	-0.104	0.905	S9, U2	0.000	0.524
U3, L4	-0.116	0.730	S9, L4	-0.050	0.571
U3, S8	0.304	0.071	D1, S8	0.272	0.071
S9, U2	-0.068	0.675	U2, L4	0.252	0.071
S9, L4	-0.192	0.952	L4, D5	0.218	0.095
S9, D5	0.336	0.063			
U2, L4	-0.072	0.722			
U2, D5	0.156	0.143			
L4, D5	0.336	0.071			

Table 4.11 Results of pairwise tests from one-way ANOSIM in which p>0.05 for spring and autumn macrofauna data aggregated to species, genera, family and phyla. Pairwise tests in which differences between sites that were not significant are listed; the fewer of these non-significant differences the better the discrimination between sites.

MEIOFAUNA

Autumn			Spring		
sites	R	p	sites	R	p
Species global R = 0.657			*Species (global R = 0.521)*		
U3, U2	0.210	0.056	L1, U3	0.130	0.111
U2, L4	0.180	0.071	L1, S8	0.509	0.095
U2, S8	0.024	0.421	D1, U2	0.308	0.063
			D1, S8	0.455	0.095
Genus (global R =0.653)			U2, L4	0.308	0.079
U3, U2	0.210	0.056	U2, S8	-0.400	1
U2, L4	0.180	0.071	L4, S8	0.245	0.190
U2, S8	0.024	0.421			
			Genus (global R = 0.316)		
			R2, B2	0.238	0.071
Family (global R = 0.645)			R2, U3	0.136	0.143
U3, U2	0.170	0.151	R2, L4	0.172	0.095
U3, L4	0.122	0.190	S9, U2	-0.092	0.738
U2, L4	0.172	0.151	S9, L4	0.048	0.317
U2, S8	0.046	0.333	S9, S8	-0.052	0.508
			S9, D5	0.128	0.111
Phylum (global R = 0.557)			D1, U2	0.244	0.071
L1, D1	0.348	0.071	U2, L4	-0.062	0.667
L1, U2	0.292	0.103			
U3, D1	0.004	0.373			
U3, U2	-0.018	0.444	*Family (global R = 0.321)*		
U3, L4	0.172	0.103	R2, B2	0.238	0.071
S9, U2	0.204	0.103	R2, U3	0.136	0.143
D1, U2	-0.064	0.063	R2, L4	0.172	0.095
D1, S8	0.296	0.635	S9, U2	-0.092	0.738
U2, S8	0.214	0.056	S9, L4	0.048	0.317
			S9, S8	-0.052	0.508
			S9, D5	0.128	0.111
			D1, U2	0.244	0.071
			U2, L4	-0.062	0.667
			U2, S8	-0.094	0.714
			Phylum (global R = 0.353)		
			R2, B2	0.204	0.079
			R2, S9	0.252	0.095
			R2, D1	0.228	0.087
			R2, L4	-0.004	0.437
			B2, U3	0.108	0.143
			L1, D1	-0.080	0.690
			S9, U2	-0.104	0.786
			S9, L4	-0.042	0.595
			S9, S8	-0.076	0.730
			S9, D5	0.176	0.111

4.3.4 Univariate measures

The overall power of univariate diversities to distinguish between macrofaunal communities at sites of different metal concentrations was lower than that identified using multivariate measures. For the autumn macrofauna, eveness measures did not vary significantly between sites ($F= 2.04$, $p<0.078$) (Table 4.12). Even though the spring and autumn meiofauna, and spring macrofauna, eveness measures showed significant variation between sites, there was no clear relationship with copper concentration (Fig. 4.6). For all data sets, variation in Shannon-Weiner diversity was highly significant across sites (Table 4.12). Both spring and autumn macrofaunal diversities were lower at sites of high Cu concentrations compared with sites of low Cu concentrations (Fig. 4.7). Highest diversities of autumn meiofauna were found at sites of intermediate contamination (Fig. 4.7). Although the spring meiofaunal diversities tended to be lower at sites of high Cu concentration, there was no clear trend.

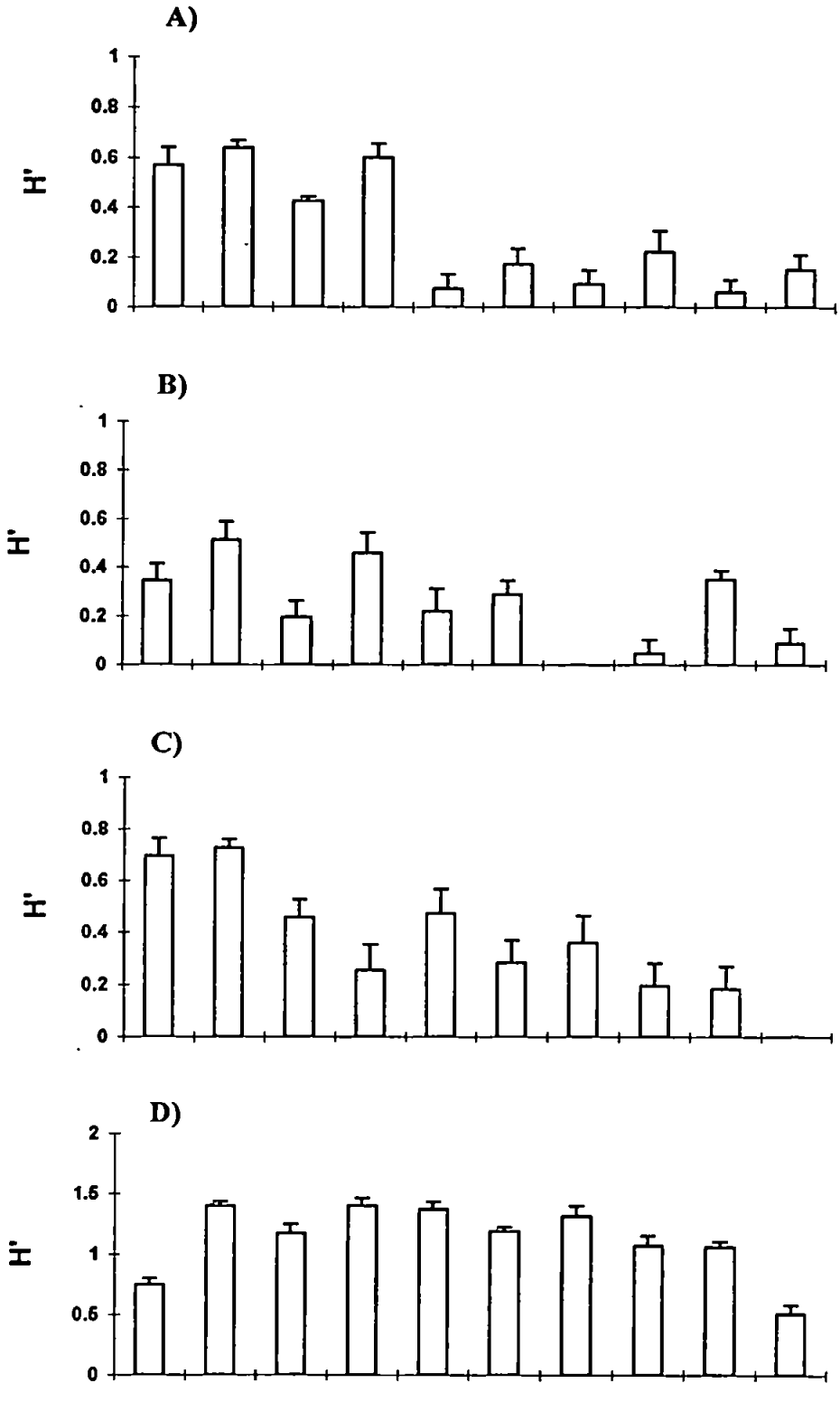


Figure 4.6 Shannon-Weiner diversity for **A)** spring macrofauna; **B)** spring meiofauna, **C)** autumn macrofauna and **D)** autumn meiofauna from the Rivers Lynher and Seaton (n=5 for each site; vertical bars = +1SD). See Figure 2.2 for the location of sites. Sites are arranged in order of increasing Cu concentration.

Table 4.12 F-ratios and significant levels for one-way ANOVA tests for differences in Shannon-Weiner diversity H' and eveness, J. The F-ratio is the ratio of variability in the site means to the variability among replicates within each site. The number of pairs of sites found to be not significantly different out of 100 possible pairs (insignificant pairs) are also shown.

Univariate measures	Autumn macrofauna			Spring macrofauna			Autumn meiofauna			Spring meiofauna		
	F	p	Insignificant pairs	F	p	Insignificant pairs	F	p	Insignificant pairs	F	p	Insignificant pairs
H'	7.06	< 0.01	23	13.09	< 0.01	19	4.91	< 0.01	26	6.42	< 0.01	26
J	2.04	0.078	32	2.55	0.0374	37	9.31	< 0.01	25	2.94	0.0178	28

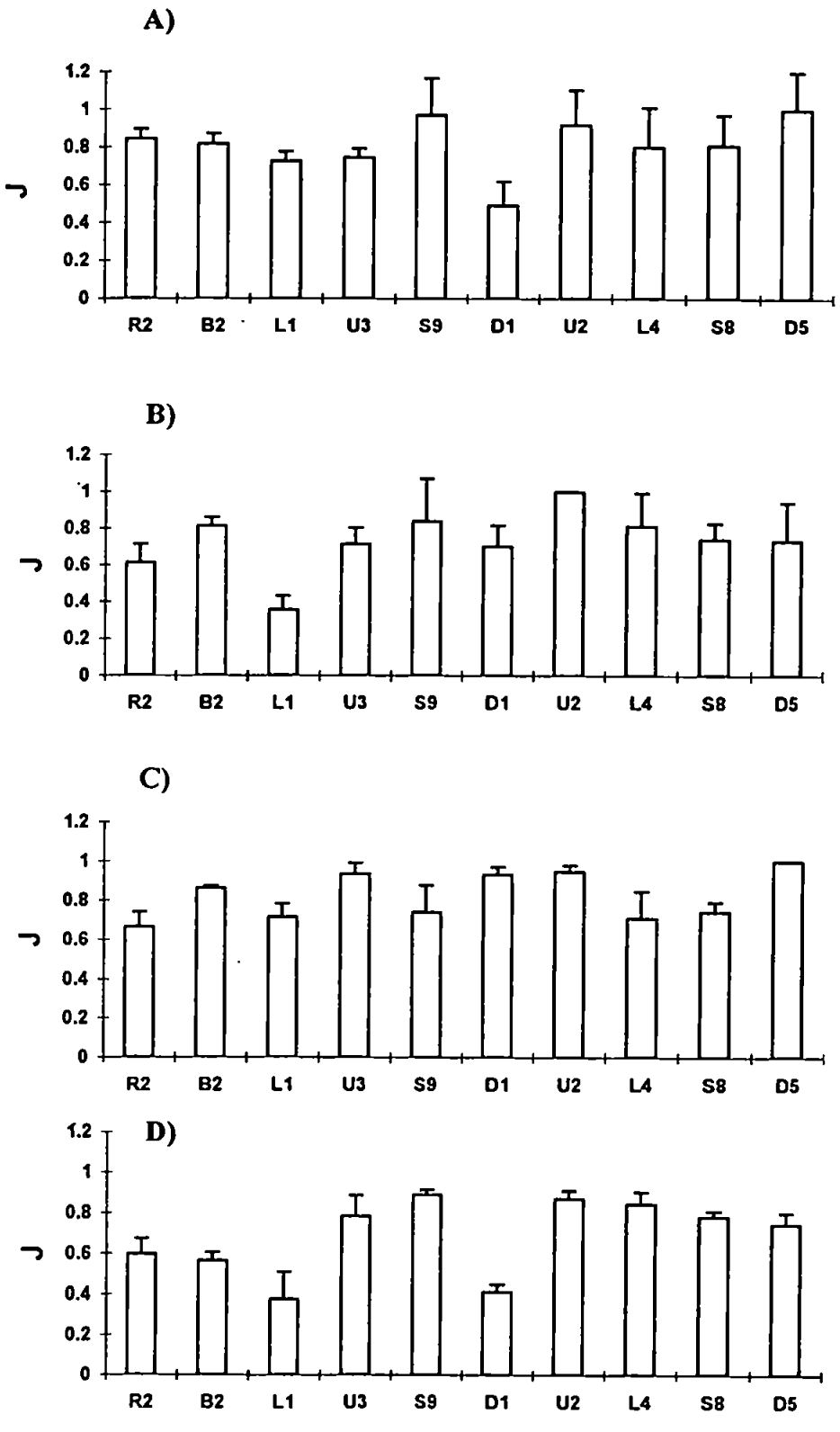


Figure 4.7 Eveness (Pielou's J) for **A)** spring macrofauna; **B)** spring meiofauna, **C)** autumn macrofauna and **D)** autumn meiofauna from the Rivers Lynher and Seaton (n=5 for each site; vertical bars = +1SD). See Figure 2.2. for the location of sites. Sites are arranged in order of increasing Cu concentration.

4.3.5 Index of Multivariate Dispersion

The values of dispersion for spring and autumn macrofauna and meiofauna did not indicate increased variability at sites of high Cu concentrations compared with sites of low Cu concentrations (Table 4.13). Thus, the variability, in terms of dispersion, is not consistent with the metal gradient.

Table 4.13 Relative Index of Multivariate Dispersion for ten sites on the tributaries of the Rivers Lynher and Seaton. Sites are arranged in order of Cu concentrations.

	Spring		Autumn	
Sites	Meiofauna	Macrofauna	Meiofauna	Macrofauna
R2	1.07	1.31	0.30	0.64
B2	0.99	1.02	0.50	0.31
L1	0.87	0.80	1.43	0.88
U3	1.02	0.77	1.67	1.64
S9	1.31	0.95	1.26	0.98
D1	0.45	0.86	1.18	1.07
U2	0.72	1.21	1.28	1.43
L4	1.33	1.48	1.17	1.28
S8	1.30	0.79	0.48	1.03
D5	0.93	0.81	0.74	0.74

4.4 Discussion

4.4.1 Comparison of meio- and macrofaunal community patterns

This chapter has demonstrated significant correlations between meiofaunal and macrofaunal community structures across sites representing a gradient in trace metal concentrations. Both faunal components reflected inter-site differences in metal concentration in a similar way in both spring and autumn. Detailed analyses of the four subsets of the metazoan community (i.e. macrofauna, meiofauna, total macrofauna and total metazoans) confirmed that the metal gradient correlated strongly with community patterns across taxonomic groups. The similarity matrices underlying all faunal data sets correlated, weakly, with the PCA ordination for metal data, although the strongest correlations were found with the spring meiofauna and total metazoa. Copper, either alone or in combination with other environmental variables, also correlated with the community structures of all four subsets.

One major benefit of including the meiofauna in marine monitoring programmes has been to obtain a full understanding of the effects of contaminants on benthic communities, as the meiofaunal component does not necessarily respond in the same way as the macrofaunal component (Austen *et al.*, 1989; Somerfield *et al.*, 1995). The same may be true for freshwater systems. Although macrofauna and meiofauna reflected inter-site differences in metal concentrations in a similar way in the present study, distinctive meiofaunal communities were also found at U2 and L4 in spring and autumn respectively. The macrofaunal communities in these seasons did not separate out these two sites. The distinct autumn meiofaunal community at L4 was associated with high Al and low pH conditions at this site, whilst the absence of all meiofauna, except for nematodes in spring, highlighted the exceptionally high levels of Zn at U2 in this season (Chapter 3). The spring meiofaunal and the total metazoan community (the latter which included the meiofauna) also

correlated better with the trace metal data than the macrofaunal components. This was likely due to the PCA for metal data also separating U2 from other sites due to the high Fe at U2.

Despite the broadly similar response to metal contamination across the different components of the community, the present study revealed differences in the extent to which faunal subsets discriminated between sites of different metal concentrations (i.e. the number of site pairs shown by ANOSIM to have significantly different communities). In addition, there were differences in the correlation value between different faunal subset community patterns and Cu concentrations. Notably, improved discrimination between sites and highest correlations with Cu concentrations occurred when the temporary meiofauna was included with the macrofauna, or the entire benthic invertebrate community was used. The resulting higher diversity of animals at sites, when using the entire benthic invertebrate community, is likely to lead to better site separation (Newell *et al.*, 1990b). Temporary meiofauna represents a large proportion of macrofauna individuals (Appendix II), so the improved resolution caused by their inclusion may be due entirely to higher abundances leading to a clearer trend. The improved discrimination may also be due to a higher sensitivity amongst smaller instars. Other studies suggest this may be the case. For example, the 96 h LC_{50} for *Chironomus tentans* larvae to Cu was between 12 and 27 times higher for first instar compared with the fourth instar larvae (Gauss *et al.*, 1985). An increased effect of Cu on the recruitment of insect populations to a perennial stream in California was demonstrated by Leland *et al.* (1989), when the dosing coincided with a major hatch, or at a time when there were normally high densities of early instars. Higher sensitivity of smaller instars could be due to larger surface:volume ratios, higher initial lipid content or a greater weight-specific metabolism, all of which could facilitate uptake of the contaminant (Powlesland & George, 1986).

In the autumn samples, the meiofauna discriminated between sites, and correlated with Cu concentrations, better than the macrofauna. The macrofaunal community pattern appeared to be influenced by variables other than metals (e.g. DOC and discharge) to a greater degree than the meiofauna. In spring, on the other hand, there was poorer discrimination of sites, and a lower correlation with Cu, by the meiofaunal community compared with the macrofaunal community. This difference was likely to be due to low meiofaunal abundances in spring, resulting in less defined inter-site differences in community structure. Rundle (1990) also recorded a distinction between meiofaunal community structure at acidic and neutral sites in all seasons except winter, when low meiofaunal abundances made the pattern unclear. Despite the fact that the meiofauna was not always the best faunal component in discriminating between sites, the inclusion of as many faunal components as possible appears to be needed to detect the more subtle effects of Cu on benthic communities.

4.4.2 Taxonomic levels

A major criticism of using communities for water quality assessment is that the processing and identification of samples can be very time consuming and, therefore, expensive, especially if species-level identification is required. This drawback is particularly relevant for the meiofauna, as their identification is often a laborious procedure that necessitates mounting the animals onto slides and considerable taxonomic expertise to discriminate to species. The present study demonstrated that sites were separated in a similar way when the macrofaunal and meiofaunal data were aggregated into species, genus, family and phylum. This was the case for all fauna except for the autumn meiofaunal data set which correlated to the family level but not to the phylum level. Rundle & Attrill (1995) also showed that the influence of pH on stream meiofaunal community structure could be detected after data

were aggregated to genus and family level. In the marine environment, the lower discriminatory power of meiofaunal data, compared with macrofaunal data, has also been reported, when data were aggregated to higher taxonomic levels (Warwick *et al.*, 1990; Somerfield & Clarke, 1995). Despite this, aggregations to family level would reduce the time and expertise required considerably, as the identification could be performed using a stereo microscope without the necessity of mounting specimens. Spring meiofaunal and macrofaunal and autumn macrofaunal data, also correlated with the PCA for metal data after aggregation to higher taxonomic levels. Furthermore, the highest correlation of the spring meiofaunal data with the metal data was found at the phyla level, with both the faunal and metal data sets clearly separating U2 from the other sites. Other studies have also shown the response of a pollution event to be even more 'clear-cut' at the higher taxonomic level than the species level (Dauvin, 1984; Warwick, 1988a, b). Warwick (1993) suggested that, as species are normally adapted to a narrow range of environmental variables, species replacements will occur from site to site due to changes in natural environmental variables. The proportions of higher taxa, however, may not be altered by natural environmental variables. Thus, if there is a degree of coherence among species within these higher taxa with respect to their response to perturbation, the response will be more evident above the natural environmental noise. In the present study there was an absence of all meiofauna, except Nematoda, at U2, apparently in response to the high Zn levels at this site.

Another criticism of community-based surveys is that they are only useful for detecting local pollution disturbance and are not applicable on a regional or global scale. This problem has been overcome by aggregating to a higher taxonomic level than species. For example, Rundle & Attrill (1995) examined the influence of pH on stream meiofauna in streams in southern England and Wales, and found that regional differences in community

structure at the species level tended to override the influence of pH. At the family level, however, there was a gradient of community separation from acidified sites in southern England to streams draining coniferous plantations in Wales to less acidic streams in both regions (Rundle & Attrill, 1995). Therefore, the smaller regional differences in community structure at family level appeared to lead to some convergence in the biological response of geographically isolated streams to pH. Similarly, the effects of trace metals on stream meiofaunal communities in the region used in the present study may be comparable with other regions only if aggregated to higher taxonomic levels.

Another problem when monitoring with communities is assessing the degree to which a community at a contaminated site has been disturbed in relation to other sites. Warwick & Clarke (1993) aggregated marine macrofaunal data to phylum level from several case studies involving various types of disturbance, to overcome species differences in geographically-separate communities. These authors demonstrated that this meta-analysis of marine macrobenthic data could be used to place marine benthic communities along a gradient of disturbance providing a template against which the extent of disturbance at other sites could be assessed. In a similar way, a template of the effects of metals on stream meiofaunal communities may be built up after further regional studies on the effects of metals on meiofauna. This would allow water managers to evaluate water quality by comparing the meiofaunal communities in their region with the template. The sites categorised as the most disturbed by Warwick & Clarke (1993), however, were influenced by organic pollutants and further studies are required to investigate whether disturbance due to elevated trace metal concentrations on meiofaunal communities would cause the same effect across regions.

Caution is required when using higher taxonomic levels to identify community responses to contamination. In the current study, the ability of the faunal data to discriminate between sites of different trace metal contamination decreased as the macrofaunal and meiofaunal abundances were aggregated to higher taxonomic levels. This is in agreement with a study comparing the ability of macrofaunal species data to ordinate and classify 268 unpolluted running water sites in Great Britain compared with family data (Furse *et al.*, 1984). Although there was a strong correlation between species and family level responses to the same environmental gradients (Furse *et al.*, 1984), species-level data gained more reliable categorisation of running waters by their physicochemistry (i.e. higher between-site variation). Species data sets contain a higher information content (i.e number of taxa) and have more precise environmental requirements (Furse *et al.*, 1984). Hence, higher taxonomic levels may not be sufficiently robust for detecting subtle differences in stream contamination levels.

4.4.3 Univariate measures and the Index of Multivariate Dispersion

There has been some debate in the literature as to the utility of univariate measures in detecting contamination in aquatic systems (Sheehan, 1984; Metcalfe, 1989; Cairns & Pratt, 1993). The assumption made is that more species occur at unimpacted than impacted ecosystems, and that the total number of individuals is distributed more evenly amongst the species in unimpacted than impacted systems (Cao *et al.*, 1994). In some cases, univariate measures have been used successfully to identify disturbed communities (lower diversity indicating high stress) (Sheehan, 1984; Norris & Georges, 1993). Conversely, diversity can increase at sites of higher contamination (Platt & Lambshead, 1985; Moore & Pearson, 1986). In the present study, Shannon-Weiner diversity indices indicated that macrofaunal diversity was lowest at sites of high metal concentrations, irrespective of season. Although lower numbers of meiofaunal species were found in spring, the highest diversities of

meiofauna were found at sites of intermediate metal concentration in autumn. This difference in the response of macrofaunal and meiofaunal diversities to disturbance was also shown by Somerfield *et al.* (1995) in their study of the effect of dredging disposal on the marine benthos. Lower diversities of copepods and macrofauna were found at sites impacted by dredging but copepod data did not reveal any clear pattern. Despite these differences in faunal patterns when using univariate measures, multivariate methods demonstrated that the pattern in copepod community structure correlated significantly with that shown by other components of the benthos (Somerfield *et al.*, 1995). Macrofaunal and meiofaunal community patterns, in the present study, also correlated despite the differing response of their faunal diversity to metal contamination.

Another criticism of univariate measures has been their apparent lack of sensitivity to detect slight or moderate pollution (Beckett, 1978; Pindar & Farr, 1987; Barton, 1992; Olsgard & Gray, 1995). The present study supports this criticism. The multivariate methods used here were more sensitive and more discriminatory in detecting changes in trace metal contamination than the univariate measures. This was not unexpected in view of the greater information included in multivariate analyses techniques. As with marine systems (Warwick & Clarke, 1991), multivariate measures may be a more powerful analytical tool for monitoring freshwater systems than univariate measures. Multivariate techniques provide comparable and sensitive results whether using the macrofauna or the meiofauna. Even so, an index, which places a value on the extent of the stress placed on the community, is still required. For both macrofaunal and meiofaunal communities, the Index of Multivariate Dispersion failed to distinguish sites of high Cu concentrations from sites of low Cu concentrations in this study. The variability of types of species and abundances amongst replicates did not increase at perturbed compared with unperturbed sites.

Conclusion

In summary, the correlation between meiofaunal community structure and trace metal contamination was similar to that of the macrofaunal community. Including the meiofauna and the temporary meiofauna in the analysis provided a better discrimination of sites of different metal concentrations and a better correlation with Cu concentrations. The meiofaunal community highlighted differences in the water chemistry that were not detected by the macrofaunal community. Aggregation of meiofaunal and macrofaunal data to higher taxonomic levels allowed the detection of the trace metal gradient. Thus, the ease of monitoring using meiofauna may be improved considerably by identifying to taxonomic levels higher than species, without loss of sensitivity. For meiofaunal and macrofaunal communities, univariate measures, and the index of multiple dispersion, were poor discriminators of sites of different metal concentrations. The implications regarding the potential of using the meiofaunal community as a monitoring tool in streams are discussed further in Chapter 6.

CHAPTER 5

Lethal and sub-lethal responses of *Bryocamptus zschokkei* to Cu

5.1 Introduction

Although monitoring pollutants using communities provides a fully integrated response of the benthos to pollutants (Chapter 1), this approach has been criticised because it is labour intensive and because pollution-induced changes at the community level are apparent only when the damage has already been done (Mackay *et al.*, 1989). Thus, regulators prefer to use single-species toxicity tests that can be undertaken quickly and which use the responses of the biota to predict the effects of chemicals on the receiving waters. Many thousands of chemicals are being developed and these require evaluating prior to their release into the freshwater environment, therefore, rapid, predictive, inexpensive tests are needed to assess the effects of these inputs. From a systems viewpoint, effects of a pollutant at the ecosystem level cannot be predicted easily from studies conducted on its component parts (Buikema & Voshell, 1992). Even so, single-species tests are likely to be the most popular method of predicting the effect of toxic substances on the ecosystem for the foreseeable future.

Most toxicity tests commonly employed to assess the health of aquatic systems use the survival of the test animal as the measured response (test endpoint) (Maltby & Calow, 1989). Although this response is quickly and easily identified in acute lethal toxicity tests, measuring mortality may not be the best approach, as many studies have shown that contaminants have sub-lethal effects (i.e. on physiology, reproduction, growth and behaviour) at concentrations lower than that which cause lethal effects (Chapter 1; Abel, 1996). Sub-lethal endpoints may, therefore, provide an early warning of deleterious contaminants. In particular, alterations to reproduction and development have been shown, in laboratory experiments, ultimately to alter population size [e.g. for the marine copepod *Tisbe furcata* (Bechmann, 1994) and the mysid *Mysidopsis bahia* (Gentile *et al.*, 1982)].

The choice of organism used in toxicity tests is also critical when attempting to extrapolate laboratory tests to the field (Cairns & Pratt, 1987). Traditionally, test organisms have been chosen primarily because of their availability and robustness in laboratory cultures, rather than their ecological relevance (Gray, 1989). For example, standardised test procedures, developed for use by regulatory agencies to predict the effects of effluents, have focused on tests that are quick and economical, using animals of known stock and acclimation history (Buikema & Voshell, 1992). This bias has limited the test species to mainly cladocerans (mostly *Daphnia* spp.) and fathead minnows (Buikema & Voshell, 1992). An alternative approach, suggested recently (Gray, 1989), is to select species known to be sensitive to specific pollution gradients, thereby, reflecting more accurately the response of the community to contaminants.

In the marine environment, the meiobenthos has aroused interest as suitable organisms for measuring contamination. Meiobenthic species have comparatively short life cycles, and require minimal space and equipment, allowing relative ease of laboratory culture (Chapter 1). Harpacticoid copepods have received particular attention as potential toxicity test organisms and, of the 68 toxicity tests using meiofauna (Coull & Chandler, 1992), 57% were conducted on this order of copepods. The development of laboratory culturing techniques has allowed the use of several harpacticoid species in chronic toxicity testing. Measured responses, evaluating the effects of toxicants on reproductive parameters in harpacticoids, have included brood size (Ustach, 1979), total number of offspring (Bengtsson & Bergstrom, 1987; Williams, 1997), naupliar survival (Dalla Venezia *et al.*, 1980), post-embryonic development (LeDean & Devineau, 1987; Williams, 1997), changes in population size (Hoppenheit & Sperling, 1977; Brand, 1986) and the use of life tables (Bechmann, 1994; Williams, 1997).

In the survey of stream meiofaunal communities across a gradient of metal contamination (Chapter 3), the abundance of the harpacticoid copepod *Bryocamptus zschokkei* correlated negatively with Cu concentrations. *Bryocamptus zschokkei* was consistently important in contributing to between site differences in community structure (Chapter 3). Thus, based on these field measurements, *B. zschokkei* was selected for further study as a potential sensitive toxicity test organism of Cu in freshwater systems.

Bryocamptus zschokkei has several attributes that make it a potentially good bioassay organism. It is abundant all year (Rundle, 1993), hence individuals are always present to initiate laboratory cultures. It is present in North America and Europe (Gurney, 1932; Dussart, 1967; O'Doherty, 1985; Fryer, 1993), allowing its use in a wide geographical area. It has a relatively short life cycle (O'Doherty, 1985), allowing sub-lethal measures (e.g. the number of offspring produced) to be assessed quickly. It has direct benthic development (eggs are carried in a brood sac), facilitating the study of all stages of its life cycle. Its benthic development also allows the possibility of using it as a sediment toxicity test organism.

Evidence that stream chemistry influences the abundance of *B. zschokkei* through changes in fecundity was established by Rundle (1993) who found more *B. zschokkei* bearing eggs at sites of high than low pH. *Bryocamptus zschokkei* may also play an important role in the detrital dynamics of head water streams. Previously, stream harpacticoids have been shown to be important consumers of bacteria (Perlmutter & Meyer, 1991). As such, they may influence substantially the quality of detritus available for stream macroinvertebrate consumption. The absence of *B. zschokkei* from streams may, therefore, have a significant effect on stream dynamics.

The aim of this chapter was to examine the potential of *B. zschokkei* as a toxicity test organism for detecting Cu effects on streams. The effects of Cu on the survival, development and fecundity of *B. zschokkei* were measured in the laboratory. The acute toxic effects of Cu on individuals from contaminated and reference (control) sites were also compared, to assess whether field populations develop tolerance to Cu.

5.2 Materials and methods

5.2.1 Collection of animals

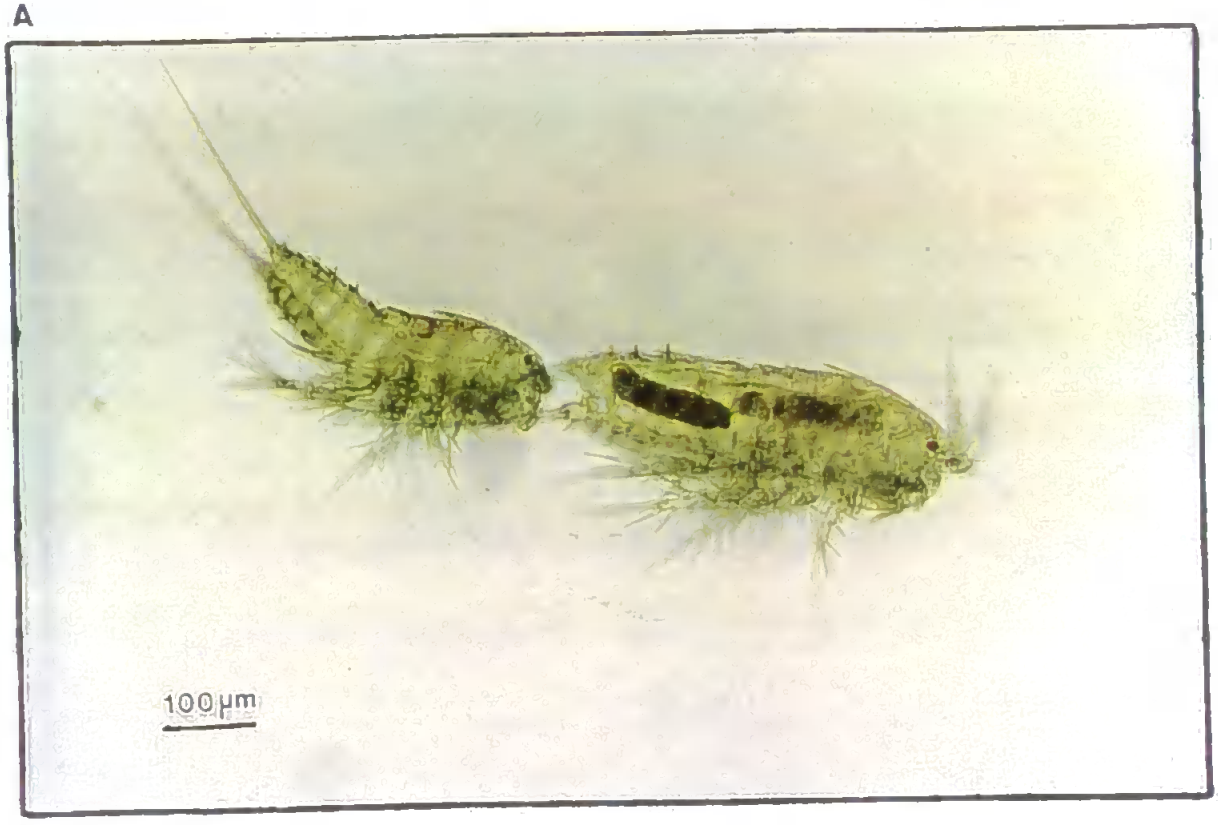
Copepods were obtained from kick samples collected at R2 (Fig. 2.2) in April 1996 (for sub-lethal toxicity tests) and in May 1996 (for acute toxicity tests). As the metal concentrations at site R2 were low (Tables 2.1 and 2.2), it was a suitable site for obtaining animals that had not been exposed previously to high metal concentrations. High abundances of *Bryocamptus zschokkei* could also be found throughout the year at R2 (Appendix II). Samples were returned to the laboratory within 2 h of collection, and passed through 500 μm and 125 μm mesh sieves. The fraction retained on a 125 μm sieve was elutriated three times to separate organic matter (i.e. detritus and copepods) from mineral particles. Ovigerous females, and copulating pairs of harpacticoids (Plate 5.1), were removed from the detritus in a petri dish under a stereo microscope (20x) using a Pasteur pipette. Individual *Bryocamptus zschokkei* were separated from the other harpacticoid species (*Bryocamptus praegeri*), under a magnification of 20x, using differences in the arrangement of the furcal setae; *B. praegeri* has divergent setae, whereas those of *B. zschokkei* are parallel (Plate 5.2).

5.2.2 Preparation of test solutions

Exposure solutions were prepared using stream water collected from R2 (control site) in April 1996, and filtered through 0.45 μm nitro-cellulose filter paper (Whatman Ltd.). This filtering process removed the protozoans, algae, fungi and other metazoans (Vijverberg, 1989). Test concentration solutions, made by adding the required volume of $CuNO_3$ (Spectrosol) to filtered stream water, were stored in propylene plastic bottles until use.

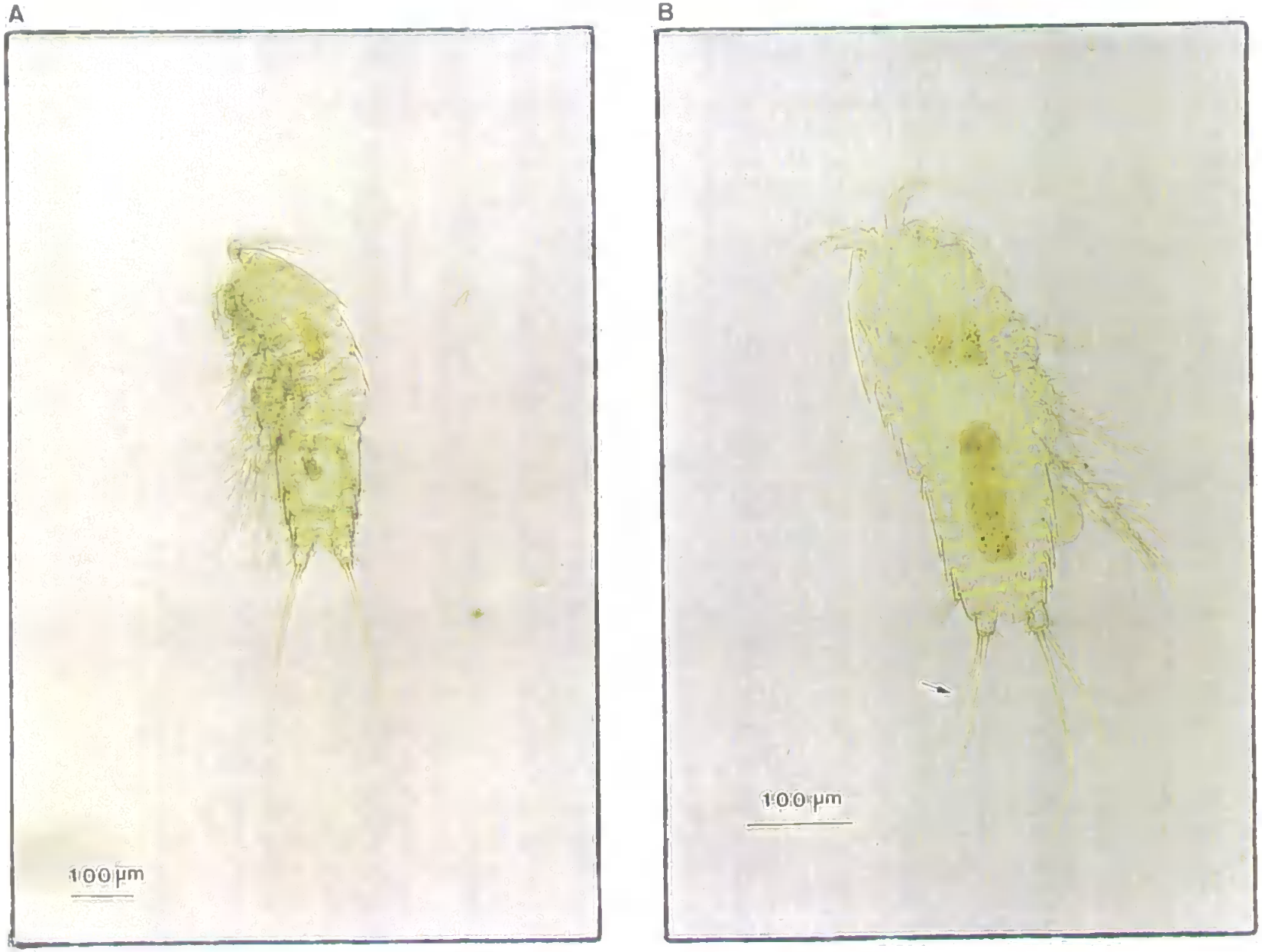
Plate 5.1 A) *Bryocamptus praegeri*, pair. The male holds on to the furcal rami of the female using modified antennules; **B)** Ovigerous female *Bryocamptus zschokkei*. (egg sac is highlighted by arrow).

A



B

Plate 5.2 A) Female *Bryocamptus zschokkeii*. **B)** Female *Bryocamptus praegeri*, (arrow indicates the divergent furcal setae of *B. praegeri*, a key feature that distinguishes this species from *B. zschokkei*).


A
100 µm
B
100 µm

5.2.3 Experimental procedures

5.2.3.1 Acute toxicity tests

Ovigerous females only were used in the acute toxicity tests as they were identified easily by the presence of a brood sac and provided a standard life-history starting point. Seven Cu test solutions were used (0, 56, 100, 180, 320, 560 and 1000 μg l^{-1} Cu), chosen as they represented the range of Cu concentrations measured at sites used in the survey of meiofaunal communities (Table 2.1). Thereby, the effects of Cu recorded in the laboratory could be related to the inter-site differences in the abundance of *B. zschokkei* observed in the field (Chapter 6).

Experimental test 'vessels' consisted of tissue culture wells (volume = 10 ml), each containing 5 ml of test solution and a single copepod. Each well was one of 12 in a multi-well, polystyrene tissue culture plate with a closely-fitting lid. Ten copepods were tested at each Cu concentration. Mortality, measured after 24, 48, 72 and 96 h, was defined as the absence of movement by the copepod after agitation of the test solution by stirring. As exposure concentrations were set in a geometric pattern, with the same number of individuals exposed to each concentration, LC_{50} values could be calculated at different time intervals by the moving average angle method (Stephan, 1977). This method is used commonly in toxicity tests (Williams, 1997). The conductivity, pH, and concentrations of DOC, Ca, Mg and Cu in combined replicates of each test solution were measured within 24 h of the trials (Appendix III).

5.2.3.2 Sub-lethal toxicity tests

Culture techniques for obtaining test organisms - Female *B. zschokkei* produce broods continuously, often from a single insemination, and the number of offspring produced varies depending on the age of the female (O'Doherty, 1986). To ensure that fecundity was assessed from brood one, and that females were of the same age and had identical mating histories, sub-lethal tests were performed on newly-mated copepod pairs. These animals were obtained in the following way. Firstly, mating pairs (or ovigerous females) were collected from site R2 and placed in groups of five in wells of a polystyrene tissue culture plate (Fig. 5.1a). The wells contained 5 ml of filtered stream water. Pairs were identified easily as the male harpacticoid holds onto the female with specially modified antennules (Plate 5.1). Previously, conditioned leaves (i.e. leaves obtained from streams) have been used successfully in laboratory feeding trials and growth experiments using harpacticoids (O'Doherty, 1985; Perlmutter & Meyer, 1991). Therefore, leaf discs (0.5 cm diameter) were cut from beech leaves using the end of an acid-washed glass test tube and used as a food source for the copepods in the present study. The beech leaves, collected from the stream bed at R2, were in a comparable state of decay. Trials with an alternative food (the alga *Chlorella sp.*) were also carried out, however, these were unsuccessful, and all animals aborted their broods within a week and died within two weeks. Experimental units were maintained in the dark at 15°C. After a few days, eggs began to hatch. Nauplii obtained from mating pairs were transferred randomly in groups of 5-10 to new cells containing 5 ml of control water (Fig. 5.1b). To prevent microbial build up, and to renew test solution concentrations, 3 ml of water was replaced every three days. After 20-30 days, development to the adult stage had occurred and mating pairs had formed (Fig. 5.1c).

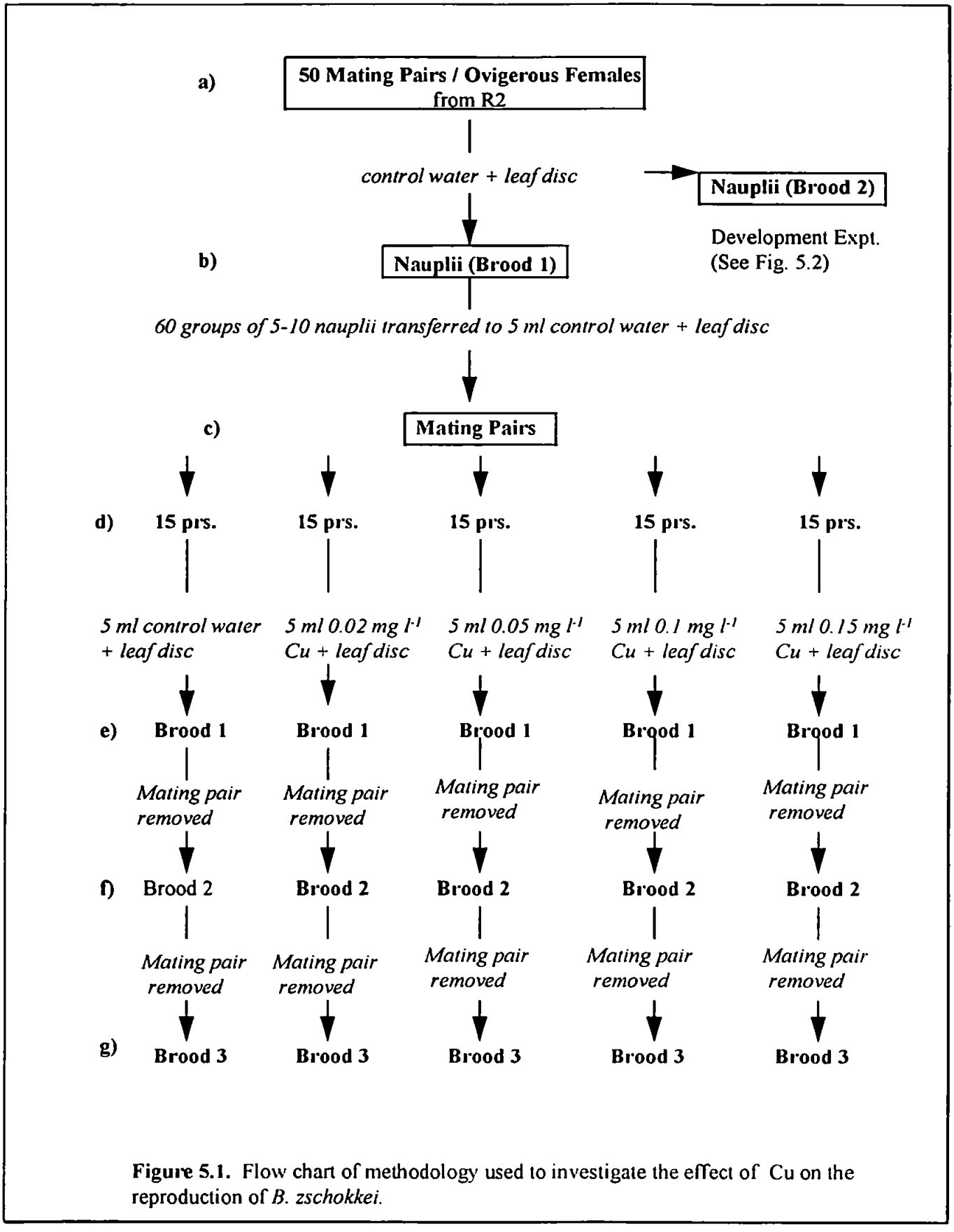


Figure 5.1. Flow chart of methodology used to investigate the effect of Cu on the reproduction of *B. zschokkei*.

Table 5.1 The concentration ($\mu g\ l^{-1}$) of Cu in the test solution used for the sub-lethal toxicity test (values are the concentrations of 15 combined replicates of 5 ml of test solution).

Prepared Cu concentration	Initial measured Cu concentration	Measured Cu concentration 7 days from preparation (leaf disc absent)	Measured Cu concentration 7 days from preparation (leaf disc present)
0	6.32	6.13	2.92
20	31.2	24.9	1.30
50	50.1	45.6	5.47
100	96.7	97.6	15.21

Fecundity - At the onset of mating, pairs were transferred to a cell (volume = 10 ml) containing a leaf disc and 5 ml of test solution. As Cu concentrations decreased if a leaf disc was present in the test solutions, presumably due to the leaf adsorbing Cu, leaf discs were placed in the test solution for 48 h before being placed in a cell with the animals. This procedure allowed the toxicant to come into equilibrium with the discs (Naylor *et al.*, 1989). Five concentrations of Cu (0, 20, 50, 100 and 150 $\mu g\ l^{-1}$) were used (Table 5.1; Fig. 5.1d). These concentrations represented concentrations at which *Bryocamptus zschokkei* was present in the field (Table 3.1). To prevent microbial build up in the water, and to renew test solution concentrations, 3 ml of the test solution were replaced every three days. Fifteen replicate pairs were exposed singly to each Cu concentration for sixty days. This exposure time was chosen to assess the effects of Cu on more than one brood of offspring within the time available for the experiment. Experiments were carried out in the dark at 15°C. Initially, animals were observed daily using a stereo microscope with fibre optic illumination and the time taken for brood one nauplii to hatch was recorded (Fig. 5.1e). Five days after the first nauplii hatched, the mating pairs were moved to a new well to ensure that broods were isolated. The times to the production of the second and third broods were

also recorded (Fig. 5.1f), with mating pairs again being removed once hatched. Offspring that reached the adult stage during the experiment were moved to new wells so that their offspring did not contribute to the total number of surviving offspring. Several measures of fecundity were obtained from these trials, including:

1) the total number of surviving offspring,

2) the total number of surviving nauplii, copepodids and reproductive adults (adults),

3) the number of surviving nauplii, copepodids and adults in each brood, and

4) the time taken for the production of each brood.

Development and survival of immature life stages - The effects of Cu on the survival and development of immature life stages were investigated using 24 h-old nauplii, and stage one copepodids. These were taken randomly from the second brood of the first generation animals (Fig. 5.2a). Groups of ten individual nauplii and copepodids were placed in cells (volume = 5 ml) containing 3 ml of test solution (0, 20, 50, 100 and 150 $\mu g\ l^{-1}$) and a leaf disc; the latter had been exposed previously to the test solution for 48 h (Fig. 5.2b). Copper concentrations were chosen to represent the Cu levels where *B. zschokkei* was present in the field. Two ml of the test solution was replaced every three days, again, to prevent microbial build up and to renew the test solution Cu concentrations. Initially, ten groups were to be exposed to each treatment, however, difficulties with obtaining enough animals from the second brood of the first generation, led to the number of groups per treatment ranging from six to nine. Observations were made daily, and the percentage survival and time taken to develop to the first copepodid stage (for nauplii) and reproductive adult (for

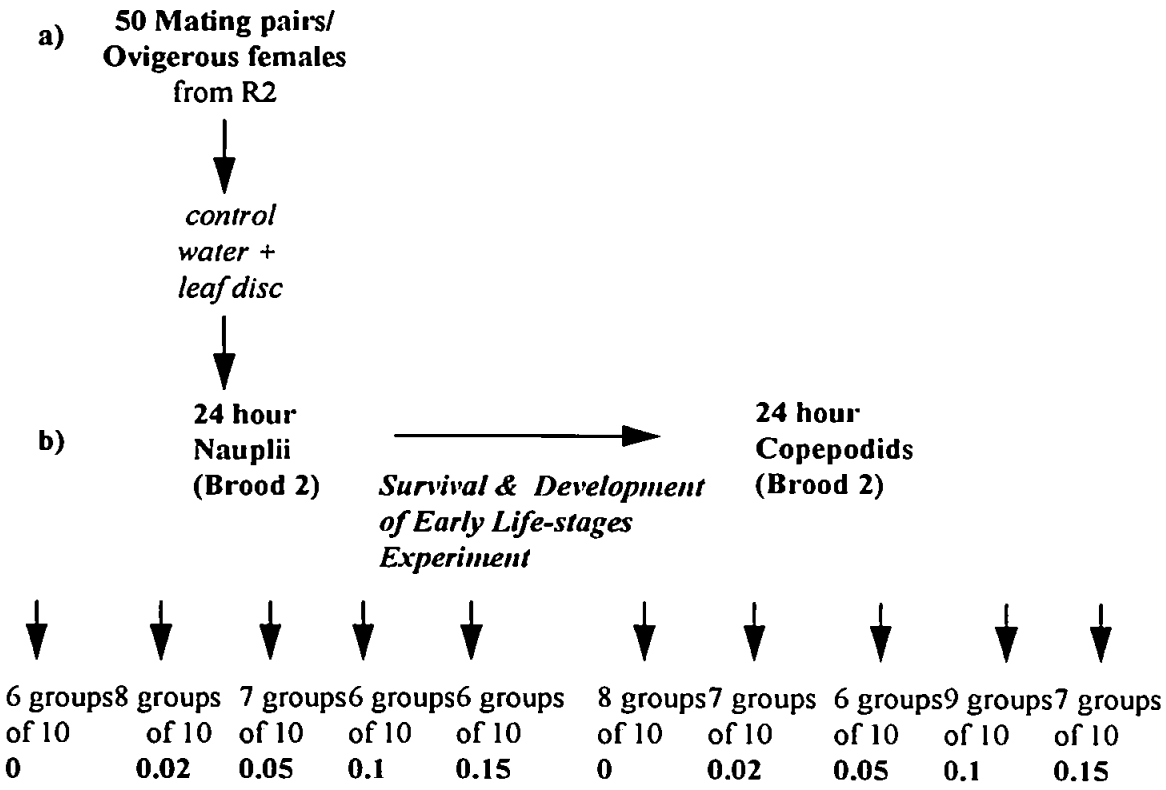


Figure 5.2 Flow chart of methodology used to investigate the effect of Cu on the survival and development of early life stages of *Bryocamptus zschokkei*.

copepodids) were recorded. Developmental time was taken as the time for 50% of surviving individuals to progress to the next life stage.

5.2.3.3 Tolerance

To investigate whether the Cu tolerance of animals collected from the metal-contaminated site (U2) was higher than that for individuals taken from uncontaminated sites, LC_{50} values for animals from site U2 were calculated using the method described in Section 5.2.3.1. LC_{50} tests were also performed on animals taken from a site on the River Yealm (OS 61° 5′ N 60° 8′ W), where there was no mining, or any other obvious source of contamination and the surface water Cu concentration measured 16 μg l^{-1} Cu (summer). This concentration was comparable to that recorded at R2 (13 μg l^{-1} Cu). Therefore, the River Yealm site provided a measure of variability of the toxic effect of Cu on two populations of copepods taken from different sites of similar water chemistry (see Appendix III for measurements of pH, conductivity, hardness and DOC taken at the River Yealm, U2 and R2).

5.2.4 Statistical analyses

The effects of Cu on the development times, and the number of surviving *Bryocamptus zschokkei* offspring, were determined using one-way ANOVA. The test was followed by a multiple range test (Fisher's least significant different process) to test for significant differences between pairs of treatments. Data that failed to meet the assumptions for analysis of variance (normal distribution and equal variances) were analysed using the non-parametric Kruskal-Wallis technique, followed by the Mann-Whitney test to identify significant differences between treatment pairs (Daniel, 1990). The data for percentage survival of different life stages were transformed using arc sine square root transformation prior to analysis, so that the data conformed to the assumption of homogeneity of variances.

5.3 Results

5.3.1 Acute toxicity test

Acute toxicity tests demonstrated that Cu had a direct toxic effect on the survival of ovigerous female *Bryocamptus zschokkei* collected at R2 (Fig. 5.3). At low Cu concentrations (up to 180 μg l^{-1} Cu), more than 70% of the copepods survived after 96 h of exposure. At Cu concentrations higher than 180 μg l^{-1} Cu, harpacticoid mortality increased with length of exposure until 72 h (Table 5.2, Fig. 5.3). The 72 h and 96 h LC_{50} value for *B. zschokkei* collected at the site were identical (290 μg l^{-1} Cu) (Table 5.2).

Table 5.2 LC_{50} values for Cu for ovigerous female *Bryocamptus zschokkei* taken from sites R2, U2 and the River Yealm. LC_{50} values were calculated using the moving average angle method (Stephan, 1977).

Site	Time (h)	LC_{50} for Cu in μg l^{-1} (95% confidence intervals)
R2	24	700 (570-940)
	48	400 (290-600)
	72	290 (210-410)
	96	290 (210-410)
U2	24	800 (700-920)
	48	680 (570-840)
	72	680 (570-840)
	96	660 (540-870)
Yealm	24	511 (411-667)
	48	448 (358-569)
	72	424 (336-534)
	96	313 (236-413)

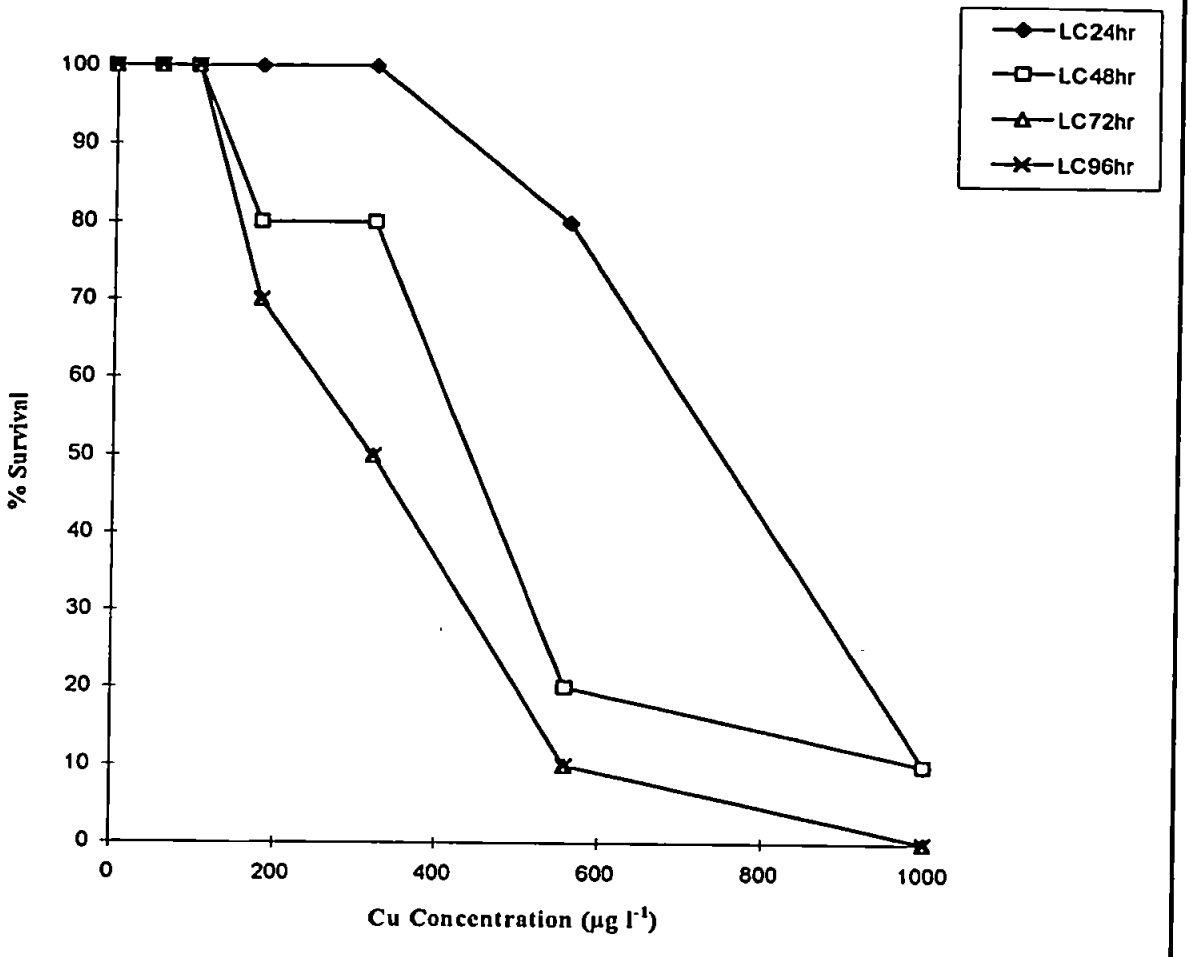


Figure 5.3 Toxicity curves for ovigerous female *Bryocamptus zschokkei* exposed to various Cu concentrations. Animals collected from site R2 (see Fig. 2.2 for site locations).

5.3.2 Sub-lethal toxicity tests

5.3.2.1 Effects on fecundity

Although some females produced egg sacs at 150 μg l^{-1} Cu, no eggs hatched at this concentration (Fig. 5.4), due to the females dying within 11 days of exposure to Cu. Some females in other treatments failed to produce any broods in other treatments (Fig. 5.4). As there was no clear difference between 0 and 100 μg l^{-1} Cu treatments for the percentage of females failing to produce broods, these females were excluded from further analyses. This reduced the variability in the number of surviving offspring within a treatment, and allowed differences between treatments to be viewed more clearly. There was no clear relationship between Cu concentration and the total number of broods produced per female for 0 and 100 μg l^{-1} Cu treatments, although the percentage of females producing a third brood decreased between Cu concentrations of 0 and 50 μg l^{-1} Cu (Fig. 5.4).

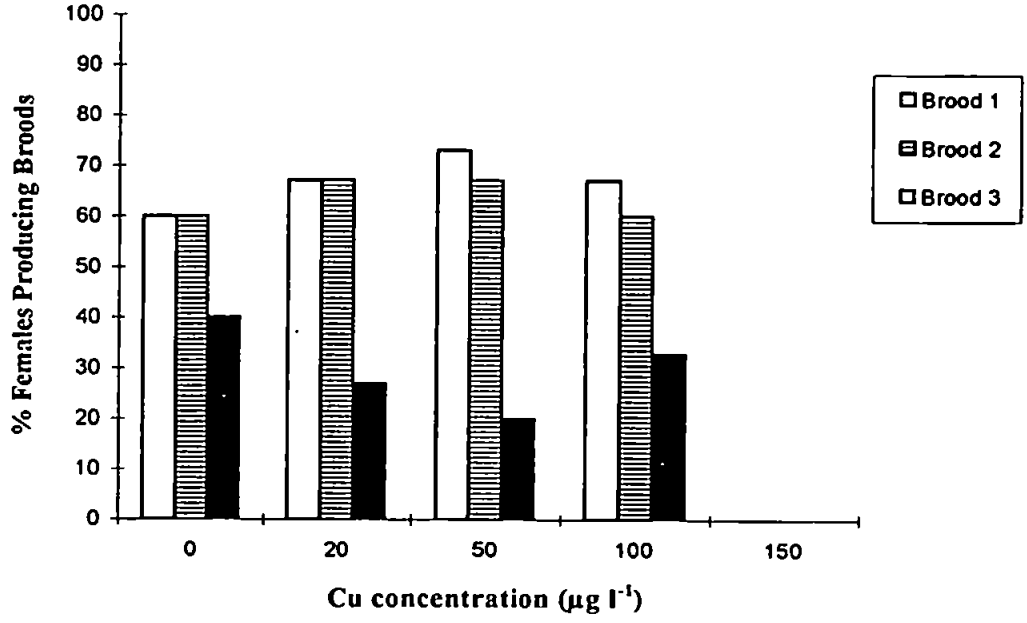


Figure 5.4 Brood Production: percentage females producing one, two and three broods at different Cu concentrations.

The fecundity of *B. zschokkei* was affected significantly at the high Cu concentrations. Significantly lower total numbers of surviving offspring were produced at 100 μg l^{-1} Cu compared with the control (Fisher's least significant different process, $p<0.05$) (Fig. 5.5). There were also significant differences in the numbers of all three life stages (i.e. adults, copepodids and nauplii) at the end of the experiment. The mean number of offspring produced, in each case, at 100 μg l^{-1} Cu was significantly lower than the number produced by females in the control (Fig. 5.5). Fewer offspring (though not significantly so) were produced by females exposed to 20 and 50 μg l^{-1} Cu compared with the control. In each case, this reduced total number of offspring was due to the reduced numbers within broods, rather than to the number of broods produced as there was no significant difference between the mean number of broods produced per female amongst treatments after 60 days (Fig. 5.6A).

To assess whether there was a temporal component to the effect of Cu on fecundity, the offspring data for each brood were analysed separately. For all life stages present in brood two and three, there were fewer surviving offspring in the Cu solutions compared to the control, although the only significant differences were between the treatments at 100 μg l^{-1} Cu and the control (Fig. 5.6). For brood two, the number of surviving copepodids and total offspring was significantly reduced at 100 μg l^{-1} Cu compared with the control (Fisher's least significant different process, $p<0.05$). For brood three, significantly fewer nauplii were recorded at 100 μg l^{-1} Cu compared to the control (Fisher's least significant different process, $p<0.05$) (Fig. 5.6). A significantly lower number of adult offspring was also found at 100 μg l^{-1} Cu compared to the control in brood one (Fisher's least significant different process, $p<0.05$) (Fig. 5.6). There was, however, no apparent relationship with Cu for the number of surviving copepodids in brood one (Fig. 5.6).

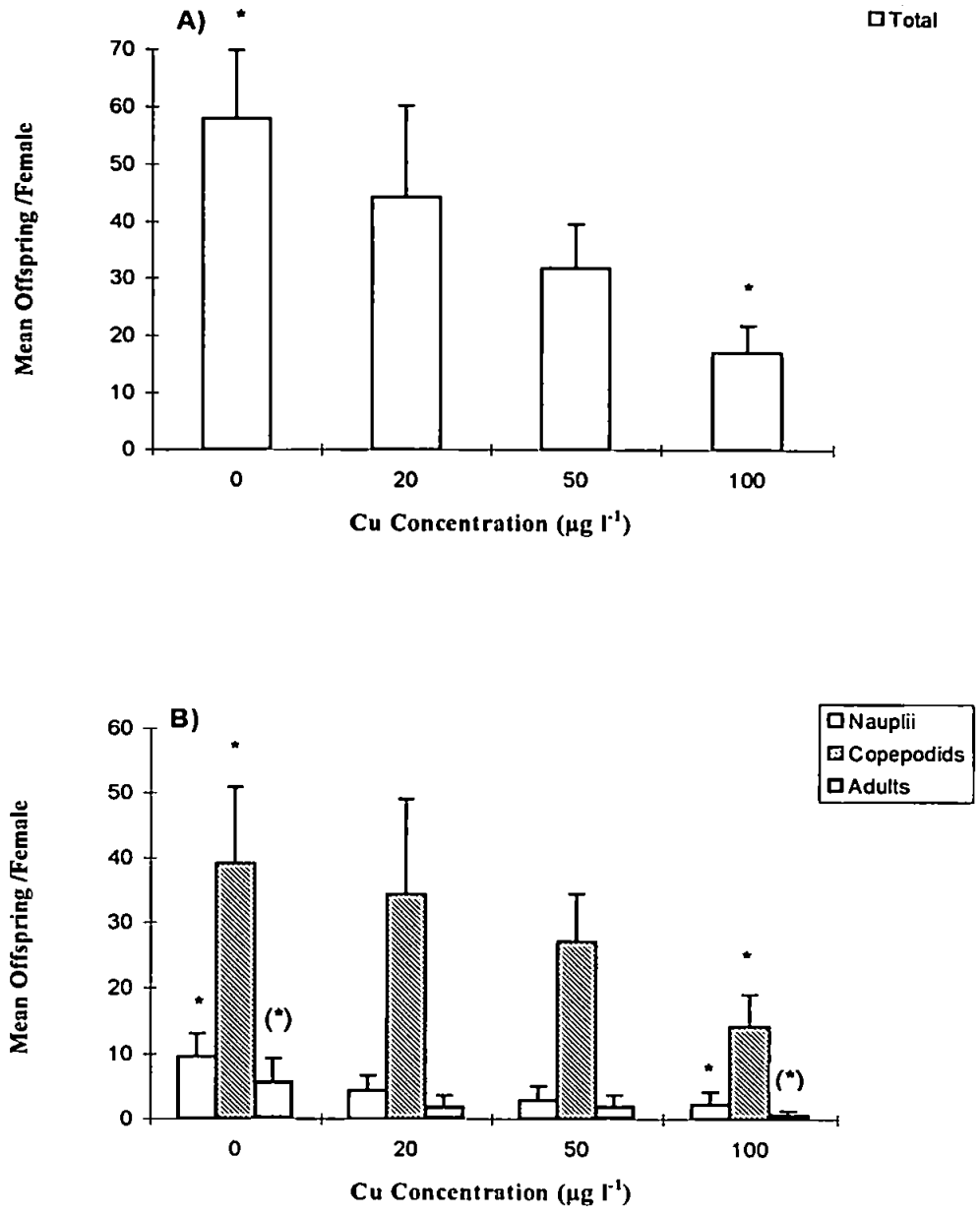


Figure 5.5 Offspring Production: the mean number of **A)** total surviving offspring and **B)** surviving adults, copepodids and nauplii present per female 60 days after parent pairing. Females that did not contribute to the surviving offspring have been excluded. Significant difference (* $p < 0.05$; (*) $p < 0.1$) compared with 0 µg l^{-1} Cu. (n = 15 for each concentration, vertical bars = + 1 SD).

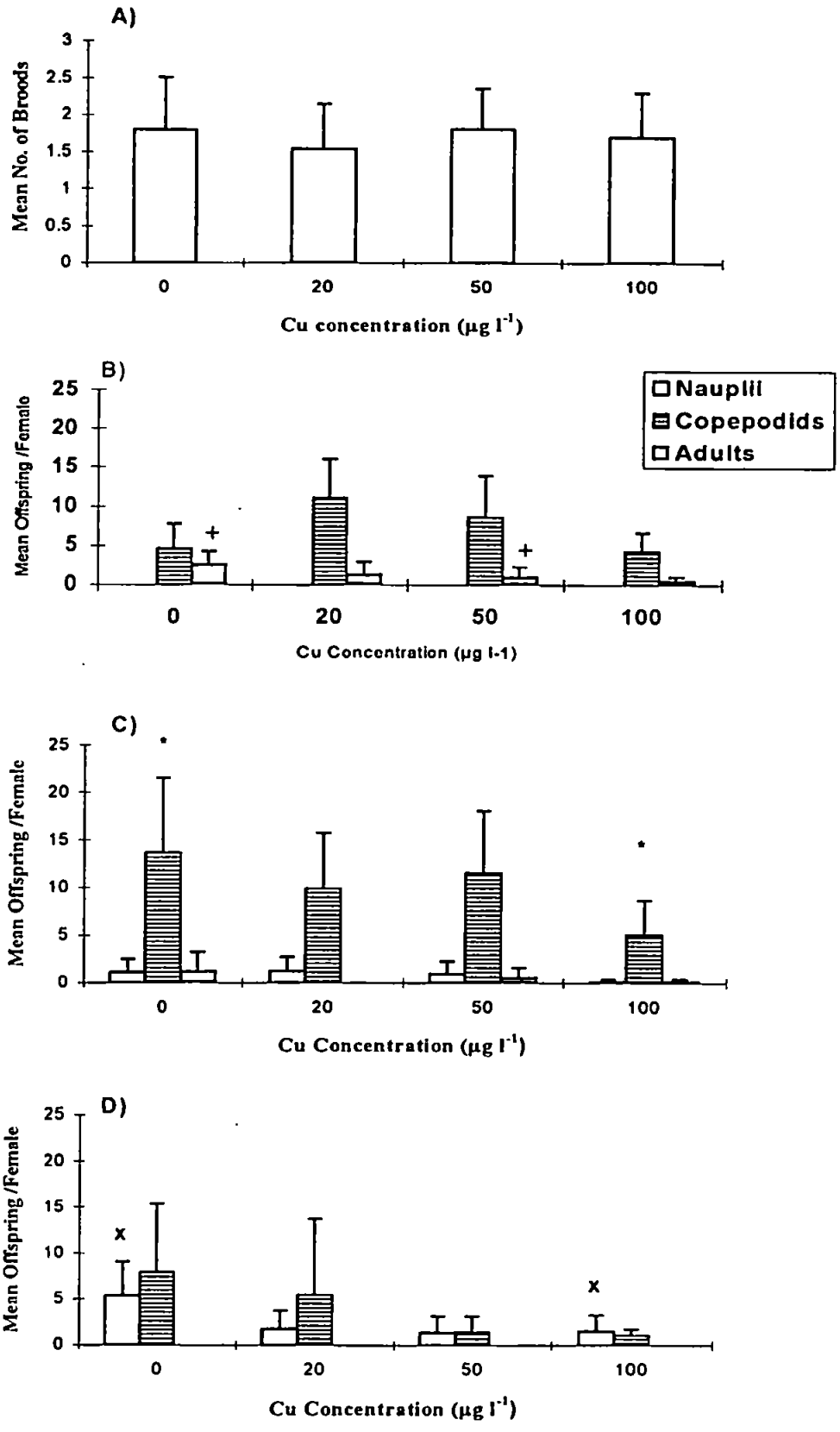


Figure 5.6 Offspring production per brood: **A)** the mean number of broods per female *Bryocamptus zschokkei*; and the mean number of surviving offspring per female present 60 days after pairing in **B)** brood 1, **C)** brood 2 and **D)** brood 3. Significant difference ($p<0.05$) denoted by * for total number of offspring, + for number of adults and x for number of nauplii. (n =15 for each concentration, vertical bars = +1 SD).

Compared to copepod pairs in the control and 20, 50 and 100 μg l^{-1} Cu test solutions, pairs exposed to 150 μg l^{-1} Cu took significantly longer than control animals to produce their first brood [11.1 days (± SD) compared with 5 days (± SD) (Fishers least significant difference process $p<0.05$)] (Fig. 5.7). The time interval between broods of animals exposed to other Cu concentrations were not significantly different (range 4.6-5.79, 14.1-19 and 8.33-13.25 days for brood one, two and three, respectively).

5.3.2.2 Effects on survival and development of immature life stages

The times taken for eggs to hatch were not affected significantly by Cu (Fig. 5.8). In the experiment assessing the survival and development of nauplii and stage one copepodids, there were 100% mortalities of nauplii and stage one copepodids in 150 μg l^{-1} Cu. Hence, all subsequent analyses of naupliar and copepodid survival and development were restricted to the 0-100 μg l^{-1} Cu treatments. No significant differences between remaining treatments were found for the time to develop and the survival of nauplii and copepodids to the stage one copepodid and the reproductive adult stage, respectively (Fig. 5.8). Total development time (i.e. from egg to ovigerous female) of copepods ranged from 37-44 days, regardless of the treatment to which they were exposed.

5.3.3 Tolerance

The 96 h LC_{50} value for individuals of *B. zschokkei* from U2 (660 μg l^{-1} Cu) was approximately twice that for animals taken from the control sites R2 (290 μg l^{-1}) and the River Yealm (313 μg l^{-1} Cu) (Table 5.2, Fig. 5.9). Differences in 96 h LC_{50} values could not be statistically evaluated but, based on the confidence interval data, show the LC_{50} 96 h value for U2 (range 540-870 μg l^{-1} Cu) was consistently different from that for R2 and the River Yealm (range 210-410 and 236-413 μg l^{-1} Cu, respectively). This suggests that

animals from U2 were more tolerant of high concentrations of Cu than animal from R2 and the River Yealm.

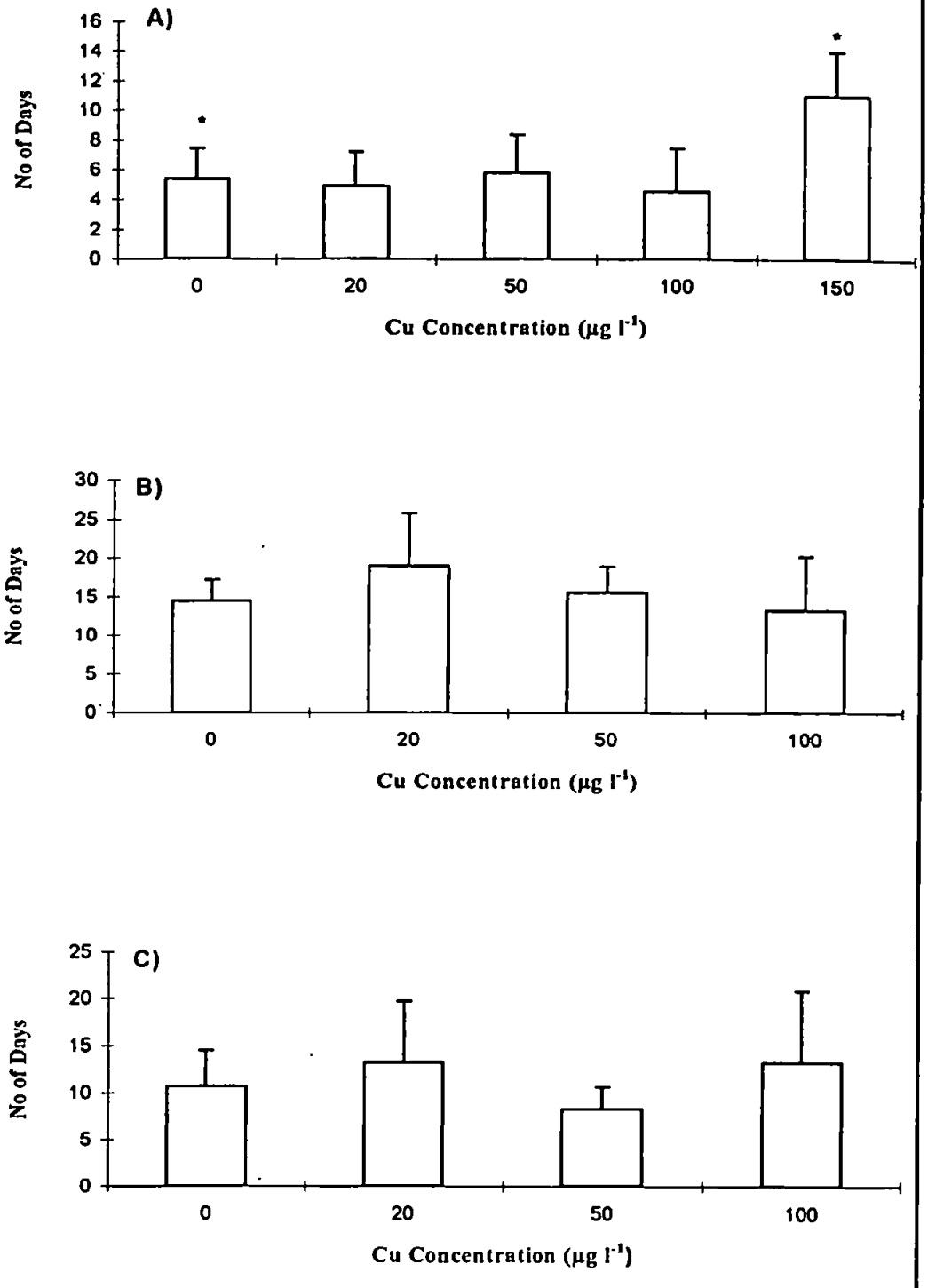


Figure. 5.7 Brood development:- time taken between: **A)** pairing and the appearance of the first brood; **B)** first and second broods; and **C)** second and third broods, for *Bryocamptus zschokkei* exposed to different concentrations of Cu (* indicates significant difference ($p < 0.05$) between treatments). (n varies from 6 to 9 for each concentration, vertical bars = + 1 SD).

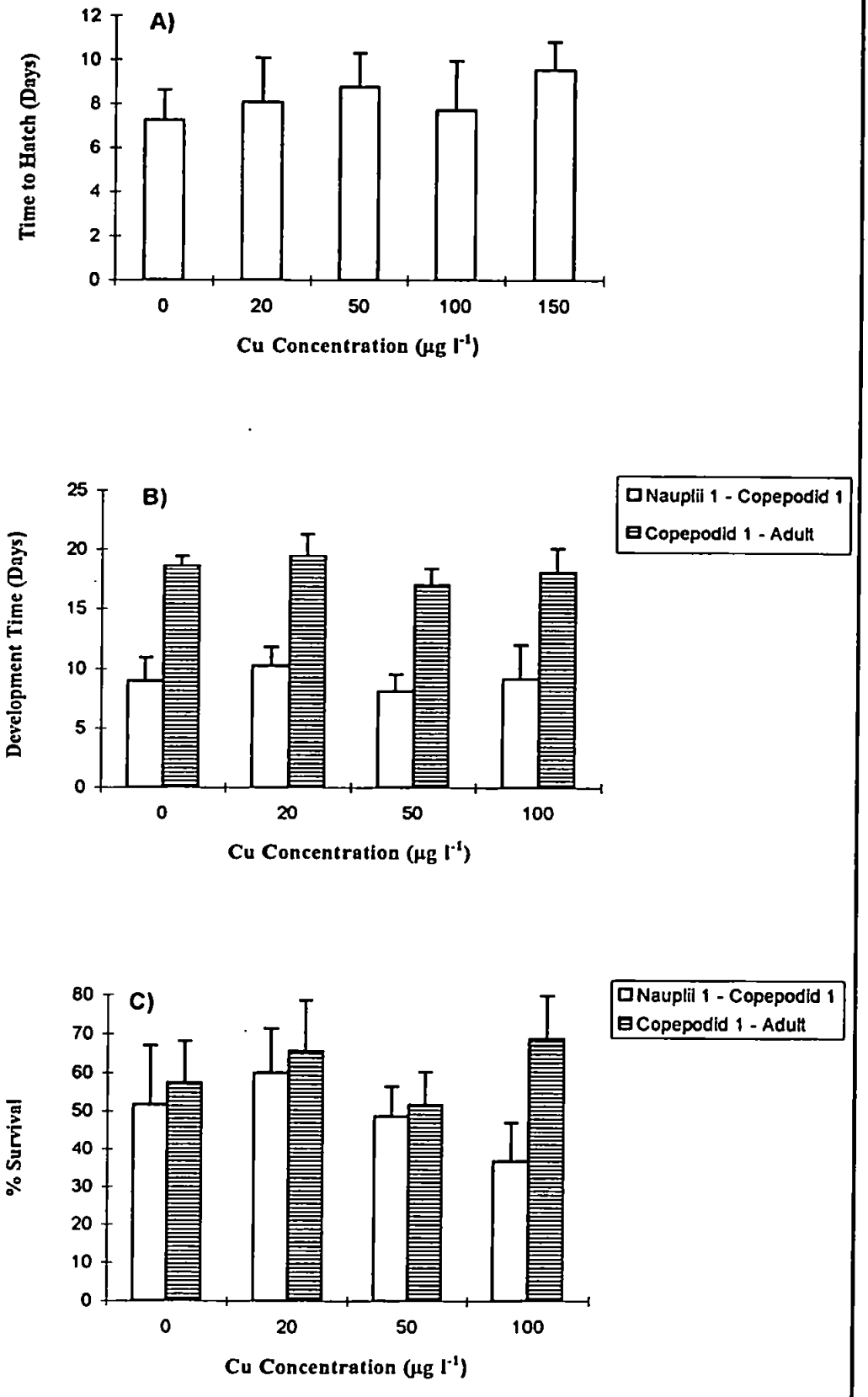


Figure 5.8 Development parameters:- time taken (days) for *Bryocamptus zschokkei* **A)** eggs to hatch, **B)** development from stage one nauplii to stage one copepodid (c1) and from c1's to adult stage; and **C)** the percentage survival of nauplii to c1's and c1's to the adult stage (n varies from 6 to 9, vertical bars = + 1 SD).

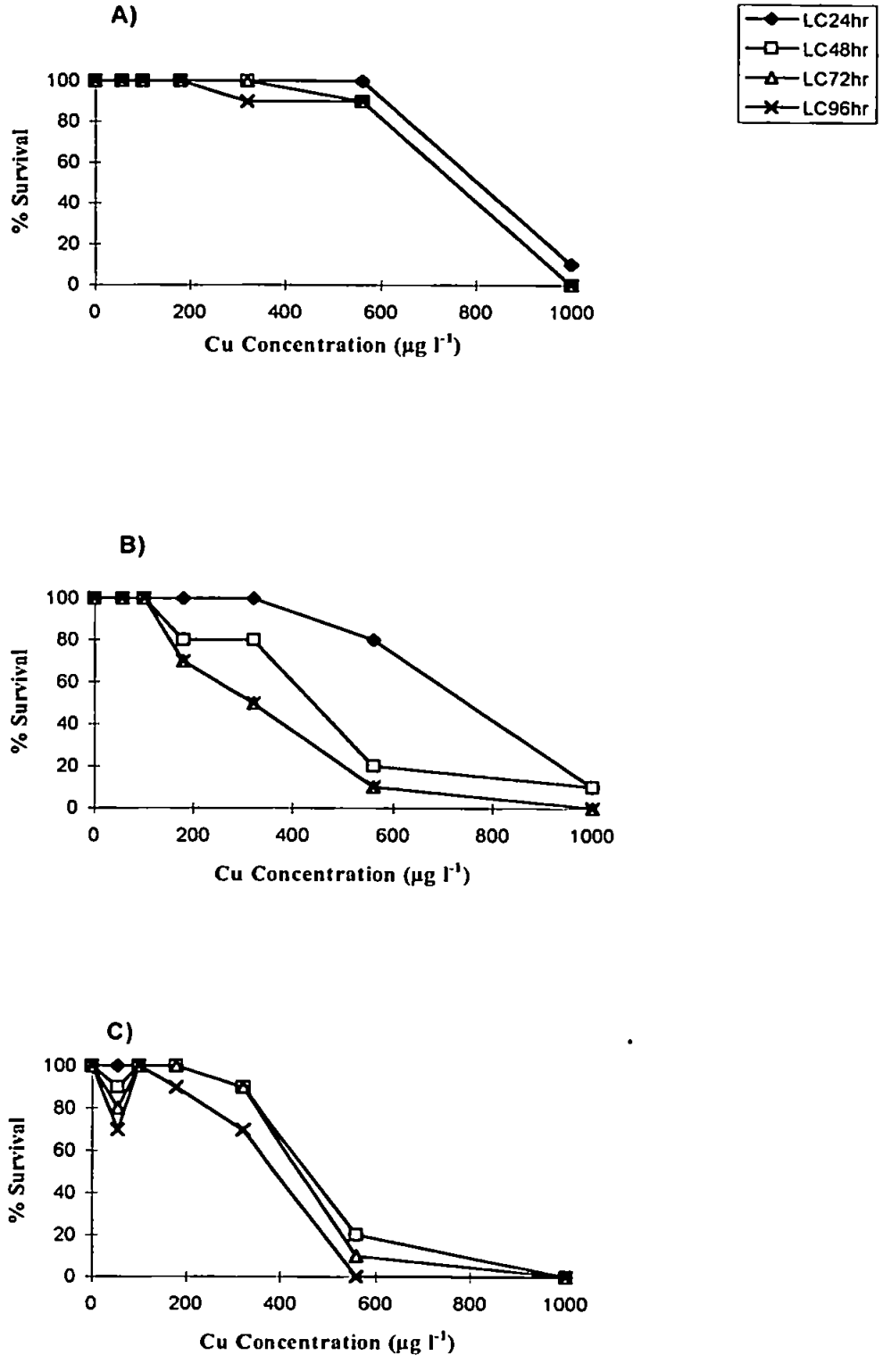


Figure 5.9 Toxicity curves for ovigerous female *Bryocamptus zschokkei* exposed to various concentrations of Cu. Animals collected from **A)** site U2, **B)** site R2 and **C)** the River Yealm.

5.4 Discussion

5.4.1 Lethal effects

Acute toxicity tests demonstrated a clear lethal effect of Cu on *Bryocamptus zschokkei,* with 50% mortality of ovigerous females occurring after 96 h at Cu concentrations of 290 $\mu g\ l^{-1}$. LC_{50} values for Cu for other species of freshwater invertebrates allow some comparison of these results with other (Table 5.3). Such comparisons must be treated with caution, however, as differences in the hardness, DOC and pH of treatment water will affect the toxicity of Cu to freshwater biota (Meador, 1991; Jin *et al.*, 1991; Winner & Owen, 1991) and these differ in the publications cited. It should be noted also that in the present study, Cu concentrations at the end of toxicity tests were lower than the initial concentration (Appendix III). To ensure a constant exposure concentration of Cu throughout the experiment, animals could have been transferred daily to new test solutions, although this may have caused the animals additional stress. Alternatively, a flow-through system could have been used, although these systems are expensive and may not be practical. Despite these difficulties, the LC_{50} value for *Bryocamptus zschokkei* is an order of magnitude higher than that for *Daphnia pulex,* suggesting that the cladoceran is more sensitive to Cu than the copepod (Table 5.3). This is in agreement with research on other copepods, such as the planktonic cyclopoid copepod *Cyclops,* which was more tolerant of Cu than *Daphnia spp* (Badouin & Scoppa, 1974; McIntosh & Keveren, 1974) (Table 5.3). As daphnids are predominantly found in still waters (Fitter & Manuel, 1994) they were not present in the streams used in the present study, and cannot be used to predict the effect of contaminants on stream communities. Furthermore, although many studies have aimed to identify the most sensitive species, under the assumption that by protecting this species, all other species will be protected. This may not be the best approach. Cairns (1986) has argued that the most sensitive species may be markedly more sensitive than all other species in the

Table 5.3 Effect of Cu on aquatic invertebrates expressed as LC_{50}. Unless stated otherwise hardness and alkalinity expressed as mg l^{-1} $CaCO_3$. Table adapted from Kelly (1988).

Species	Life stage	Test Conditions	Metal Salt Added	Response Criteria	Metal Concentration ($\mu g\ l^{-1}$)	
Daphnia pulex	Multiaged	Static	$CuSO_4$	96-h LC_{50}	28	McIntosh and Keveren (1974)
Cyclops sp.	Multiaged	Hardness 112-157 pH 8.0-10.1		96-h LC_{50}	>225	Badouin & Scoppa (1974)
Cyclops abyssorum prealpinus	Adult	Static	$CuCl_2$	48-h LC_{50}	2500	
Eudiaptomus padanus padanus		Hardness 0.6 meq l^{-1}		48-h LC_{50}	500	
Daphnia hyalina		pH 7.2		48-h LC_{50}	5	
Gammarus fasciatus	Multiaged	Static Hardness 206 pH 7.75	$CuSO_4$	48-h LC_{50}	210	Judy (1979)
Asellus meridianus		Static River Hayle	$CuSO_4$	48-h LC_{50}	1650, 1700, 250	Brown (1977a)
		Hardness 25 R. Gannel		48-h LC_{50}	1900	
		R. Bradwell			1200	
Tubifex tubifex		Static				Brkovic-Popovic and Popovic (1977
		Hardness 34.2, pH 7.2	$CuSO_4$	48-h LC_{50}	210	
		Hardness 261, pH 7.32		48-h LC_{50}	890	
Nais sp.		Static	CuSO4	96-h LC_{50}	90	Rehwoldt *et al.* (1973)
Chironomous sp.		Hardness		96-h LC_{50}	30	

community, perhaps by many orders of magnitude. Thereby, regulatory agencies may impose actions that incur unnecessary costs for the protection of a single species that may not even be present in the system under concern. Test species with intermediate, or low, sensitivity to contaminants may also be used to rank highly contaminated sites, where more sensitive animals would fail to detect differences (Transpurger & Drew, 1996). Thus, above all other criteria, the species chosen as a toxicity test organism should be relevant to the system that requires protection. In streams, *Bryocamptus zschokkei* fulfils this role.

As the Cu concentrations in the streams used in the present study all exceeded the recommended UK Environmental Quality Standards for Cu (NRA, 1994), species more sensitive to Cu may be found in other areas. For example, there were many species of harpacticoids present in streams in mid-Wales and the Ashdown Forest (e.g. *Atheyella crassa, Canthocamptus staphylinus* and *Bryocamptus echinatus*) (Rundle & Hildrew, 1990; Rundle & Ormerod, 1991) that were not present in the streams in the south west study site, and any of these may be more sensitive than *B. zschokkei* to Cu. Despite this, *B. zschokkei* can be considered a good toxicity test organism for predicting the effect of increasing Cu levels on the meiofaunal communities at sites where Cu concentrations are already high. The harpacticoid was consistently important in explaining between site differences in meiofaunal community structure (Chapter 3).

A lethal effect (at 150 $\mu g\ l^{-1}$ Cu) was observed on mating pairs of *B. zschokkei* in sub-lethal experiments, with all the pairs dying within eleven days of Cu exposure. This result demonstrated one of the failings of the LC_{50} approach in that acute exposure is not necessarily indicative of the effects of chronic exposure with which the fauna of polluted waters is more commonly exposed. The LC_{50} value was designed as a measure that could be used to compare the toxicity of different chemicals or to compare the toxic effect of a

chemical on one species relative to another (Abel, 1996). The median value was selected because it is the most statistically reliable measure. The time endpoint is selected for convenience, although it may also be limited by the fact that the animals used are usually starved. Many investigations of lethal toxicity have been criticised for not continuing experiments for long enough to establish the lethal threshold (a concentration so low that it will never cause the death of half the animals). For example, Sprague (1969) found that, of 375 publications measuring lethal toxicity, only 211 showed a lethal threshold within four to seven days. Thus, it may have been appropriate, in the present study, to have prolonged the time of exposure in LC_{50} tests, whilst also feeding the animals during the experiment.

Another general failing of acute toxicity tests may be with the choice of life-history stage. Using ovigerous female *B. zschokkei* allows a standard life-history starting point, but other life-history stages may be more sensitive to Cu. From the experiment investigating the survival and development of early life stages, there were indications that the naupliar stage of *B. zschokkei* was more sensitive to Cu concentrations than indicated by the LC_{50} for ovigerous females. A lower (though not significant) percentage of nauplii reached the first copepodid stage when exposed to 100 $\mu g\ l^{-1}$ Cu compared to the control. Other studies of marine meiobenthic copepods have also found, consistently, that the naupliar stage was the most sensitive life stage to heavy metals (Verriopoulos & Moraitou-Apostolopoulou, 1982; Verriopoulos & Hardouvelis, 1988; O'Brien, *et al.*, 1988; Hutchinson, *et al.* 1994). Verriopoulos & Moraitou-Apostolopoulou (1982) suggested that the older life-history stages are more resistant due to their thicker cuticles reducing the entry of metals into the copepod body. The regulation of metal ions is also less effective in young invertebrates than adults (Bryan & Hummerstone, 1971) and it appears that the formation of the mechanism of detoxification may not yet be fully developed in young life-history stages (Bernard & Lane, 1961; Bryan, 1974). Further work is required on the relative sensitivities of the different life

stages, as the effects on these early life-history stages may have large implications for the population dynamics of *B. zschokkei*.

5.4.2 Sub-lethal effects

5.4.2.1 Fecundity

The present study demonstrated that the total number of offspring produced 60 days after parent pairing was significantly lower at 100 $\mu g\ l^{-1}$ Cu compared to the control. Fewer offspring (though not significantly so) were also produced by females exposed to 20 and 50 $\mu g\ l^{-1}$ Cu than the controls. In comparison, no mortality of *B. zschokkei* was observed at 100 $\mu g\ l^{-1}$ Cu after 96 h in the acute toxicity tests. Thus, fecundity appears to be a more sensitive measure of Cu toxicity to *B. zschokkei* than the LC_{50}. Several ecotoxicological studies with marine meiofauna have showed sub-lethal effects of toxicants (e.g. offspring production) at concentrations lower than those which cause mortality (see review by Coull & Chandler, 1992). The copper concentration for the lowest 96 h LC_{50} for nauplii and adult female *Tisbe battagliai* was 11 times higher than the concentration shown to have no observable effect on reproduction and survival after eight days of Cu exposure (Hutchinson *et al.*, 1994). Sub-lethal concentrations of copper caused a reduction in the number of offspring produced by *Tisbe holothuriae* (Moraitou-Apostolopoulou *et al.*, 1983). These sub-lethal effects may, ultimately, confer an effect on population size. Between 0 and 100 $\mu g\ l^{-1}$ Cu, there was no significant effect of Cu on the brood interval, although the time taken for the first brood to be produced was significantly longer at 150 $\mu g\ l^{-1}$ Cu than for the control. Therefore, the reduced fecundity at higher Cu concentrations was due to a lower number of offspring per brood rather than a reduction in brood production.

Some caution is required when interpreting the results of the number of surviving offspring produced by successive broods in the present study. Earlier broods were exposed longer to

the treatment solution than later ones, which may explain why a lower number of surviving offspring was evident at all stages for brood two and three but not for brood one, when compared to the control. Williams (1997) excluded the first brood from analyses in his study on the effects of pentachlorophenol on the reproduction of the harpacticoid *Tisbe battaglia*. In this case, it was argued that the production of the first brood would have been influenced by the previous culture regime from where the ovigerous females were taken. The lack of effect on brood one in the present experiment may, therefore, be due to the shorter length of exposure of the adults to the test solution prior to the production of this brood. This suggests offspring production may be influenced by parent fitness prior to the production of broods. Therefore, to clearly detect the effect of different treatments on offspring production, brood one could be excluded. Alternatively, the lack of effect on this first brood may be considered as part of the effect of exposing a *B. zschokkei* population to Cu, and, therefore, perhaps the brood should be included for realism.

A drawback of using sub-lethal responses, rather than acute toxic measures, is the time taken (60 days) to reach the test endpoint. As the present study showed Cu to have a significant effect on all three broods, on at least one of the life stages within each brood, it may not be necessary for the test to run for 60 days for an effect to become evident. To reduce further the time needed to obtain the test endpoint for sub-lethal effects on *B. zschokkei*, parameters (i.e. biomarkers) indicative of the effects of Cu on offspring could be used (i.e. the critical life-stage bioassay). This approach requires an understanding of the mechanisms underpinning a reduction in the number of offspring. Thus, the entire life cycle requires investigating, so that measures which are indicative of effect on the population may be identified. An effect on the early life-history stages was evident in the present study, with a significantly lower number of nauplii found in the third brood at 100 $\mu g\ l^{-1}$ Cu than for the control. The apparent decrease in nauplii survival at 100 $\mu g\ l^{-1}$ Cu compared to the control,

in the experiment investigating the effects of Cu on the survival and development of the early life stages, also suggests an effect of Cu on this early life stage. No effect of Cu on the survival of copepodids was observed in this latter experiment. The effect of Cu on the reproduction of *B. zschokkei* may also be explained by the effect of the metal on parent fitness (i.e. a decrease in the number of viable eggs produced by females or a delayment in egg production). The delay in the appearance of the first brood in females exposed to 150 $\mu g\ l^{-1}$ Cu certainly suggests an effect on the reproductive status of the female at high Cu concentrations, but no effect at 0-100 $\mu g\ l^{-1}$ when other sub-lethal effects occurring. The effect of Cu on the percentage of eggs hatching still requires investigating to verify whether Cu is affecting parent fitness in this way.

5.4.2.2 Development

Whereas studies on marine copepods have reported development of immature life stages to be affected by metal toxicity (D'Agostino & Finney, 1974; Moraitou-Apostolopoulou *et al.*, 1983), the development rates of the nauplii and copepodids of *B. zschokkei* were not affected by Cu. The presence of the leaf disc in the cells used in the *B. zschokkei* experiments, however, made observations of nauplii and copepodids difficult. Nauplii obtained from the second brood of the first generation, used to initiate the experiment following the survival and development of nauplii and copepodids, may have been older than 24 h (if they were missed during a daily inspection of a cell). Thus, in some cases, the development time may have been underestimated. There was also a lack of effect on the time taken for the first brood to be produced and nauplii to hatch at Cu concentrations between 0 and 100 $\mu g\ l^{-1}$ Cu. This may have been due to measurements being taken only a few days after the parents were exposed to the treatment, when the effect of exposure may not yet have been apparent. If the parents had been exposed to Cu for longer, they may have become more stressed leading to an effect on development of these early life stages.

Further investigations are required, therefore, to confirm that Cu was not having an effect on embryonic and post-embryonic development.

5.4.3 Linking effects on individuals and populations

Sub-lethal endpoints may be more sensitive than lethal endpoints but their relevance to population dynamics still requires validation. Evidence that sub-lethal effects of metals do ultimately reduce population size after prolonged exposure has been demonstrated previously for copepods. For example, Verripoulos & Hardouvelis (1988) exposed the copepod *Tisbe holothuriae* to three sub-lethal concentrations of Zn (0.07, 0.01 and 0.007 ppm) for four subsequent generations. An effect on population dynamics was observed at concentrations of 0.07 and 0.01 ppm Zn, however, at 0.07 ppm Zn, no effect was observed on the first generation. These latter animals could not produce a second generation, mainly due to the lower number of egg sacs produced and the lower percentage of animals producing egg sacs. For 0.01ppm Zn, the effect was not observed until the fourth generation, with the percentage of animals producing egg sacs decreasing significantly from generation to generation. Further work exposing *B. zschokkei* to sub-lethal concentrations of Cu over a number of generations is required to reveal whether there is an effect on population growth.

Complete life-cycle tests, where cohorts of the test organism are exposed from birth to death, have also been used successfully to highlight the effects of the toxicant on specific life stages of numerous invertebrates. Biological information can then be used to calculate population parameters such as intrinsic rate of natural growth (r_m) (Daniels & Allen, 1981 for marine copepods; Gentile *et al.*,1982 for mysid shrimps; Janssen *et al.*, 1993 for rotifers; Meyer *et al.*, 1987 for cladocerans). The intrinsic rate of increase integrates age-specific survival and reproduction (age at first reproduction, reproductive frequency, brood size and

reproductive period), thereby, providing a more ecologically relevant criterion than separate measures of survival and reproduction. The results of life table experiments on *Tisbe furcata* exposed to 0.9 μM Cu demonstrated that significant negative effects on demographic parameters (total production of nauplii, life span and reproductive period for fertile females), and a reduction in the percentage of fertile females, led to a 61% reduction of r_m (Bechmann, 1994). As *B. zschokkei* has been found to live as long as 370 days (O'Doherty, 1985), shorter exposure durations than from birth to death could be used to estimate r_m (Allan & Daniels, 1982; Daniels & Allan, 1982; Meyer *et al.*, 1987).

It should be noted also that there was a wide variation in the number of surviving offspring produced by individual females in the fecundity experiments. These high levels of variability may have masked the effects of Cu. For example, there was a low number of surviving copepodid offspring for copepods exposed to 50 and 100 μg l^{-1} Cu compared with 0 and 20 μg l^{-1} Cu; these differences were not significant due to the high variation amongst individuals. High variability in measures of survival and reproduction have also been reported for other microcrustaceans such as the harpacticoids *Tisbe holothuriae* (Williams, 1997), *Tisbe furcata* (Bechmann, 1994), and the cladocerans *Daphnia magna* (Martinez-Jeronimo *et al.*, 1994) and *Bosmina longirostris* (Koivisto and Ketola., 1995). Standardised procedures using *Daphnia spp.* as a test organism involve the selection of animals of limited genetic heterogeneity, an approach that has been advised to minimise the variation in individual response (Baird *et al.*, 1989). This approach has been criticised (Forbes & Depledge, 1992), however, as biological variability is an important component of a population's ecological and evolutionary response to pollution stress (Forbes & Forbes, 1994). Even so, it is important to be aware that even in test solutions where the number of offspring produced were not significantly lower than the control, an effect on the population may eventually occur. Exposure of *Tisbe furcata* to 0.5 μM Cu resulted in an effect on

demographic parameters, which even though they were non-significant, led to a 10% reduction of r_m (Bechmann, 1994). Williams (1997) demonstrated that *Tisbe holothuriae* exposed to concentrations of PCP, that did not result in a significant effect on offspring production, still contributed to a high percentage reduction in the intrinsic growth rate calculated for the population.

Finally, the effect of pollutants such as trace metal levels on populations in the field can occur through several mechanisms including alterations to biotic interactions. These mechanisms also need to be understood if effects in the laboratory are to be related to the field (Chapter 6.).

5.4.4 Tolerance

One important factor to consider when predicting the effects of trace metals, such as Cu, on species in the field, is whether pre-exposure of populations has led to some degree of tolerance. Some evidence for tolerance was found in this study. The LC_{50} value for *B. zschokkei* individuals from site U2, a stream with high Cu concentrations, was clearly higher than that for individuals from streams with low Cu concentrations (R2 and the River Yealm). Hence, individuals from the population at U2 appeared to show some tolerance to acute levels of Cu compared to animals from sites of low Cu concentration. Numerous marine and freshwater studies have shown that crustaceans from metal-contaminated sites are less susceptible to metals than animals from clean sites (see review by Klerks & Weis, 1987). Tolerance can be due to either physiological acclimation or genetic adaptation. Physiological acclimation of *Gammarus pulex* individuals to cadmium after pre-exposure to sub-lethal concentrations of cadmium and zinc in the laboratory has been reported by Stuhlbacher and Maltby (1992). In the latter case, increased cadmium tolerance in acute toxicity tests was shown to be associated with an increase in the body concentration of a

metallothionein-like protein, to which the metals bound. This resulted in the metals being sequestered in a less toxic form. Genetic adaptation of *Asellus meridianus* to lead was shown by Brown (1977a). Isopods from contaminated sites were more tolerant than those from uncontaminated sites and this persisted into the F2 generation after animals had been reared in clean water. It would be illuminating to elucidate whether the resistance of *B. zschokkei* to copper has a genetic basis or is due to physiological acclimation. This could be accomplished by rearing *B. zschokkei* from contaminated and uncontaminated sites in clean water and repeating exposure experiments on the F2 generation.

Evidence that the acquired tolerance of copepods to metals has a subsequent effect on population dynamics has been demonstrated by several workers. Moraitou-Apostolopoulou *et al.* (1983) followed the effect of exposure to sub-lethal concentrations of Cu on the marine benthic harpacticoid copepod *Tisbe holothuriae* over five generations. A prolonged exposure of Cu resulted in the sub-lethal effect of increased maturation time to be less pronounced from the F3 generation (Moraitou-Apostolopoulou *et al.*, 1983). The sub-lethal effect of Cd and Zn on populations of *Tisbe holothuriae* were also shown to be counteracted by an acclimation process over the generations by Hoppenheit (1977) and Verriopoulus and Hardouvelis (1988), respectively.

5.4.5 Possible improvements in the experimental procedure

The experimental protocols developed in this chapter used conditioned beech leaves as a food source following methods described by O'Doherty (1985). It was, however, difficult to standardise the amount of food provided for the animals using a leaf disc as the degree of decay and the density of bacteria on these leaves were likely to be variable. Differences in the quality and quantity of food may have been partly the reason for the high variability in the number of offspring produced by animals exposed to the same test concentration.

Furthermore, the number of surviving offspring had to be assessed at the end of the experiment rather than counting the number of nauplii which hatched out, as it was difficult and highly time consuming to find the animals while a leaf disc was present.

As previous workers have shown the quality and quantity of food provided can have a significant effect on the lethal and sub-lethal toxicity of chemicals to biota, it is important that, if *B. zschokkei* is to be used as a toxicity test organism in the future, the food source must be standardised. Perlmutter & Meyer (1991) demonstrated that the stream harpacticoid *Attheyella* was feeding on bacteria living on birch leaves. Bengtsson (1978) also used a bacterial culture to successfully feed the estuarine benthic harpacticoid *Nitocra spinipes* during experiments assessing the effect of metal toxicity on reproduction. Thus, the isolation and culture of freshwater bacteria as an appropriate natural food source for *B. zschokkei* may help standardisation. Without the presence of the leaf disc, observations of the copepods would also be made easier. Improvements in the methodology would, thereby, allow direct measurements of the number of offspring produced rather than measuring the number of surviving offspring produced over a period of time.

As most toxic pollutants of aquatic systems have a strong affinity for particulate matter they are often associated with sediments [note the higher interstitial concentrations of metals compared to surface water concentrations found in this study (Chapter 2)]. Thus, to provide a greater degree of environmental realism, and increase the ability to predict environmental effects, there has been a growing interest in developing sediment toxicity tests (Transpurger & Drews, 1996). As *B. zschokkei* is an interstitial species it may also have potential to be developed as a toxicity test organism for assessing the effects of contaminants associated with the sediment.

5.4.6 Conclusion

In summary, this chapter demonstrated that Cu had toxic effects on the survival and reproduction of *Bryocamptus zschokkei*. Acute toxicity tests gave a rapid result, although, the effects on survival occurred at a higher Cu concentration than that found in the chronic tests. Sub-lethal concentrations of Cu resulted in the harpacticoids producing fewer offspring, though the time taken to give the measured response was lengthy. Improvements in the methodology, and further experimental work, are required to find quick, sub-lethal, measurable responses indicative of changes in the population growth of *B. zschokkei*. Animals already exposed to chronic concentrations of Cu exhibited greater tolerance to Cu. Thus, one factor that is important to consider when predicting the effects of metals on a system is the previous exposure of the populations to the toxicant. Other factors that need to be considered if toxicity tests using *B. zschokkei* are to be used to predict the effects of Cu on stream communities in the field are discussed in Chapter 6.

CHAPTER 6

General discussion and conclusions

The primary objective of this thesis was to assess the use of lotic meiofauna for monitoring metal contamination at the individual and community level of biological organisation. In this concluding chapter, the potential benefits of including stream meiofaunal communities in monitoring programmes are evaluated. Possible techniques for overcoming some of the difficulties encountered when working with this component of the stream fauna are discussed. The use of both lethal and sub-lethal responses of the harpacticoid copepod *Bryocamptus zschokkei* as indicators of Cu toxicity are also appraised as are the potential advantages of using this harpacticoid copepod as a toxicity test organism.

6.1 The potential use of meiofaunal communities to monitor metal contamination

The survey of meiofaunal communities in metal-contaminated streams presented in Chapter 3 demonstrated a clear relationship between community structure and Cu concentrations. Subsequent analyses demonstrated that the correlation between meiofaunal community structure and trace metal contamination was similar to that of the macrofaunal community (Chapter 4). Thus, the information gained from using the meiofaunal community may be of equal value to that from using macrofaunal community structure for monitoring metals and other pollutants. Although further validation work is required before meiofaunal communities could practically be used by regulatory bodies, meiofauna exhibit a number of advantages that may make their inclusion in monitoring programmes worthwhile. Firstly, more information may be gained by including the meiofauna, alongside macrofauna, when monitoring the impact of contaminants on freshwater systems. The inclusion of both macrofauna and meiofauna in environmental impact surveys has been advocated in marine systems as meiofauna have been found to provide additional information on the effects of pollutants (Austen *et al.*, 1989; Somerfield *et al.*, 1995). In the present study, there was a distinct meiofaunal community at L4 in autumn associated with high Al and low pH conditions at this site, while the absence of all meiofauna, except for

nematodes in spring, highlighted the exceptionally high levels of Zn at U2 in this season. These site differences in chemistry were not highlighted by the macrofaunal community. The inclusion of the temporary meiofauna with the macrofauna, or the use of the entire metazoan community (i.e. meiofauna macrofauna and temporary meiofauna) when assessing the impact of metal contamination, also improved the discrimination between sites of varying metal contamination (Chapter 4). Thus, the use of the entire metazoan community may be useful in detecting more subtle differences in contamination among sites.

Stream meiofauna may have other potential advantages for pollution monitoring not mentioned in this study. The shorter generation times of meiofauna and their intimate association with the sediment suggests that meiofauna may respond more rapidly and have greater sensitivity to pollution inputs than many macrofauna (Warwick, 1993). These attributes led Moore & Pearson (1987) to suggest that meiofauna might play an important role in the detection of subtle alterations to ecosystems due to chronic pollution. Meiofauna may serve as a sensitive tool either to ascertain the spatial extent of the impact of a pollution source, or as an early warning of community change in response to anthropogenic disturbance. In the present study, Cu concentrations at all the sites exceeded the recommended UK Environmental Quality Standards for Cu (NRA, 1994), therefore, further survey work is required to investigate whether meiofaunal community structure is altered at lower metal concentrations than that affecting macrofauna. Stream microcosms or transfer experiments could also be undertaken to investigate the short-term response of meiofauna to elevated metal levels, thereby, assessing whether the response of meiofaunal communities to metal contamination is more rapid than that of the macrofauna.

Difficulties that have been encountered using benthic macroinvertebrate communities as monitors are also likely to apply to meiofauna. The high cost of monitoring at the species level, due to time-consuming sampling and processing, is a constraint of particular relevance to meiofauna, which often require the preparation of permanent mounts for identification under high power magnification. The taxonomy of meiofauna is also considered difficult and keys are lacking for some groups (e.g. Nematoda). This study, however, demonstrated that meiofaunal communities at higher taxonomic levels, such as family, still clearly illuminated the change of community structure along the metal gradient. Thus, identification to a higher taxonomic level may be adequate when detecting contamination, which will reduce the effort of processing samples and the risk of misidentifications.

Perhaps the ideal approach, if meiofauna were to be used on a widespread basis by regulatory bodies, is the generation of an index of contamination. Diversity measures and the index of multivariate dispersion, however, were poor discriminators of sites of different metal concentrations (Chapter 4). Another method is to use biotic indices (Chapter 1). Biotic indices summarise the information in a way that can be understood by decision managers and the concerned public, giving an indication of contamination levels. A very simple biotic index using the ratio of nematodes to copepod densities (N:C ratio) was suggested by Raeffaeli & Mason (1981) to detect organic pollution in the marine environment. The present study demonstrated that the sensitivities of cyclopoids and harpacticoid copepods differed, with the cyclopoids being generally more tolerant of metal contamination (Chapter 3). Thus, a similar ratio based on the sensitivity of harpacticoids, and the tolerance of cyclopoids to metals could be used to detect metal contamination in freshwater systems. However, the use of such ratios may be rather crude and the marine N:C ratio provoked much criticism when published, in that the influence of sediment grain

size and other environmental factors on individual species did not allow the index to be applied universally (Boucher, 1980; Coull *et al*, 1981; Lambshead, 1984; Huys *et al.*, 1992). Modifications that took into account various feeding types and habitat adaptations of nematodes and copepods were used to improve the validity and general applicability of this index (Warwick, 1981; Raeffaeli, 1987). A similar difficulty might arise with the use of a copepod index. Despite the general tolerance of cyclopoids to high metal concentrations, some species of cyclopoids were sensitive to elevated metal concentrations (Chapter 3). Thus, although such an index might be successful at assessing contamination for these sites it may not be useful in other regions where different species of copepods are present.

Once more is known about individual stream meiofaunal tolerances to metals, a more sophisticated scoring system of tolerant and intolerant groups could be designed. However, the difficulties encountered in distinguishing the effect of metal contamination on stream macroinvertebrates, from the influence of other environmental factors (e.g. Gower *et al.*, 1994), and the confounding effect of mixtures of metals, may also apply to meiofauna.

Finally, if stream meiofaunal communities are to be used in routine monitoring, a better knowledge of the types of communities to be expected at uncontaminated streams is required to provide a baseline against which pollution effects can be gauged. A system akin to RIVPACS (Chapter 1), where the degree to which observed fauna deviates away from a predicted fauna is used as an indication of disturbance at a site, would then allow potentially stressed sites to be pinpointed.

In summary, the advantages of using meiofaunal communities in monitoring are:-

1) They provide additional ecological information (as shown by the present study).

2) Their inclusion with macrofauna provides a better discrimination of sites of different contamination levels (as shown by the present study).

3) They are ubiquitous.

4) Their short generation times may result in a more rapid response to contamination.

5) They have an intimate association with the sediment.

Disadvantages of using meiofaunal communities are:-

1) The identification of meiofauna is perceived as difficult.

2) Sample processing is time consuming.

3) No score has been designed for rapid assessment in freshwaters.

6.2 The potential of *Bryocamptus zschokkei* as a toxicity test organism

This thesis has presented the first evaluation of a freshwater benthic copepod, the harpacticoid *Bryocamptus zschokkei,* as a toxicity test organism. This organism was chosen as it was of high ecological relevance in terms of its response to Cu contamination; *B. zschokkei* was both sensitive to Cu and reflected changes occurring in the meiofaunal community structure in response to Cu in the field (Chapter 3). SIMPER analyses demonstrated that the abundance of *Bryocamptus zschokkei* also contributed highly to the separation of sites by the total metazoan community structure in spring and autumn (Fig. 6.1). Thus, field data suggested that it might be an ideal toxicity test organism for predicting the long-term effects of Cu on the stream benthic community.

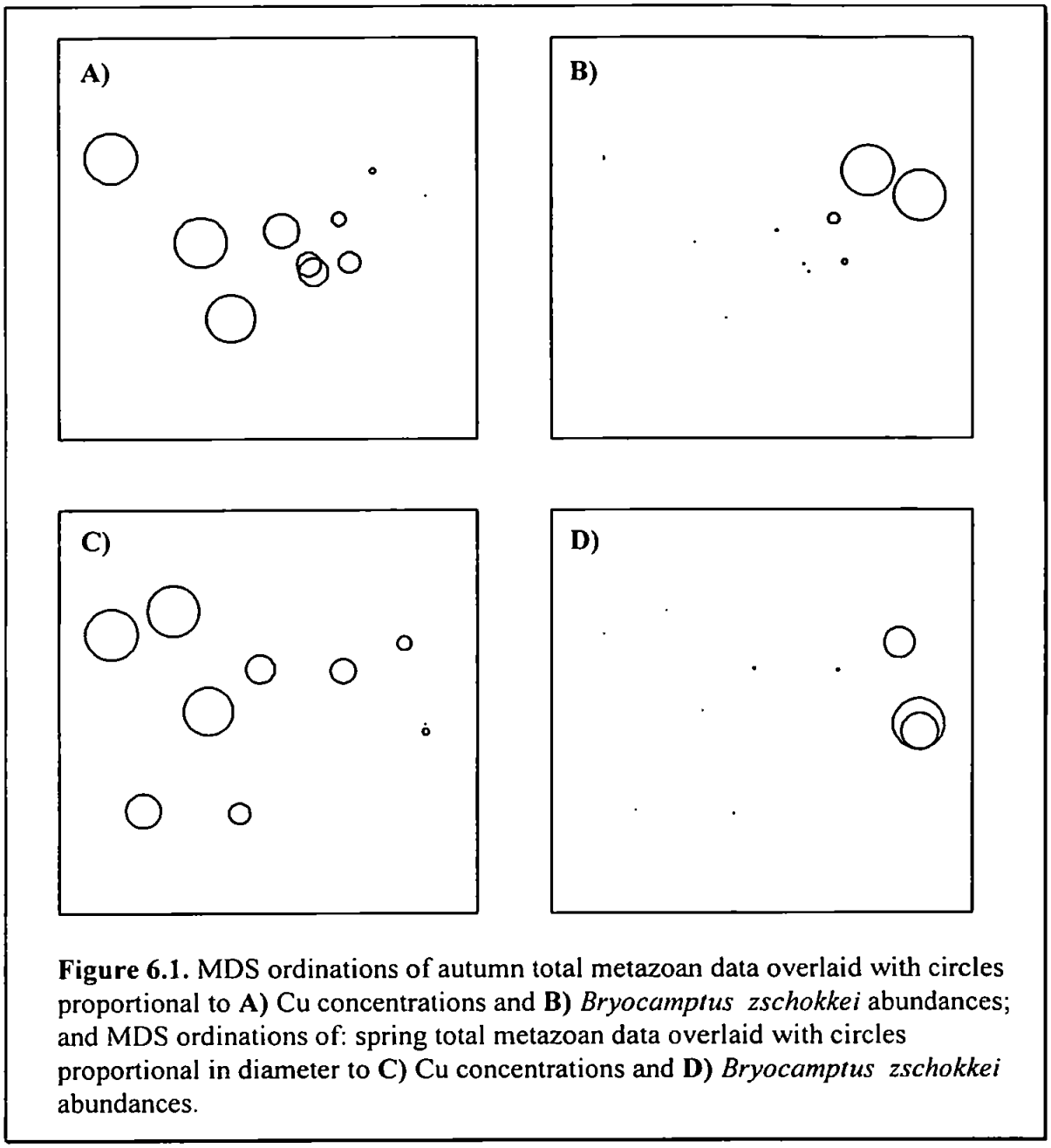


Figure 6.1. MDS ordinations of autumn total metazoan data overlaid with circles proportional to **A)** Cu concentrations and **B)** *Bryocamptus zschokkei* abundances; and MDS ordinations of: spring total metazoan data overlaid with circles proportional in diameter to **C)** Cu concentrations and **D)** *Bryocamptus zschokkei* abundances.

Although *Bryocamptus zschokkei* was sensitive to Cu contamination in the field, a comparison of the LC_{50} values for Cu for *B. zschokkei* with the LC_{50} values for other freshwater invertebrates demonstrated that other animals had a greater sensitivity to Cu (Chapter 5). Even so, the main criterion for choosing a predictive test organism should be that the test organism reflects changes in the community structure. Therefore, *B. zschokkei* should still be considered as a useful organism for protecting a system where Cu levels are already elevated.

As has been found for many other toxicity test organisms (Abel, 1996), Cu had an effect on the fecundity of *B. zschokkei* at lower concentration than those which caused mortality. A significantly lower number of offspring was produced at 100 $\mu g\ l^{-1}$ Cu compared to the control, whilst the LC_{50} 96 h value for ovigerous female *B. zschokkei* was 240 $\mu g\ l^{-1}$Cu. This reduction in the number of offspring produced by *B. zschokkei* exposed to Cu was an easily identifiable response. Even so, while the death of an organism is an unequivocal toxic action, the biological significance of sub-lethal toxic effects is frequently difficult to assess. Verification is required that responses at the individual level actually results in an adverse effect on the population. Too often studies fail to attempt to link 'biomarkers' to adverse effects at higher levels of biological organisation (Munkittrick & McCarty, 1995). The link between individual and population response in *B. zschokkei* could be achieved, either by comparing effects of Cu on fecundity to the effects of Cu on the population in the laboratory, or by using a complete life-cycle study to identify responses that can be related to the intrinsic rate of growth of the population. Although the short generation times of *B. zschokkei* allowed sub-lethal measures to be assessed relatively quickly sub-lethal responses were still relatively time consuming compared to acute measures. In the present study, the number of offspring produced by *B. zschokkei* was not recorded until after 60 days compared with the 96 hour exposure of *B. zschokkei* to Cu. The duration and scale of

experiments could be considerably reduced by identifying the most sensitive part of the life cycle. Results from the present study corroborated those from many others (Abel, 1996) in that the effect on the early life stage appeared to be the most critical with lower (though not significant) numbers of nauplii surviving to the copepodid stage when exposed to 100 μg l^{-1} Cu compared with the control. There was also a significantly lower number of nauplii found in the third brood at 100 μg l^{-1} Cu compared with the control, although this may have been due to an effect on parent fitness.

The direct benthic development of *Bryocamptus zschokkei* facilitated the study of all stages of the life cycle, however, the presence of the leaf disc in sub-lethal experiments made it difficult, and highly time consuming, to find animals. Thus, improvements of the culturing methodology of the harpacticoid are required. As stated in Chapter 5, the isolation and culture of freshwater bacteria as an appropriate natural food source for *B. zschokkei* would make observations of the copepods easier while allowing standardisation of the food source.

It is important that the effects of toxicants at the individual level in the laboratory are related to the effects at the community level in the field if the responses of *Bryocamptus zschokkei* in the laboratory are to be used to predict the effects of Cu on the rest of the stream biota. As the study measured the impact of metal contamination on meiofaunal communities in the field, the Cu concentrations that induced a response in *B. zschokkei* in the laboratory could be compared with the Cu concentration measured at sites where a lower *B. zschokkei* abundance and an altered community structure were recorded.

Figure 6.2 shows the abundance of *B. zschokkei* recorded at sites of various Cu concentrations in autumn. It is evident that lower abundances of *B. zschokkei* than that

found at the control sites were found at sites where surface water Cu concentrations were lower than 100 μg l^{-1}. At L1 (mean Cu concentration of 40 μg l^{-1}) *B. zschokkei* abundances were consistently lower than that found at R2 in spring, summer and autumn (Chapter 3).

The stream community structure was also altered at sites of a lower Cu concentration than predicted by either the lethal or sub-lethal responses of *B. zschokkei* to Cu in the laboratory. At sites where Cu concentrations were lower than 100 μg l^{-1}, the metazoan community was significantly different from the community at R2 (Chapter 4). For example, in spring, summer and autumn the metazoan community at L1 (mean Cu concentration 40 μg l^{-1}) was significantly different from R2.

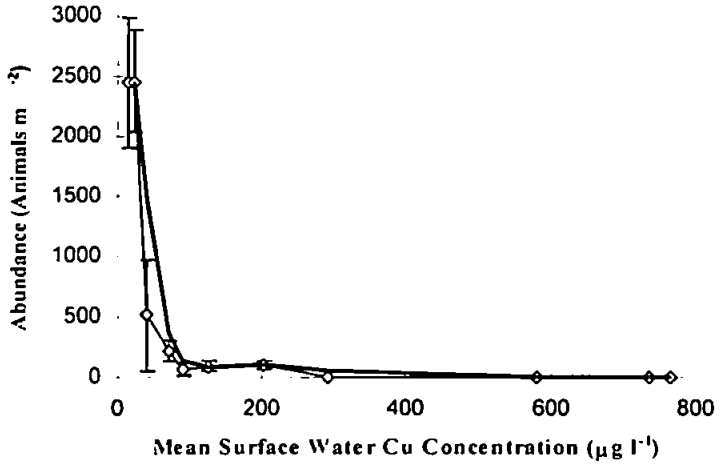


Figure 6.2 The mean abundance of *Bryocamptus zschokkei* found at sites of different Cu concentrations in autumn. The data is overlaid with a moving average trendline which smooths fluctuations in the data and shows the trend more clearly.

A comparison of the Cu concentrations that induced a response in *B. zschokkei* in the laboratory with the Cu concentration measured at sites where a lower *B. zschokkei* abundance and an altered community structure were recorded are summarised in Figure 6.3. Clearly, a lower abundance of *B. zschokkei* and an alteration of the benthic community structure compared with 'uncontaminated' waters, occurred at lower Cu concentrations in the field than predicted by the lethal and sub-lethal toxicity tests. As Parkhurst (1995)

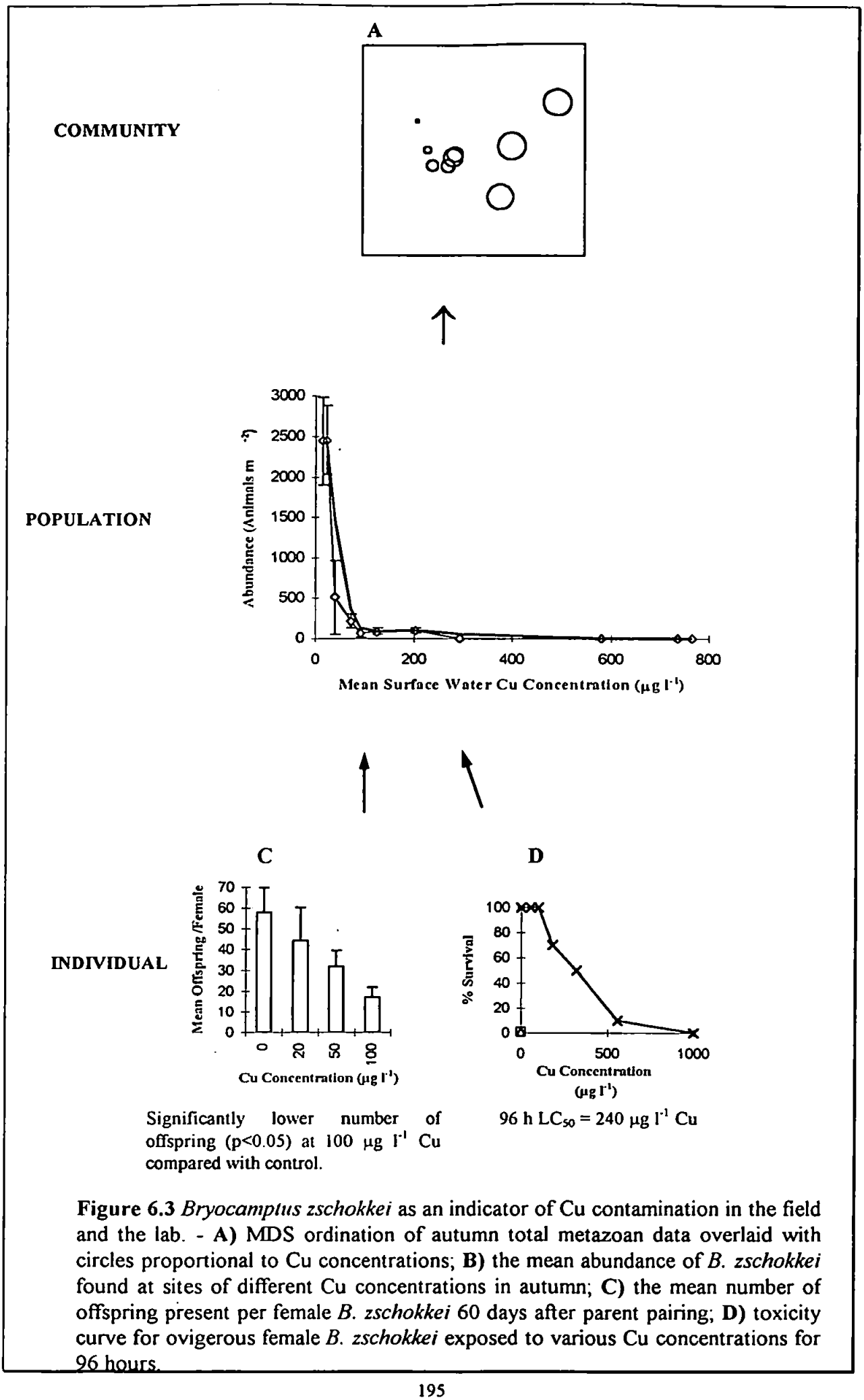


Figure 6.3 *Bryocamptus zschokkei* as an indicator of Cu contamination in the field and the lab. - **A)** MDS ordination of autumn total metazoan data overlaid with circles proportional to Cu concentrations; **B)** the mean abundance of *B. zschokkei* found at sites of different Cu concentrations in autumn; **C)** the mean number of offspring present per female *B. zschokkei* 60 days after parent pairing; **D)** toxicity curve for ovigerous female *B. zschokkei* exposed to various Cu concentrations for 96 hours.

noted, however, determining correspondence is not the same thing as validating a prediction. An understanding of the additional biotic and abiotic modifying factors associated with toxicants in the field is required to be able to link effects on individual toxicity test organisms in the laboratory to effects on the stream biota in the field.

Established laboratory toxicity test procedures rarely take into account the many environmental and biotic factors which will also influence the toxicity of metals on the biota of the receiving waters (Chapter 1). It is unlikely that Cu will be acting in isolation but that other metals will also be involved. Environmental factors other than metals will also beinfluencing the abundance of *B. zschokkei*, either directly or by affecting the bioavailability of Cu [e.g. DOC, temperature and food quality (Chapter 3; O'Doherty, 1986)]. The concentration of pollutants in receiving waters are also rarely constant, as demonstrated by the variable metal concentrations measured in four seasons in the stream tributaries of the Rivers Lynher and Seaton (Chapter 2). The rate at which a pollutant is introduced into the receiving waters also plays an important role in governing the community response (Smith *et al.*, 1979), and a higher community resilience is expected where the change is gradual rather than sudden, as populations may have had time to acquire tolerance. The sub-lethal effects of Cd and Zn on populations of *Tisbe holothuriae* were shown to be counteracted by an acclimation process over several generations (Hoppenheit, 1977; Verriopoulus & Hardouvelis, 1988). Experiments using *B. zschokkei* may demonstrate similar increased resilience of the population to sub-lethal Cu concentration over generations.

In the present study, the acute toxicity of Cu to individuals of *Bryocamptus zschokkei* from a population at a contaminated site was found to be lower than its effect on those animals taken from a control site (R2). It appeared, therefore, that pre-exposure to Cu led to the

development of metal tolerance in *B. zschokkei*. This illustrates another general problem with toxicity tests, if the pre-exposure of the field population to a contaminant has not been considered a more severe effect on a population may be predicted from toxicity tests. It is, therefore, important that previous metal exposure of the population of *B. zschokkei* in the receiving waters is considered when predicting the effects of metals. To elucidate whether the resistance of *B. zschokkei* to Cu has a genetic basis or is due to physiological acclimation, *B. zschokkei* could be reared from contaminated and uncontaminated sites in clean water and then exposure experiments repeated to assess whether tolerance is retained in the F2 generation.

In the field, predation and competition factors will also be influencing the *B. zschokkei* population structure. The influence of such biotic factors are as yet unknown for the majority of lotic meiofauna. The lack of knowledge of the consequences of the indirect effects of predation and competition are perhaps the biggest difficulty in linking levels when predicting the effects on community structure from effects at the population level. Further work manipulating densities of key species in mesocosms and natural systems is required to understand these competitive and predator-prey interactions.

Thus, even though there was an ecological basis to the choice of using *B. zschokkei* as a toxicity test organism for Cu, there are still many major caveats in linking effects of contaminants in the laboratory to field effects. Improvements to the culturing methodology of *B. zschokkei* are also still required to facilitate the assessment of the sub-lethal response of the harpacticoid to contaminants. Even so, *B. zschokkei* still has many attributes which make it a potentially useful toxicity test organism. It is relatively easy to culture and requires minimal space. Furthermore, its short generation times and direct benthic development facilitate the study of the effects of contaminants on its reproduction and

development. These attributes have aroused interest from ZENECA Limited (Brixham Environmental Laboratory) and investigations into the effects of oestrogenic compounds on this harpacticoid are at present in progress with further development of its use as a toxicity test organism. Finally, as an interstitial species, *B. zschokkei* may have potential as a toxicity test organism for assessing the effects of contaminants associated with the sediment. Toxicity analysis of the sediment is particularly important as most toxic pollutants of aquatic systems have a strong affinity for particulate material and eventually become associated with the sediment.

In summary, the advantages of using *B. zschokkei* as a toxicity test organism are :-

1) The harpacticoid is an ecologically-relevant toxicity test organism of streams.

2) It has an identifiable sub-lethal response to metals (as shown by the present study).

3) It has a short life cycle.

4) It has direct benthic development.

5) It requires minimal space and equipment.

6) It has potential as a sediment toxicity test organism.

Disadvantages of using *B. zschokkei* as a toxicity test organism are:-

1) It has relatively low sensitivity to Cu (as shown by the present study).

2) Its sub-lethal responses are still relatively time consuming to measure.

3) It is time consuming to locate animals in culture vessels using the present methodology.

4) Comparatively low *B. zschokkei* population abundances occurred at Cu concentrations in the field than indicated by responses of *B. zschokkei* exposed to Cu in the lab. suggesting the relevance of the latter may not be high.

5) There is a lack of knowledge of the additional abiotic and biotic factors influencing *B. zschokkei* in the field.

6.6 CONCLUSIONS

Stream meiofaunal communities as monitors of heavy metal contamination

The correlation between meiofaunal community structure and trace metal contamination was similar to that of the macrofaunal community. Cu was the single variable best explaining inter-site differences for both communities. Even so, the meiofaunal community highlighted differences in water chemistry that were not detected by the macrofaunal community. The combination of meiofauna, macrofauna and temporary meiofauna in a combined metazoan data set improved the discrimination of sites of different metal concentrations. The detection of the gradient in metal contamination using meiofaunal community data aggregated to the family level data may reduce the effort of processing samples.

The harpacticoid copepod, *Bryocamptus zschokkei* as a toxicity test organism of copper

Bryocamptus zschokkei shows potential as an ecologically-relevant toxicity test organism of streams for Cu, being both sensitive to Cu whilst changes in its abundance in the field reflect the differences between site community structure. However, the concentrations of Cu found to effect survival and reproduction in the laboratory were lower than the concentration that appeared to effect *B. zschokkei* populations or community structure in the field. Despite this, the harpacticoid is an ecologically-relevant toxicity test organism of streams and short generation times and direct benthic development facilitate the study of the effects of contaminants on its reproduction and development.

CHAPTER 6

General discussion and conclusions

The primary objective of this thesis was to assess the use of lotic meiofauna for monitoring metal contamination at the individual and community level of biological organisation. In this concluding chapter, the potential benefits of including stream meiofaunal communities in monitoring programmes are evaluated. Possible techniques for overcoming some of the difficulties encountered when working with this component of the stream fauna are discussed. The use of both lethal and sub-lethal responses of the harpacticoid copepod *Bryocamptus zschokkei* as indicators of Cu toxicity are also appraised as are the potential advantages of using this harpacticoid copepod as a toxicity test organism.

6.1 The potential use of meiofaunal communities to monitor metal contamination

The survey of meiofaunal communities in metal-contaminated streams presented in Chapter 3 demonstrated a clear relationship between community structure and Cu concentrations. Subsequent analyses demonstrated that the correlation between meiofaunal community structure and trace metal contamination was similar to that of the macrofaunal community (Chapter 4). Thus, the information gained from using the meiofaunal community may be of equal value to that from using macrofaunal community structure for monitoring metals and other pollutants. Although further validation work is required before meiofaunal communities could practically be used by regulatory bodies, meiofauna exhibit a number of advantages that may make their inclusion in monitoring programmes worthwhile. Firstly, more information may be gained by including the meiofauna, alongside macrofauna, when monitoring the impact of contaminants on freshwater systems. The inclusion of both macrofauna and meiofauna in environmental impact surveys has been advocated in marine systems as meiofauna have been found to provide additional information on the effects of pollutants (Austen *et al.*, 1989; Somerfield *et al.*, 1995). In the present study, there was a distinct meiofaunal community at L4 in autumn associated with high Al and low pH conditions at this site, while the absence of all meiofauna, except for

Appendix I

Metal concentrations (μg l^{-1}) for twelve sites on the tributaries of the Rivers Lynher and Seaton in spring (sp) and summer (su)1995, autumn (au) 1994 and winter (wi)1996 and the mean (x) and standard deviation (sd) of the four values (* no value)

A) Copper

	Surface						Interstitial					
	Sp	Su	Au	Wi	*x*	*sd*	Sp	Su	Au	Wi	*x*	*sd*
T1	7	0	17	16	10	*8*	6	0	20	*	9	*10*
R2	20	5	18	11	13	*7*	44	22	34	62	40	*17*
B2	13	11	26	37	22	*12*	16	3	31	47	24	*19*
S9	39	*	144	94	92	*52*	246	*	*	479	362	*165*
L1	26	23	49	62	40	*19*	156	262	94	649	290	*249*
U3	52	47	70	116	71	*32*	86	76	142	167	118	*44*
D1	104	48	112	247	128	*85*	152	92	252	242	185	*77*
U2	182	149	180	302	203	*68*	389	411	490	406	424	*45*
D3	278	235	352	307	293	*49*	562	1183	576	284	651	*379*
L4	588	573	563	602	582	*17*	101	101	618	563	346	*283*
S8	574	1205	687	478	736	*324*	1823	1787	1783	1890	1821	*50*
D5	742	566	829	928	766	*153*	1763	882	1622	878	1286	*473*

B) Zinc

	Surface						Interstitial					
	Sp	Su	Au	Wi	*x*	*sd*	Sp	Su	Au	Wi	*x*	*sd*
T1	56	68	123	156	101	*47*	97	113	271	*	160	*96*
R2	78	74	175	148	119	*50*	158	167	114	199	159	*35*
B2	134	31	48	246	115	*98*	123	40	151	134	112	*50*
S9	80	*	25	52	52	*28*	92	*	*	71	81	*15*
L1	54	46	95	163	89	*54*	172	152	277	317	229	*80*
U3	110	272	49	65	124	*102*	96	129	70	96	98	*24*
D1	45	18	84	106	63	*39*	52	24	91	239	101	*96*
U2	1121	773	742	860	874	*172*	1083	800	869	1069	955	*142*
D3	368	415	458	133	344	*145*	296	61	278	125	190	*115*
L4	209	269	420	197	274	*103*	192	58	444	173	217	*163*
S8	227	423	421	424	374	*98*	396	417	446	248	377	*88*
D5	186	119	313	250	217	*83*	201	126	329	247	226	*85*

C) Iron

	Surface						Interstitial					
	Sp	Su	Au	Wi	*x*	*sd*	Sp	Su	Au	Wi	*x*	*sd*
T1	175	151	107	193	157	*37*	136	116	140	*	131	*13*
R2	156	130	84	186	139	*43*	867	297	157	1705	757	*703*
B2	88	83	97	109	94	*11*	176	69	581	229	264	*222*
S9	76	*	59	101	79	*21*	136	*	*	128	132	*5*
L1	163	91	89	184	132	*49*	236	252	97	217	200	*70*
U3	102	77	43	337	140	*134*	176	93	86	492	212	*191*
D1	45	30	32	98	51	*32*	232	41	134	364	193	*138*
U2	286	212	304	780	396	*259*	478	127	196	1146	487	*465*
D3	136	115	58	124	108	*35*	156	201	44	50	113	*78*
L4	76	85	90	599	212	*258*	98	87	1492	1160	709	*725*
S8	68	78	86	73	76	*8*	156	114	180	119	142	*31*
D5	76	78	25	85	66	*28*	67	31	80	296	118	*120*

D) Aluminium

	Surface						Interstitial					
	Sp	Su	Au	Wi	*x*	*sd*	Sp	Su	Au	Wi	*x*	*sd*
T1	437	308	460	402	402	*67*	56	354	46	*	152	*175*
R2	102	187	103	80	118	*47*	967	460	163	1693	821	*670*
B2	236	413	169	152	243	*119*	621	569	444	677	578	*99*
S9	314	*	347	329	330	*17*	538	*	*	528	533	*7*
L1	478	466	365	520	457	*66*	569	582	43	602	449	*271*
U3	375	364	440	438	404	*40*	372	476	40	630	380	*250*
D1	286	266	280	386	305	*55*	436	327	101	890	439	*332*
U2	536	186	236	938	474	*346*	421	136	88	1229	469	*528*
D3	389	286	534	398	402	*102*	479	549	119	407	389	*189*
L4	1023	1002	697	1615	1084	*384*	1321	422	1138	1559	1110	*490*
S8	865	1280	745	683	893	*269*	1062	1740	1194	343	1085	*575*
D5	256	346	90	289	245	*110*	562	272	534	917	571	*265*

Physicochemical variables for twelve sites on the tributaries of the Rivers Lynher and Seaton in spring and summer 1995, autumn 1994 and winter 1996 and the mean (x) and standard deviation (sd) of these four values (* no value).

A) pH

	Spring	Summer	Autumn	Winter	x	*sd*
T1	6.4	6.8	6.4	7.4	**6.8**	*0.47*
R2	6.0	6.8	6.3	7.4	**6.6**	*0.61*
B2	5.2	5.8	6.0	6.8	**6.0**	*0.66*
L1	6.4	6.8	6.1	7.3	**6.7**	*0.52*
U3	5.4	5.8	5.7	6.9	**6.0**	*0.66*
S9	5.8	*	5.8	7.6	**6.4**	*1.04*
D1	6.2	6.5	6.3	7.4	**6.6**	*0.55*
U2	5.6	6.0	6.0	7.4	**6.3**	*0.79*
D3	6.0	6.8	6.3	7.1	**6.6**	*0.49*
L4	4.4	5.6	4.8	5.6	**4.8**	*0.60*
S8	5.6	5.8	5.7	7.0	**6.0**	*0.66*
D5	5.7	6.6	5.9	6.6	**6.2**	*0.47*

B) Conductivity ($\mu S\ cm^{-1}$)

	Spring	Summer	Autumn	Winter	x	*sd*
T1	148	172	186	128	**159**	*26*
R2	203	172	262	173	**203**	*42*
B2	83	62	106	69	**80**	*19*
L1	201	160	207	153	**180**	*28*
U3	117	81	140	94	**108**	*26*
S9	133	*	173	97	**134**	*38*
D1	158	126	191	130	**151**	*30*
U2	132	92	160	103	**122**	*31*
D3	113	101	116	111	**110**	*7*
L4	149	101	154	105	**127**	*28*
S8	155	122	170	117	**141**	*26*
D5	140	121	102	101	**116**	*18*

C) Hardness (mg $CaCO_3\ l^{-1}$)

	Spring	Summer	Autumn	Winter	x	*sd*
T1	28	13	35	36	**28**	*13*
R2	323	11	56	33	**106**	*22*
B2	215	34	14	23	**72**	*10*
L1	27	13	40	63	**36**	*25*
U3	12	19	23	44	**24**	*14*
S9	14	*	28	47	**29**	*13*
D1	20	38	26	43	**32**	*9*
U2	13	18	28	50	**27**	*16*
D3	29	32	26	30	**29**	*3*
L4	13	30	40	36	**30**	*5*
S8	17	15	58	32	**30**	*21*
D5	21	20	23	30	**24**	*5*

D) Discharge ($m^3\ s^{-1}$)

	Spring	Summer	Autumn	Winter	x	*sd*
T1	0.060	0.015	0.078	0.236	**0.097**	*0.096*
R2	0.010	0.000	0.017	0.008	**0.009**	*0.007*
B2	0.051	0.007	0.060	0.040	**0.040**	*0.023*
L1	0.200	0.023	0.187	0.176	**0.146**	*0.083*
U3	0.075	0.024	0.056	0.074	**0.057**	*0.024*
S9	0.000	0.000	0.011	0.021	**0.008**	*0.010*
D1	0.186	0.063	0.248	0.261	**0.189**	*0.091*
U2	0.094	0.033	0.051	0.082	**0.065**	*0.028*
D3	0.009	0.007	0.008	0.011	**0.009**	*0.002*
L4	0.012	0.000	0.007	0.015	**0.008**	*0.006*
S8	0.093	0.030	0.078	0.125	**0.082**	*0.039*
D5	0.034	0.001	0.039	0.025	**0.025**	*0.017*

Physicochemical variables for twelve sites on the tributaries of the rivers Lynher and Seaton in spring and summer 1995, autumn 1994 and winter 1996 and the mean (x) and standard deviation (sd) of the four values (* no value).

A) Temperature

	Spring	Summer	Autumn	Winter	x	*sd*
T1	11	11.1	10.6	7.9	**10.2**	*1.5*
R2	10	12.3	11.9	8.8	**10.8**	*1.6*
B2	10.6	11.5	11.3	8.6	**10.5**	*1.3*
L1	11.2	12.3	11.8	8.8	**11.0**	*1.6*
U3	10.5	12	11.1	9.2	**10.7**	*1.2*
S9	10.4	*	11.7	8.5	**10.2**	*1.6*
D1	10.6	12.3	11.8	9.2	**11.0**	*1.4*
U2	11.1	12.6	11.5	9.4	**11.2**	*1.3*
D3	11.1	12.4	11.6	9.1	**11.1**	*1.4*
L4	10.1	12.3	11.8	8.8	**10.8**	*1.6*
S8	11.4	12.6	11.7	8.5	**11.1**	*1.8*
D5	11.5	12.6	11.9	8.6	**11.2**	*1.8*

B) Interstitial DOC (mg l^{-1})

	Spring	Summer	Autumn	Winter	x	*sd*
T1	*	8.6	8.5	*	**8.5**	*0.1*
R2	7.4	3.3	5.5	7.2	**5.9**	*1.9*
B2	31.4	4.9	3.5	5.1	**11.3**	*13.5*
L1	9.0	9.0	7.5	9.0	**8.6**	*0.75*
U3	4.1	3.7	2.9	4.6	**3.8**	*0.72*
S9	5.7	*	3.8	5.6	**5.0**	*1.07*
D1	6.6	4.7	4.4	4.5	**5.1**	*1.04*
U2	7.8	2.4	2.5	4.8	**4.4**	*2.54*
D3	4.1	2.2	3.2	5.3	**3.7**	*1.32*
L4	4.1	2.6	3.0	6.0	**3.9**	*1.52*
S8	4.0	2.2	2.6	5.5	**3.5**	*1.5*
D5	3.8	2.1	2.5	4.2	**3.2**	*1.5*

C) Surface DOC (mg l^{-1})

	Spring	Summer	Autumn	Winter	x	*sd*
T1	7.6	14.6	8.8	15.2	**11.5**	*3.9*
R2	7.4	9.2	6.7	8.5	**7.9**	*1.1*
B2	15.2	11.9	4.2	16.7	**12.0**	*5.6*
L1	9.0	12.8	6.8	5.4	**8.5**	*3.2*
U3	4.1	7.0	2.8	9.6	**5.9**	*3.0*
S9	5.7	*	2.9	9.3	**5.9**	*3.2*
D1	6.1	7.2	4.0	7.3	**6.1**	*1.5*
U2	7.8	5.3	2.7	5.1	**5.2**	*2.1*
D3	3.8	5.5	3.4	4.6	**4.3**	*0.9*
L4	4.1	5.5	4.2	7.4	**5.3**	*1.5*
S8	4.0	5.3	2.4	9.5	**5.3**	*3.0*
D5	4.8	5.3	3.9	6.8	**5.2**	*1.2*

APPENDIX I

Replicate interstitial samples (I1 and I2) taken in winter. * = no value

	Copper			Zinc			Aluminium			Iron		
	I1	I2	Mean	I1	I2	Mean	I1	I2	Mean	I1	I2	Mean
R2	34	90	62	257	140	199	130	3256	1693	3233	178	1705
B2	35	59	47	170	98	134	240	1115	677	89	370	229
L1	870	427	649	114	520	317	583	620	602	293	217	255
U3	*	167	167	*	96	96	*	630	630	*	492	492
S9	479	*	479	*	71	71	*	528	528	*	128	128
U2	*	406	406	*	1069	1069	*	1229	1229	*	1146	1146
D3	263	305	284	131	120	125	415	400	407	68	31	50
L4	461	665	563	143	203	173	2693	422	1559	440	1879	1160
S8	*	1890	1890	*	248	248	*	343	343	*	119	119
D5	*	878	878	*	247	247	*	917	917	*	296	296
T1	*	*	*	*	*	*	*	*	*	*	*	*

APPENDIX II Mean abundances of meiofaunal species (m^{-2}) at ten sites on the tributaries of the Rivers Lynher and Seaton in spring 1995.

	R2	B2	L1	U3	S9	D1	U2	L4	S8	D5
Bryocamptus zschokkei	933	667	551	18	44	36	0	0	0	0
Bryocamptus praegeri	44	169	71	0	0	0	0	0	0	0
Bryocamptus pygmaeus	0	0	9	0	9	0	0	9	9	0
Paracyclops fimbriatus	80	0	0	0	18	0	0	0	0	0
Eucyclops serrulatus	0	0	0	0	9	0	0	0	98	0
Diacyclops languidoides	9	9	80	0	36	80	0	44	142	240
Diacyclops bisetosus	0	9	9	0	0	0	0	0	18	9
Speocyclops demetiensis	0	0	0	0	0	0	0	0	27	0
Graeteriella unisetiger	0	0	0	0	0	0	0	0	0	0
Alona quadrangularis	0	0	18	0	0	0	0	0	0	0
Chydoris sphaericus	0	0	0	0	0	0	0	0	0	0
Candonidae	9	18	36	0	0	0	0	0	9	0
Feltria minuta	53	98	0	9	18	0	0	0	0	0
Lobohalacarus weberi	0	9	9	0	0	0	0	0	0	0
Soldanellonyx monardi	0	0	27	0	0	0	0	0	0	0
Limnohalacarus wackeri	0	0	9	0	0	0	0	0	0	0
Porohalacarus alpinus	0	0	0	0	0	0	0	0	0	0
Nematodes	2249	329	533	62	1129	373	18	89	569	36
DENSITY	3377	1307	1351	89	1262	489	18	142	871	284
SPECIES RICHNESS	7	8	11	3	7	3	1	3	7	3

APPENDIX II

Mean abundances of meiofaunal species (m^{-2}) at ten sites on the tributaries of the River Lynher and Seaton in summer 1995.

	T1	R2	B2	L1	U3	D1	U2	D3	S8	D5
Bryocamptus zschokkei	8221	3666	5706	4000	2862	11	0	0	0	9
Bryocamptus praegeri	2222	967	98	9	0	0	0	0	0	0
Bryocamptus pygmaeus	0	0	0	0	0	22	0	0	0	0
Paracyclops fimbriatus	0	111	62	53	747	144	0	0	0	9
Eucyclops serrulatus	0	0	9	0	1093	0	329	0	356	0
Diacyclops languidoides	9	78	0	169	0	44	107	427	18	36
Diacyclops bisetosus	0	0	0	0	0	11	71	116	9	36
Speocyclops demetiensis	0	0	0	0	0	178	53	9	0	0
Graeteriella unisetiger	0	0	0	0	0	0	0	0	0	0
Acanthocyclops vernalis	0	0	0	142	2154	0	0	0	0	0
Alona quadrangularis	0	0	0	700	0	0	0	0	0	0
Chydoris sphaericus	0	0	0	0	0	0	0	0	0	0
Candonidae	27	67	302	0	0	0	9	0	0	0
Lobohalacarus weberi	0	0	0	0	0	0	0	0	0	0
Soldanellonyx monardi	0	0	0	0	0	0	0	0	0	0
Limnohalacarus wackeri	0	0	0	0	0	0	0	0	0	0
Porohalacarus alpinus	116	100	0	0	0	0	0	0	0	0
Feltria minuta	0	0	0	0	0	0	9	0	53	36
Hygrobates sp.	151	22	0	222	18	0	0	0	0	0
Torrenticola sp.	116	0	0	36	0	0	0	0	0	0
Atractides sp.	0	0	0	0	0	0	0	9	0	0
Sperchon sp.	9	0	0	0	0	0	0	0	0	0
Hydrachnellae sp A	9	11	0	0	0	0	0	9	0	0
Hydrachnellae sp B	160	0	36	196	0	0	0	0	0	0
Nematodes	551	133	36	427	320	178	36	9	71	595
DENSITY	11591	5155	6248	5953	7193	589	613	578	507	720
SPECIES RICHNESS	11	9	7	10	6	6	7	7	5	6

APPENDIX II Mean abundances of meiofaunal species (m^{-2}) at ten sites on the tributaries of the Rivers Lynher and Seaton in autumn 1994.

	R2	B2	L1	U3	S9	D1	U2	L4	S8	D5
Bryocamptus zschokkei	2444	2444	516	213	62	89	107	0	0	0
Bryocamptus praegeri	0	44	27	0	18	53	36	53	27	0
Bryocamptus pygmaeus	3680	693	36	18	27	62	18	0	0	0
Paracyclops fimbriatus	27	53	36	53	36	116	80	62	9	0
Eucyclops serrulatus	0	0	0	80	71	53	18	0	27	391
Diacyclop languidoides	9	27	27	9	71	89	44	267	160	80
Diacyclops bisetosus	0	0	0	9	0	62	0	36	0	0
Speocyclops demetiensis	0	0	27	36	0	0	124	0	0	0
Graeteriella unisetiger	0	0	9	0	0	18	0	0	0	0
Alona quadrangularis	0	0	9	0	0	18	0	0	0	0
Chydoris sphaericus	0	0	0	0	18	0	0	0	0	0
Candonidae	0	0	27	0	0	9	0	0	0	0
Feltria minuta	0	62	0	0	9	0	9	0	0	0
Lobohalacarus weberi	0	9	44	18	9	0	0	27	0	0
Soldanellonyx monardi	0	0	0	0	18	0	0	9	0	0
Limnohalacarus wackeri	0	9	27	0	0	0	0	0	0	0
Porohalacarus alpinus	0	0	0	0	0	0	0	0	62	9
Nematodes	453	453	284	187	3591	2826	284	178	400	27
DENSITY	6613	3795	1067	622	3928	3395	720	631	684	507
SPECIES RICHNESS	5	9	12	9	11	11	9	7	6	4

APPENDIX II Mean abundances of macrofaunal species (m^{-2}) at ten sites on the tributaries of the rivers Lynher and Seaton in spring 1995.

	R2	B2	S9	L1	U3	L1	U2	L4	S8	D5
Leuctra nigra	0	0	0	0	0	0	0	0	0	0
Leuctra inermis	0	0	0	18	0	0	0	0	0	0
Leuctra hippopus	0	124	0	0	0	0	0	0	0	0
Leuctra fusca	0	0	0	0	0	0	0	0	0	0
Isoperla grammatica	18	9	0	0	0	0	0	0	0	0
Amphinemura sulcollis	44	53	240	89	0	0	0	0	0	0
Nemoura erratica	0	0	0	0	0	0	0	0	0	0
Protonemura meyeri	0	9	18	0	0	0	0	0	0	0
Nemurella pictetii	0	0	0	0	0	0	0	0	0	0
Isogenus nubecula	0	0	0	0	0	0	0	0	0	0
Chloroperla torrentium	27	142	44	53	0	0	0	0	0	0
Baetis rhodani	524	240	18	542	0	27	0	0	0	0
Rhithrogena semicolorata	53	9	0	0	0	0	0	0	0	0
Hydropsyche siltalai	44	44	0	27	0	0	0	0	0	0
Hydropsyche pellucidula	0	0	0	0	0	0	0	0	0	0
Rhyacophila dorsalis	0	0	0	9	0	36	0	0	0	9
Plectrocnemia conspersa	0	0	0	0	0	0	0	0	0	0
Potomophylax cinculatus	0	9	0	44	0	0	0	0	0	0
Tinodes sp.	9	0	0	0	0	0	0	0	0	0
Phagocata vitta	0	0	9	0	0	27	9	18	27	0
Polycelis felina	160	62	0	0	18	0	0	0	0	0
Simulium ornatum	0	18	0	9	0	0	0	0	0	0
Dicranota sp.	9	18	62	36	0	18	0	9	0	0
Chironomidae	98	418	489	204	107	809	62	89	53	18
Oligochaeta	80	426	0	116	0	0	0	27	0	0
Limnius volkmari	0	0	0	0	0	0	0	0	0	0
Gyrinus sp	0	0	0	0	0	0	0	0	0	0
Pisidium obtusata	0	0	0	0	0	0	0	0	0	0
Asellus aquaticus	0	0	0	0	0	0	0	0	0	0
Notonecta sp.	9	0	0	0	0	0	0	0	0	0
DENSITY	1075	1582	880	1147	124	915	71	142	80	27
SPECIES RICHNESS	12	14	7	11	2	5	2	4	2	2

APPENDIX II Mean abundances of macrofaunal species (m^{-2}) at ten sites on the tributaries of the Rivers Lynher and Seaton in autumn 1994.

	R2	B2	S9	L1	U3	D1	U2	L4	S8	D5
Leuctra nigra	62	178	0	0	0	0	0	0	0	0
Leuctra inermis	44	169	0	0	18	0	0	0	0	0
Leuctra hippopus	53	0	9	0	0	0	0	0	0	0
Isoperla grammatica	142	649	329	9	44	0	9	0	9	0
Amphinemura sulcicollis	124	9	0	0	0	0	0	0	0	0
Nemoura erratica	267	516	0	0	151	0	0	0	0	0
Chloroperla torrentium	0	44	0	0	0	0	0	0	0	0
Protonemura meyeri	0	178	9	0	0	0	0	0	0	0
Isogenus nubecula	0	9	0	0	0	0	0	0	0	0
Leuctra fusca	0	0	0	0	0	0	0	9	0	0
Nemurella pictetii	116	0	53	9	0	80	0	0	0	0
Baetis rhodani	151	0	0	0	0	0	0	0	0	0
Rhithrogena semicolorata	160	0	0	0	0	0	0	0	0	0
Hydropsyche siltalai	0	0	0	9	0	9	0	0	0	0
Hydropsyche pellucidula	0	0	18	0	0	9	0	0	0	0
Rhyacophila dorsalis	0	0	0	0	0	0	9	0	9	0
Plectrocnemia conspersa	0	62	0	0	0	0	0	0	0	0
Potomophylax cingulatus	124	0	0	9	0	0	0	0	0	0
Tinodes sp.	0	0	36	0	36	9	18	44	187	0
Phagocata vitta	258	0	9	0	27	9	0	0	0	0
Polycelis felina	27	0	0	0	0	0	9	0	0	0
Simulium ornatum	27	0	0	0	0	0	18	9	9	0
Dicranota sp.	0	0	0	0	0	0	9	0	0	0
Culicoides sp.	18	0	53	9	9	9	0	0	0	0
Pedicia rivosa	240	0	27	0	18	9	62	9	27	18
Chironomidae	2853	382	0	27	702	0	36	231	0	0
Oligochaeta	0	0	0	18	0	0	0	0	0	0
Limnius volckmari	0	0	0	0	9	0	0	0	0	0
Gyrinus sp	9	0	0	0	0	0	0	0	0	0
Pisidium obtusata	9	0	0	0	0	0	0	0	0	0
Asellus aquaticus	0	0	0	0	0	0	0	0	0	0
Noctonecta sp.	0	0	0	0	0	0	0	0	0	0
DENSITY	4684	2195	542	89	1013	133	169	302	240	18
SPECIES RICHNESS	18	10	9	7	9	7	8	5	5	1

APPENDIX II Mean abundances of temporary meiofaunal species (m^{-2}) at ten sites on the tributaries of the Rivers Lynher and Seaton in spring.

	R2	B2	S9	L1	U3	D1	U2	L4	S8	D5
Leuctra nigra	0	0	9	9	0	9	0	0	0	0
Leuctra inermis	0	0	0	0	0	0	0	0	0	0
Leuctra hippopus	0	0	0	0	0	0	0	0	0	0
Isoperla grammatica	9	0	0	0	0	0	0	0	0	0
Amphinemura sulcicollis	89	0	9	36	0	0	0	0	0	0
Nemoura erratica	0	0	0	0	0	0	0	0	0	0
Chloroperla torrentium	0	0	0	0	0	0	0	0	0	0
Protonemura meyeri	0	0	0	0	0	0	0	0	0	0
Isogenus nubecula	0	0	0	0	0	0	0	0	0	0
Leuctra fusca	0	0	0	0	0	0	0	0	0	0
Nemurella pictetii	0	0	0	0	0	0	0	0	0	0
Baetis rhodani	44	35	18	80	18	0	0	0	0	0
Rhithrogena semicolorata	0	0	0	0	0	0	0	0	0	0
Hydropsyche siltalai	0	0	0	0	0	0	0	0	0	0
Hydropsyche pellucidula	0	0	0	0	0	0	0	0	0	0
Rhyacophila dorsalis	0	0	0	0	0	0	0	0	0	0
Plectrocnemia conspersa	0	0	0	0	0	0	0	0	0	0
Potomophylax sp	0	0	0	0	0	0	0	0	0	0
Tinodes sp.	0	0	0	0	0	0	9	0	0	0
Phagocata vitta	0	0	0	0	0	0	0	0	0	0
Polycelis felina	0	0	0	0	0	0	9	0	18	0
Simulium ornatum	18	160	9	338	18	9	18	178	0	0
Dicranota sp.	18	0	9	89	0	9	0	0	0	0
Culicoides sp.	0	0	0	0	0	0	0	0	0	0
Pedicia rivosa	0	0	0	0	0	0	0	0	0	0
Chironomidae	978	827	169	809	133	560	169	453	267	453
Oligochaeta	293	36	0	133	9	0	0	53	0	0
Limnius volkmari	0	0	0	0	0	0	0	0	0	0
Gyrinus sp	0	0	0	9	0	0	0	0	0	0
Pisidium obtusata	0	0	0	0	0	0	0	0	0	0
Asellus aquaticus	0	0	0	0	0	0	0	0	0	0
Notonecta	0	0	0	0	0	0	0	0	0	0
DENSITY	1449	1057	222	1502	178	587	204	684	284	453
SPECIES RICHNESS	7	4	6	8	4	4	4	3	2	1

APPENDIX II Mean abundances of temporary meiofaunal species (m^{-2}) at ten sites on the tributaries of the Rivers Lynher and Seaton in autumn 1994.

	R2	B2	S9	L1	U3	D1	U2	L4	S8	D5
Leuctra nigra	0	0	0	0	0	0	0	0	0	0
Leuctra inermis	0	0	0	0	0	0	0	0	0	0
Leuctra hippopus	0	0	0	0	0	0	0	0	0	0
Isoperla grammatica	0	0	0	0	0	0	0	0	0	0
Amphinemura sulcicollis	18	0	18	36	0	0	0	0	0	0
Nemoura erratica	0	0	0	0	0	53	0	0	0	9
Chloroperla torrentium	36	89	0	36	62	27	18	0	0	0
Protonemura meyeri	0	9	0	0	0	0	0	0	0	0
Isogenus nubecula	0	0	0	0	0	0	0	0	0	0
Leuctra fusca	0	0	0	0	0	0	0	0	0	0
Nemurella pictetii	0	0	0	0	0	0	0	0	0	0
Baetis rhodani	98	0	0	44	9	27	27	0	0	0
Rhithrogena semicolorata	9	0	0	0	0	0	0	0	0	0
Hydropsyche siltalai	0	0	0	0	0	0	0	0	0	0
Hydropsyche pellucidula	0	0	0	0	0	0	0	0	0	0
Rhyacophila dorsalis	9	9	0	0	0	9	0	0	0	0
Plectrocnemia conspersa	0	0	0	0	0	0	0	0	0	9
Potomophylax cingulatus	0	0	0	0	0	0	0	0	0	0
Tinodes sp.	44	0	0	18	0	0	0	0	0	0
Phagocata vitta	0	0	9	0	0	27	18	18	9	0
Polycelis felina	27	0	0	0	0	0	0	0	0	0
Simulium ornatum	18	18	18	18	0	0	0	0	9	9
Dicranota sp.	9	9	27	9	0	9	18	0	9	0
Culicoides sp.	0	0	0	0	0	0	0	0	0	0
Pedicia rivosa	0	0	0	0	0	0	0	0	0	0
Chironomidae	1564	480	151	418	98	116	267	27	53	9
Oligochaeta	1324	533	160	649	142	391	71	196	18	0
Limnius volckmari	0	0	0	0	0	0	0	0	0	0
Gyrinus sp	0	0	0	0	0	0	0	0	0	0
Pisidium obtusata	0	0	0	0	0	0	0	0	0	0
DENSITY	3155	1147	382	1227	311	658	418	240	98	36
SPECIES RICHNESS	11	7	6	8	4	8	6	3	5	4

APPENDIX III

The chemistry of the test solutions *Bryocamptus zschokkei* were exposed to in the acute toxicity tests (Measurements were taken at the end of the toxicity tests)

	R2 Cu Concentration of Test Solution (µg l^{-1})							U2	YEALM
	0	56	100	180	320	560	1000		
pH	7.4	7.4	7.4	7.3	7.3	7.4	7.3	6.3	6.4
Conductivity	159	166	170	171	171	186	193	122	59
Cu (µg l^{-1})	0	24	26	67	72	371	983	203	16
Hardness (mg l^{-1})	36	33	31	30	35	31	33	27	160
DOC(mg l^{-1})	6.92	7.04	6.70	6.33	5.05	2.80	3.85	5.2	8.01

APPENDIX IV

Results of sub-lethal tests for assessing the effect of Cu on the fecundity and development of *Bryocamptus zschokkei*

a) Fecundity at control concentration (0 $\mu g\ l^{-1}$ Cu)

	Date Paired	Date eggs.	Date of first nauplii	Brood 1 (N)	Brood 1 (C)	Brood 1 (A)	Brood 1 (Total)	Brood 2 (N)	Brood 2 (C)	Brood 2 (A)	Brood 2 (Total)	Brood 3 (N)	Brood 3 (C)	Brood 3 (A)	Brood 3 (Total)	Total (N)	Total (C)	Total (A)	Tota off-spri
1	28.04	01.05	07.05	0	0	8	8	0	30	16	46	22	53	0	75	22	83	24	106
2	29.04	05.05	12.05	0	13	1	14	0	23	0	26	16	9	0	25	16	45	1	62
3	30.04	08.05	14.05	0	2	0	2	0	12	0	12	12	7	0	19	12	21	0	33
4	01.05	05.05	10.05	0	15	6	21	0	2	0	2	0	0	0	0	0	17	6	23
5	02.05	10.05	12.05	0	16	9	25	0	34	0	34	7	1	0	8	7	51	9	67
6	03.05	10.05	20.05	0	0	5	5	0	6	0	6	15	20	0	35	15	26	5	46
7	05.05	09.05	17.05	0	2	6	8	9	44	2	55	0	0	0	0	9	46	8	63
8	05.05	08.05	17.05	0	8	4	12	0	21	0	21	8	28	0	36	8	57	4	69
9	05.05	08.05	14.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	08.05	16.05	23.05	0	12	0	12	7	33	0	40	0	0	0	0	7	45	0	52
11	02.05	06.05	17.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	04.05	08.05	20.05	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
13	05.05	09.05	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0605	14.05	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	01.05	08.05	13.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
x				0	4.6	2.6	11.9	1.07	13.67	1.2	26.6	5.33	7.87	0	22	6.4	26.1	03.8	34.8
sd				0	6.27	3.36	7.38	15.11	15.7	4.13	18.4	7.54	15.11	0	24.6	7.26	26.8	6.4	34.4

b) Fecundity at 20 µg l^{-1} Cu concentration.

	Date Paired	Date eggs.	Date of first nauplii	Brood 1 (N)	Brood 1 (C)	Brood 1 (A)	Brood 1 (Total)	Brood 2 (N)	Brood 2 (C)	Brood 2 (A)	Brood 2 (Total)	Brood 3 (N)	Brood 3 (C)	Brood 3 (A)	Brood 3 (Total)	Total (N)	Total (C)	Total (A)	Total off-spring
1	30.04	07.05	1305	0	6	7	13	0	38	0	38	11	65	0	76	11	109	7	127
2	01.05	06.05	12.05	0	13	11	21	0	12	0	12	0	3	0	3	0	28	11	39
3	01.05	08.05	14.05	0	12	0	12	0	15	0	15	4	8	0	12	4	35	0	39
4	03.05	10.05	14.05	0	10	0	10	7	10	0	17	0	0	0	0	7	20	0	27
5	05.05	06.05	19.05	0	26	0	26	0	28	0	28	0	0	0	0	0	54	0	54
6	05.05	12.05	28.05	0	20	0	20	1	3	0	4	0	0	0	0	1	23	0	24
7	05.05	10.05	17.05	0	16	2	18	0	23	0	23	11	5	0	16	11	44	2	57
8	06.05	04.05	28.05	0	16	0	16	0	9	0	9	0	0	0	0	0	25	0	25
9	06.05	07.05	13.05	0	30	0	30	0	6	0	6	0	0	0	0	0	36	0	36
10	06.05	02.05	19.05	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
11	07.05	05.05	21.05	0	0	0	0	10	4	0	14	0	0	0	0	10	4	0	14
12	08.05	04.05	21.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	30.04			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	02.05	09.05	21.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	02.05	10.05	13.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
x				**0**	**11.07**	**1.33**	**15.4**	**1.2**	**9.87**	**0**	**15.1**	**1.73**	**5.4**	**0**	**9.73**	**3.43**	**28.2**	**1.33**	**29.5**
sd				**0**	**9.84**	**3.24**	**9.74**	**3.03**	**11.7**	**0**	**11**	**3.9**	**16.7**	**0**	**22.4**	**4.47**	**28.6**	**3.24**	**33.5**

c) Fecundity at 50 µg l^{-1} Cu concentration

	Date Paired	Date of Eggs	Date First Nauplii	Brood 1 (N)	Brood 1 (C)	Brood 1 (A)	Brood 1 (Total)	Brood 2 (N)	Brood 2 (C)	Brood 2 (A)	Brood 2 (Total)	Brood 3 (N)	Brood 3 (C)	Brood 3 (A)	Brood 3 (Total)	Total (N)	Total (C)	Total (A)	Total Off-spring
1	30.04	03.05	19.05	0	7	0	7	0	9	0	9	0	2	0	2	0	18	0	18
2	30.04	03.05	14.05	0	10	0	10	0	0	0	0	0	1	0	1	0	11	0	11
3	30.04	05.05	12.05	0	5	10	15	0	25	0	25	0	0	0	0	0	30	10	40
4	02.05	09.05	17.05	0	12	3	15	0	47	0	47	0	0	0	0	0	59	3	62
5	05.05	14.05	19.05	0	0	0	0	0	14	8	22	12	5	0	17	12	19	8	39
6	05.05	08.05	14.05	0	11	0	11	0	15	0	15	8	13	0	21	8	39	0	47
7	05.05	10.05	16.05	0	32	0	32	0	13	0	13	0	0	0	0	0	45	0	45
8	05.05	13.05	19.05	0	1	0	1	0	23	0	23	0	0	0	0	0	24	0	24
9	06.05	12.05	23.05	0	4	0	4	3	11	0	14	0	0	0	0	3	15	0	18
10	06.05	12.05	19.05	0	2	0	2	10	16	0	26	0	0	0	0	10	18	0	28
11	29.04	05.05	*	0	32	2	34	0	0	0	0	0	0	0	0	0	32	2	34
12	29.04	03.05	12.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	07.05	19.05	28.05	0	12	0	12	1	0	0	1	0	0	0	0	1	12	0	13
14	30.04	*	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	06.05	10.05	23.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
x				0	8.53	1	11.9	0.93	11.53	0.533	16.25	1.33	1.4	0	3.42	22.43	23.22	1.64	25.3
sd				0	10.6	2.65	11	2.63	13.1	2.07	13.6	3.60	3.48	0	7.36	4.2	16.7	3.27	19.0

d) Fecundity at 100 µg l^{-1} Cu concentration

	Date Paired	Date First Eggs	Date First Nauplii	Brood 1 (N)	Brood 1 (C)	Brood 1 (A)	Brood 1 (Total)	Brood 2 (N)	Brood 2 (C)	Brood 2 (A)	Brood 2 (Total)	Brood 3 (N)	Brood 3 (C)	Brood 3 (A)	Brood 3 (Total)	Total (N)	Total (C)	Total (A)	Total Off-spring
1	30.04	09.05	17.05	0	12	0	12	0	19	0	19	0	3	0	3	0	34	0	34
2	01.05	01.05	19.05	0	4	4	8	0	15	0	15	2	4	0	6	2	23	4	29
3	02.05	06.05	13.05	0	4	0	4	0	5	0	5	0	0	0	0	0	9	0	9
4	05.05	09.05	14.05	0	14	0	14	0	6	0	6	0	0	0	0	0	20	0	20
5	05.05	10.05	17.05	0	3	0	3	0	21	0	21	0	0	0	0	0	24	0	24
6	07.05	12.05	19.05	0	2	1	3	0	2	0	2	10	3	0	13	10	7	1	18
7	06.05	12.05	20.05	0	7	0	7	2	3	0	5	0	0	0	0	2	10	0	12
8	02.05	09.05	15.05	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1
9	05.05	08.05	25.05	0	11	0	11	0	0	0	0	0	0	0	0	0	11	0	11
10	08.05	08.05	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	07.05	12.05	19.05	0	3	0	3	0	2	0	2	10	3	0	13	10	8	0	18
12	07.05	09.05	16.05	0	4	0	4	0	4	2	6	1	1	0	2	1	9	2	12
13	29.04	07.05	10.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	30.04	02.05	07.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	30.04	09.05	12.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
x				0	5.82	0.45	6.27	0.18	7	0.18	7.36	3.45	7.6	0	3.45	2.08	13	0.59	15.6
sd				0	4.56	1.21	4.47	0.603	7.63	0.603	7.49	3	7.2	0	5.08	3.78	10.1	1.24	10.3

Percentage survival and development time of a) stage one nauplii (n1) to stage one copepodids (C1) and b) stage one copepodids (C1) to the reproductive adult stage

a)

0 µg l^{-1}		20 µg l^{-1}		50 µg l^{-1}		100 µg l^{-1}	
% Survival	Development Time (C1-A)	% Survival	Development Time (C1-A)	% Survival	Development Time (C1-A)	% Survival	Development Time (C1-A)
90	12	90	13	50	7	60	8
40	11	70	9	30	11	40	7
10	4	60	16	50	10	30	15
30	6	40	9	50	3	60	17
60	14	30	9	40	9	10	2
80	7	60	7	40	7	20	6
		90	12	80	10		
		40	7				
x = 51.7	x = 9	x = 60	x = 10.25	x = 48.6	x = 8.14	x = 36.7	x = 9.17
sd = 30.6	sd = 3.9	sd = 22.7	sd = 3.15	sd = 15.7	sd = 2.73	sd = 20.7	sd = 5.71

b)

0 µg l^{-1}		20 µg l^{-1}		50 µg l^{-1}		100 µg l^{-1}	
% Survival	Development Time (C1-A)	% Survival	Development Time (C1-A)	% Survival	Development Time (C1-A)	% Survival	Development Time (C1-A)
90	18	80	23	40	14	90	22
80	18	80	23	70	19	40	19
60	21	80	23	60	18	70	24
30	18	10	18	30	19	40	14
70	18	60	18	70	19	70	14
50	16	70	18	40	13	100	20
40	20	80	13			90	13
40	20					70	21
						50	16
x = 57.5	x = 18.6	x = 65.7	x = 19.4	x = 51.7	x =17	x = 68.9	x = 18.1
sd = 21.2	sd = 1.6	sd = 25.7	sd = 3.78	sd = 17.2	sd = 2.76	sd = 22.0	sd = 3.98

TAXONOMIC KEYS USED FOR IDENTIFICATION OF MACROFAUNA AND MEIOFAUNA

Croft, P.S. (1986). A key to the major groups of British freshwater invertebrates. *Field Studies* **6,** 531-379.

Edington, J.M. and Hildrew, A.G. (1981). *Caseless caddis larvae of the British Isles.* Freshwater Biological Association Scientific Publication No. 43.

Fitter, R. and Manuel, R. (1995). *Collins photo guide to lakes, rivers, streams and ponds of Britain and North-West Europe.* HarperCollins, Hong Kong.

Green & Macquety (1987). *Halacarid mites.* Synopses of the British Fauna No.36.

Gurney, R. (1933). *British Freshwater Copepoda. Vols I, II, III:* Ray Society, London.

Hynes, H.B.N. (1977). *A key to the nymphs of the British stoneflies (Plecoptera). with notes on their ecology and distribution.* Freshwater Biological Association Scientific Publications No. 17.

Macan. T.T. (1979). *A key to the nymphs of the British species of Ephemeroptera with notes on their ecology.* Freshwater Biological Association Scientific Publications No. 20.

Reynoldson, T.B. (1978). *A key to the British species of freshwater triclads (Turbellaria, Paludicola).* Freshwater Biological Association Scientific Publications No. 23.

Viets, K. (1936). *Die Tierwelt Deutschlands und der Angrenzenden Meeresteile Spinnentiere oder Arachnridea VII : Wassermillen oder Hydracharina.* Jener G. Fischer.

Wallace, I.D., Wallace, B. and Philipson, G.N. (1990). *A key to the case bearing caddis larvae of Britain and Ireland.* Freshwater Biological Association Scientific Publications No. 51.

REFERENCES

Abel, P.D. (1996). *Water Pollution Biology.* Taylor & Francis Ltd.

Abel, T.H. and Barlocher, F. (1984). Effects of cadmium on aquatic hyphomycetes. *Applied and Environmental Microbiology* **48,** 245-251.

Allan, J.D., (1995). *Stream ecology: Structure and function of running waters.* Chapman & Hall.

Allan, J.D. and Daniels, R.E. (1982). Life table evaluation of chronic exposure of *Eurvemora affinis* (Copepoda) to kepone. *Marine Biology* **66,** 179-184.

Antonietti, R. and Sartore, F. (1996). Optimisation of parameters used for freshwater survey. *Water Research* **30,** 1535-1538.

Armitage, P.D. Moss, D., Wright, J.F. and Furse, M.T. (1983). The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water Research* **17,** 333-347.

Austen, M.C., McEvoy, A.J., Warwick, R.M. (1994). The specificity of meiobenthic community responses to different pollutants: results from mesocosm experiments. *Marine Pollution Bulletin* **28,** 557-563.

Austen, M.C. Warwick, R.M. and Rosado, M.C. (1989). Meiobenthic and macrobenthic community structure along a putative pollution gradient in southern Portugal *Marine Pollution Bulletin* **20,** 398-404

Baird, D.J., Barber, I., Bradley, M., Calow, P. and Amadeu, M.V.M. (1989). The Daphnia bioassay: a critique. *Hydrobiologia* **188/189,** 403-406.

Barton, D.R. (1992). A comparison of sampling techniques and summary indices for assessment of water quality in the Yamaska River, Quebec, based on benthic macroinvertebrates. *Environmental Monitoring Association* **21,** 225-244.

Baudouin, M.F. and Scoppa, P. (1974). Acute toxicity of various metals to freshwater zooplankton. *Bulletin of Environmental Contamination and Toxicology* **12,** 745-751.

Bechmann, R.K. (1994). Use of life tables and LC50 tests to evaluate chronic and acute toxicity effects of Cu on the marine copepod *Tisbe furcata* (Baird). *Environmental Toxicology and Chemistry* **13,** 1509-1517.

Beckett, D.C. (1978). Ordination of macroinvertebrate communities in a multistressed river system. *In Energy and Environmental Stress US Department of Energy* (Thorp, J.H. and Gibbons, J.W., eds.), pp 748-770. CONFS-771114, NTIS, US Department of Commerce, Springfield, Virginia.

Bengtsson, B.E. (1978). Use of harpacticoid copepod in toxicity tests. *Marine Pollution Bulletin* **9,** 238-241.

Bengtsson, B.E. and Bergstrom, B. (1987). A flow through fecundity test with *Nitocra spinipes* (Harpacticoida, Crustacea) for aquatic toxicity. *Ecotoxicology and Environmental Safety* **14,** 260-268.

Bernard, F.S. and Lane, C.E. (1961). Absorption and excretion of Cu ions during settlement and metamorphosis of the barnacle, *Balanus amphitrire*. Biol. Bull. **121,** 438-448.

Birge, W.J. and Black, J.A. (1985). Research needs for rapid assessments of chronic toxicity. In *Aquatic Toxicology and Hazard Assessment: Eighth Symposium, ASTM STP 891* (Bahner, R.C. and Hansen, D.J., eds.), pp 51-60. American Society for Testing and Materials, Philadelphia.

Bongers, T. and Van der Haar, J. (1990). On the potential of basing an ecological typology of aquatic sediments on the nematode fauna: An example from the River Rhine. *Hydrobiol. Bull.* **24,** 37-45.

Borchadt, M.A. and Bott, T.L. (1995). Meiofaunal grazing of bacteria and algae in a Piedmont stream. *Journal of North American Benthological Society* **14,** 278-298.

Borgman, U. (1980). Interactive effects of metals in mixtures on biomass production kinetics of freshwater copepods. *Canadian Journal of Fisheries and Aquatic Science,* **37,** 1295-1302.

Borgman, U. (1983). Metal speciation and toxicity of free metal ions to the aquatic biota. In *Aquatic Toxicology* ed. Nriagu, J. , John Wiley & Sons.

Bott, T.L. & Kaplan, L.A. (1989). Densities of benthic protozoa and nematodes in a Piedmont stream. *Journal of North American Benthological Society* **8,** (2) 187-196.

Boucher, G. (1980). Impact of Amoco Cadiz oil spill on intertidal and sublittoral meiofauna. *Marine Pollution Bulletin* **11,** 95-100.

Brand, G.W., Fabris, G.J. and Arnott, G.H. (1986). Reduction of population growth in *Tisbe holothuriae* Humes (Copepoda: Harpacticoida) exposed to low cadmium concentrations. *Australian Journal of Marine and Freshwater Research* **37,** 475-479.

Bray, J.R. and Curtis, J.T. (1957). An ordination of the upland forest communities of Southern Wisconsin. *Ecol. Monogr.* **27,** 325-349.

Brkovic-Popvic, I. and Popovic, M. (1977). Effects of heavy metals on survival and respiration rate of tubificid worms. Part 1: Effects on survival. *Environmental Pollution* **13,** 65-71.

Brown, B.E. (1977a). Observations on the tolerance of the isopod *Asellus meridanus* Rac. To copper and lead *Water Research* **10,** 555-559.

Brown, B.E. (1977b). Effects of mine drainage on the River Hayle, Cornwall. A) Factors affecting concentrations of copper, zinc and iron in water, sediments and dominant invertebrate fauna. *Hydrobiologia* **52,** 221-233.

Bryan, G.W. (1974). Adaptation of an estuarine polychaete to sediments containing high concentrations of heavy metals. I. General observations and adaptation to Cu. *Journal of Marine Biological Association U.K.* **51,** 845-863.

Bryan, G.W. and Gibbs, P.E. (1983). Heavy metals in the Fal Estuary Cornwall: A study of long term contamination by mining waste and its effects on estuarine organisms. *Marine Biological Association U.K. Occasional Publication No. 2, 111pp.*

Bryan, G.W. and Hummerstone, L.G. (1971). Adaptation of polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of heavy metals. I. General observations and adaptation to Cu. *Journal of Marine Biological Association ,U.K.* **51,** 845-863.

Buikema, A.L. Jr. and Voshell, J.R. Jr. (1993). Toxicity studies using freshwater benthic macroinvertebrates. In *Freshwater biomonitoring and benthic macroinvertebrates.* (Rosenberg, D.M. & Resh, V.H., Eds.). pp.1-9 Chapman & Hall.

Burton, T.M., Standforf, R.M. and Allan, J.W. (1985). Acidification effects on stream biota and organic matter processing. *Canadian Journal of Fisheries and Aquatic Science* **42,** 669-675.

Cairns, J., Albaugh, D.W. Busey, F. and Chancy, M.D. (1968). The sequential comparison index - a simplified method for non biologists to estimate relative differences in biological diversity in stream pollution studies. *J. Wat Pollut. Control. Fed.* **40,** 1607-1613.

Cairns, J., Jr. (1986). The myth of the most sensitive species. *Bioscience* **36,** 670-672.

Cairns, J., Jr, and Pratt, J.R., (1989) The scientific basis of bioassays. *Hydrobiologia* **188/189,** 5-20.

Calow, P. (1989). The choice and implementation of environmental bioassays. *Hydrobiologia* **188/189,** 61-64.

Camargo, J.A. (1993). Macroinvertebrate surveys as a valuable tool for assessing freshwater quality in the Iberian Peninsula. *Envir. Monit, Assn.* **24,** 71-90.

Campbell, P.G.C. and Stokes, P.M. (1985). Acidification and toxicity of metals to aquatic biota. *Canadian Journal of Fisheries and Aquatic Sciences* **42,** 2034-2049.

Cao, Y., Bark, A.W. and Williams, W.P. (1996). Measuring the response of macroinvertebrate communities to water pollution: a comparison of multivariate approaches, biotic and diversity indices. *Hydrobiologia* **341,** 1-19.

Carpenter, J., Odum, W.E. and Mills, A. (1983). Leaf litter decomposition in a reservoir affected by acid mine drainage. *Oikos* **41,** 165-172.

Chandler, J.R. (1970). A biological approach to water quality management. *Water Pollution Control* **69,** 415-422.

Chapman, P.M. (1987). Oligochaete respiration as a measure of sediment toxicity in Puget Sound. *Hydrobiologia* **155,** 249-258.

Clarke, K.R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18,** 117-143.

Clarke, K.R. and Ainsworth, M. (1993). A method of linking multivariate analyses of changes in community structure to environmental variables. *Marine Ecological Progress Series* **92,** 205-219.

Clarke, K.R. and Green, R.H. (1988). Statistical design and analysis for a 'biological effects' study. *Marine Ecological Progress Series* **46,** 213-226.

Clarke, K.R. and Warwick, R.M. (1994a). Changes in marine communities - an approach to statistical analysis and interpretation. *Plymouth Marine Laboratory, Natural Environmental Research Council.*

Clarke, K.R. and Warwick, R.M. (1994b). Similarity-based testing for community pattern: the 2-way layout with no replication. *Marine Biology* **118,** 167-176.

Clements, W.H. (1994). Benthic invertebrate community response to heavy metals in the Upper Arkansas River Basin, Colorado. *Journal of North American Benthological Society* **13,** 1 30-44.

Clements, W.H., Cherry, D.S. and Cairns, J. Jr. (1988). Impact of heavy metals on insect communities in streams: a comparison of observational and experimental results *Canadian Journal Fisheries and Aquatic Science* **45,** 2017-2025.

Clements, W.H., Cherry, D.S. and Cairns, J. Jr. (1989). The influence of copper exposure on predator-prey interactions in aquatic insect communities. *Freshwater Biology* **21,** 483-488.

Clements, W.H., Cherry, D.S. and Van Hassel, J.H. (1992). Assessment of the impact of heavy metals on benthic communities at the Clinch River (Virginia): evaluation of an index of community sensitivity. *Canadian Journal of Fisheries and Aquatic Science* **49,** 1686-1694.

Coull, B.C. and Chandler, G.T. (1992). Pollution & meiofauna field, laboratory & mesocosm studies. *Oceanography & Marine Biology Annual Review* **30,** 191-271.

Coull, B.C., Hicks, G.R.F. and Wells, J.B.J. (1981). Nematode/copepod ratios for monitoring pollution: a rebuttal. *Marine Pollution Bulletin* **12,** 378-381.

Cusimano, R.F., Brakke, D.F. and Chapman, G.A. (1986). Effects of pH on the toxicities of cadmium, copper and zinc to Steelhead trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* **43,** 1497-1503.

D'Agostino, A and Finney, C. (1974). The effect of Cu and cadmium on the development of *Tigriopus japonicus*. In *Pollution and Physiology of Marine Organisms*, (Vernberg, F.J and Vernberg, W.B., eds), pp 445-463. Academic Press, New York, NY.

Dalla Venezia, L., Fossato, V.U. and Scarfi, S. (1980). Characteristics of suspensions of PCB Aroclor 1254 and Corexit 7664 and their short- and long-term effects on *Tisbe bulbisetosa*. *V^es Journees D Etude Pollutions*, 613-620.

Daniel, W.W. (1990). *Applied Non-Parametric Statistics.* PWS-Kent Publishing Company, Boston.

Daniels, R.E. and Allan, J.D. (1981). Life table evaluation of chronic exposure to a pesticide. *Canadian Journal of Fisheries and Aquatic Sciences* **38,** 485-494.

Darlington, S.T. (1987). The impact of copper in a contaminated stream with particular reference to *Plectronemia conspersa* (Curtis) (Trichoptera). *Ph.D. Thesis., University of Plymouth, UK.*

Dauvin, J.C. (1984). Dynamique d'ecosystemes macrobenthiques des fondes sedimentaires de la baie de Morlaix et leur perturbation par les hydrocarbures de L'Amoco Cadiz. *Phd thesis , Pierre et Marie Curie University.*

Devore, J. and Peck, R. (1990). *Introductory Statistics.* West Publishing Company, St Paul, MN, USA.

Diamond, J.M., Winchester, D.G., Mackler, D.G. and Gruber, D. (1992). Use of the mayfly *Stenonema modestum* (Heptageniidae) in subacute toxicity assessments. *Environmental Toxicology and Chemistry* **11,** 415-425.

Dines H.G., 1956. The Metalliferous Mining Region of South-West England. *Vols 1 and 2 HMSO, London 795 pp.*

Duddridge, J.E. and Wainwright, M. (1980). Heavy metal accumulation by aquatic fungi and reduction in viability of *Gammarus pulex* fed Cd $^{2+}$ contaminated mycelium. *Water Research* **14,** 1605-1611.

Dussart, B. (1969). *Les copépodes des eaux continentales d'europe occidentale.* N. boubée & Cie, Paris.

Egglishaw, H.J. and Morgan, N.C. (1965). A survey of the bottom fauna of streams in the Scottish highlands. Part II. The relationship of the fauna to the chemical and geological conditions. *Hydrobiologia* **26,** 173-183.

Fitter, R. and Manuel, R. (1995). *Collins photo guide to lakes , rivers, streams and ponds of Britain and North-West Europe.* HarperCollins, Hong Kong.

Forbes, V.E. and Depledge, M.H. (1992). Predicting population response to pollutants: the significance of sex. *Functional Ecology* **6,** 376-381.

Forbes, V.E. and Forbes, T.L. (1994). *Ecotoxicology in Theory and Practice.* Chapman and Hall, London, 247 pp.

Fitter, R. and Manuel, R. (1995). *Collins photo guide to lakes , rivers, streams and ponds of Britain and North-West Europe.* HarperCollins, Hong Kong.

Fryer, G. (1980). Acidity and species diversity in freshwater crustaceans faunas. *Freshwater Biology* **10,** 42-46.

Fryer, G. (1993). Variation in acid tolerance of certain crustaceans in different natural waters. *Hydrobiologia* **250,** 119-125.

Furse, M.T., Moss, D., Wright, J.F. & Armitage, P.D. (1984). The influence of seasonal and taxonomic factors on the ordination and classification of running-water sites in Great Britain and on the prediction of their macro-invertebrate communities. *Freshwater Biology* **14,** 257-280.

Furse, M.T., Wright, J.F., Armitage, P.D. and Moss, D. (1981). An appraisal of pond-net samples for biological monitoring of lotic macro-invertebrate communities. *Water Research* **15,** 679-689.

Gauss, J.D., Woods, P.E., Winner, R.W. and Skillings, J.H. (1985). Acute toxicity of copper to three life stages of *Chironomus tentans* as affected by water hardness - alkalinity. *Environmental Pollution Series A* **37,** 149-157.

Gentile, J.H., Gentile, S.M., Hairston, N.G. Jr. and Sullivan, B.K. (1982). The use of life tables for evaluating the chronic toxicity of pollutants to *Mysidopsis bahia*. *Hydrobiologia* **93,** 179-187.

Gerhardt, A. (1993). Review of the impact of heavy metals on stream invertebrates with special emphasis on acid conditions. *Water Air & Soil Pollution* **66,** 289-314.

Giere, O. (1993) *Meiobenthology - the microscope fauna in aquatic sediments.* Springer Verlag.

Giere, O, Eleftheriou, A. and Murison, D.J. Abiotic factors. In *Introduction to the study of Meiofauna* (Higgins, R.P. and Thiel, H. (Eds), pp 71-78. Smithsonian Institution Press, Washington, DC.

Gledhill, T. (1985). Water mites - predators and parasites. Annual Report of the Freshwater Biological Association **53,** 45-59.

Gower, A.M., Myers, G, Kent, M. and Foulkes, M.E. (1994). Relationship between the macroinvertebrate community & environmental variables in metal contaminated streams in SW England. *Freshwater Biology* **32,** 199-221.

Gray, J.S., (1989). Do bioassays adequately predict ecological effects of pollutants? *Hydrobiologia* **188/189,** 397-402

Green & Macquety (1987). *Halacarid mites.* Synopses of the British Fauna No.36.

Gurney, R. (1933). *British Freshwater Copepoda. Vols I, II, III*: Ray Society, London.

Hall, R.J., Driscoll, C.T. and Likens, G.E. (1987). Importance of hydrogen ions and aluminium in regulating the structure and function of stream ecosystems: an experimental test. *Freshwater Biology* **18,** 17-43.

Ham, S. (1982). The Crustacea of some chalk streams in southern England. *Hydrobiologia* **97,** 193-201.

Heip, C. (1980). Meiobenthos as a tool in the assessment of marine environmental quality. *Rapp P-V Reun Cons. Int. Explr. Mer* **179,** 182-187.

Hicks, G.R.F. (1991) Monitoring with meiofauna: a compelling option for evaluating environmental stress in tidal inlets. *Water. Qual. Centre Publ.* **21,** 387-391.

Hildrew, A.G. and Giller, P.S. (1995). Patchiness, species interactions and disturbance in the stream benthos. *Aquatic Ecology, Scale, Pattern and Process* (Ed. By P.S. Giller, A.G. Hildrew & Raffaelli, D.G.), pp. 21-62. Blackwell Sciences Ltd.

Hildrew, A. G. And Townsend, C.R. and Francis, J.E. (1984). Community structure in some southern English streams: the influence of species interactions. Freshwater Biology **14,** 297-310.

Hill, M.O. (1979). *TWINSPAN-A FORTRAN program for arranging multivariate data in an ordered two-way table by classification of the individuals and the attributes.* Ecology & Systematics, Cornell University, Ithaca, New York 90 pp.

Hockin, D.C. (1983). The effects of organic enrichment upon a community of meiobenthic harpacticoid copepods. *Marine Environmental Research* **10,** 45-58.

Hodda, M. And Nicholas, W.L. (1986). Nematode diversity and industrial pollution in the Hunter River estuary. *Marine Pollution Bulletin* **17,** 251-254.

Hoppenheit, M. (1977). On the dynamics of exploited populations of *Tisbe holothuriae* (Copepoda, Harpacticoida) V. The toxicity of cadmium: response to sublethal exposure. *Helgolander wissenschftliche Meeresuntersuchungen* **29,** 503-523.

Hoppenheit, M. And Sperling, K.R. (1977). On the dynamics of exploited populations of *Tisbe holothuriae* (Copepoda, Harpacticoida) IV. The toxicity of cadmium:response to lethal exposure. *Helgolander wissenschaftliche Meeresuchungen* **29,** 328-336.

Howarth, R.S. and Sprague, J.B., (1978). Copper lethality to rainbow trout in waters of various hardness and pH. *Water Research* **12,** 455-462.

Hummon, W.D., Evans, W.A., Hummon, M.R., Doherty, F.G., Wainberg, R.H. and Stanley, W.S. (1989). Meiofaunal abundance in sandbars of acid mine polluted, reclaimed, and unpolluted streams in south-eastern Ohio. In *Energy and environmental stress in aquatic ecosystems.* H.H.(Thorp, H.H. and Gibbons, J.W., eds.). pp 188-203. DOE Symposium Series (CONF-771114), Nat. Tech. Info. Service, Springfield.

Huston. M. (1979). A general hypothesis of species diversity. *The American Naturalist* **113,** 81-97.

Hutchinson, T.H., Williams, T.D. and Eales, G.J. (1994). Toxicity of cadmium, hexavalent chromium and copper to marine fish larvae (*Cyprinodon variegatus*) and copepods (*Tisbe battagliai*). *Marine Environmental Research* **38,** 275-290.

Huys, R., Herman, P.M.J., Heip, C.H.R. and Soetaert, K. (1992). The meiobenthos of the North Sea: density, biomass trends and distribution of copepod communities. *ICES J. Mar. Sci.* 49, 23-44.

Hynes, H.B.N. (1970). *The biology of polluted waters.* 202 pp. Liverpool University Press, Liverpool.

International Organization for Standardization (ISO) (1979). *Assessment of the biological quality of rivers by a macroinvertebrate 'score'.* ISO/TC 147/SC5/WG 6 N 5, 18p.

Janssen, C.R., Ferrando Rodrigo, M/D. And Persoone, G. (1993). Ecotoxicological studies with the freshwater rotifer *Brachionus calyciflorus* I. Conceptual framework and applications. *Hydrobiologia* **255/256,** 21-32.

Jennett, J.C. and Foil, J.L. (1979). Trace metal transport from mining, milling and smelting watersheds. *Journal of the Water Pollution Control Federation* **51,** 378-404.

Jin, H.J., Zhang, Y.M. and Yang, R, (1991). Toxicity and distribution of copper in an aquatic microcosm under different alkalinity and hardness. *Chemosphere* **22,** 577-596.

Johnson, C.A. and Thornton, I., (1987). Hydrological and chemical factors controlling the concentrations of Fe, Cu, Zn and As in a river system contaminated with acid mine drainage. *Water Research* **21,** 359-65.

Joshi, H.S.K., Shishodia, S.N., Kumar, D.K., Saikia, D.K., Nasriyal, B.P., Mathur, R.P., Pande, P.K., Mathur, B.S. and Puri, N. (1995). Ecosystem studies on upper region of Ganga river, India. *Envrir. Monit. Assn.* **35,** 181-206.

Judy, R.D. (1979). The acute toxicity of Cu to *Gammarus fasciatus*, a freshwater amphipod. *Bulletin of Environmental Contamination and Toxicology* **21,** 219-224.

Kansanen, (1981). Effects of heavy pollution on the zoobenthos in Lake Vanajavesi, southern Finland, with special reference to the meiozoobenthos. *Ann. Zool. Fennici* **18,** 243-251.

Keller, M. (1985). Distribution quantitative de la meiofaune dans l'aire d'epandage de l'agglomeration marseillaise sur les populations meiobenthiques. *C. R. Acad. Sci., Ser III,* **229,** 756-768.

Kelly, M., (1988). *Mining and the freshwater environment.* Elsevier Applied Science London and New York.

Kiffney, P.M. and Clements, W.H. (1994) Effects of heavy metals on macroinvertebrate assemblage from a Rocky Mountain stream in experimental microcosms. *Journal of North American Benthological Society* **13,** 4 511-523.

Klerks P.L. and Weis, J.S. (1987). Genetic adaptation of heavy metals in aquatic organism: a review. *Environmental Pollution* **45,** 173-205.

Koivisto, S. And Ketola, M. (1995). Effects of Cu on life-history traits of *Daphnia pulex* and *Bosmina logirostris. Aquatic Toxicology* **32,** 255-269.

Koivisto, S., Ketola, M. and Walls, M. (1992). Comparison of five cladoceran species in short- and long term copper exposure. *Hydrobiologia* **248,** 125-136.

Kontogiannis, J.E. and Barnett, C.J. (1973). The effect of oil pollution on survival of the tidal pool copepod *Tigriopus californicus*. *Environmental Pollution* **4**, 69-79.

Kullberg, A. (1994). Mobilisation and transport of dissolved organic matter and its biological effects in acidified, humic freshwaters. *Ph.D. Thesis, Lund University, Sweden.*

Kruskal, J.B. and Wish, M. (1978). *Multidimensional scaling.* Sage Publications, Beverley Hills, California.

Lambshead, P.J.D. (1984). The nematode/copepod ratio. Some anomalous results from the Firth of Clyde. *Marine Pollution Bulletin* **15**, 256-259.

Lancaster, J. and Hildrew, A.G. (1993). Characterising in stream flow refugia. *Canadian Journal of Fisheries and Aquatic Science* **50**, 1663-1675.

Lancaster, J. and Robertson, A.L., (1995). Benthic microcrustacea in lotic food webs. *Freshwater Biology* **34**, 123-134.

LaPoint, T.W., Melancon, S.M. and Morris, M.K. (1984). Relationships among observed metal concentrations, criteria & benthic community structural responses in 15 streams. *Journal Water Pollution Control Federation* **9**, 1031-1039.

LeBlanc, G.A. (1985). Effects of Cu on the competitive interactions of two species of Cladocera.. *Environmental Pollution Series A*, **27**, 309-322.

Le Dean, L. And Devineau, J. (1987). In search of standardisation: A comparison of toxicity bioassays on two marine crustaceans (*Palaemon serratus* and *Tigriopus brevicornis). Rev. Trav. Peches marit* **49**, 187-198.

Leland, H.V. and Carter, J.L. (1985). Effects of copper on production of periphyton, nitrogen fixation and processing of leaf litter in a Sierra Nevada, California stream. *Freshwater Biology* **15**, 155-173.

Leland, H.V., Fend, S.V., Dudley, T.L. and Carter, J.L. (1989). Effects of copper on species composition of benthic insects in a Sierra Nevada, California, stream. *Freshwater Biology* **21**, 163-179.

Lloyd, R. (1972). Problems in determining water quality criteria for freshwater fisheries. *Proc. R. Soc. Lond.* **180**, 429-449.

Lotufo, G.R. (1997). Toxicity of sediment-associated PAHs to an estuarine copepod: effects on survival feeding, reproduction and behaviour. *Marine Environmental Research* **47**, 149-167.

Mackay, D.W., Holmes, P.J. and Redshaw, C.J. (1989). The application of bioassay techniques to water polution problems - The United Kingdom experience. *Hydrobiologia* **188/189**, 77-86.

Macek, K.J. and Sleight, B.H. (1977). Utility of toxicity tests with embryos and fry of fish in evaluating hazards associated with the chronic toxicity of chemicals to fishes. In: *Aquatic Toxicology and Hazard Evaluation. Proceedings of the First International Symposium on Aquatic Toxicology and Hazard Evaluation. Proceedings of the First International*

Symposium on Aquatic Toxicology. American Society for Testing and Materials, Philidelphia, STP No. 634.

McIntosh, A.W. and Keveren, N.R. (1974). Toxicity of Cu to zooplankton. *Journal of Environmental Quality* **3,** 166-170.

Mckim, J.M. (1977). Evaluation of tests with early life stages of fish for predicting long-term toxicity. *J. Fish. Res. Bd. Can.* **34,** 1148-1154.

McMurtry, M.J. (1984). Avoidance of sublethal doses of copper and zinc by tubificid oligochaetes. *Journal of Great Lakes Research* **10,** 267-272.

Maltby, L. and Booth, R. (1991). The effect of coal-mine effluent on fungal assemblages and leaf breakdown. *Water Research* **25,** 247-250.

Maltby, L. and Calow, P. (1989). The application of bioassays in the resolution of environmental problems; past, present and future. *Hydrobiologia* **188/189,** 65-76.

Mantoura, R.F.C., Dickson, A. and Riley, R.P. (1978). The complexation of metal, with humic materials in natural waters. *Estuarine and Coastal Marine Sciences* **6,** 387-408.

Martinez-Jeronimo, F., Villasenor, R., Rios, G. and Espinosa, F. (1994). Effect of food type and concentration on the survival, longevity and reproduction of *Daphnia magna*. *Hydrobiologia* **287,** 207-214.

Mason, C.F. (1996). *Biology of Freshwater pollution,* 3rd edn. Longman, London.

Mantoura, R.F.C., Dickson, A. and Riley, J.P. (1978). The complexation of metals with humic materials in natural waters. *Estuarine and Coastal Marine Science* **6,** 387-408.

Marcotte, B.M. (1983). *The imperatives of copepod diversity, perception, cognition competition and predation.* In: Schram F.R. (ed) Crustacean Phylogeny. Balkemas Rotterdam pp 47-72.

Meador, J.P. (1991). The interaction of pH, dissolved organic carbon, and copper in the determination of ionic copper and toxicity. *Aquatic Toxicology* **19,** 13-31

Metcalfe, J.L. (1989). Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status in Europe. *Environmental Pollution* **60,** 101-139.

Meyer, J.S., Ingersoll, C.G. and McDonald, L.L. (1987). Sensitivity analysis of population growth rates estimated from cladoceran chronic toxicity tests. *Environmental Toxicology and Chemistry* **6,** 115-126.

Meyer, J.L.; Tate, C.M.; Edwards, R.T.; Crocker, M.T. (1988). *Trophic significance of dissolved organic carbon in streams. In:* Swank, W.,T.; Crossley, D.A. Jr. *ed.* Ecological studies, Vol. 66: Forest hydrology and ecology at Coweeta pp. 269-278. New York, Springer-Verlag.

Moore, C.G. and Bett, B.J. (1989). The use of meiofauna in marine pollution impact assessment. *Zoological Journal of the Linnean Society* **96,** 263-280.

Moore, C.G. and Pearson, T.H. (1986). Response of a marine benthic copepod assemblage to organic enrichment. In, *Procedings of the Second International Conference on Copepoda*, Ottawa, Canada, pp. 369-373.

Moore, J.W. and Ramamoorthy, S. (1984). *Heavy metals in natural waters.* Applied Monitoring and Impact Assessment. 268 pp. Springer-Verlag, New York.

Moraitou-Apostolopoulou, M., Kiortsis, V., Verriopoulos, G. and Platanistioti, S. (1983). Effects of copper sulphate on *Tisbe holothuriae* Humes (Copepoda Harpacticoida) and development of tolerance to copper. *Hydrobiologia* **99,** 145-150.

Mount, D.I. and Norberg, T.J. (1984). A seven day life-cycle cladoceran toxicity test. *Environmental Toxicology and Chemistry* **3,** 425-434.

Munkittrick, K.R. and McCarty, L.S. (1995). An integrated approach to aquatic ecosystem health: top-down, bottom-up of middle-out? *Journal of Aquatic Ecosystem Health* **4,** 77-90.

Naylor, C., Maltby, L. And Calow, P. (1989). Scope for growth in *Gammarus pulex* (Crustacea, Amphipoda): A critical review. *Freshwater Biology* **14,** 443-489.

Newman, M.C. and McIntosh, A.W. (1991). Metal ecotoxicology: concepts & applications. *Lewis Publishers Inc.*

Newell, P.F., Newell, R.C. and Trett, M.W. (1990a). Environmental impact of an acid-iron effluent on macrobenthic and meiofaunal assemblages of St. Lawrence river. *The Science of the Total Environment* **97-98,** 771-781.

Newell, P.F., Newell, R.C. and Trett, M.W. (1990b). Assessment of the impact of liquid wastes on benthic invertebrate assemblages. *The Science of the Total Environment* **97/98,** 855-867.

Nienke, G.E. and Lee, G.F. (1982). Sorption of zinc by Lake Michigan sediments. Implications for zinc water quality criteria standards. *Water Research* **16,** 1373-1378.

Norberg, T.J. and Mount, D.I. (1985). A new fathead minnow (*Pimephales promelas)* subchronic toxicity test. *Environmental Toxicology and Chemistry* **4,** 711-718.

Norris, R.H. and Georges, A. (1993). Analysis and interpretation of benthic macroinvertebrate survey. In *Freshwater biomonitoring and benthic macroinvertebrates* (Rosenberg, D.M. & Resh, V.H., eds.) pp 234-286. Chapman & Hall, New York.

Norris, R.H., Lake, P.S. and Swain, R. (1982). Ecological effects of mine effluents on the South Esk River, North eastern Tasmania. II. Trace metals. *Australian Journal of Marine and Freshwater Research* **32,** 165-173.

NRA (1994). *Seaton, Looe and Fowey catchment management consultation report.*

O'Brien, P., Feldman, H., Grill, E.V. and Lewis, A.G. (1988). Cu tolerance of the life history stages of the splashpool copepod *Tigriopus californicus* (Copepoda, Harpacticoida). *Marine Ecological Progress Series* **44,** 59-64.

O'Doherty, E.C., (1985). Stream dwelling copepods: their life history and ecological significance. *Limnology and Oceanography* **30,** 554-564.

Olsgard, F. and Gray, J.S. (1995). A comprehensive analysis of the effects of offshore oil and gas exploration on the benthic communities of the Norwegian continental shelf. *Marine Ecological Progress Series* **122,** 277-306.

Ormerod, S.J. and Edwards, R.W. (1987). The ordination and classification of macroinvertebrate assemblages in the catchment of the River Wye in relation to environmental factors. *Freshwater Biology* **17,** 533-546.

Ormerod, S.J., Boole, P., McCahon, C.P., Weatherly, N.S., Pascoe, D. and Edwards, R.W. (1987). Short term acidification of a Welsh stream: comparing the biological effects of a Welsh stream : comparing the biological effects of hydrogen ions and aluminium. *Freshwater Biology* **17,** 341-356.

Pagenkopf, G.F., Russo, R.C. and Thurston, R.V. (1974). Effect of complexation on toxicity of copper to fishes. *Journal of the Fisheries Research Board of Canada* **31,** 462-465.

Palmer, M.A., (1990). Temporal and spatial dynamics of meiofauna within the hyporheic zone of Goose Creek, Virginia. *Journal of the North American Benthological Society* **9,** 17-25.

Parkhurst, B.R. (1995). Are single species toxicity test results valid indicators of effects to aquatic communities?. In *Ecological Toxicity Testing: Scale, Complexity and Relevance* (Cairns, J.Jr. and Niederlehner, B.R., eds.) pp 105-121 Lewis Publishers, Boca Raton, Florida.

Pennak, R.W. (1988). Ecology of the freshwater meiofauna. In *Introduction to the study of Meiofauna* (Higgins, R.P. and Thiel, H. (Eds), pp 39-60. Smithsonian Institution Press, Washington, DC

Pennak, R.W. and Ward, J.V. (1986). Interstitial faunal communities of the hyporheic and adjacent groundwater biotopes of a Colarado mountain stream. *Arch. Hydrobiol. Suppl.* **74,** 356-396.

Perlmutter, D.G. and Meyer, J.L. (1991). The impact of a stream-dwelling harpacticoid copepod upon detritally associated bacteria. *Ecology* **72,** 2170-2180.

Perryman, S.A.M. (1996). The effect of heavy metal contamination on estuarine benthic fauna at varying levels of biological organisation. *PhD Thesis, University of Plymouth, UK.*

Pindar, L.C.V. and Farr, S. (1987). Biological surveillance of water quality 3 The influence of organic enrichment on the macroinvertebrate fauna of small chalk streams. *Arch. Hydrobiol.* **109,** 619-637.

Pieper, H.G. (1976). Die tierische Besiedlung des hyporheisshcen interstitials eines Urgebirgsbaches unter dem Einfluss von allochthoner Nahrstoffzufuhr. *International Journal of Speleology* **8,** 53-68.

Platt, H.M. and Lambshead, P.J.D. (1985). Neutral model analysis of patterns and their use in the detection of environmental perturbations. *Hydrobiologia* **118,** 59-66.

Powlesland, C. and George, J. (1986). Acute and chronic toxicity of nickel to larvae of *Chironomus riparis* (Meigen). *Environmental Pollution (series A)* **42,** 47-64.

Raffaeli, D. (1987). The behaviour of the nematode/copepod ratio in organic pollution studies. *Marine Environmental Research* **23,** 135-152.

Raffaeli, D.G. and Mason, C.F. (1981). Pollution monitoring with meiofauna, using the ratio of nematodes to copepods. *Marine Pollution Bulletin* **12,** 158-163.

Read, P.A., Anderson, K.J., Matthews, J.E., Watson, P.G., Halliday, M.C. and Shiells, G.M. (1983). Effects of pollution on the benthos of the Firth of Forth. *Marine Pollution Bulletin* **14,** 12-16.

Resh, V.H. and Jackson, J.K. (1992) Introduction to freshwater biomonitoring and benthic mavroinvertebrates. In *Freshwater biomonitoring and benthic macroinvertebrates.* (Rosenberg, D.M. & Resh, V.H., Eds.). pp.1-9 Chapman & Hall.

Rehwoldt, R., Lasko, L., Shaw, C., Wirhowski, E. (1973). The acute toxicity of some heavy metals towards benthic organisms. *Bulletin of Environmental Contamination and Toxicology* **6,** 445-448.

Richardson, B.J. and Martin, M. (1994). Marine and estuarine toxicity testing: A way to go? Additional sittings from northern and southern hemisphere perspectives. *Marine Pollution Bulletin* **28,** 138-142.

Robertson, A.L. (1990). The population dynamics of Chydoridae and Macrothricidae (Cladocera: Crustacea) from the River Thames, U.K. *Freshwater Biology* **24,** 375-389.

Robertson, A.L., Lancaster, J. and Hildrew, A.G. (1995). Stream hydraulics & the distribution of microcrustacea - a role for refugia. *Freshwater Biology* **33,** 469-484.

Roline, R.A. (1988). The effects of heavy metal pollution of the upper Arkansas river on the distribution of aquatic macroinvertebrates. *Hydrobiologia* **160,** 3-8.

Rosenberg, D.M. and Resh, V.H. (1992). Introduction to freshwater biomonitoring and benthic mavroinvertebrates. In *Freshwater biomonitoring and benthic macroinvertebrates.* (Rosenberg, D.M. & Resh, V.H., Eds.). pp.1-9 Chapman & Hall.

Rundle, S.D. (1988). The Micro-arthropods of some southern English streams. *Ph.D. Thesis*

Rundle, S.D. (1990). Micro-arthropod seasonality in streams of varying pH. *Freshwater Biology* **24,** 1-21.

Rundle, S.D. (1993). Temporal and demographic patterns of microcrustacean populations in upland Welsh streams of contrasting pH. *Archives of Hydrobiologia* **128,** 91-106

Rundle, S.D. and Attrill, M.J. (1995). Comparison of meiobenthic crustacean community structure across freshwater acidification gradients. *Arch. Hydrobiol.* **211,** 1-16.

Rundle, S.D. and Hildrew, A.G., (1990). The distribution of micro-arthropods in some southern English streams: the influence of physicochemistry. *Freshwater Biology* **23,** 411-431.

Rundle, S.D. and Ormerod, S.J., (1991). The influence of chemistry and habitat features on the microcrustacea of some upland Welsh streams. *Freshwater Biology* **26,** 439-451.

Rutt, G.P., Weatherley, N.S. and Ormerod, S.J. (1990). Relationships between the physicochemistry and macroinvertebrates of British upland streams: the development of modelling and indicator systems for predicting fauna and detecting acidity. *Freshwater Biology* **24,** 463-480.

Rutt, G.P., Pickering, T.D. and Reynolds, N.R.M. (1993). The impact of livestock-farming on Welsh streams: the development and testing of a rapid biological method for use in the assessment and control of organic pollution from farms. *Environmental Pollution* **81,** 217-228.

Samoiloff, M.R. (1987). Nematodes as indicators of toxic environmental contaminants. In *Vistas on Nematology* (Veech, J.A. and Dickson, D.W., Eds) pp 433-439.

Sandulli, R and de Nicola, M. (1991). Responses of meiobenthic communities along a gradient of sewage pollution. *Marine Pollution Bulletin* **22,** 463-467.

Sarvala, J. (1979). Effect of temperature on the duration of egg, nauplius and copepodite development of some freshwater benthic copepods. *Freshwater Biology* **9,** 515-534.

Schmidtz, A. and Nadel, R. (1995). Influence of 3,4-dichloroaniline (3,4-DCA) on benthic invertebrates in indoor experimental streams. *Ecotoxicol. Envir, Saftey* **30,** 63-71

Shambrook, H.R.. (1986). The Caradon and Phoenix mining area. *Office Liason Ltd., Sheffield*

Sheehan, P.J. (1984). Effects on community and ecosystem structure and dynamics. In *Effects of pollutants at the ecosystem level.* (Sheehan, P.J., Miller, D.R., Butler, G.C. and Bourdeau, P., eds.), pp 51-100. John Wiley & Sons, Chichester, New York.

Sherman, K.M. and Coull, B.C. (1980). The response of meiofauna to sediment disturbance. *J. Exp. Mar. Biol. Ecol.* **46,** 59-71.

Sherman, K.M., Reidenauer, J.A., Thistle, D. and Meeter, D.A. (1983). Role of a natural disturbance in an assemblage of marine free-living nematodes. *Mar. Ecol. Prog. Ser.* **11,** 23-30.

Shiozawa, D.K. (1985) The seasonal community structure & drift of microcrustaceans in Valley Creek, Minnesota. *Can. J. Zool.* **64,** 1655-1664.

Slooff, W. (1983). Benthic macroinvertebrates and water quality assessment: Some toxicological considerations. *Aquatic Toxicology* **4,** 73-82.

Smith, A.E. (1973). A study of the variation with pH of the solubility and stability of some metal ions at low concentrations in aqueous solution. Part 1. *Analyst* **98,** 65-68.

Somerfield, P.J. and Clarke, K.R. (1995). Taxonomic levels in marine community studies, revisited. *Marine Ecological Progress Series* **127,** 113-119.

Somerfield, P.J., Gee, J.M. and Warwick, R.M. (1994). Soft sediment meiofauna community structure in relation to a long term heavy metal gradient in the Fal estuary system. *Marine Ecol. Prog. Series* **105,** 79-88.

Somerfield, P.J., Rees, H.L. and Warwick, R.M. (1995). Interrelationships in community structure between shallow-water marine meiofauna and macrofauna in relation to dredgings disposal. *Marine Ecology Progress Series* **127,** 103-112.

South West Water. (1982). Chemical survey of River Lynher catchment. *Report SSW/12Q/O2/84/CL/DSN. South West Water.*

Spear, P.A. & Pierce, R.C. (1979). *Copper in the aquatic environment - chemistry distribution and toxicology.* Associate Committee on Scientific Criteria for Environmental Quality NRCC No. 16454.

Sprague, J.B. (1969). The measurement of pollutant toxicity to fish. I - Bioassay methods for acute toxicity. *Water Research* **3,** 793-821.

Stephan, C.E. (1977). Methods for calculating an LC50. In *Aquatic Toxicology and Hazard Evaluation, ASTM STP 634* (Mayer, F.L. and Hamelink, J.L., eds.), pp 65-84. American Society for Testing and Materials, Philidelphia.

Stephenson, R.R.. (1983). Effects of water hardness, water temperature, size of the test organism on the susceptibility of the freshwater shrimp, *Gammarus pulex* (L.) to toxicants. *Bull. Environ. Contam. Toxicol.* **31,** 459-466.

Steinberg, C.E.W. and Wright, R.F. (1994). *Acidification of freshwater ecosystems: implications for the future.* Wiley, Chichester.

Stuhlbacher, A. and Maltby, L. (1992). Cadmium resistance in *Gammarus pulex* (L.). *Archives of Environmental Contamination and Toxicology* **22,** 319-324.

Surber, E.W. (1970). Procedure in taking stream bottom samples with the stream square foot bottom sampler. *Proc. Conf. Of South East Association of Game Fisheries Commission* **23,** 587-591.

Suren, A.M. (1992). Meiofaunal communities associated with bryophytes and gravels in shaded and unshaded alpine streams in New Zealand. *New Zealand Journal of Marine and Freshwater Research* **26,** 115-125.

Suter, G.W. (1990). Seven-days tests and chronic tests-letter to editor. *Environmental Toxicology and Chemistry* **9,** 143.

Teschner, (1963). Die biologie, verbreitung und okologie der grundwasser milbe *Lobohalacarus weberi quadriporus* (Walter, 1947) Limnohalacaridae, Acari. *Arch. Hydrobiol.* **59,** 71-102.

Thistle, D. (1980). The response of a harpacticoid copepod community to a small-scale natural disturbance J. *Mar. Res.* **38,** 381-395.

Thorp, V.J. and Lake, P.S. (1974). Toxicity bioassays of cadmium on selected freshwater invertebrates and the interaction of cadmium and zinc on the freshwater shrimp, *Paratya tasmaniensis* Riek. *Australian Journal of Marine and Freshwater Research* **25,** 97-104.

Townsend, C.R., Hildrew, A.G. and Francis, J. (1983). Community structure in some southern English streams: the influence of physicochemical factors. *Freshwater Biology* **13,** 521-544.

Transpurger, W. and Drews, C. (1996), Toxicity analysis of freshwater and marine sediments with meio- and macrobenthic organisms: a review. *Hydrobiologia* **328,** 215-261.

Transpurger, W.C., Steinberg, C. And Bongers, T. (1995). Nematoden in der okotoxikologischen Forschung: Playdoyer fur eine vernachlassigte, jedoch sehr aussagekraftige Tiergruppe. UWSE-Z Umweltchem. *Okotoxicol.* **7,** 74-83.

Tuffery, G. and Verneaux, J. (1968). Methode de determination de la qualite biologique des eaux courantes. Exploitation codifee des inventaires de la faune du fond. Ministere de l'Agriculture (France), Centre National d'Etudes Techniques et de Recherches Technologiques pour L'Agriculture, les Forets et l'Equipment Rural, Section Peche et Pisciculture, 23 p.

Turekian, K.K. (1971). Rivers, tributaries, and estuaries. In: *Impingement of Man on the Oceans* Hood, (D.W. ed.). pp. 9-74. Wiley-Interscience, New York.

USEPA (US Environmental Protection Agency). (1979). Guidence for the premanufacture testing: discussion of policy issues, alternative approaches and test methods. *Federal Register* 44: 16240.

Ustach, J.F. (1979). Effects of sublethal oil concentrations on the copepod, *Nitocra affinis*. *Estuaries* **2,** 273-276.

Van Damme, D., Heip, C. and Willems, K.A. (1984). Influence of pollution on the harpacticoid copepods of two north sea estuaries. *Hydrobiologia* **112,** 143-160.

Verriopoulos, G. and Hardouvelis, D. (1988). Effects of sublethal concentration of Zn on survival and fertility in four successive generations of *Tisbe*. *Marine Pollution Bulletin* **19,** 162-166.

Verriopoulos, G. and Moraitou-Apostolopoulou, M. (1982). Differentiation of the sensitivity to Cu and cadmium in different life stages of a copepod. *Marine Pollution Bulletin* **13,** 123-125.

Viets, K. (1936). *Die Tierwelt Deutschlands und der Angrenzenden Meeresteile Spinnentiere oder Arachnridea VII : Wassermillen oder Hydracharina.* Jener G. Fischer.

Vijverberg, J. (1989). Culture techniques for studies on the growth, development & reproduction of copepods & cladocerans under laboratory & in situ conditions : a review. *Freshwater Biology* **21,** 317-373.

Wade, K.R., Ormerod, S.J. and Gee, A.S. (1989). Classification and ordination of macroinvertebrate assemblages to predict stream acidity in upland Wales. *Hydrobiologia* **171,** 59-78.

Warwick, R.M. (1981). The nematode/copepod ratio and its use in pollution ecology. *Marine Pollution Bulletin* **12,** 329-333.

Warwick, R.M. (1984). Species size distributions in benthic communities. *Oecologia (Berl.)* **61,** 32-41.

Warwick, R.M. (1988a). Analysis of community attributes of the macrobenthos of Friefjord/Langesundfjord at taxonomic levels higher than species. *Marine Ecological Progress* **46,** 167-170.

Warwick, R.M. (1988b). The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. *Marine Pollution Bulletin* **19,** 259-268.

Warwick, R.M. (1993). Environmental impact studies on macrobenthic communities: pragmatical considerations. *Australian Journal of Ecology* **18,** 63-80.

Warwick, R.M. and Clarke, K.R. (1991). A comparison of some methods for analysing changes in benthic community structure. *Journal of the Marine Biological Association of U.K.* **71,** 225-244.

Warwick, R.M. and Clarke, K.R. (1993a). Increased variability as a symptom of stress in marine communities. *Journal of Experimental Marine Biology and Ecology* **172,** 215-226.

Warwick, R.M. and Clarke, K.R. (1993b). Comparing the severity of disturbance: a meta-analysis of marine macrobenthic community data. *Marine Ecological Progress Series* **92,** 221-231.

Warwick, R.M., Clarke, K.R., Suharsono (1990). A statistical analysis of coral community responses to the 1982-3 El Nino in the Thousands Islands, Indonesia. *Coral Reefs* **8,** 171-179.

Watton & Hawkes, H.A. (1984). The performance of an invertebrate colonisation sampler in biological surveillance of lowland rivers. In *Freshwater Biological Monitoring* (Pascoe, D. and Edwards, R.W., eds.), pp 15-24. Pergamon Press, Oxford.

Weatherley, N.S. & Ormerod, S.J. (1987). The impact of acidification on macroinvertebrate assemblages in Welsh streams : towards an empirical model. *Environmental Pollution* **46,** 223-240.

Whitman, R.L. and Clark, W.J. (1984). Availability of dissolved oxygen in interstitial waters of a sandy creek. *Hydrobiologia* **92,** 651-658.

Wiederholm, T., Wiederholm, A.M. and Milbrank, G. (1987). Bulk sediment bioassays with five species of freshwater oligochates. *Water, Air and Soil Pollution* **36,** 131-154.

Wilhm, J.L. and Dorris, T.C. (1968). Biological parameters for water quality criteria. *Bioscience* **18,** 477-481.

Williams, L.G., Joyce, J.C. and Monk, J.T., (1973). Stream-velocity effects on the heavy metal concentrations. *J. Amer. Wat Works Ass* **65**, 275-278.

Williams, T., (1997). Life-cycle parameters of *Tisbe battagliai* (Copepoda: Harpacticoida) as indicators of chronic toxicity. *PhD thesis, University of Plymouth, UK*

Willis, M. (1985). Analysis of the effects of Zn pollution on the macroinvertebrate populations of the Afon Crafnant, North Wales. *Environmental Geochemistry and Health* **7**, 98-109.

Willoughby, L.G. and Mappin, R.G. (1988). The distribution of *Ephemerella ignita* (Ephemeroptera) in streams: the role of pH and food resources. *Freshwater Biology* **19**, 145-155.

Wilson, A.L., (1976). Concentrations of trace metals in river waters: a review. *Water Research Centre technical Report TR16 WRC Environment, Medmenham.*

Winner, R.W. and Owen, H.A. (1991). Toxicity of copper to *Chlamydomonas-reinhardtii* (Chlorophyceae) and *Ceriodaphnia-dubia* (Crustacea) in relation to changes in water chemistry of a freshwater pond. *Aquatic Toxicology* **21**, 157-170.

Winner, R.W., Boesel, M.W. and Farret, M.P. (1980). Insect community structure as an index of heavy metal pollution in lotic ecosystems. *Canadian Journal of Fisheries and Aquatic Science* **37**, 647-655.

Woodweis, F. (1964). The biological system of stream classification used by the Trent River Board. *Chemistry and Industry* **11**, 443-447.

Wright, J.F., Furse, M.T. and Armitage, P.D. (1994). Use of macroinvertebrate communities to detect environmental stress in running waters. *In Water quality and stress indicators in marine and freshwater ecosystems: Linking levels of organisation (Individuals, populations, communities) (Sutcliffe,* D.W., ed.), pp. 15-34. Freshwater Biological Association.

Wright, J.F., Moss, D., Armitage, P.D. and Furse, M.T. (1984). A preliminary classification of running-water sites in Great Britain based on macro-invertebrate species and the prediction of community type using environmental data. *Freshwater Biology* **14**, 221-256.

Zitiko, V. (1994). Principal component analysis in the evaluation of environmental data. *Marine Pollution Bulletin* **28**, 718-722.

Zullini, A. and Ricci, C. (1980). Bdelloid rotifers and nematodes in a small Italian stream. *Freshwater Biology* **10**, 67-72.