INTRASPECIFIC VARIATION IN THE RESPONSES TO COPPER BY TWO ESTUARINE INVERTEBRATES

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Doctor of Philosophy
in the University of Hull

by

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ABSTRACT

Copper tolerance has been investigated in *Nereis diversicolor* and *Corophium volutator* from three different estuaries; the Humber, Alaw and Dulas, and intraspecific variation in the responses to copper of both these animals were examined. Sediment concentrations of copper from Dulas were the highest (224μg g⁻¹) compared to the Humber (70μg g⁻¹) and Alaw (6.2μg g⁻¹) estuaries.

A high ability to tolerate copper based on 96 hour LC₅₀ values for dissolved copper (1.75 mg l⁻¹ in *Corophium* and 0.59mg l⁻¹ in *Nereis*) and high body copper concentrations (450μg g⁻¹ in *Corophium* and 698μg g⁻¹ in *Nereis*) were found in animals from the Dulas estuary. Moderate tolerance (1.34mg l⁻¹ in *Corophium* and 0.34mg l⁻¹ in *Nereis*) and moderate body copper concentrations (140μg g⁻¹ in *Corophium* and 100μg g⁻¹ in *Nereis*) were found in species from the Humber, compared to animals from the Alaw which showed very low copper tolerance (0.8mg l⁻¹ in *Corophium* and 0.26mg l⁻¹ in *Nereis*) with very low body copper concentrations (52μg g⁻¹ in *Corophium* and 65μg g⁻¹ in *Nereis*). These copper concentrations and degree of tolerance in *Corophium* and *Nereis* from the three estuaries reflected the levels in the sediments. Tolerance could not be acquired after exposure of juvenile and adult *Nereis* to a range of sublethal copper concentrations over a 30 day period. Tolerance in adult worms from Dulas was not lost after exposure to ‘clean’ conditions for 30 days supporting the suggestion that it may be genetic. In the Humber estuary there was generally little spatial or temporal variability shown in a suite of metal concentrations in the sediments, *Corophium* and *Nereis* recorded at 3-monthly intervals over a 12 month period.

Interspecific and intraspecific differences were found in the uptake and accumulation of copper. Copper tolerant *Corophium* accumulated significantly lower amounts of copper (relative to their control concentrations) compared to the less tolerant populations after exposure to external dissolved copper concentrations. The opposite was true for the tolerant population of *Nereis* accumulating the highest amount of copper relative to their control levels.

The high levels of total body copper found in the tolerant populations of both *Corophium* and *Nereis* suggests that the metal is being sequestered in a non-toxic way, but an exclusion mechanism and/or an excretion mechanism may also be in operation. Localisation of this metal was investigated in the tolerant population of *Nereis* and the accumulated copper was found to be tissue specific. The ability to tolerate copper was probably due to increased deposition of copper in membrane-bound structures located in the cells of the nephridial tubules. Copper was not found in the nephridial area of the non tolerant worms from the Alaw estuary.
Intraspecific variation was found to occur in the survival and body copper concentrations of *Corophium* and *Nereis* after exposure to different natural sediments in experimental situations. The physicochemical nature of each of the sediments affected the bioavailability of copper which caused different responses in *Corophium* and *Nereis*. Patterns and similarities between the biological responses and physico-chemical parameters were examined and simple predictive models were constructed to explain the variation found in the responses of *Corophium* and *Nereis*. The LC$_{50}$ value was used as an index of tolerance and was found to be important in explaining variation in the survival and copper accumulation in *Corophium* and *Nereis*.

These results were used to discuss the importance of intraspecific variation in *Corophium* and *Nereis* in the monitoring and management of metals in estuaries. This would produce sensitive and responsive management tools for individual estuaries rather than a blanket approach. This may be appropriate in some situations, particularly where certain metals are problematical as with the case of copper in the Humber estuary.
GENERAL INTRODUCTION

Heavy metal contamination and pollution occurs in estuaries where metals from natural and anthropogenic sources become trapped. The main contributors to heavy metal pollution in coastal and estuarine waters are acidic mine drainage waters, smelting works, industrial water discharges, sewage sludge, the atmosphere, shipyard paints and electricity power stations (Bryan, 1984). The distribution, speciation and reactivity of chemical components within an estuary are controlled by the dynamics of sediments, changes in salinity and other parameters that occur in such environments.

Due to precipitation and adsorption, a high percentage of heavy metals is removed from the aqueous phase and deposited on the bed of the estuary at concentrations many orders of magnitude greater than those in the overlying water (Allen, 1986). The scrubbing processes of precipitation, chelation and adsorption onto particulate materials ensure that only small amounts of metals escape to the open sea (Turekian, 1977).

Heavy metals have been classified according to the risks they pose to aquatic organisms. The classification is not just based on the intrinsic toxicity of the metals but also on their availability. Metals considered to represent a high risk to the biota as they are both very toxic and relatively accessible are; Be, Co, Ni, Cu, Zn, As, Se, Pd, Ag, Cd, Sn, Te, Pt, Au, Hg, Tl, Pb and Bi (Forstner and Wittmann, 1981).

Levels of metals in estuarine sediments remain relatively stable for long periods and are good indicators of contamination compared to estuarine water (Langston, 1985). Sediment analysis can be used to estimate temporal and spatial changes, related to fixed point discharges and may also indicate polluting sources which may no longer be active. However, analysis of water and sediment metal levels do not provide a reliable indication of the bioavailability of metals (Bryan and Gibbs, 1983). Hence, analysis of biological material seems an appropriate method of assessing contamination if the natural variability in accumulated metal can be taken into account.

To provide direct evidence for ecological impact by metals a different approach is required. Luoma (1977), advocated the study of tolerance to toxicants as a means of determining if trace contaminants are affecting organisms in a given situation. Essentially, if a population is tolerant of a toxicant, then the toxicant or a close chemical relative must have had an impact on that population. The tolerance approach has the added benefit of specificity in determining which contaminants are having the more significant effects.

One of the initial aims of this study was to examine copper tolerance in *Nereis diversicolor* (O.F. Moller) and *Corophium volutator* (Pallas) from three different estuaries; the
Humber, Alaw and Dulas. An overall aim of this study was to investigate intraspecific variation in both these animals in their responses to copper.

Luoma (1977) proposed that the following four criteria must be met if tolerant characteristics are shown: - (i) tolerance results from Darwinian selection for tolerant genotypes, it cannot be induced over the life time of the individuals, (ii) physiological mechanisms specific for a single toxicant or group of toxicants are involved rather than the selection for vigorous strains, (iii) tolerance reduces the overall fitness of the population. When the selection pressure is removed the populations revert, in a few generations to dominance by intolerant genotypes and (iv) the degree of tolerance is related to the level of exposure to the toxicant.

Chapter 1 of this thesis deals with Luoma point (iv). Copper levels were measured in *Corophium* and *Nereis* from the three estuaries and were related to sediment levels and copper tolerances. A monitoring study was also carried out investigating the temporal and spatial distribution of a suite of metals in *Corophium, Nereis* and sediment in the Humber. Luoma’s point (i) was also addressed in this chapter by the use of experiments to investigate the possibility of inducing copper tolerance by exposure to sub-lethal levels of copper within a 30 day period.

In Chapter 2 intra-specific differences in the accumulation of copper in *Nereis* and *Corophium* from the different populations are investigated. In Chapter 3 the localisation of this copper is described.

Intra-specific differences in the responses of *Nereis* and *Corophium* to natural sediments is investigated in Chapter 4. The bioavailability of copper in these sediments to the organisms is also examined. In Chapter 5, Multivariate Statistics and Multiple Regression were used on the data generated in Chapter 4. Patterns and relationships in the physico/chemical variables describing the test sediments and the biological variables describing the organisms responses were examined. Both Chapter 4 and 5 are concerned with the occurrence of intra-specific variation and its implications to the management of metals in coastal environments.

Chapter 6 includes an attempt to give a holistic overview of intraspecific variation in *Corophium* and *Nereis*; and its importance in the monitoring and management of metals in different estuaries.
SITE DESCRIPTION AND GENERAL METHODS

i) DESCRIPTION OF STUDY AREAS

Dulas Bay

Dulas Bay, Anglesey (OS grid ref. SH 482885) (Figure 1) is polluted by acid mine drainage from Parys Mountain via the Afon Goch (Foster et al., 1978; Boult et al., 1994). Parys Mountain has a long history of mining, dating back to the Bronze Age and Roman times (Swallow, 1990). During the 18th and 19th centuries it was extensively exploited for copper. Ore was initially mined from shafts, but due to their early collapse, opencast mining took over (Walton and Johnson, 1992). Forty thousand tonnes per year of copper ore were exported via the adjacent port of Amlwch, making Parys Mountain the world’s largest copper mine at that time (Swallow, 1990; Westhead, 1991). Mining ceased at Parys Mountain in 1911 because of lower production costs elsewhere in the world (Westhead, 1991). During the 150 years of operation, over 130,000 tonnes of copper metal had been extracted (Swallow, 1990). Drainage of the old mine adits and erosion of the spoil heaps continues, maintaining the high concentrations of metals in the Afon Goch (Foster et al., 1978 and Boult et al., 1994).

Much of Parys Mountain is covered in mine spoil (approximately 200 ha), and large areas of sulphide rich rocks are open to erosion and subsequent oxidation. The Afon Goch rises from Parys Mountain and flows in a southerly direction for 11 km before reaching Dulas Bay. The sulphide minerals are dominated by pyrite, together with chalcopyrite, sphalerite and galena (Walton and Johnson, 1992). The resulting acidic water (average pH at the head of the stream is 2.4 (Boult et al., 1994)) dissolves large quantities of trace metals including iron, copper, zinc, manganese and aluminium. Typical dissolved metal concentrations in the acid mine stream are (in mg/l) Fe 193, Mn 11. Cu, 19 and Zn 30 (Boult et al., 1994). Downstream, much of the iron is lost from solution as hydrated iron oxide, giving the waters a characteristic orange-brown hue (Boult et al., 1994), and hence the name of the stream (Afon Goch is Welsh for Red River).

This water enters Dulas bay which is restricted at its seaward end by a large spit of land, thus preventing rapid dilution and dispersion of the heavy metals beyond the estuary. Dulas bay is approximately 1.25 km long and 0.6 km at its widest point. For about 3 hours before and after low tide the bay is devoid of seawater, and the river flows in a narrow channel (width approximately 8m) cut in the sediment. The channel meanders from its point of entry on the north shore, and then back to the north sere, before flowing out into the Irish Sea. With the rising tide, the outward flow of the river is eventually reversed and the bay fills with a mixture of river and seawater, rapidly emptying again on the ebb tide. The sediments at the head of the
estuary are fine-grained black muds, the oxidised layer being only a few millimetres thick at most. Further down the estuary, the sediments become coarser and the oxidised layer is much thicker (approximately 10cm deep).

Elevated metal levels have been found in various seaweeds and invertebrates collected from the estuary (Walker, 1976; Icely and Nott, 1980; Rainbow et al., 1989; Webb, 1990), confirming that Dulas Bay is still heavily contaminated.

**Alaw estuary**

The Alaw estuary on the north-west coast of Anglesey (OS grid ref. SH 302815) (Figure 1) was chosen as a control site because there was no evidence to suggest metallic contamination (the Afon Alaw drains into the estuary from Llyn Alaw, a reservoir in the centre of the island).

The Alaw estuary is approximately 1.45 km long and 0.55 km at its widest point. The seaward end of the estuary is restricted by a large spit of land. The Afon Alaw runs in a narrow channel (width approximately 6m) cut in the southern half of the estuary, before meandering across to the north shore, around the spit and into Holyhead Bay. The estuary is empty of seawater for approximately 3 hours before and after low tide. The sediments at the head of the estuary are fine-grained muds, with the oxic layer being about 2 cm deep. Further down the estuary the sediments become sandier and the oxic layer is approximately 8 cm deep. On the south side of the estuary, an area of rocky shore extends from the spit westwards as far as the Stanley Embankment (a distance of approximately 1.5 km).

**Humber estuary**

The Humber has the largest catchment area (26,000 km$^2$) of any estuary in the United Kingdom (Figure 2) and is the principal fresh water input from England into the North Sea. It is formed by the confluence of the rivers Ouse and Trent at Trent Falls and runs approximately 62km East and South East to enter the North Sea between Spurn Point and Donna Nook. One fifth (10.8 million) of the population of the U.K. live within the catchment which includes the cities of Birmingham, Bradford, Derby, Leeds, Leicester, Nottingham, Sheffield and Stoke-on-Trent and contains 60% of the country's coal production and 40% of crude steel production. The main freshwater inputs therefore contain high levels of industrially-derived metals.

The outer estuary supports a small scale commercial fishery and is also important as a nursery area for flatfish (JNCC, 1995). Between Trent Falls and the Humber Bridge an within Spurn Bright extensive marshes and mudflats provide habitats for large numbers of geese, ducks and waders, many of which are migrants which use the estuary as a feeding ground. Most of the rural coastline is designated as a Site of Special Scientific Interest (S.S.S.I.) in
recognition of its importance to wildlife, particularly bird populations. Six nature reserves are directly associated with the estuary.

The tidal waters of the estuary also directly receive trade effluent high in metal content. On the north bank at East Clough there was a metal smelter and there are a variety of chemical complexes nearer the estuary mouth, including two plants producing titanium dioxide pigments. The estimated inputs of metals into the Humber estuary from both freshwater sources and direct trade/swage effluents are: cadmium, $20\text{kg day}^{-1}$; copper, $430\text{ kg day}^{-1}$; lead, $400\text{ kg day}^{-1}$; zinc, $2500\text{ kg day}^{-1}$ and arsenic $1100\text{ kg day}^{-1}$ (Edwards et al, 1987; NRA, 1994). Metal input into the Humber, particularly through direct discharge into the tidal waters has occurred mostly over the last 100 years.

The Environmental Quality Standards (EQS) set by the NRA for metals are $\text{Cd}:2.5\mu\text{g l}^{-1}$, $\text{Cu}:5\mu\text{g l}^{-1}$, $\text{Pb}:25\mu\text{g l}^{-1}$, $\text{Zn}:40\mu\text{g l}^{-1}$ and $\text{As}:25\mu\text{g l}^{-1}$. The levels of Cd, Cr, Ni, Pb, Zn, Hg, As and Fe are well below their respective EQS levels at all sites sampled. However, dissolved copper levels at all sites sampled by the NRA were above the EQS, although the data shown were for total copper, which is not all toxic in nature as copper can be present in estuary waters in many forms (NRA,1994). Work by the Water Research Centre (WRc 1990) has suggested that less than 1% of copper in saline water is in the non-complexed form and is readily bio-available. Therefore the copper failures are not considered to be of serious concern (NRA, 1994).

Despite this metal and other pollution problems, such as oxygen depletion, the Humber estuary as a whole is only considered as moderately polluted. The National Water Council produced a classification system in the River Water Quality Survey (1980) based on Class A, good, B, fair, C, poor, and D, bad. The tidal Ouse was classed as C improving to class B at Trent falls. The Trent and upper Humber comes under class B, whereas the rest of the North Bank is class A. The South bank is class B down stream to Cleethorpes due to effluent discharges from industrial plants and domestic sewage input as well as the effect of the Coriolis Force moving such effluents towards the southern shore.

ii) SAMPLING

The geographical location of the sites studied are shown in Figures 2. 3 and 4 and are described in Table 1. The main site sampled in the Humber was at Paull (Figure 2). All sampling sites were located in the upper shore and sampling was always carried out at low water. The three main sites (salinity 15-20) maintained a population of Nereis and Corophium throughout the three years of study. The sampling dates are shown in Table 2.
At least four separate samples of surface sediments (top 2-5cm) were collected during low tide on each visit and placed in acid washed polypropylene containers. Samples were returned to the laboratory where they were stored in a freezer at \(-20^\circ C\) until they were required for metal analysis.

Depending on the nature of the site sampled, the worms were either picked by hand after digging in the intertidal sediments or collected from underneath rocks. They were returned to the laboratory in plastic buckets containing natural sediments. Intact worms of approximately 30-40mm in length were chosen. Numbers collected were dependent on requirements for analysis or experimentation.

After collection the worms were returned to the laboratory where they were sieved and cleaned using a 1mm sieve and jetted tap water. The worms were then placed in aerated seawater at a salinity of 17 and a constant \(12^\circ C\) in glass aquaria. All studies were conducted in a constant temperature room with a regime of 12 hours light and 12 hours dark. To avoid the worms damaging each other and becoming entangled, glass tubes (5mm diameter x 100mm) were added to each tank. The worms were maintained in these conditions for a period of 96 hours. The aquaria were aerated with gentle bubbling air. This acclimation period allowed the worms to evacuate their guts and to remove any adhering sediments. No food was provided during this cleaning period. The worms were then removed for subsequent experimentation. Only healthy worms (approximately 30-40mm long) were used in the experiments. Damaged and green worms which may have been approaching spawning were rejected.

*Corophium* were collected by sieving the surface sediment using a 2mm sieve. Sediment containing *Corophium* can be identified by the trails they leave on the sediment surface. The animals were returned to the laboratory in plastic buckets containing some natural sediment. Numbers collected were dependent on requirements for analysis or experimentation. The *Corophium* were then cleaned by being further sieved (gently) through a 1mm sieve. These animals were then acclimated as described for *Nereis* for 48 hours, then removed and prepared for subsequent experimentation.
Figure 1. Map showing location of Dulas Bay and the Alaw estuary
Figure 3. Map of Dulas Bay showing location of sampling site (■).

Figure 4. Map of the Alaw estuary showing location of sampling site (■).
Table 1. Descriptions of sites sampled and the studies undertaken.

<table>
<thead>
<tr>
<th>SITES</th>
<th>APPROXIMATE SALINITY</th>
<th>SEDIMENT</th>
<th>STUDIES UNDERTAKEN</th>
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<td>HUMBER SITES</td>
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<tr>
<td>PAULL</td>
<td>17</td>
<td>Cobbles overlying mud</td>
<td>T, C, H, I, M, A, R,</td>
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<td>BROUGH</td>
<td>9</td>
<td>Soft mud</td>
<td>H</td>
</tr>
<tr>
<td>BARTON</td>
<td>11</td>
<td>Mud with rocky covering</td>
<td>H</td>
</tr>
<tr>
<td>DULAS BAY</td>
<td>15</td>
<td>Soft mud</td>
<td>T, C, M, I, A, L, R,</td>
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<tr>
<td>ALAW ESTUARY</td>
<td>17</td>
<td>Soft mud with gravel</td>
<td>T, C, M, I, A, L, R,</td>
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Table 2. Sampling dates for studies carried out

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<td>T  Toxicity testing</td>
<td>Spring 1994</td>
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<tr>
<td>C  Copper analysis in animals and sediments</td>
<td>Spring 1994</td>
</tr>
<tr>
<td>H  Analysis of a suite of metals in animals and sediments</td>
<td>July 1994-July 1995(3-monthly)</td>
</tr>
<tr>
<td>I  Induction experiments</td>
<td>Spring 1995</td>
</tr>
<tr>
<td>M  Analysis of a suite of metals in sediments</td>
<td>Spring 1995</td>
</tr>
<tr>
<td>A  Accumulation experiments</td>
<td>Spring 1995</td>
</tr>
<tr>
<td>L  Light microscope and E.M. work</td>
<td>Spring 1995</td>
</tr>
<tr>
<td>R  Responses to natural sediments</td>
<td>Spring 1996</td>
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CHAPTER 1
METAL LEVELS AND TOLERANCE TO COPPER IN THREE ESTUARIES; WITH A SPATIAL AND TEMPORAL STUDY IN THE HUMBER ESTUARY

INTRODUCTION

Copper is an essential trace metal in invertebrates and is required for the metabolic functioning of certain proteins. Copper also forms part of the co-factors involved in oxidation-reduction cycles (White and Champ, 1983). In Corophium (and all crustaceans) the metabolic requirement for copper is higher than in Nereis as it is required for the functioning of the blood pigment haemocyanin (White and Rainbow, 1982). In excess concentrations, however, copper is extremely toxic. One hypothesis is that these poisons exert their toxic effects by inactivating vital processes which occur at the animals' surface. The other theory is that the poisons are absorbed by the animal and act internally by inhibiting metabolic changes (Moore, 1985).

The metal content of a marine invertebrate can be divided into two components (Rainbow, 1985): metal absorbed into the body (potentially subject to physiological control) and metal passively adsorbed onto the body surface (the cuticle in the case of crustaceans which is beyond metabolic control). The interaction of copper with salinity and temperature has been reported as being synergistic, with copper being considerably more toxic as salinity decreases (McLusky et al, 1986). Certain metals may have an antagonistic or synergistic effect with one another (Bryan, 1976).

Many aquatic animals can regulate their body copper levels (White and Rainbow, 1982) but beyond a critical limit the regulation process breaks down and the organisms begin to accumulate the metal (discussed further in Chapter 3). If an animal is exposed to high levels of metal in its environment it may result in becoming metal tolerant (Wright, 1986). Mechanisms of tolerance may vary and can manifest themselves quite differently in terms of metal uptake and ability to accumulate the metal. A tolerant animal may have developed an increased binding capacity for a particular metal, and may therefore show increased metal accumulation, compared with it's non tolerant counterpart (eg. Brown, 1977). Conversely, tolerance may be associated with decreased permeability to a particular metal and may result in a lower body burden than a non tolerant animal (Wright, 1986).

Exposure to sub-lethal levels of a pollutant can lead to physiological acclimation or behavioural changes within the life span of the exposed animal. However, exposure to a toxicant can also give rise to natural selection for an increased resistance, resulting in tolerance to the pollutant, tolerance being defined as genetic (or genotypic) adaptation (Weis and Weis, 1989). Physiological (or phenotypic) acclimation gives increased resistance which will not
necessarily be passed on to the next generation but genetic adaptation will be transmitted to offspring.

If natural selection occurs only the more tolerant animals will survive and reproduce. However, what is seen in the environment is a collection of phenotypes, the product of the effects of the environment or the expression of the genotype of an individual. These effects can make genetically different individuals appear phenotypically similar.

Bryan and Hummerstone (1971) placed high copper (high body burden) *Nereis* in low copper sediments (less than 50µg g⁻¹ copper) and low copper *Nereis* in high copper sediments (more than 1000µg g⁻¹ copper). After exposure for 45-76 days copper toxicity tests were carried out on the respective worms. The results of these tests showed that the high copper *Nereis* were copper tolerant and that such tolerance was not induced in the non-tolerant worms by exposure to high copper sediment concentrations within the time scale used. In addition to this the copper tolerance was not lost when the tolerant worms were exposed to clean sediments. Hence, these workers postulated that this tolerance was under genetic control.

Annelids have been shown to demonstrate both tolerance and acclimation to metals. Pesch and Hoffman (1982) reported that *Neanthes arenaceodentata* could display copper resistance after exposure to copper. But this induced effect was readily lost, hence the authors concluded that acclimation was involved. Klerks and Levinton (1989) discovered genetic tolerance to cadmium and nickel in *Limnodrilus hoffmeisteri*. They estimated that this tolerance had developed within 30 years. Hately (1989) found genetic tolerance in *Nereis diversicolor* for both copper and zinc. Brown (1976) in her study involving *Asellus meridianus* found a genetic component was involved in tolerance to copper, while Frazer (1980) showed that lead tolerance in *Asellus aquaticus* could be induced in laboratory conditions when exposed to low levels of lead (0.1mg l⁻¹) for exposure periods of 5 days. Acquired tolerance is likely to be temporary and metal specific (Wang, 1987).

Many organisms including fish, mussels, plants, algae and bacteria can exhibit physiological acclimation to metals, if they are exposed to sub-lethal metal concentrations. Few examples of genetic tolerance to metals, in higher organisms have been reported. Fish have been shown to develop resistance due to exposure (acclimation). Pascoe and Beattie (1979) treated Rainbow Trout alevins with cadmium and they showed resistance when compared to non-acclimated fish. Dumcan and Klawerkamp (1983) reported that White Suckers resistance to cadmium increased as a consequence of previous exposure.

The aims of this chapter are two-fold. 1) To investigate intraspecific differences in copper body burdens and degree of copper tolerance in *Corophium* and *Nereis* from estuaries with different metal contamination (the Alaw, Humber and Dulas). 2) To report a temporal and
spatial study on a suite of metals in sediments, *Corophium* and *Nereis* from the Humber Estuary.
MATERIALS AND METHODS

i) COPPER LEVELS IN THREE ESTUARIES

a) Copper in sediment

Ten sediment samples were collected (the sampling protocol can be found in General Methods) for later metal analysis from the Alaw, Humber and Dulas estuaries in June 1994. Sediment samples stored at -20°C were allowed to defrost and dried for 24 hours in a drying oven at 100°C, desegregated with a pestle and mortar and sieved to <180μm. Known amounts (1g) of dried sediment were digested on hot plates in concentrated nitric acid (ANALAR) for four hours. This digestion process extracts approximately 70-80% of total copper, which includes the organically bound copper. This digestion method does not extract the total copper including copper in the sediment matrix which would not be biological available to the organism. Reference standards and blanks were used and were subjected to the same digestion procedures as the samples to avoid any digestion related artefacts. After the digestion process the liquid was filtered through Whatman No. 1 filter paper into 20ml volumetric flasks and made up to 20ml with deionised water.

Samples were analysed using a Perkin Elmer, P40 Emmission Inductively Coupled Plasma (ICP) Spectrophotometer. The instrument was recalibrated separately for sediment samples from the Alaw, Humber and Dulas estuaries due to the range of metal levels differing by several orders of magnitude. Sediments from the Alaw and Dulas had similar physical properties (see Chapter 4), whereas sediments from the Humber had smaller particles. In this study it was not thought necessary to standardise the samples in terms of their sediment size fractions as the metal levels in each sediment were very different. Also, the effect of physical and chemical variation in sediments on the bioavailability of a metal is further discussed in Chapter 4.

b) Copper in animals

Acclimated Nereis (see General Methods) from the three estuaries were taken from the aquarium (after a period of 96 hours) and stored at -20°C in a freezer. Acclimated Corophium from the three estuaries were taken from the aquarium (after a period of 48 hours) and frozen in the same way as Nereis. All animals were collected at the same time as the sediment. The samples were removed from the freezer and defrosted for 3 hours. To give the required dry weight for analysis, the samples of Nereis and Corophium consisted of 2-4 and 6-8 adult (see General Methods) animals respectively. These samples were then placed in pre-weighed
conical flasks, placed in an oven at 105°C for 24 hours, then cooled in a desiccator for 2 hours and the dry weight was determined.

ANALAR concentrated nitric acid (10ml) was added to each sample and a glass ball was placed on the flask to minimise evaporation. The samples were digested for 6 hours on hot plates at 80°C. The ball was removed and the acid was evaporated off until the sample was near dryness. Samples were made up to 10ml with deionised water. Reference standards and blanks were used and were subjected to the same digestion procedures as described to avoid any digestion related artefacts. Ten samples of *Corophium* and *Nereis* from each site were analysed for copper by an VG Elemental PQ2+ Inductively Coupled Plasma - Mass Spectrophotometer (I.C.P. M.S.). Standard operating conditions were followed throughout (instructions Plasma 40 Emission Spectrophotometer, Perkin/Elmer, Norwalk Connecticut, U.S.A, 1987). Each analysis was replicated three times.

**ii) TOXICITY EXPERIMENTS**

Generally copper is more toxic to estuarine organisms in extreme salinities and high temperatures (McLusky et al 1986). The three sites studied in this work had average salinities in the range 7.0-30.0. Therefore a standard experimental salinity of 17 and a low temperature of 12°C were used in all experiments. *Nereis* and *Corophium* were acclimated for approximately 96 hours at this salinity and temperature. The final copper concentrations were made up from a standard copper solution of 1000mg l⁻¹ Cu. These dilutions were renewed every 24 hours and no food was supplied during these tests. The animals were checked every 24 hours and the dead animals were counted and removed. Animals were deemed to be dead when they failed to respond to mechanical stimulation. The experiments were replicated so that at least 40 *Corophium* and 40 *Nereis* from each estuary were tested at each concentration of copper. The 96 hour acute toxicity levels (96 hour LC₅₀) for copper were determined by Probit Analysis (Kinnear et al 1990) using SPSS.

*Nereis*

Only healthy worms (approximately 30-40mm long) were used in the toxicity tests. Damaged and green worms which may have been approaching spawning were rejected. The static acute toxicity tests were conducted in glass crystallising dishes (140mm diameter x 70mm) with five glass tubes to reduce the entangling of worms. Groups of ten worms were put in each crystallizing dish. The copper concentrations used were 0.2, 0.4, 0.6, 0.8 and 1.0mg l⁻¹ Cu. Ten worms from each site were kept in uncontaminated water as controls.
Corophium

Only healthy adult intermoult amphipods were used in the toxicity tests. The static acute toxicity tests were conducted in glass crystallising dishes (100mm diameter x 40mm). Groups of ten amphipods were put in each crystallising dish. The copper concentrations used were 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4mg l\(^{-1}\)Cu. Ten amphipods from each site were kept in uncontaminated water as controls.

iii)INDUCTION/LOSS OF TOLERANCE EXPERIMENTS

To examine if tolerance to copper could be induced in both adult (30-40mm long) and juvenile (10-20mm long) Nereis diversicolor from the Alaw and the Humber estuaries, worms were exposed to sub-lethal levels of copper (15 and 30\(\mu\)g l\(^{-1}\)) for a period of 30 days prior to being challenged with an exposure concentration of 150\(\mu\)g l\(^{-1}\)Cu.

\textit{Induction.} 60 Alaw, Humber and Dulas worms were acclimated for 96 hours in 17.5 salinity water at 12°C. 20 worms from each site were then exposed to copper levels of 15 and 30\(\mu\)g l\(^{-1}\) in glass crystallising dishes at 12°C. The remaining 20 worms from each site were kept in clean sea water as controls. The copper solutions were changed every 3 days and dead or damaged worms were removed wherever necessary. The worms were fed 24 hours prior to the water change on powdered commercial rat food every three days. This induction regime was carried out in duplicate.

\textit{Challenge.} After 30 days, 10 worms from each concentration were exposed to a challenge concentration of 150\(\mu\)g l\(^{-1}\) Cu and the other 10 remained in uncontaminated sea water as controls. The animals were checked daily and dead animals were counted and removed. The copper solutions were changed daily. This experiment was duplicated.

To examine if tolerance was easily lost from the copper tolerant Dulas worms, adult worms where maintained in clean conditions for 30 days prior to being challenged with a lethal dose. 20 worms were maintained in control conditions (0\(\mu\)g l\(^{-1}\) Cu) for the 30 day period, then challenged with a copper concentration of 400\(\mu\)g l\(^{-1}\) Cu. The animals were checked daily and dead animals were counted and removed. The copper solutions were changed daily. This experiment was duplicated.

iv) DISTRIBUTION OF METALS IN THE HUMBER ESTUARY

Samples of animals (Corophium and Nereis) and sediments were collected from three sites (Paull, Brough and Barton) on the Humber and were analysed for a suite of metals. These samples were collected at 3-monthly intervals over a period of one year from July '94 to July
'95. Measurements of a suite of metals were also carried out on sediments from the Alaw, Humber and Dulas estuaries that were collected in June '95.

These samples were treated and dried in the same way as described in Section i,a and b. However, the actual digestion and analyses were carried out by the Environment Agency using the following protocol.

a) Sediment

Aliquots of dry sediment (2.5g) were digested (cold) in 10ml of 50% nitric acid overnight followed by refluxing for 2 hours on dry-block using 50ml tubes and air condensers. The resulting digest was then cooled, filtered and made up to 50ml with deionised water. The samples were then analysed for Cr, Cu, Pb, Ni and Zn by the Inductively Coupled Plasma - Optical Emission Spectrophotometer (ICP - OES) using the Thermo Jarrel-Ash Atomscan 25. Cadmium was analysed by the more sensitive technique of Inductively Coupled Plasma - Mass Spectrometer (ICP - MS) using the Perkin-Elmer Elan 5000 to improve detection limits.

b) Animals

The whole dry sample was weighed (approximately 5-50mg of Corophium, and 50-100mg of Nereis). Samples were cold digested overnight with 20% nitric acid followed by a period of boiling in all-plastic containers. Digests were cooled and diluted to 100ml without filtration. The samples were analysed for all six metals using the Elan 5000 in order to meet the required detection limits.
RESULTS

i) METAL LEVELS IN THE THREE ESTUARIES

The mean values for copper concentrations in *Corophium* sampled from the Alaw, Humber and Dulas estuaries are shown in Figure 1.0. Copper concentrations in *Corophium* were the highest (450µg g<sup>-1</sup>Cu) in organisms from the Dulas estuary, and the lowest (52µg g<sup>-1</sup>Cu) from the Alaw estuary. *Corophium* from the Humber estuary had intermediate copper concentrations of 140µg g<sup>-1</sup>. The mean values for copper concentrations in *Nereis* sampled from the same three estuaries are shown in Figure 1.1. Copper concentrations in *Nereis* were the highest (698µg g<sup>-1</sup>) in organisms from the Dulas estuary, and the lowest (65µg g<sup>-1</sup>) from the Alaw estuary. *Nereis* from the Humber estuary had intermediate copper concentrations of 100µg g<sup>-1</sup>. The mean values for total copper concentrations in sediments sampled from the three estuaries are shown in Figure 1.2. The concentrations of copper in the organisms reflected the concentrations in the sediments. The highest copper concentrations were found in Dulas sediments (224µg g<sup>-1</sup>), the lowest in Alaw sediments (6.2µg g<sup>-1</sup>) and concentrations in Humber sediments were 70µg g<sup>-1</sup>. These are similar to the mean copper concentrations in sediments collected one year later in June'95 (Figure 1.3) which shows the concentrations of a suite of metals in sediments.

The relative abundances of the metals in sediments (Figure 1.3) from the Alaw were Zn > Cr > Ni > Pb > Cu > Cd; Zn > Cr > Pb > Cu > Ni > Cd from the Humber and Zn > Cu > Pb > Cr > Ni > Cd from Dulas. The highest metal concentration found in Alaw sediment was for zinc (38µg g<sup>-1</sup>) compared to the other metals which ranged from 0.03-10.9µg g<sup>-1</sup>. Zinc concentrations were also much higher in Humber sediments (279µg g<sup>-1</sup>) compared to copper, lead, nickel and chromium which ranged from 37.7-102.6µg g<sup>-1</sup> and cadmium which was 0.5µg g<sup>-1</sup>. Both copper (171µg g<sup>-1</sup>) and zinc (402µg g<sup>-1</sup>) in Dulas sediments were found in much greater concentrations than the other metals which ranged from 0.06-10.8µg g<sup>-1</sup>. Cadmium was the metal found in the lowest concentrations (0.03-0.5µg g<sup>-1</sup>) in all sediments.

ii) RELATIVE TOLERANCES TO COPPER

The LC<sub>50</sub> values of copper for *Corophium* from the Alaw, Humber and Dulas estuaries are shown in Figure 1.4. *Corophium* from the Dulas estuary showed the greatest ability to tolerate copper producing the highest LC<sub>50</sub> value (1.75mg l<sup>-1</sup>Cu) compared to *Corophium* from the Alaw (0.8mg l<sup>-1</sup>Cu) and the Humber (1.34mg l<sup>-1</sup>Cu).
The LCSO values for copper in *Nereis* from the three estuaries are shown in Figure 1.5. *Nereis* from the Dulas estuary showed the greatest ability to tolerate copper producing the highest LCSO value (0.59mg l\(^{-1}\)Cu) compared to *Nereis* from the Alaw (0.26mg l\(^{-1}\)Cu) and the Humber (0.3mg l\(^{-1}\)Cu). There is no overlap between the 95% confidence limits for the LCSO values of the tolerant and non-tolerant populations of *Corophium* and *Nereis*.

iii) ACQUISITION/LOSS OF TOLERANCE

The LT50 values for adult *Nereis* from all three estuaries are summarised in Table 1.0 and for juvenile *Nereis* in Table 1.1. These values were determined after exposure to a 'challenge' copper concentration of 150µg l\(^{-1}\) after a 30 day induction period to 15, 30 and 0µg l\(^{-1}\). In all induction conditions both juvenile and adult *Nereis* from Dulas had the highest LT50 value, *Nereis* from Alaw had the lowest and Humber *Nereis* had an intermediate value. These experiments demonstrated that pre-exposure concentrations of 15 and 30µg l\(^{-1}\)Cu over a period of 30 days failed to induce copper tolerance in juvenile and adult *Nereis* from the three estuaries. In juvenile and adult *Nereis* from the Alaw and the Humber, the opposite was indicated as pre-exposed worms were more susceptible to the toxic effects of the metal than unexposed worms.

The LT50 values for adult *Nereis* from Dulas after exposure to a test copper concentration of 400µg l\(^{-1}\)Cu before and after a 30 day acclimation period to clean water (0mg l\(^{-1}\)Cu) are summarised in Table 1.2. The LT50 values after acclimation were slightly lower than before acclimation and the 95% confidence limits overlapped.

iv) DISTRIBUTION OF METALS IN THE HUMBER ESTUARY

Copper concentrations in sediment, *Corophium* and *Nereis* sampled from three sites on the Humber estuary at 3-monthly intervals over a period of one year can be seen in Figure 1.6. There was little spatial variability shown for copper, with concentrations in the sediment, *Corophium* and *Nereis* being in the same order of magnitude at each site. The copper concentrations at all sites ranged from 18-103µg g\(^{-1}\) in sediment, 75-194µg g\(^{-1}\) in *Corophium* and 26-194µg g\(^{-1}\) in *Nereis*. Concentrations of copper in *Corophium* were the highest at Paull (139-194µg g\(^{-1}\)), and least from Barton (75-85µg g\(^{-1}\)). Copper concentrations were also the lowest in *Nereis* from Barton compared to worms from Brough and Paull. These values did not reflect the range of concentrations of copper in sediment sampled from the same site. The
copper concentrations in sediment from Paull showed the least temporal variability (37-81μg g⁻¹) and sediment from Barton showed the greatest (18-99μg g⁻¹).

No indication of any patterns in the copper concentrations in the sediment, Corophium or Nereis at each of the sites due to seasonal variation can be observed in Figure 1.6. Temporal changes in the sediment copper content do not appear to relate to the copper concentrations in the organisms. Generally, the copper concentrations in the organisms were greater than in the sediment. However, Nereis sampled in October ‘94 at Barton, Brough and Paull had lower copper concentrations than those in the sediment from the same site at the same time. Nereis from Barton sampled in January ‘95 and July ‘95 also had lower copper concentrations than in the sediment. When both organisms were sampled at the same time Corophium always had higher levels of copper than Nereis.

Zinc concentrations in sediment, Corophium and Nereis sampled from three sites on the Humber estuary at 3 monthly intervals over a period of one year can be seen in Figure 1.7. There was some spatial variability in the zinc concentrations in the sediments. The sediment from Barton had the greatest range of concentrations (103-618μg g⁻¹) compared to sediment from Brough (219-479μg g⁻¹) and Paull (180-384 μg g⁻¹). Concentrations of zinc in Nereis and Corophium (Figure 1.6) did not indicate much spatial variability with concentrations at all sites ranging from (97-172 μg g⁻¹) in Corophium and (185-337 μg g⁻¹) in Nereis. Both Nereis and Corophium sampled from Paull generally had the highest range of zinc concentrations over the sampling period compared to organisms from Brough and Barton.

There was no indication of any seasonal variation in Figure 1.7 in the concentration of zinc in the sediment or organisms. The concentration in Nereis and Corophium at each site do not appear to reflect any increased metal load in the sediment at any one sampling time as no patterns can be observed. When both organisms were sampled at the same time Nereis always had higher levels of zinc (unlike copper) than Corophium. Generally, zinc concentrations in the organism were not greater than in the sediment (as was the case for copper).

Lead concentrations in sediment, Corophium and Nereis sampled from three sites on the Humber estuary at 3 monthly intervals over a period of one year can be seen in Figure 1.8. There was little spatial variability shown for lead, with concentrations in the sediment, Corophium and Nereis being at similar levels at each site. The lead concentrations at all sites ranged from 47-212μg g⁻¹ in sediment, 3-17μg g⁻¹ in Corophium and 0.5-6μg g⁻¹ in Nereis.

The range of concentrations in the sediment at Brough was the smallest (76-94μg g⁻¹) compared to Barton and Paull, apart from sediment sampled in October ‘94 which contained the highest concentrations of lead (212μg g⁻¹). The highest concentrations of lead were found in
Corophium also sampled from Brough at the same time and appear to reflect the high sediment loads. However, this was not the case for Nereis.

No indication of any patterns in the lead concentrations in the sediment, Corophium or Nereis at each of the sites due to seasonal variation can be observed in Figure 1.8. Generally, the lead concentrations in Corophium are greater than in the Nereis and the concentrations in the sediment greatly exceeds that in the organisms. There is some indication that the concentrations of lead in Corophium reflect the temporal variation in the sediment lead concentrations.

Cadmium concentrations in sediment, Corophium and Nereis sampled from three sites on the Humber estuary at 3 monthly intervals over a period of one year can be seen in Figure 1.9. There was no spatial variability shown for cadmium with concentrations in the sediment, Corophium and Nereis being at similar levels at each site. The cadmium concentrations at all sites ranged from 0.5-0.8μg g⁻¹ in sediment, 0-1.9μg g⁻¹ in Corophium and 0.4-1.5μg g⁻¹ in Nereis. Generally, the cadmium concentrations in the sediments from each of the sites were similar to those in the organisms. However, the highest concentrations of cadmium were found in Corophium from Barton and Paull.

There was no temporal variation in the cadmium levels in the sediment (Figure 1.9), and any temporal variation in the organisms' cadmium levels does not reflect the sediment loads. There is no evidence of any seasonal variation in the sediment or the organisms.

Chromium concentrations in sediment, Corophium and Nereis sampled from three sites on the Humber estuary at 3 monthly intervals over a period of one year can be seen in Figure 1.10. There is some evidence of spatial variability in the concentrations of chromium in the sediments. Sediments from Barton had the least chromium content (34-71μg g⁻¹) compared to concentrations in sediment from Brough (63-99μg g⁻¹) and Paull (72-95μg g⁻¹). Concentrations of chromium in Corophium and Nereis do not indicate much spatial variability with concentrations ranging from (3-8μg g⁻¹) in Corophium and (1-4.6μg g⁻¹) in Nereis.

There is no indication of any seasonal variation in Figure 1.10 in the concentration of chromium in the sediment or organisms. The concentration in Nereis and Corophium at each site do not appear to reflect any increased metal load in the sediment at any one sampling time as no patterns can be observed. Generally, concentrations of chromium in Corophium are greater than in Nereis and the concentrations in the sediment exceeds that in the organisms.

Nickel concentrations in sediment, Corophium and Nereis sampled from three sites on the Humber estuary at 3 monthly intervals over a period of one year can be seen in Figure 1.11. There is no indication of spatial variability in the concentrations of nickel in the sediments or Nereis with concentrations ranging from (29.8-45μg g⁻¹) in sediment and (3.3-7μg g⁻¹) in
Nereis. There was some spatial variation in nickel concentrations in *Corophium* with concentrations being the highest at Paull (4.9-7μg g⁻¹) compared to Barton (1.2-1.8μg g⁻¹) and Brough (1.05-2.9μg g⁻¹).

The range of concentrations at each site in the sediment, *Nereis* or *Corophium* (Figure 1.11) was small indicating that there is little temporal variability. The concentrations in *Nereis* were generally larger than in *Corophium*, and the concentrations in the sediment exceeded those in the organisms.
DISCUSSION

i) METAL LEVELS AND TOLERANCE TO COPPER IN THREE ESTUARIES

Metal levels in the sediments and biota (*Corophium volutator* and *Nereis diversicolor*) from three estuaries are discussed in this first section. Sediment metal levels rank the estuaries in order of contamination; Dulas > Humber > Alaw. Levels in the Alaw were similar to concentrations found in 'average' shale (Krauskopf, 1967) and are typical of a clean estuary (Bryan, 1984).

The metal levels in the Humber sediments were higher than these 'background' levels and are indicative of significant metal contamination. The concentrations of copper, zinc, lead and arsenic (not measured in this study) in the Humber are typical of an estuary polluted by industrial and domestic sewage (Jaffe and Walters, 1977). Hamilton *et al* (1979) found similar levels of these metals in the Severn estuary. Significant anthropogenic enrichment of sediment levels in the Humber has occurred (within the last 100 years) for many metals notably arsenic (x6), lead (x5), copper (x5) and zinc (x4), (Middleton and Grant, 1990).

Metal levels in the Dulas estuary are similar to those found in Restronguet Creek which is one of the most highly contaminated estuaries in Britain (Bryan, 1984). As with Restronguet Creek (Bryan *et al* ,1983) continuing metal input results from the drainage of old copper and tin mines.

Copper levels in *Corophium volutator* and *Nereis diversicolor* from the three estuaries reflected the levels in the sediment from each estuary. Organisms from the Alaw had accumulated the lowest body burden of copper and organisms from Dulas had the greatest burden. *Corophium* and *Nereis* from the Humber had more accumulated copper than organisms from the Alaw but the levels were several orders of magnitude lower than organisms from Dulas. Iceley and Nott (1980) also found high levels of copper in *Corophium* from Dulas estuary. Body burden has been shown to be roughly related to sediment copper levels in *Nereis diversicolor* (Luoma and Bryan, 1982). However, relating copper concentrations in native organisms to total sediment concentrations provides little information on the extent of biological impact of the metals at a site. Ideally, biological indicators should be good accumulators of metals and reflect changes in environmental availability (Bryan and Langston, 1992). The limitations of *N. diversicolor* as an indicator organism have been discussed by other workers (Howard and Brown, 1983; Bryan *et al*, 1980). Concentrations in the worm do tend to reflect changes in the availability of certain metals (e.g. silver, cadmium, copper and lead) (Bryan and Hummerstone, 1971, 1973 and 1977).

A number of factors are known to cause variations in metal concentrations in marine organisms (Bryan, 1984). For example, the size and reproductive state of *Corophium* and
Nereis may modify the variation in the total metal levels, smaller worms have been shown to have higher copper concentrations than larger worms (Howard and Brown, 1983). However, in the present study the organisms and sediment were sampled at the same time to reduce seasonal effects and only organisms of the same size were used. It is possible, that the organisms from each estuary were at different reproductive states, although this was not apparent externally.

In this next section the relative copper tolerances of each population of Corophium and Nereis from the three estuaries is discussed. In order to assess tolerance levels acute static bioassays were used throughout this study. Static toxicity tests have been used to determine maximum allowable concentrations of toxic substances for environmental management purposes. Small samples of animals used for these bioassays have been assumed to represent the species concerned and the results have been extrapolated to field conditions. This method has been criticised (Luoma et al 1983; Kimball and Levin 1985) on the grounds that organisms accumulate waste products, deplete oxygen and alter pH of the experimental solutions used, hence affecting the results. No extrapolation to field conditions was needed in this study as the point of interest was the relative performances of different populations of Corophium and Nereis. Therefore, all test animals were exposed to the metals under identical experimental conditions and optimal survival conditions (low temperature, and mid-salinity; Fernandez, 1983). McLusky et al (1986) and Sahu (1989) have shown that toxicity of metals was greatest at extremes of salinity and low oxygen levels.

LC$_{50}$ determinations rank both Corophium and Nereis in order of copper tolerance; Dulas > Humber > Alaw. Copper tolerance in both animals related to the levels found in the environment and to a lesser extent to the levels in the organisms. Both Nereis and Corophium from Dulas had higher body copper levels and copper tolerance compared to the organisms from the Alaw and the Humber. These animals have apparently developed a copper tolerance to their high environmental levels.

The Humber population of Corophium has also responded to the elevated copper levels by developing an increased tolerance, compared to animals from the Alaw which has very low environmental levels. Copper tolerances in Nereis from Alaw and the Humber are similar which was not the case for Corophium from these two estuaries. This would suggest that the Humber population of Nereis has not acquired a tolerance to copper. Hately (1989) also concluded that there was no evidence of either zinc or copper tolerance in Nereis diversicolor from the Humber estuary. Copper and zinc tolerance in N. diversicolor was also found to be roughly related to environmental levels (Hately, 1986). It is difficult to state at what concentration tolerance becomes apparent. Bryan (1976) found copper tolerant animals from the Hayle estuary living in just 700µg g$^{-1}$ copper whereas in this study copper tolerant animals were found in sediments of approximately 200µg g$^{-1}$ Cu.
Vowles (1994) suggested that the sediment arsenic levels gave more information on the degree of arsenic sensitivity in *N. diversicolor* from the Humber than worm arsenic levels. However, due to the variability of the estuarine environment, many factors such as the form of metal, temperature, salinity, pH, dissolved oxygen, biology of the organism and the presence of other metals, affect the relationship between toxicity and metal concentrations. He found that tolerance to copper and zinc were not shown to relate to the measured environmental levels as sites with vastly different sediment and worm levels exhibited similar sensitivity levels.

When interpreting the significance of resistance of an organism to a particular pollutant care must be taken as the mechanisms of tolerance may not always be specific. For example, it has been demonstrated that co-tolerance exists when an organism becomes resistant to one toxic substance and may then confer resistance to another pollutant, although the organism may not have been exposed to high levels of this second substance. Co-tolerance has been discovered in *Asellus* (Brown, 1978) and in *Nereis* (Bryan, 1976).

Tolerance to copper in *Nereis diversicolor* has been found to be partly based on a decreased permeability to the metal as well as to an efficient excretory mechanism (Bryan and Hummerstone, 1973). However, it is more probable that the excess metal is detoxified and stored in the epidermis, nephridia or other tissues (Pirie et al 1985). The mechanism and form of copper tolerance will be discussed in a later chapter.

When dealing with areas polluted by a complex of toxicants, a survey for specific resistance can, in theory, be used as a tool to decide which toxic compounds have affected which populations (Hately et al. 1989). The evidence for a negative effect on an ecosystem holds especially if it can be demonstrated that the increased resistance has a genetic basis as this implies that the pollutant has either killed the more sensitive individuals or reduced their number of offspring (Klerks and Levinton, 1989).

Variation in tolerance has been suggested as the basis for the mapping of pollutant effects (Hately et al 1989). The tolerance of Cu in Restronguet creek worms was found to extend for 3km while Zn tolerance only extended for 1 km (Hately et al 1989). Klerks and Levinton (1989) showed differences in metal tolerance between *Limnodrilus hoffmeisteri* collected from sites with different metal concentrations that were only 200m apart. This indicates very strong selection or very limited gene flow. Luoma et al (1983) found that large scale geographical isolation of populations was found not to be a prerequisite for the development of intraspecific differences in tolerance by the bivalve *Macoma balthica* and the copepod *Acartia clausi*.

Anthropogenic trace metal enrichment is often localised in the area of specific discharges. Luoma and Cloern (1982) found metal concentrations in sediments and organisms to be spatially and temporally heterogeneous. Localised adaptations to contamination may
increase the fitness in a heterogeneously-stressed environment. Mortality along steep gradients of metal levels could restrict gene flow between non-isolated populations (Levington 1980). Survival in polluted areas would only occur for tolerant animals. Therefore, populations that do not appear to be geographically isolated and are very close together could give very different responses to metal exposure.

Exposure of *Nereis* from the Alaw and Humber to a range of sublethal copper concentrations over a 30 day period failed to induce tolerance to this metal (within the duration of this experiment). In fact, it would seem that the exposure to sub-lethal levels of copper had adversely affected the worms so that when they are finally exposed to the test concentration they did not survive as long as the non exposed worms. Hately (1989) failed to induce (over a 30 day period) copper tolerance in *N. diversicolor* and Bryan and Hummerstone (1971) failed to induce copper tolerance in *N. diversicolor* after exposure to sediments containing high levels of copper for a 76 day period. Klerks and Levinton (1989) identified a case of development of resistance in *Limnodrilus hoffmeisteri* to sediments with high levels of cadmium and nickel. They estimated that resistance could have developed in 1-4 generations. Dulas estuary has been exposed to high metal levels for approximately 200 years, due to local mining activities which began in the late 18th century. In the Humber, exposure to high levels of metals is thought to have occurred over the last 100 years (Middleton and Grant, 1990).

Peck and Hoffman (1982) did manage to induce copper tolerance in the polychaete *Neanthes arenaceodentata* by pre-exposure over a 28 day period. However, their results showed that the control worms performed better than the worms that were pre-exposed to the two lower concentrations. The authors therefore concluded that a minimum threshold concentration of between 0.028 and 0.016 mg l⁻¹ copper existed, below which no adaptation occurred. Pascoe and Bettie (1979) acclimated Rainbow Trout alevins by pre-exposure to sublethal levels of cadmium. Such acclimations are thought to be due to the induction of metallothioneins (or metallothionein like proteins, MTP's). Metallothioneins have not been reported in polychaetes (the mechanism of tolerance to copper in *Nereis* is discussed in Chapter 3).

The induction periods made no measurable difference to the copper tolerant *Nereis* from Dulas as there were insufficient mortalities to determine a LT₅₀ value regardless of whether the worms were pre-exposed to a sublethal concentration of copper or not. Results from these experiments support other toxicity tests done in this study which have shown that in terms of copper tolerance; Alaw *Nereis*<Humber *Nereis*<Dulas *Nereis*. Worms from Dulas did not appear to lose their tolerance after 30 days of exposure to copper free conditions. Hence, from these investigations it is clear that in the short term tolerance to copper in *Nereis*
*Nereis diversicolor* from all three estuaries (regardless of any intraspecific differences) can neither be easily lost (in tolerant worms) or acquired (in non-tolerant worms).

To investigate if the tolerance to copper in *Nereis diversicolor* is acquired phenotypically or is a genotypic response, the worms from Dulas would have to be tested for heritable components. Hately (1989) found through non-specific matings of *Nereis* that both copper and zinc tolerance had significant heritable components. Klerks and Levinton (1989) demonstrated that elevated resistance to cadmium, nickel and cobalt remained after 2 generations in *Limnodrilus hoffmeisteri* and Brown (1976) found this was also the case with lead tolerance in *Asellus meridians*. They also found that the second generation was slightly less tolerant to heavy metal stress than the field-derived worms. Klerks and Weis (1987) explained this by possible reduction of selection pressure due to laboratory conditions. However, as tolerance remained in the second generation, Klerks and Weis's (1987) investigations ruled out the possibility of maternal effects (cytoplasmic transmission) as tolerance remained in the F2 generation. It is probable that copper tolerance has a heritable nature due to a genotypic response.

ii) DISTRIBUTION OF METALS IN THE HUMBER ESTUARY

The present results show that the range of mean values of Cu, Zn, Pb, Cd, Cr and Ni in the sediments were similar to the ranges reported in Humber sediments by the Environment Agency (NRA, 1994). However, the range of values of copper and zinc in *Nereis diversicolor* in this study were found to be slightly higher compared to those measured by the Environment Agency (NRA, 1994) whereas concentrations of cadmium were found to be similar.

There was little variation shown in copper either spatially or temporally, with concentrations in the sediment, *Corophium* and *Nereis* being in the same or similar orders of magnitude at each site and at each sampling time. However, the highest concentrations observed during the monitoring period were in *Corophium* from Paull. This may be attributed to the fact that the copper concentrations in sediments from Paull (compared to the other sites) showed the least variability over the sampling period. These consistent copper concentrations in the sediment may have exerted a greater biological impact on the organism than fluctuating levels as the copper in the sediment would be consistently more available for uptake by the organisms.

Sediment and organisms from Paull would receive sewage effluent discharged from Hull Sewage Works not far upstream from Paull. The copper load discharged into the estuary is largely dominated by those of Hull sewage and a titanium dioxide plant on the south bank of the Humber (NRA, 1994). The copper load into the estuary from sewage and industry were
approximately 15kg day\(^{-1}\) and 20kg day\(^{-1}\) respectively (NRA, 1994). Copper concentrations in sediment at Barton showed the greatest temporal variability and both *Corophium* and *Nereis* from Barton had the least copper concentrations during the sampling period, thus contributing further evidence that these fluctuating levels of copper in Barton sediment are less available for uptake by the organisms compared to Paull sediment.

Generally, the copper concentrations in the sediment were found to be lower than in the organisms. This would indicate that these organisms have accumulated the copper. It would be difficult to determine if this copper is having adverse physiological or populational effects on the organisms without sublethal toxicity and bioassay testing. Generally, no patterns could be observed between the copper concentrations in the sediments, *Corophium* and *Nereis* at each sampling time at each site. However, any relationships may have been masked due to the copper concentrations in the sediment and organisms not differing greatly at each of the sites. If the organism was regulating the metal in any way, the concentration in its body would not relate to that in the sediment apart from at lower environmental levels. The blood pigment in *Corophium* is haemocyanin which contains copper whereas the pigment (erythrocyanin) in *Nereis* contains iron. This increased requirement for copper in *Corophium* may explain why the copper concentration in these organisms was always higher than in *Nereis*.

There was some spatial and temporal variation in the concentrations of zinc in the sediment. Sediment sampled from Barton showed the largest temporal variability (as for copper) in concentrations compared to Paull and Brough. However, this is not reflected by the concentrations in organisms from the same site. These fluctuating levels could be attributed to the sediment at this site being easily mobilised. The zinc load into the estuary from sewage and industry were approximately 100kg day\(^{-1}\) and 1200kg day\(^{-1}\) respectively (NRA, 1994).

*Corophium* and *Nereis* from Paull had the highest zinc concentrations which suggests that the available metal has had a continuous impact on the biota for some time. The lack of relationship between the levels of zinc in the sediment, *Corophium* and *Nereis* could (as with copper) indicate possible regulation of this metal.

Generally the zinc load was greater in the sediment than in *Corophium* suggesting that some sort of excretion or exclusion mechanism may take place. *Nereis* generally had higher levels of zinc than *Corophium* and similar levels to their sediment suggesting that they can accumulate the metal rather than excrete it.

Spatially the lead concentrations in sediments and the organisms did not differ greatly. The lead load into the estuary from sewage and industry were approximately 10kg day\(^{-1}\) and 25kg day\(^{-1}\) respectively (NRA, 1994). The highest concentration of lead was found in the sediment from Brough and was reflected by the highest concentration in *Corophium* from the same site at the same time. There was also some evidence of the lead concentrations in
sediment at Paull relating to the concentrations in *Corophium* from this site. Lead is not an essential metal to these organisms, and it appears to be less available to *Nereis* than *Corophium* as the concentration in *Nereis* were always lower than in *Corophium*. Generally, the levels in the sediments greatly exceeded those in the organisms. Either the lead is not in an available form to these organisms or some sort of exclusion or excretion mechanism is in operation.

Concentrations of cadmium in the sediments did not differ much spatially or temporally. This was not reflected by the concentrations in the organisms as the cadmium concentrations did show some temporal and spatial variation. Concentrations of cadmium in the sediments, *Corophium* and *Nereis* were similar. The cadmium load into the estuary from sewage and industry were approximately 0.1kg day\(^{-1}\) and 0.2kg day\(^{-1}\) respectively (NRA, 1994).

Chromium and nickel concentrations in the sediments were several orders of magnitude higher than in *Corophium* and *Nereis*. Accumulation of these metals does not seem to occur, indicating that either these metals are not very available to these organisms or that some sort of exclusion mechanism is taking place. Spatial variability in this metal does not seem to occur but there is some evidence of temporal variation.

Environmental Quality Standards (EQSs) for metals are set as the annual average concentration of either the total or the dissolved fraction. They provide the maximum concentration of specific substances permitted in the water column. All metals investigated in this study apart from copper complied with the designated EQS level set for the estuary. However, work by the WRc (1990) has suggested that less than 1% of copper in saline water is in the non complexed form and is readily bioavailable. Therefore, the Environmental Agency does not consider these copper failures to be of great concern (NRA, 1994).

No environmental quality standards for metals in sediments have been set by the Environmental Agency. Metals in sediments in the estuary are monitored by the Environmental Agency, but as a complimentary technique to the monitoring of metals within the water column. Although sediments do move within the estuarine environment they are much less mobile than the water column, and thus analysis of sediments should provide a better historical record. However, the direct biological availability of metals in sediments is largely limited to those that ingest them, although they do transfer into solution, particularly under anaerobic conditions.

The concentrations of metals in sediments is much greater than in water, therefore the results obtained tend to be analytically more reliable. However, results expressed in terms of concentration (\(\mu g \, g^{-1}\)) are very dependent on the size fraction of sediment analysed and variations in sample type may introduce considerable variations.

Organic muddy sediments generally contain the highest concentration of metals and sandy sediments the lowest. It is important, to compare samples of similar particle size if any valid conclusions are to be drawn. Generally, no spatial or temporal trend could be observed
for the metal content of sediments within the Humber estuary, which could indicate that the sediments are relatively well mixed. The sediment samples analysed in this study were all muddy sediments and *Corophium* and *Nereis* were used as indicators of the bioavailable fraction of the metals at the three sites.

There is evidence that a considerable proportion of the metals discharged to the Humber are retained in the sediments which are accumulating within the system (Grant and Middleton, 1990). A proportion will be transported either in solution or adsorbed onto suspended sediment into the North Sea. Considerable reductions in metal loads discharged in effluents were made during the last decade but it may be some time before this is reflected in sediment loads.

In the short term, Humber sediment levels appear stable as there was little variation between samples taken one year apart. In the long term levels have been shown to be steadily increasing (Grant and Middleton, 1990). A sample of *Scrobicularia* clay from the ancient bed of the estuary was used as an indicator of background levels (Middleton and Grant, 1990) and significant anthropogenic enrichment of sediment levels has occurred for many metals notably arsenic (x6), lead (x5), copper (x5) and zinc (x4). In the cases of arsenic, copper and zinc the majority of the enrichment is probably relatively recent (ie. within the last 100 years) (Middleton and Grant, 1990).

### iii) SUMMARY

The copper concentrations in *Corophium* and *Nereis* from the three estuaries reflected the levels in the sediment from each estuary. The relative copper tolerances in these organisms also reflected the level of copper contamination in the animals environment. The concentrations of accumulated copper and the tolerance to copper rank both these animals as follows: Dulas>Humber>Alaw.

Exposure of juvenile and adult *Nereis* from the Alaw and Humber to a range of sublethal copper concentrations over a 30 day period failed to induce tolerance to this metal. Tolerance in adult worms from Dulas was not lost after exposure to ‘clean’ conditions for 30 days.

The range of values (μg g⁻¹) measured in the study of metal distribution in the Humber estuary can be summarised as follows:

<table>
<thead>
<tr>
<th>SEDIMENT</th>
<th>COROPHIUM NEREIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>20-100</td>
</tr>
</tbody>
</table>
The main points observed in the study of metal distribution in the Humber estuary can be summarised as follows;

1) The spatial distribution of metal in each parameter (sediment, *Corophium* and *Nereis*) did not differ greatly.

2) There was generally no spatial or temporal pattern of metal distribution in the parameters.

3) Metal concentrations were generally higher in animals than in sediments.

4) Metal concentrations were generally higher in the sediment than the animals, but accumulation in the animals did occur.

5) Metal concentrations were a lot higher in sediments than in animals.

6) Concentrations in the three parameters did not differ greatly.

7) Concentrations were generally higher in *Nereis* compared to *Corophium*.

8) Concentrations were generally higher in *Corophium* compared to *Nereis*.

9) Concentrations in *Corophium* and *Nereis* did not differ greatly.
Figure 1.0: Mean copper concentration (showing standard error bars) in three populations of *Corophium*

![Graph showing copper concentration in three populations of *Corophium*.](image)

Population:
- Alaw
- Humber
- Dulas

Figure 1.1. Mean copper concentration (showing standard error bars) in three populations of *Nereis*

![Graph showing copper concentration in three populations of *Nereis*.](image)

Population:
- Alaw
- Humber
- Dulas

Figure 1.2. Mean copper concentration (showing standard error bars) in three estuarine sediments

![Graph showing copper concentration in three estuarine sediments.](image)

Sediment:
- Alaw
- Humber
- Dulas

NOTE. All the above samples were collected in June 1994.
Figure 1.3. Metal levels in three estuarine sediments (bars indicate standard errors) sampled in June 1995.
Figure 1.4: LC50 values with 95% confidence limits for three populations of Corophium

Figure 1.5: LC50 values with 95% confidence limits for three populations of Nereis
Table 1.0
LT50 in hours (with 95% confidence limits) for adult Nereis from three estuaries challenged by 0.15 mg/l Cu. This followed a 30 day induction period exposure to 0.00, 0.015 and 0.03mg/l Cu.

<table>
<thead>
<tr>
<th>ESTUARY</th>
<th>Induction Concentrations (mg/l Cu)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>ALAW</td>
<td></td>
</tr>
<tr>
<td>HUMBER</td>
<td></td>
</tr>
<tr>
<td>DULAS</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 1.1
LT50 in hours (with 95% confidence limits) for juvenile Nereis from three estuaries challenged by 0.15 mg/l Cu. This followed a 30 day induction period exposure to 0.00, 0.015 and 0.03mg/l Cu.

<table>
<thead>
<tr>
<th>ESTUARY</th>
<th>Induction Concentrations (mg/l Cu)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>ALAW</td>
<td></td>
</tr>
<tr>
<td>HUMBER</td>
<td></td>
</tr>
<tr>
<td>DULAS</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 1.2
LT50 in hours (with 95% confidence limits) for Dulas Nereis challenged by 0.4mg/l copper before and after a 30 day acclimation period to clean water.

<table>
<thead>
<tr>
<th>LT50 VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Acclimation</td>
</tr>
<tr>
<td>After Acclimation</td>
</tr>
</tbody>
</table>

N/A Insufficient mortalities to compute LT50 values
Figure 1.6. Mean copper concentrations (showing standard error bars) at three sites on the Humber

Barton

\[ \mu g/g \ (dry \ weight) \]

\[ 0 \rightarrow 120 \]

Sediment  Corophium  Nereis

Brough

\[ \mu g/g \ (dry \ weight) \]

\[ 0 \rightarrow 220 \]

Sediment  Corophium  Nereis

Paull

\[ \mu g/g \ (dry \ weight) \]

\[ 0 \rightarrow 250 \]

Sediment  Corophium  Nereis

July'94  Oct'94  Jan'95  April'95  July'95
Figure 1.7. Mean zinc concentrations (showing standard error bars) at three sites on the Humber.
Figure 1.8. Mean lead concentrations (showing standard error bars) at three sites on the Humber

**Barton**

**Brough**

**Paull**

![Graphs showing lead concentrations at three sites on the Humber](image-url)
Figure 1.9. Mean cadmium concentrations (showing standard error bars) at three sites on the Humber

**Barton**

- Sediment
- Corophium
- Nereis

**Brough**

- Sediment
- Corophium
- Nereis

**Paull**

- Sediment
- Corophium
- Nereis

Legend:
- July'94
- Oct'94
- Jan'95
- April'95
- July'95
Figure 1.10. Mean chromium concentrations (showing standard error bars) at three sites on the Humber

**Barton**

- **Sediment**
- **Corophium**
- **Nereis**

**Brough**

- **Sediment**
- **Corophium**
- **Nereis**

**Paull**

- **Sediment**
- **Corophium**
- **Nereis**

**Legend:**
- July'94
- Oct'94
- Jan'95
- April'95
- July'95
Figure 1.11. Mean nickel concentrations (showing standard error bars) at three sites on the Humber

Barton

μg/g dry weight

Sediment Corophium Nereis

Brough

μg/g dry weight

Sediment Corophium Nereis

Paull

μg/g dry weight

Sediment Corophium Nereis

July'94  Oct'94  Jan'95  April'95  July'95
CHAPTER 2
ACCUMULATION OF COPPER IN NEREIS DIVERSICOLOR AND COROPHIUM VOLUTATOR FROM THREE ESTUARIES

INTRODUCTION

Some metals are essential to life, e.g. copper, zinc and iron. These elements are vital components of enzymes and respiratory pigments (White and Champ, 1983). Carbonic anhydrase, carboxy-peptidase A and B and several dehydrogenases contain zinc; pyruvate and carboxylase contains manganese, haemocyanin contains copper and haemoglobin contains iron. Consequently, marine invertebrates must somehow deliver a variety of trace metals to their tissues in sufficient quantities to meet diverse metabolic and respiratory requirements. Therefore, the well being of organisms is dependent on an appropriate metal supply and metal deficiency or accumulation in reactive states beyond a threshold concentration gives rise to detrimental effects (Depledge and Rainbow, 1990).

Among marine invertebrates there appear to be two contrasting methods of essential trace metal accumulation (Weeks and Rainbow, 1991): i) increasing body concentrations proportional to increasing metal bioavailabilities, often with an accompanying means of detoxifying the newly accumulated metal, and ii) the regulation of the body metal concentration at an approximately constant value over a wide range of metal bioavailabilities (Rainbow et al, 1990).

Nereis is known to accumulate metals such as Ag, Cd, Pb and Cu and these appear to reflect the availabilities of metals in the environment (Luoma and Bryan 1982, Bryan and Gibbs 1983). Tolerance to metals in Nereis diversicolor is due to a lowered permeability to Cu coupled with an increased ability to deposit Cu in the tissues in tertiary lysosomes: metallothioneins do not seem to be involved (Bryan and Gibbs, 1983; Pirie et al, 1985). However, excretion of the metal was found to be the mechanism of control of lead and zinc in earthworms (Ireland, 1976).

Marine organisms may have the ability to detoxify trace metals through sequestration on proteins and/or in membrane-bound vesicles (Depledge and Rainbow, 1990). This detoxification capacity is finite, and under conditions of high metal uptake or severe stress the capacity to sequester these metals can be exceeded. Under these conditions excess metals would spill over and have direct impact on sites of toxic action, such as the enzyme-containing pool.
The tolerance of *Corophium volutator* to high concentrations of copper in the environment may be partially attributable to the formation of intracellular granules within the cells of the alimentary canal. In common with several species of decapods, isopods and amphipods, the production of granules in the cells of the gut is restricted to the 'hepatopancreas' (Icely and Nott, 1980).

The more physiologically advanced members of the crustacean group (decapoda) are capable of the regulation of body levels of essential metals. As an example, the littoral prawn *Palaemon elegans* is able to regulate body concentrations of zinc (Rainbow and White, 1989), and copper (White and Rainbow, 1982). Radiotracers have confirmed that zinc regulation in *P. elegans* is achieved by balancing the rate of zinc excretion to match the rate of zinc uptake (White and Rainbow, 1984a,b). Other work has provided evidence of regulation for a range of other decapod Crustacea, for example, the brown shrimp *Crangon crangon* (Amaiard *et al*., 1985).

Other crustaceans have also adopted storage accumulation strategies. Rainbow and White (1989) found net accumulation of copper at all experimental concentrations and no evidence of regulation in the amphipod *Echinogammarus pirloti* during an exposure period of twenty eight days. Two species of talitrid amphipods (*Orchestia gammarellus* and *Orchestia mediterranea*) showed a net accumulation when exposed to a range of zinc and copper concentrations in solution.

Barnacles do not regulate body concentrations of zinc or copper to a constant concentration (Rainbow and White, 1989), but accumulates metals in proportion to metal availability. Such a strategy requires an associated mechanism for the detoxification and subsequent storage of such metals, which would otherwise inevitably reach metabolically available toxic concentrations. In barnacles the principal means of rendering copper metabolically inactive is by binding with metallothionein to produce insoluble deposits rich in copper and sulphur in the parenchyma cells which surround the midgut (Rainbow, 1987). Similar systems for the detoxification of high concentrations of copper and zinc have been described for woodlice (Hopkin, 1989). No crustacean appears to regulate the body concentrations of non-essential metals such as cadmium (Rainbow and White, 1989).

Estuarine environments receive a large variety of xenobiotic inputs which may tend to overload normal physiological mechanisms. Continued survival of organisms is due to protective mechanisms of biotransformation or detoxification present in the cells (Moore *et al*, 1986). Moore (1985) and Moore *et al* (1986) linked cellular and subcellular damage in marine organisms to xenobiotic perturbation of cells and investigated protective mechanisms of biotransformation and detoxification involving NADPH-dependent cytochrome, P-450 monoxygenases and metallothioneins respectively. They also discussed the use of such effects
as an 'early warning' biomonitoring system incorporating the initial effects of a pollutant on cell function,

Depledge and Rainbow (1990) state that the key determinants of trace metal body concentrations in marine invertebrates are bioavailability from seawater and from food. However, the nature of the trace metal (essential or non-essential and the chemical properties) and the physiological state of the organism, strongly influences the subsequent handling, distribution, tissue accumulation and excretion.

Peddicord (1984) discussed the meaning of bioaccumulation as a measure of marine pollution effects. To determine the specific levels that cause particular ecological effects in a given species, it is necessary to distinguish between change and effect. Since environmental consequences of particular levels of bioaccumulation cannot be determined, it is not possible to judge the acceptability of pre-change baseline conditions. It could be that the levels against which change is determined is sufficient to cause, or already have caused, adverse environmental impact. To be meaningful in measuring effects of marine pollution, consequences of bioaccumulation are going to have to be stated in terms of demonstrably adverse biological responses to specified levels of bioaccumulation.

Bioavailability and toxicity may be substantially altered by site-specific variations in water chemistry (e.g., salinity, pH, dissolved organics) or by the presence of other contaminants. This latter point is crucial in complex effluent situations where a myriad of trace metals and organics results in complex matrices which make modelling of uptake and toxicity of individual compounds difficult. The major problem is determining the relevance of a specific tissue or body burden to the fitness of an organism or community (Depledge and Rainbow, 1990). These correlations are made further difficult because organisms have the ability to detoxify and acquire tolerance to a wide range of environmental contaminants.

The major factors influencing metal concentrations in the tissues of marine invertebrates stated by Depledge and Rainbow (1990) are: 1) the environmental bioavailability of trace metals; 2) the diffusion conductance of the boundary surfaces of the animal (including the gut); and 3) the extent to which metals are retained within the animal. The aims of the present study were to investigate this third factor by comparing the total copper accumulation, the actual amount of copper accumulated and rate of accumulation over specific time periods by exposure to increasing sublethal levels of copper, in populations of Nereis and Corophium with different abilities to tolerate copper.
MATERIALS AND METHODS

i) COPPER ACCUMULATION EXPERIMENTS WITH NEREIS

Two hundred worms of similar size (30-40mm in length) were collected from both the Alaw and the Humber estuary. Two hundred and forty worms were collected from the Dulas estuary and all worms were acclimated as described earlier (see General Methods) for ninety-six hours. It was important to keep to a minimum any metal loss due to the acclimation period in clean water whilst at the same time ensuring that the worms had emptied the contents of their guts. Copper concentrations of 0, 0.05, 0.1 and 0.15 mg l⁻¹ were used in experiments for Alaw and Humber worms and concentrations of 0, 0.05, 0.1, 0.15 and 0.3 mg l⁻¹ were used in experiments for Dulas worms. The highest levels of these respective ranges represent approximately 50% of the 96 hour LC₅₀ recorded for worms from each of these sites.

Forty worms from each site were exposed to each of the above concentrations, separated into batches of ten in individual crystallising dishes. All experiments were carried out at 14 °C, pH 7.8 and at a salinity of 17. The copper solutions were changed every 24 hours. These test conditions were maintained for a period of 96 hours. Two batches of worms (usually 3-4 individuals) were required for analysis, and were pooled from each copper treatment at 24, 48 and 96 hours. Any dead animals were removed and discarded. The worms were then immediately frozen at -20 °C and stored for subsequent metal analysis. The digestions and metal analyses were carried out, using the same protocol described in Chapter 1 (Materials and Methods, section i,b).

To calculate the actual amounts of copper accumulated in each population of Nereis the mean total copper concentration from the control treatments in each test condition was subtracted from the mean total copper concentration at each copper treatment. However, after 96 hours in the control treatments worms from Dulas lost a large proportion of their body copper content (Table 2.2). This reduced value did not represent the animals usual ‘baseline’ copper concentrations. Therefore it was thought more accurate, (when calculating the amount of copper accumulation at 96 hours) to subtract the mean control value at 48 hours from the mean total copper concentration at 96 hours. The rates of accumulation in the three populations of Nereis were subsequently calculated.

ii) COPPER ACCUMULATION EXPERIMENTS WITH COROPHIUM

The protocol was the same for Corophium as described for Nereis. However, eighty organisms from each site were exposed to each of the concentrations, separated into batches of
twenty, because of the increased number of *Corophium* needed to give the required dry weight for metal analysis and the difficulty at this sampling time (Spring 1995) of collecting high numbers of these organisms from the Dulas estuary, it was decided that the experiment would only be maintained for 48 hours and that the copper concentrations would be 0.05 and 0.3mg l⁻¹ for *Corophium* from all three estuaries. The highest concentration represents approximately 50% of the 96 hour LC₅₀ recorded for the organisms from the Humber which was intermediate to that from the Alaw and Dulas.

The amounts of copper accumulated in each population of *Corophium* were calculated (as for *Nereis*) by subtracting the mean total copper concentration from the control treatments in each test condition from the mean total copper concentration at each copper treatment after 24 and 48 hours. This method for standardising the mean total copper concentrations was carried out in all test conditions as *Corophium* from Dulas in the control treatments did not lose a great proportion of their body copper contents. The rates of accumulation in each population of *Corophium* were also calculated.

A Three Way Analysis of Variance (ANOVA) test was carried out using SPSS to determine if the origin of the organism (ie. the population), the exposure concentration and the exposure period were significant ($p \leq 0.05$) in explaining the variability in the levels of accumulated copper in both *Corophium* and *Nereis*. Transformation of the data was not necessary as it was assumed the data had a distribution close to normal as the variance of the data for each group were similar.

Higher order interactions (ie. combined effects of these variables) were also examined for the *Corophium* data set but could not be analysed for the *Nereis* data set. As the number of external concentrations differed between populations it was impossible to examine the higher order interactions using the three way ANOVA. In order to examine these interactions it would have been necessary to omit the copper accumulation data associated with the fourth exposure concentration for Dulas *Nereis*. This would have altered the data set and would have affected which of the variables significantly explained the variation in the levels of accumulated copper in *Nereis*.

A One Way Anova was then performed on the data to establish if there were any significant differences ($p \leq 0.05$) in the different combinations (groups) of exposure concentrations, exposure period and origin of population. Posterior testing (using Least Significant Differences) was then performed on these groups to establish at which concentrations, exposure periods and population of organism combinations these significant differences occurred.
RESULTS

i) **NEREIS**

a) **TOTAL COPPER CONCENTRATION**

The total body copper concentrations in the three populations of worms before experimental treatment are shown in Tables 2.0, 2.1 and 2.2 (length of exposure 0 hours). Worms from the Alaw had the lowest Cu concentration of 39.66 $\mu$g g$^{-1}$ compared to worms from the Humber (143$\mu$g g$^{-1}$) and Dulas (1048$\mu$g g$^{-1}$). After 96 hours of exposure to all concentrations (not including the control) the total copper concentrations ranged from 130.67-248.06 $\mu$g g$^{-1}$ in Alaw worms, 195.76-221.85 $\mu$g g$^{-1}$ in Humber worms and 1104-1311.19 $\mu$g g$^{-1}$ in Dulas worms.

The levels of copper in worms from the Alaw (Table 2.0 and Figure 2.0) increased as the exposure time increased, particularly after exposure to the highest concentration of 0.15mg l$^{-1}$ Cu. There was no consistent pattern to be observed in the levels of copper in worms from the Humber (Table2.1 and Figure 2.1) after exposure to the different Cu concentrations at an increasing exposure period. However, when exposed to the highest concentration (0.15mg l$^{-1}$ Cu), after an initial decrease the body copper concentration increased with time. A high level of variability can be observed in the total copper concentrations in worms from the Humber (Figure 2.1). At all exposure concentrations the copper levels in Dulas worms (Table 2.2 and Figure 2.2) increased after 24 hours then began to level off at 48 hours. These worms in the control environment (0mg l$^{-1}$ Cu) lost body copper as the exposure time increased.

b) **COPPER ACCUMULATION**

Values of the actual amounts of copper accumulated in each of the three populations can be seen in Tables 2.0, 2.1 and 2.2. The population of worm (based on the estuary of origin), the copper concentrations used and the exposure period were all found to be statistically significant (p<0.05) in explaining the variation in copper accumulation in *Nereis* (Table 2.3).

Table 2.4 shows the results of the analysis of variance (and posterior testing) on the combinations (or groups) of concentrations, exposure periods and population of organisms. Levels of accumulated copper in worms from Dulas (at all exposure concentrations) and Alaw (at the highest concentration) after 48 and 96 hours of exposure had significantly (p<0.05) higher accumulated copper compared to any other exposure time and concentration.
combination. All the Humber worm Cu concentrations (at every Cu conc/exposure time combination) were also found to be significantly different to the above mentioned group.

There were no significant differences in the level of copper accumulation in all three populations of worms at any exposure concentration after only 24 hours of exposure. This indicates that initially the amount of copper that can be accumulated is similar regardless of the organism's tolerance or the external concentration. However, it was only worms from Dulas that had a negative accumulation rate (Table 2.2) at 24 hours.

c) COPPER ACCUMULATION RATES

After 96 hours of exposure to all exposure concentrations (not including the control) the rates of copper accumulation ranged from 20.78-50.93μg g⁻¹ in Alaw worms, -2.37-13.9μg g⁻¹ in Humber worms and 33.78-85.46μg g⁻¹ in Dulas worms. Initially (at 24 hours) worms from the Alaw showed the highest accumulation rates compared to worms from the Humber and the Dulas (Tables 2.0, 2.1 and 2.2). After 96 hours of exposure the highest accumulation rates were found in worms from Dulas, with the lowest found in Humber worms.

The accumulation rate in Alaw worms (Figure 2.3) increased with time when exposed to the highest concentration. The rate of accumulation at all concentrations showed a similar pattern where it reduced at 48 hours then increased again. There was no consistent pattern shown in the accumulation rate for Humber worms at all concentrations (Figure 2.4). After 24 hours of exposure to most of the concentrations, Nereis from Dulas (Figure 2.5) had a negative accumulation rate indicating that they were losing metal. The rate of accumulation at every concentration increased in these worms until 48 hours, then decreased.

ii) COROPHIUM

a) TOTAL COPPER CONCENTRATION

The total body copper concentrations in the three populations of Corophium before experimental treatment are shown in Table 2.5 (length of exposure 0 hours). Corophium from the Alaw had the lowest Cu concentration of 120.08μg g⁻¹ compared to those from the Humber (162.36μg g⁻¹) and Dulas (442.4μg g⁻¹).

The levels of copper in Corophium from the Alaw (Table 2.5 and Figure 2.6) increased with exposure time at both concentrations (0.05 and 0.3mg g⁻¹). A similar pattern can be seen for Humber organisms (Figure 2.7). Corophium from Dulas in control conditions lost copper between 24 and 48 hours (Figure 2.8), whereas copper levels increased with time at the exposure concentrations. At 48 hours the highest external concentration (0.3mg l⁻¹) always
produced the highest copper levels in all the organisms. However, at this concentration (at 48 hours) the levels of copper were lower in Dulas animals (682.35 \mu g \text{g}^{-1}) than from either Alaw (697.95 \mu g \text{g}^{-1}) or Humber (851.3 \mu g \text{g}^{-1}) animals even though the natural body copper levels in organisms from Dulas were originally the highest.

b) COPPER ACCUMULATION

Values of the actual amounts of copper accumulated in each of the three populations can be seen in Table 2.5. The estuary from which the Corophium originated (population), the copper concentrations used and the exposure period were all found to be statistically significant (p>0.05) in explaining the variation in copper accumulation in Corophium (Table 2.6). Interactions (combined effects) between the variables were not found to be statistically significant in explaining the variables.

Table 2.7 shows the results of the analysis of variance (and posterior testing) on the combinations (or groups) of concentrations, exposure periods and population of organisms. At both concentrations and at both times, the accumulation in Dulas animals was significantly lower than animals from the Alaw and the Humber at the same concentrations and times. There was no difference between the Cu accumulated in Alaw and Humber Corophium at 48 hours at the highest concentration. There was no statistical difference between the level of accumulated copper at 24 hours and 48 hours when exposed to the highest concentration (0.3mg l$^{-1}$) in animals from the Alaw. The same is true for animals from the Humber. However, the level of accumulated copper in Dulas animals was significantly higher at 48 hours compared to 24 hours at this same concentration.

c) COPPER ACCUMULATION RATES

After 24 and 48 hours of exposure to both concentrations the rate of copper accumulation was the lowest in organisms from Dulas and highest in organisms from the Humber (Table 2.5). At the highest concentration the rate of copper accumulation seemed to decrease with time for organisms from the Alaw and the Humber, but not change greatly in animals from Dulas. After 48 hours of exposure to this concentration the rate of copper accumulation was the lowest in Dulas animals (138.62 \mu g \text{g}^{-1}) compared to animals from the Humber (339.6 \mu g \text{g}^{-1}) and the Alaw (284.45 \mu g \text{g}^{-1}). The rate of accumulation was greater at the highest concentrations for every population at 24 and 48 hours.
DISCUSSION

*Nereis* and *Corophium* from the metal contaminated Dulas estuary had the highest basal copper body burden and tolerated the greatest amount of copper compared to organisms from the Humber and the Alaw estuaries. (see Chapter 1). The Alaw estuary is considered the 'clean' estuary and the organisms collected from this estuary had the lowest basal body copper levels and the least ability to tolerate copper. Organisms from the Humber had intermediate levels of basal body copper and were more able to tolerate copper than organisms from the Alaw but not as tolerant as those from Dulas (see Chapter 1).

i) ACCUMULATION IN *NEREIS*

The total levels of copper in the tolerant worms from Dulas did not continue to increase with exposure time after 24 hours, at any of the experimental concentrations, with the rates of copper accumulation decreasing after 48 hours. This indicates a possible ability to regulate the levels of copper in the organisms from this estuary. Howard and Brown (1983) found that *N. diversicolor* from the Tees estuary appeared to be able to regulate uptake of copper, zinc and iron from 50% sea water at concentrations below 0.01, 1.0 and 5.0 µg ml⁻¹ respectively. For copper and zinc these concentrations were exceeded in the interstitial waters of surface sediments grossly polluted with metals but not in uncontaminated sediments (Bryan and Hummerstone, 1973). They concluded that worms from high zinc sediments are better able to regulate zinc than those from uncontaminated estuaries.

Posteriori testing was carried out to establish at which population, concentration and exposure period combinations the significant differences in the amounts of accumulated copper occurred. The actual amounts of accumulated copper in Dulas worms after exposure to every concentration at 48 and 96 hours were significantly higher than in any other time/concentration combination (with the exception of Alaw worms exposed to 0.15 mg g⁻¹ Cu). As these worms from Dulas can tolerate high levels of copper (Chapter 1) it would seem probable that these organisms have adapted a mechanism for accumulating the copper and sequestering it in a non toxic form.

Studies on the bioavailability and accumulation of heavy metals in sediments have been reviewed by Bryan and Langston (1992) who have said that *Nereis* is found in some of the most heavily polluted sediments and is demonstrably far more tolerant of Cu (and Zn) than the same species from cleaner areas (Bryan and Hummerstone, 1971, 1973). Tolerance is due to a lowered permeability to copper, coupled with an increased ability to deposit Cu in the tissues in

Negative accumulation rates were calculated for the Dulas worms at every exposure concentration at 24 hours, with worms in the control conditions losing approximately half their body copper levels. This meant that initially these organisms were losing some of their basal body copper. This could be because the copper in the experimental solutions were less available to these worms, ie. of a lower concentration or in a different form than copper in their natural environment. Howard and Brown (1983) discovered that Nereis from the Tees estuary lost approximately half the copper they had taken up when placed in sea water for a similar period of time to which they were exposed to copper in solution.

Total body copper levels and the rates of accumulation in worms from the Alaw increased with exposure time at all external concentrations indicating that these organisms do not have an ability to regulate their body copper levels. There was no significant difference found between the amounts of accumulated copper in Alaw and Dulas worms when exposed to their highest respective concentrations (0.15mg l⁻¹Cu) and 0.3mg l⁻¹Cu) at 48 and 96 hours. Both these populations of worms accumulated the highest amounts of Cu at these highest respective exposure concentrations. These concentrations represented approximately half the population’s LC50 value. As these worms are not tolerant to copper (Chapter 1) this would suggest that these organisms have not adapted a mechanism for accumulating copper in a non-toxic way and the metal would accumulate in the tissues causing physiological damage. As the rate of copper accumulation in worms from the Alaw when exposed to this concentration at 48 hours was increasing, this concentration over a longer exposure period would probably be lethal.

There was no consistent pattern found for the total copper levels or the rates of accumulation of this metal in the worms from the Humber. This may indicate a large natural variability in the ability of these organisms to acquire an ability to regulate this metal. There is some evidence for potential regulation when exposed to the lower copper levels. However, at the highest exposure concentration the total body copper levels increased with time and the accumulation rate was the greatest at 96 hours.

The population of Nereis (differing with respect to its origin), the external copper concentration and the period of exposure were all found to be statistically significant in explaining the variability in the actual amounts of copper which had been accumulated. Howard and Brown (1983) also found that different populations of Nereis might be adapted to regulate at levels within the ranges of metal concentrations normally found in the interstitial waters of the different sediments in that organism’s habitat. Differences between the rates of accumulation of copper in populations of the same organisms (compared to other metals) may
depend on its chemical form in the water, but also on differences between the ways in which they are adsorbed and then transported inwards.

The uptake of metals by *Nereis diversicolor* from the environment may be via the epidermis, ingested sediments or food. Bryan and Hummerstone (1971, 1973) proposed the uptake of Cu and Zn by *Nereis* to be predominantly from interstitial waters. In this experiment the uptake of the metal would be almost solely via the epidermis as there was no sediment and the organisms were not fed. However, uptake through the intestinal tract can not be ruled out as the experimental solution may have been ingested.

No significant differences were found between the levels of accumulated copper in the different organisms at any of the exposure concentrations at 24 hours. This would indicate that initially, the amount of copper that can be accumulated is similar regardless of the physiological differences these *Nereis* may have.

Generally, the rate of accumulation of copper in all worms was found to increase as the exposure concentrations increased. This would be expected due to the increased number of dissolved copper ions that would be available for uptake by the organisms. The level of copper in worms also tends to be proportional to that in the sediment (Bryan and Langston, 1991) with relationships being observed in the polychaetes *Nereis diversicolor*, *Perinereis cultrifera* and *Nephtys hombergi* by Luoma and Bryan (1982) and Bryan and Gibbs (1983; 1987).

**ii) ACCUMULATION IN COROPHIUM**

Organisms from the Dulas in the control exposure lost total body copper over the exposure period. However, their total body copper increased when exposed to both the exposure concentrations (0.05 and 0.3 mg l⁻¹ Cu). At both exposure concentrations at 24 and 48 hours the actual amount of copper accumulated in *Corophium* from Dulas was significantly lower than in organisms from the Alaw and the Humber.

Dulas organisms are the most tolerant to copper and have the highest body copper levels naturally. Therefore, although copper is obviously stored in a non toxic form, in the case of amphipods, isopods and decapods it is bound to granules in the hepatopancreatic ceaca (Icely and Nott, 1980). It also seems likely that excretion of this metal occurs. Wright (1986) found that the uptake patterns for copper and lead in *Gammarus marinus* were quite different from that of zinc. Significant uptake of both copper and lead was seen in all populations. However unlike zinc, chronic lead and copper exposure is not associated with an increased capacity for the metal. The reverse may be true, and there is some evidence that an exclusion mechanism may operate in populations from chronically contaminated environments.
The total body copper levels in amphipods from the Alaw and Humber estuaries increased with the exposure time at both concentrations, indicating that in the 48 hours of exposure there was no evidence of an ability to regulate the copper levels. *Corophium* from both the Alaw and the Humber estuaries showed greater sensitivity to copper (see Chapter I) compared to those from Dulas, but the actual levels of copper that they accumulated over the exposure period were found to be significantly higher than those in organisms from Dulas. This was also found in a study carried out by Riesh (1993) who showed that *Corophium insidiosum* had the greater sensitivity and bioaccumulation of copper, zinc and other metals compared to *Elasmopus bampo* (which were less sensitive) over a 20 day period.

At the highest exposure concentration the rates of copper accumulation in Dulas *Corophium* were similar at both exposure times. For organisms from the Alaw and the Humber the rates decrease slightly. Frazier and George (1983) have illustrated that oysters from a relatively pristine environment take up trace metals faster than those from a chronically contaminated environment.

There may be a danger in attempting to predict a heavy metal accumulation strategy from consideration of uptake rates alone. Amphipods (as peracarid malacostracans) have morphologies not greatly dissimilar to that of decapod malacostracans (known to regulate Cu and Zn), with most of the cuticle being tanned and permeable surfaces being restricted for example to thoracic gills. It is also important to consider the other half of the regulation equation - that of the excretion rate (Rainbow and White, 1989).

The population (with respect to its origin), the exposure concentration and the exposure period were all found to be statistically significant in explaining the variability in the actual amounts of copper that were accumulated in these organisms. The methods of heavy metal accumulation vary within crustaceans among species and among metals (Bryan 1976; Rainbow, 1987, 1988; Nugegoda and Rainbow, 1988; Rainbow et al, 1990). For example, the decapod *Palaemon elegans* is able to regulate body concentrations of the essential metals zinc and copper to a constant body concentration over a wide range of ambient metal availabilities before regulation breaks down and net accumulation begins, whereas the non-essential metal cadmium is accumulated at all availabilities (Rainbow and White, 1989). The suggestion was that copper is both taken up and excreted, i.e. regulated, by the decapod *Palaemon elegans.* However, the same authors in consideration of the accumulation patterns of the amphipod *Echinogammarus pirloti* and the barnacle *Elminius modestus* concluded that, whether or not copper excretion occurs, there is still net accumulation of copper by these crustaceans from a dissolved source (Weeks and Rainbow, 1991). These results have provided a basis for Rainbow et al (1989) to use talitrid amphipods as biomonitors of the bioavailability of ambient zinc and copper concentrations in British waters.
iii) GENERAL DISCUSSION

In a physiological sense, the term regulation implies that despite perturbations in the surrounding environment (air or water), some biological processes within tissues or whole organisms can be maintained more or less constant. If the concentration of a particular trace metal rises above a certain level, sensors detect the rise and a negative feedback loop is initiated which restores the original level (Bell, 1973). This might involve enhanced metal excretion, impairment of metal uptake or some combination of the two. The other method of regulating metabolically available metal which involves the storage of the metal in a non toxic way allows metal availability at intracellular, metabolically active sites to be regulated, even though an analysis of the whole animal may suggest an extremely high metal concentration in the body. If a significant proportion of a particular metal is taken up and stored in a detoxified form (rather than excreted), then the tissue will gradually accumulate the metal. If the tissue excretion rate matches tissue uptake rate, tissue concentrations will remain more or less constant (Depledge and Rainbow, 1990).

The ability to regulate one metal does not necessarily confer the ability to regulate others. Trace metals in storage may be gradually lost from the animal or, in some cases, continue to accumulate throughout life. Alternatively, stored trace metals may be made metabolically available once more, for use in some vital process (Rainbow and White, 1989).

Depledge and Rainbow (1990) stated that it is insufficient to consider whole body metal concentrations without some knowledge of tissue concentrations and tissue loads within the organism. It is also difficult to fully appreciate the significance of a particular metal load without considering the physiological status of the organism in question. In the context of this study, the physiological status considered was that of tolerance to copper. Other physiological variables, such as size were kept constant. The natural copper body burdens in the three populations of each species were known, as were the possible locations of the tissue loads, which is investigated further in _Nereis_ in Chapter 3.

Other biological variables may be important in affecting accumulation of a metal. For example, uptake of copper from solution by _Nereis_ has been shown experimentally to result in a change from a linear to a curvilinear plot of metal content against size (Howard and Brown, 1983). Rainbow and Moore (1986) found that the concentration of copper and zinc in a selection of amphipods decreased with increasing amphipod dry weight.

Environmental variables such as salinity can also affect the accumulation of a metal (McLusky _et al_, 1986). For example, there is a lack of competition from calcium and magnesium ions at low salinities which may increase the uptake of metals. To avoid these
problems in these experiments an intermediate salinity was used and only organisms of similar size were chosen for the study. It would have been beneficial to have used a longer exposure period (particularly for Corophium) as the accumulation patterns would become clearer.

The use of aquatic organisms as biomonitors of trace metal bioavailability is well established and the prerequisite properties of biomonitors are discussed at length by Phillips (1980) and Bryan et al (1980). One essential requirement of any biomonitor is that it should be a net accumulator of the trace metal in question, not regulating the body metal concentrations over a range of ambient exposure metal exposure availabilities. However, as stated by Depledge and Rainbow (1990), it would be beneficial to understand the mechanisms underlying regulatory and storage abilities of individual tissues to fully identify the biological significance of trace metals.

iv) SUMMARY

This study has indicated that both Nereis and Corophium are net accumulators of copper from solution in that their body concentrations are affected by differences in dissolved metal availability. However, both the tolerant populations of Corophium and Nereis from the Dulas estuary show very different accumulation strategies compared to the other populations of the same species, with Nereis accumulating significantly higher amounts of copper than Corophium. Thus indicating that as well as interspecific differences in the toxicant uptake and physiological responses to accumulating the metal, there are also intraspecific differences.

From the evidence presented in this study it would appear that generally metal accumulation in the tolerant populations of Nereis was greater than in the non tolerant organisms. Perhaps due to genotypic or phenotypic adaptations to the high levels of copper in their natural habitat these organisms have an increased ability to sequester the copper in a non toxic form. This may be due to an increased number of active sites (or proteins) available for binding or an increased ability to synthesise these proteins due to some sort of 'trigger'. These worms seem to be regulating the metal, i.e. the total body copper levels do not increase after 24 hours and rates of uptake decrease after 48 hours. Active expulsion of copper cannot be ruled out as the amounts and rates of accumulation in tolerant worms level out as time period and exposure increase. Excretion of copper may take place at higher levels and periods of exposure but the length of exposure and level of copper are limited in this investigation by the effects on the worms such as starvation and toxicity. Isotopic labelling may provide the answer, if carried out over greater time periods and varied concentrations, but starvation and mortality would have to be taken into account.
Special physiological control mechanisms need not explain the differences in trace metal concentrations among the tissues of various invertebrates (Depledge and Rainbow, 1990). A likely scenario could be that physical or chemical site saturation processes were primarily responsible for the reduction of accumulation at the higher copper levels. As the concentration increases the competition for uptake sites on the surface of the organism increase, hence, once the metal reaches a critical concentration the uptake sites may become saturated, resulting in the uptake and the associated accumulation being reduced (Unlu and Fowler, 1979).

However, if this was the case the results would have shown similar patterns of reduction in accumulation with time and copper concentration in each of the species used in this study regardless of their tolerant abilities. This did not happen as the non tolerant worms from the Alaw ('clean' estuary), do not appear to regulate, with their total body copper levels increasing with exposure time and Cu concentration. The amounts of copper that were accumulated at the higher exposure concentrations were significantly higher than the amounts accumulated at lower concentrations. This was also shown at the highest exposure concentration in worms from the Humber as the total body copper levels increased with time.

The tolerant population of *Corophium* from Dulas had the highest total body copper levels but accumulated significantly lower amounts of copper compared to organisms from the Alaw and the Humber. The high levels of total body copper suggest that the metal is being sequestered in a non-toxic way but an exclusion mechanism and/or an excretion mechanism may also be in operation. *Corophium* from the Alaw and the Humber accumulated the highest amounts of copper which exceeded their basal levels. As these organisms have significantly lower LC50 values (Chapter 1) than the tolerant organisms from Dulas it would be interesting to investigate with further experiments where the metal is and what happens to it as the organisms become increasingly stressed as the exposure period continues.
Table 2.0: The mean total copper concentrations, copper accumulation and rate of copper accumulation for Alaw worms.

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<tr>
<th>Length Of Exposure (Hours)</th>
<th>Cu Conc. (mg/l)</th>
<th>Total Cu Conc. (µg/g)</th>
<th>S.E. (µg/g)</th>
<th>Cu Accum (µg/g)</th>
<th>Rate Of Cu Accum ((µg/g)/day)</th>
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Table 2.1: The mean total copper concentrations, copper accumulation and rate of copper accumulation for Humber worms.

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<th>Total Cu Conc. (µg/g)</th>
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<th>Cu Accum (µg/g)</th>
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Table 2.2: The mean total copper concentrations, copper accumulation and rate of copper accumulation for Dulas worms

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<td>384.3</td>
<td>201.88</td>
<td>100.94</td>
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<td>94.01</td>
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<td>388.23</td>
<td>194.11</td>
</tr>
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<td>(control) 0</td>
<td>745</td>
<td>145</td>
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<td>--</td>
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<td>100.9</td>
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<td>99.9</td>
<td>341.87</td>
<td>85.46</td>
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Figure 2.0: Mean total copper concentrations (showing standard error bars) in Alaw Nereis when exposed to copper concentrations relative to the exposure period.
Figure 2.1: Mean total copper concentrations (showing standard error bars) in Humber *Nereis* when exposed to copper concentrations relative to the exposure period.
Figure 2.2: Mean total copper concentrations (showing standard error bars) in Dulas Nereis when exposed to copper concentrations relative to the exposure period.
Table 2.3: Analysis of variance on the worm population (i.e. which estuary the worms were sampled from), the exposure concentration and the exposure time.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of Squares</th>
<th>DF</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig of F</th>
<th>Significance p&lt;0.05</th>
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<td>sig</td>
</tr>
<tr>
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<td>sig</td>
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</tr>
<tr>
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<td>sig</td>
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<tr>
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<td></td>
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Table 2.4
Analysis of variance for copper accumulation in worms.
One way ANOVA on Groups representing the estuary the animal came from, the external copper concentration and the exposure time.

Homogenous Subsets (highest and lowest means are not significantly different)
Homogenous Subsets by groups (as shown by Multiple Range Test (Least Significant Difference)).

| Est. | D | D | D | H | H | D | H | H | A | H | A | A | H | H | A | A | D | D | D | D | D | D | D | D |
| Time | 24 | 24 | 24 | 48 | 96 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 96 | 48 | 24 | 48 | 24 | 96 | 48 | 48 | 48 | 96 | 48 | 96 | 48 |
| Conc | 0.1 | 0.15 | 0.05 | 0.1 | 0.1 | 0.3 | 0.15 | 0.05 | 0.1 | 0.05 | 0.1 | 0.05 | 0.15 | 0.05 | 0.15 | 0.05 | 0.1 | 0.15 | 0.05 | 0.05 | 0.05 | 0.3 | 0.15 | 0.1 | 0.3 |
| Gp   | 20 | 21 | 19 | 14 | 17 | 22 | 15 | 12 | 10 | 4 | 11 | 16 | 5 | 2 | 1 | 18 | 13 | 3 | 8 | 7 | 6 | 25 | 24 | 9 | 23 | 27 | 26 | 29 | 28 | 30 |
| Mean | -189 | -148 | -77 | -22 | -9.5 | -9 | -0.2 | 1.25 | 15.5 | 24.1 | 24.9 | 28.8 | 29.8 | 35.6 | 38.1 | 55.6 | 60.7 | 73.7 | 81.2 | 86.4 | 113 | 188 | 202 | 204 | 293 | 359 | 388 | 422 | 464 | 566 |

KEY Groups within the shaded area are significantly different to groups that are not in the shaded area.
Groups within the shaded area are not significantly different to each other.
Figure 2.3: The rate of copper accumulation per day for Alaw *Nereis* when exposed to three copper concentrations

Figure 2.4: The rate of copper accumulation per day for Humber *Nereis* when exposed to three copper concentrations

Figure 2.5: The rate of copper accumulation per day for Dulas *Nereis* when exposed to three copper concentrations
Table 2.5: The mean total concentrations, copper accumulation and rate of copper accumulation for *Corophium* from three estuaries.

<table>
<thead>
<tr>
<th>Population Of Organisms</th>
<th>Length Of Exposure (Hours)</th>
<th>Cu Conc (mg/l)</th>
<th>Mn. Body Cu level (µg/g)</th>
<th>S.E.</th>
<th>Cu Accum (µg/g)</th>
<th>Rate Of Cu Accum (µg/g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALAW</td>
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<td></td>
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<td></td>
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<td></td>
<td>304.3</td>
<td>152.15</td>
</tr>
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<td>697.95</td>
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<td></td>
<td>568.9</td>
<td>284.45</td>
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<td>24</td>
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<td>48</td>
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<td>277.25</td>
<td>138.62</td>
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Figure 2.6: Mean total copper concentrations (showing standard error bars) in Alaw Corophium when exposed to copper concentrations relative to the exposure period.
Figure 2.7: Mean total copper concentrations (showing standard error bars) in Humber Corophium when exposed to copper concentrations relative to the exposure period.
Figure 2.8: Mean total copper concentrations (showing standard error bars) in Dulas Corophium when exposed to copper concentrations relative to the exposure period.
Table 2.6: Analysis of variance on the Corophium population (ie. which estuary the Corophium were sampled from), exposure concentration and the exposure time.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of Squares</th>
<th>DF</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig of F</th>
<th>Significance</th>
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</thead>
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<td>Main Effects</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>sig</td>
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<tr>
<td>2-Way Interactions</td>
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<td>23</td>
<td>45389.88</td>
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Table 2.7
Analysis of variance for copper accumulation in *Corophium*
One way ANOVA on Groups representing the estuary the animal came from, the external copper concentration and the exposure time.

Homogenous Subsets (highest and lowest means are not significantly different)
Homogenous subsets by groups as shown by Multiple Range Test (Least Significant Difference)).

<table>
<thead>
<tr>
<th>Est.</th>
<th>D</th>
<th>D</th>
<th>D</th>
<th>A</th>
<th>D</th>
<th>A</th>
<th>H</th>
<th>A</th>
<th>H</th>
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<td>48</td>
<td>24</td>
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<td>24</td>
<td>48</td>
<td>24</td>
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<tr>
<td>Conc</td>
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<td>0.05</td>
<td>0.05</td>
<td>0.3</td>
<td>0.05</td>
<td>0.05</td>
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<td>0.05</td>
<td>0.3</td>
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<td>7</td>
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<td>160.5</td>
<td>222.35</td>
<td>277.25</td>
<td>306.42</td>
<td>357.25</td>
<td>470.75</td>
<td>530.75</td>
<td>568.9</td>
<td>652.9</td>
<td>673.75</td>
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KEY       Groups within the shaded area are significantly different to groups that are not in the shaded area.

Groups within the shaded area are not significantly different to each other.
CHAPTER THREE
LOCALISATION OF COPPER IN *NEREIS DIVERSICOLOR*

INTRODUCTION

*Nereis diversicolor* from the Dulas estuary have been found to show an increased tolerance to copper compared to *Nereis* from the Alaw estuary (see Chapter one). This resistance must be due to some sort of detoxification mechanism as organisms from Dulas have very high body copper levels compared to organisms from the clean Alaw estuary. Simkiss and Mason (1983) presented some criteria that may be useful for establishing whether a system is involved in detoxification or not. a) Do the activities of this system enhance the tolerance of the organisms to the toxicant? b) Does the presence of the toxicant increase the activity of the system? c) Is the presumptive detoxification system compartmentalised or polarised in some tissue or cell so that the level of toxicant in the body is minimised? d) Is the toxicant excreted due to the activities of the system? In this section of this study the question of metal compartmentalisation is considered.

One possible detoxification mechanism involves the metallothioneins (Mts) which are low molecular weight proteins rich in cysteine. They are induced by several types of factor, including exposure to such metals as cadmium, mercury, zinc, copper, gold and silver (Kagi and Schaffer, 1988). MTs have high affinity for these metals and it is generally accepted that the binding of toxic metals to MTs constitutes a detoxification mechanism (although metal detoxification may not be MTs primary role). However, *Nereis diversicolor* along with some other invertebrates are not thought to possess metallothioneins having the characteristics defined by Kagi and Nordberg (1979). Nejmeddine *et al* (1988) found a low molecular weight protein not related to a MT (called metalloprotein II (MP II)) in *Nereis diversicolor* from contaminated sites that has the capacity to bind to cadmium and probably zinc and copper.

Another possible detoxification mechanism is the sequestering of metals in vesicles, lysosomes and other membrane-bound structures, as granules and intranuclear inclusions, or as insoluble metal precipitates (George, 1982). The sequestering of a metal will constitute a detoxification if this process keeps the metal from interacting at sensitive sites in the organism. Bryan and Hummerstone (1971) showed that copper was present in the epidermis, parapodia and in part of the nephridium of *Nereis*. Pirie *et al* (1985) found copper to be present in spherical granules mainly in the epithelial cells of the first segment (peristomium) and the cells of the nephridium in metal tolerant *Nereis*. They found that copper was present, in association with sulphur, in membrane-limited vesicles similar to tertiary lysosomes. The accumulation of different metals was tissue specific and indicated that tolerance was probably due to increased
deposition of Cu in tertiary lysosomes and not to the presence of a significant concentration of metallothionein.

Metals are accumulated to very high concentrations within discrete 'granules' or vacuoles in many different cell types in most living organisms. The tissue distribution, composition, size, morphology, function, and possibly the fate of these structures is extremely varied (Brown, 1982; Morgan and Winters, 1986). Based on largely compositional information, at least 6 different metal accumulating structures can be recognised, separated into two distinct types (Icely and Nott, 1980). The first type consists of homogenous electron dense material which always contains copper and sulphur. The second type has concentric layers of dense material which usually contain calcium, magnesium and phosphorus. A range of other metals can occur in these granules including zinc, potassium, iron and lead.

This study aimed to examine the localisation of copper in animals from a copper tolerant population of *Nereis diversicolor* from the Dulas estuary. A histochemical examination at the organ/tissue, cellular and intracellular level was used to investigate the mechanism of tolerance in this population of *Nereis*. 
MATERIALS AND METHODS

i) EXPERIMENTAL REGIME

Five Nereis from the Alaw estuary and five from the Dulas estuary were taken from the samples of worms that were being acclimated (allowed 48 hours for gut evacuation) for participation in accumulation experiments (Chapter 2). All the worms were approximately 4cm long and were sliced into 3 equal sections along the length of the worm and fixed in 70% alcohol. The area of the worm under investigation was the middle section as it has already been shown in previous studies (George and Pirie, 1985) that copper can be stored in Nereis diversicolor in the nephridia and intestinal epithelium.

A specific technique which stains copper using rubeanic acid (Uzman, 1956) was used in this study to identify the areas in which copper may be present in the section. Approximately 20 sections from the same area of each worm were examined under the light microscope to ensure that there was consistency shown in the results of the staining. Photographs using the light microscope were taken to show the transverse section of a worm with the copper represented by an indicator colour. Photographs were taken using a Zeiss photomicroscope 2.

Sections from the same area of worm were also prepared for Transmission Electron Microscopy (T.E.M.) in a Transmission Electron Microscope (JEOL 100C, U.K. Ltd.) and viewed at 80KV. The areas of cells or tissues where copper was identified were photographed.

ii) THE FIXATION AND PREPARATION OF SAMPLES

The histochemical localisation of copper with rubeanic acid has been reliably used for a long time (Uzman, 1956). This procedure shows the copper in tissues in the form of a fine granular black precipitate. Methods for section cutting and staining for light microscope work can be found in Claydon (1962). The methods for the preparation of biological samples for electron microscopy can be found in Lewis and Knight (1977). Both methods are summarised below.

Staining with Rubeanic Acid

1) Fix the sample with 70% alcohol.
2) Wash in distilled water.
3) Gradually dehydrate the sample with increasing strengths of alcohol.
4) Soak in a 1:1 mixture of histoclear (which is miscible with wax) and Paramatt Plus wax and leave to slowly penetrate into the centre of the tissue.

5) Make three changes of the wax to remove any traces of the histoclear.

6) Finally embed in fresh Paramatt wax and allow to solidify.

7) Cut sections 8μm thick using a microtome and mount onto microscope slides.

8) Stain the sections with rubeanic acid and leave to dry. Identify any dark staining using a light microscope.

**Preparation of samples for T.E.M.**

1) Fix in a solution containing 2.5% glutaraldehyde, 1% formalin, containing 0.35M sucrose for 18-24 hours.

2) The solution was buffered in 0.1M phosphate buffer (pH 7.2) to ensure that the solution has an osmolarity similar to the sample. This avoided the sample either shrinking or swelling which would damage the cellular structure.

3) The sample was embedded in a mixture of Epon and Araldite (Epoxyresin).

4) The sections were cut using glass knives on an Ultramicrotome to a thickness of approximately 90nm which made the sections appear gold in colour due to the refraction of light through them at this thickness.

5) The sections were then mounted on uncoated copper grids and stained with 5% aqueous Uranyl Acetate. They were further stained with lead citrate and allowed to dry before examination under the electron microscope.
RESULTS

i) HISTOCHEMICAL LOCALISATION OF COPPER

Transverse sections of *Nereis diversicolor* under the light microscope showing the different structures of the body can be seen in Dulas worms in Figure 3.0 (A) and in Alaw worms (Figure 3.0, B).

Figure 3.1 shows transverse sections (in colour) using the light microscope of *Nereis* from and Alaw (A) and Dulas (B) stained with rubeanic acid. The rubeanic acid stains any copper complexes a brown/black colour compared to the usual pink stain. Copper is shown to be present in the nephridial area of the Dulas worms (B) but not present in the same area of the Alaw worms (A). The staining of many sections of both worms showed consistently the presence of copper in the nephridial area of the worms from the Dulas estuary. No copper was found to be present in the sections made from the same areas of the worms from the Alaw estuary.

The nephridial area on the right side of the Dulas worm can be seen in greater detail under the light microscope in Figure 3.2. A and B. The convoluted tubules of the right nephridium can be seen clearly. The dense brown/black dots in the lower area of the nephridium indicate copper localisation. Figure 3.3 A and B show the same under higher magnification.

ii) ULTRASTRUCTURAL EXAMINATION OF COPPER

Figures 3.4-3.7 show the ultrastructure of specific areas using the Transmission Electron Microscope. Figures 3.4 and 3.5 show the cells lining the nephridial tubes and the where the copper is localised ultrastructurally and the dense copper granules are situated within. Cilia and microvilli can be seen in cross section in the tubular lumen. Figures 3.6 and 3.7 show the copper concentrations under magnification of 38000 and 62700 respectively. The copper complexes are clearly situated within intracellular membranes.
Figure 3.0: Transverse sections of *Nereis diversicolor* under the light microscope, showing the different structures of the body, stained with rubeanic acid.

Magnification: \( \times 30 \)

A: Worm from Dulas (metal contaminated) estuary.

B: Worm from Alaw (clean) estuary.

**KEY**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>d</td>
<td>dorsal vessel</td>
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<tr>
<td>g</td>
<td>gut</td>
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<tr>
<td>dm</td>
<td>dorsal longitudinal muscle</td>
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<tr>
<td>n</td>
<td>nephridium</td>
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<tr>
<td>nc</td>
<td>nerve cord</td>
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<tr>
<td>vm</td>
<td>ventral longitudinal muscle</td>
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Figure 3.1: Transverse sections under the light microscope of the right nephridial area of *Nereis* from the Alaw and Dulas estuaries stained with rubeanic acid. Any copper complexes present stain a brown/black colour compared to the usual pink stain.

Magnification ×300

A: Worm from Alaw (clean) estuary.

B: Worm from Dulas (metal contaminated) estuary.

KEY

n  nephridium

vm  ventral longitudinal muscle

c  deposition of copper
Figure 3.1: Transverse sections under the light microscope of the right nephridial area of *Nereis* from the Alaw and Dulas estuaries stained with rubeanic acid. Any copper complexes present stain a brown/black colour compared to the usual pink stain. 
Magnification ×300

A: Worm from Alaw (clean) estuary.
B: Worm from Dulas (metal contaminated) estuary.

KEY

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tr>
<td>n</td>
<td>nephridium</td>
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<tr>
<td>vm</td>
<td>ventral longitudinal muscle</td>
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<tr>
<td>c</td>
<td>deposition of copper</td>
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Figure 3.2: Transverse sections under the light microscope of *Nereis* from Dulas showing the nephridium with the denser copper complexes as black dots, stained with rubeanic acid. Magnification ×72

A: Photographic representation using the normal green filter.
B: No green filter used which shows the darker and denser copper complexes more clearly.

**KEY**

- n: nephridium
- c: deposition of copper
- g: gut
- dm: dorsal longitudinal muscle
- nc: nerve cord
- vm: ventral longitudinal muscle
Figure 3.3 A and B: Transverse sections of Nereis under the light microscope from Dulas showing the nephridium with the denser copper complexes as black dots, stained with rubeanic acid.

Magnification ×450

Note
Both these figures are photographs of the same section using different focuses. Each photograph focuses on different granules which shows that these granules can be found at different depths in the section.

KEY

- nd  convoluted nephridial ducts
- cg  copper granules
- vm: ventral longitudinal muscle
Figure 3.4: Transverse section of *Nereis* using the T.E.M. from the Dulas estuary showing the dense copper granules in the right nephridium, stained with uranyl acetate and further stained with lead citrate.

Magnification ×2500

Figure 3.5: Transverse section of *Nereis* using the T.E.M. from the Dulas estuary showing the dense copper complexes in the right nephridium, stained as above.

Magnification ×3800

KEY

t: tubular lumen

c: cilia

cg: copper granules

m: microvilli
Figure 3.6: Transverse section of *Nereis* using the T.E.M. from the Dulas estuary showing the dense copper complexes in the nephridium, stained with uranyl acetate and further stained with lead citrate.

Magnification ×38000

Figure 3.7: Transverse section of *Nereis* under the T.E.M. from the Dulas estuary showing the membrane bound copper complexes in the nephridium, stained as above.

Magnification ×62700

KEY

m: membrane
hm: dense homogenous material
DISCUSSION

The rubeanic acid staining method and the light microscope showed that copper appears as granular deposits in the nephridia of *Nereis diversicolor* collected from the Dulas estuary and that these were absent from those collected from the Alaw estuary (Figures 3.2A and B).

Bryan and Hummerstone (1971) also used the rubeanic acid method and showed that copper appeared as granular deposits in the epidermis and the nephridia of *Nereis diversicolor* collected from copper contaminated estuarine sediments. Pirie *et al.* (1985), using X-ray microanalysis found that cells in the nephridium of *Nereis diversicolor* living in Restronguet Creek contained granules with high concentrations of copper. They also reported that copper was localised in the epithelial cells of the first segment (peristomium), the outer epidermis, oesophagus and stomach. Granular accumulations of copper were found also by X-ray microanalysis within marine Crustacea (Walker, 1977) and Mollusca (George *et al.* 1978).

Other metals have been reported to accumulate in specific tissues. Jenson and Baatrup (1988) found that mercury was demonstrated histochemically in the intestine, nephridia and epidermis of *Nereis virens*. Zinc was found localised in the gut wall, dorsal epidermis and blood systems (Fernandez, 1983), and in the jaws (Bryan and Gibbs, 1980) of *Nereis diversicolor*. However, it was concluded that the zinc may be associated with organic material in the jaw and that rather than being the end point of a detoxification process this metal may have a structural role.

Gibbs *et al.* (1981) have shown that the high body copper in *Melinna palmata* is not related to the availability of copper in the surface sediments on which it feeds. Much of the copper is contained in special epidermal cells identified using rubeanic acid, in the branchiae, feeding tentacles, head and anterior thoracic segments. The copper is released from the cells in the epidermis of the branchiae and tentacles when ruptured upon seizure, which reduces the palatability, thus inhibiting predator activity.

A pair of metanephridia lies on the floor of nearly every segment of *Nereids*. Each nephrostome opens into the coelomic cavity and the convoluted nephridial duct opens on the exterior surface. The cells lining the inner surface of the nephridial duct are ciliated. The excretory tube is coiled through most of its length, and the coils are compacted within a granular oval mass (Barnes *et al.*, 1988). The external pore lies at the base of the parapodium. The internal end passes through the coelomic partition, and opens into the coelomic chamber just anterior to that in which the main body of the organ and the external pore lie. Wastes from the blood which pass through the excretory organ and microscopic particles in the coelomic fluid are wafted into the opening of the funnel by beating of the cilia.
Under the light microscope copper was observed in the ectodermal cells situated towards the lower area of the convulated tubular mass. It would appear that the copper was accumulated away from the mesodermal cells of the coelomoduct and sequestered towards the external pore. Silver grains have also been observed under the light microscope in the nephridial tubule cells of mercury-treated worms (Jensen and Baatrup, 1988).

At the ultrastructural level, the copper concentrations were localised mainly as membrane bound structures in the cells of the nephridial tubules. Mercury accumulations have also been localised predominantly within lysosome-like bodies in the nephridia and epidermis of *Nereis virens* (Jensen and Baatrup, 1988). Pirie *et al* (1985) found that copper was present in *Nereis diversicolor*, in association with sulphur, in membrane limited vesicles similar to tertiary lysosomes. This type of granule according to Icely and Nott (1980) consists of homogenous electron dense material which always contains copper and sulphur; in *Asellus meridians* they can also incorporate lead (Brown, 1978). The results of this study suggest that in accordance with Pirie *et al* (1985) the concentrations observed in *Nereis diversicolor* from the Dulas estuary are of this type. The other distinct type of granule (Icely and Nott, 1980) has concentric layers of dense material which usually contain calcium, magnesium and phosphorus; a range of other metals, which can occur in these granules includes zinc, potassium, iron and lead. Such structures were not recognised in this study.

Nejmeddine *et al* (1987) confirmed the presence of a low molecular weight protein, metaloprotein II (MP II) (estimated at about 10kD in its purified state) having the capacity to bind cadmium, zinc and copper in both control and contaminated *Nereis diversicolor*. These results may explain the relatively low bioaccumulation of cadmium in contaminated worms since an inhibitory effect of zinc on the accumulation of cadmium was experimentally shown in both *N. diversicolor* (Bryan and Hummerstone, 1973) and *N. virens* (Ray *et al*, 1979). A cadmium binding pool having a molecular weight of about 9 kD which also bound copper and zinc was described in the polychaete *Neanthes arenaceodentata* (Jenkins and Sanders, 1986).

Gel permeation chromatography of this fraction showed that Cu and Zn were predominantly associated with low molecular weight components and not metallothionein in *Nereis diversicolor* (Pirie *et al*, 1985). *N. diversicolor*, in common with some other invertebrates does not possess metallothioneins having the characteristics defined by Kagi and Nordberg (1979). Young and Roesijadi (1983) also found a low molecular weight (about 5kD) copper binding protein in another polychaete, *Eudistylia vancouveri*.

Among the metaloproteins found in invertebrates several have, in common with proteins found in *Nereis*, a molecular weight which is relatively low (5-20kD) (Nejmeddine *et al*, 1987). These molecules have a very low level of cysteine and a substantial level of aromatic amino acids and histidine.
In the field, potential metal sources for *Nereis* would be from food and water. The pathway of copper into the worm would be through cell membranes such as the epidermal cells and gut tissue. The ability to detoxify and accumulate the copper in the nephridial area of the worms from Dulas could be explained by a number of metabolic pathways. The copper would be associated with transport molecules such as metaloproteins or coelomic fluid.

It is not known at present whether the copper deposits in the nereid tubular epithelium originated from the blood or whether they had been absorbed from the tubular lumen, or originated from both these sources. The nereid metanephridium is, however, known to be an excretory organ (Fretter and Graham, 1976; Barnes, 1980) which is involved in the turnover of exogenous materials. It is possible that nephridial excretion of copper in *Nereids* does occur. The same conclusions were drawn from studies on the localisation of mercury in *Nereis virens* (Jenson and Baatrup, 1988).

In polychaetes, Dales (1954) reported that the coelomic fluid is full of free cells (coelomocytes) originating from particular tracts of epithelium lining the coelomic cavity. The shape and function of these coelomocytes are varied and they have been classified as erythrocytes, eleocytes, haemocytes which contain haemoglobin; amoebocytes, phagocytes, granulocytes and so on according to their structure and function. The MP II protein is, at least partially, localised in a category of free coelemic cells, the granulocytes (Nejmeddine et al., 1988). It is also reported that annelid amoebocytes have powers of movement and may leave the ceolom and creep through the tissues to the skin or into blood vessels.

The nephridia of annelids are controlled by sphincter muscles which are relaxed periodically for the discharge of wastes. In many annelids there is evidence that it is not only the coelomocytes which act as phagocytes, but some cells of the nephridia may act phagocytically and take up particles from the fluid drained through them. Macrophage amoebocytes which are enzymatically equipped with lysosomal phosphatase, can perform both extra and intracellular digestion of foreign particles are involved in the phagocytic clearance of pollutants (Bouchaud et al., 1992).

Copper tolerant *Corophium* from Dulas were not examined in this part of the study. However, the tolerance of *Corophium volutator* to high concentrations of copper has been correlated directly with the level of copper in the environment (Icely and Nott, 1980). *Corophium* from the Menai Straits have no granules or only a few, while those collected from the Dulas estuary may be partially attributable to the formation of intracellular granules within the cells of the alimentary canal. In common with several species of decapods, isopods and amphipods, the production of granules in the cells of the gut is restricted to the hepatopancreatic caeca.
The formation of the inclusions, containing heavy metals, in the hepatopancreatic caeca of *Corophium volutator*, *Carcinus maenus* and *Stegocephalooides christianiensis* is associated with the maturation of cells along the length of the caeca (Hopkin and Nott, 1979; Moore, 1979). The inclusions in the differentiating cells at the distal end of each caecum are smaller than those in the mature cells at the proximal end. In *Corophium*, intact granules have been identified in dying cells at the proximal end of the caeca which suggests that they are removed via the gut when the cells finally disintegrate. In this way copper could be excreted directly in an insoluble form (Icely and Nott, 1980). As copper is an essential metal it would be incorporated into a metabolic pathway for physiological use. However, when the levels of copper inside the worm become greater than that animal's ability to deal with it, then physiological damage would occur. Previous work (Chapter 2) has shown that the non-Cu tolerant worms from the Alaw continue to accumulate copper with time at an external concentration of approximately half that organisms' LC50 value. This would indicate that copper was not been stored in a non toxic form and with increased duration of exposure would probably cause the *Nereis* to die. Accumulated copper in the tolerant Dulas worms must be stored, in a non toxic form.

Klerks and Bartholomew (1991) investigated the possible physiological mechanisms by which resistance was achieved by a cadmium tolerant population of the oligochaete *Limnodrilus hoffmeisteri* from the metal polluted Foundary Cove. The resistant worms accumulated more metal compared to the control population and had significantly higher levels of a cadmium binding metallothionein-like protein than the control worms. It was therefore likely that the increased resistance was due to an increased capacity to detoxify the cadmium. This elevated protein level was shown to be genetically determined as the second generation offspring of these tolerant worms that had no pre-exposure to cadmium also had an elevated level of this cadmium binding protein.

Brown (1977) showed that differences with respect to the sequestering of metals in organelles plays a role in genetic adaptations of animals to elevated metal levels. A resistant population of *Asellus meridianus* had elevated copper content in 'granules' and 'dense spherical inclusions' compared to a non-tolerant population.

It is probable that the *Nereis* used in this study have developed a genotypic ability to tolerate high levels of copper in the natural environment. Acclimation of these worms to 'clean' conditions (Chapter 1) for 30 days did not reduce these organisms' ability to tolerate copper. This would indicate that the ability to sequester the copper in a non-toxic form was not lost due to phenotypic acclimation to clean conditions, at least in the short term.

It is important to determine genetically based differences in MT, MT like binding proteins or the ability to sequester the metal in populations from different sites. Intraspecific
differences in the genotypic ability of an organism to tolerate a metal should be taken into account in the impact assessment of bioavailable metals in marine and estuarine systems.

This copper tolerant population of *Nereis* may have acquired an ability to respond genotoxicologically and switch on genes that synthesise metalloproteins when a certain trigger (ie. a certain level of copper in the blood) is made. Perhaps these tolerant *Nereis* have more specialised coelomocytes that are associated with metalloproteins. The specific area of tissues used to store the metal granules in the nephridial mass presumably contain many specialised cells which engulf the copper and bound it in a membrane where it is stored and possibly incorporated into the lysosomal cycle.

It is not known at present whether the visualised copper deposits in the nereid tubular epithelium originated from the blood or whether they had been adsorbed from the tubular lumen, or originated from both these sources. The nereid metanephridium is, however, known to be an excretory organ (Fretter and Graham, 1976, Barnes, 1980) which is involved in the turnover of exogenous materials. It is possible, that nephridial excretion of copper in *Nereids* does occur. The same conclusions were drawn from studies on the localisation of mercury in *Nereis virens* (Jenson and Baatrup 1988).

In conclusion, the compartmentalisation of copper into membrane bound structures in the *Nereis* from Dulas is a detoxification strategy which relates to their ability to tolerate high levels of copper in their environment. It would have been useful for quantitative purposes to have carried out X-ray microprobe analysis. These resistant worms sequester Cu in individual granules in the cells of the nephridial tubules. It is possible, that a metaloprotein (not metallothionein) also plays a role in the resistance.
CHAPTER FOUR
THE RESPONSES OF COROPHIUM VOLUTATOR AND NEREIS DIVERSICOLOR TO COPPER IN NATURAL SEDIMENTS

INTRODUCTION

Measurements of levels of heavy metals in marine habitats can be made from the water, sediments or the biota (Rainbow, 1995). Many monitoring programmes only measure the dissolved heavy metal concentrations which provide an assessment of total metal present, not of the portion which is bioavailable. It is the bioavailable fraction that is potentially toxic and of ecotoxicological relevance. Heavy metals accumulate in sediments, particularly organically rich sediments, and may exceed the levels in the overlying water by between three and five orders of magnitude (Bryan and Langston, 1992). These sediment levels are easily measured and are much less susceptible to accidental contamination than the low levels found in water. Furthermore, sediments offer a degree of time integration, overcoming the worst effects of temporal variability of heavy metal availability (Rainbow, 1995). With such high concentrations, the bioavailability of sediment metal assumes considerable importance, especially in some filter feeding and burrowing organisms. There are various routes by which sediment metal can reach the biota and individual biomonitors respond differently to different sources of bioavailable metal - for example, in solution, in sediment or in food. Therefore, to gain a complete picture of total heavy metal bioavailability in a marine habitat it is necessary to use a suite of biomonitors, reflecting bioavailabilities from all available sources.

The physico/chemical nature of metals in estuaries

Trace metals are distributed among various compartments of the environment. A portion will be associated with organic and inorganic ligands in solution whereas another fraction will become associated with the particulate matter following adsorption, precipitation, co-precipitation, or uptake by living organisms. As a result of these complex physical, chemical and biological processes, a major fraction of the trace metals will eventually become associated with the bottom sediments.

The marked physico-chemical gradients encountered in estuaries result in changes in the chemical speciation of trace metals (Duinker et al., 1982). Increased salinities and sometimes pH, during estuary mixing leads to flocculation of iron oxides, humic acids, and other colloidal particles such as clays. As iron oxides and humics are efficient scavengers of trace metals (de Groot et al., 1976; Duinker et al., 1982) this results in a general shift of metals
from the dissolved to the particulate form. Deposition of this particulate material then generally follows, although fine colloidal material has a very low settling velocity and may remain in suspension for long periods of time, being transported over large distances (de Groot et al., 1976). When such removal processes operate, the metal is said to behave non-conservatively; i.e. its dissolved concentration does not decrease simply as a function of dilution (Jones, 1978). In Restronguet Creek Bryan et al. (1980) found that Cu was removed while Zn behaved conservatively. Morris et al. (1984) found that both Cu and Zn behaved non-conservatively in the Tamar estuary.

Once sediments have been deposited in estuaries the chemical environment often changes drastically to a reducing one (de Groot et al., 1976). Heavy metal compounds that are chemically stable in an oxidising environment become unstable as the oxygen level falls; the metal forms new compounds that are stable under reducing conditions. In the marine environment this generally results in the formation of relatively insoluble metal sulphides (Bryan, 1984). Thus, although reduction of hydrated Fe oxide should result in the release of adsorbed metals, they remain essentially fixed in reducing sediments as insoluble sulphides (Forstner and Wittmann, 1979). However, observations made on porewater metal concentrations tend to challenge the above theory. A considerable body of evidence points to the existence of a relatively large reservoir of interstitial dissolved trace metals within the upper layers of reducing sediments in the estuarine environment (Duchart et al., 1973; Manheim, 1976).

Remobilisation of metals from sediments may result from 1) dredging and associated activities in coastal marine areas, 2) hydraulic phenomena such as wave action and erosion, and 3) the activities of burrowed benthic organisms (bioturbation) (Swift, 1993). Bioturbation may influence the geochemistry of sediments and their interstitial waters through: 1) the pumping of metal enriched interstitial water out of the sediment to the overlying water; 2) through the action of burrowing organisms bringing oxygen into the deeper, anoxic layers, changing the solubility of trace metals; 3) redistribution and incorporation of particulate material in the sediments, including the incorporation of dead organisms that have accumulated metals in their body tissues; and 4) deposition of faecal pellets onto the sediment surface (Swift, 1993).

Partitioning of metals in sediments

The distribution of heavy metals between the various fractions of the sediments is described as elemental partition. They can be adsorbed at particle surfaces (e.g. clays, humic acids, metal oxyhydroxides); ii) carbonate-bound (e.g. discrete carbonate minerals co-precipitated with major carbonate phases); iii) occluded in iron and/or manganese oxyhydroxides (e.g.
discrete nodules, cement between particles, coatings on particles); iv) bound up with organic matter in either living or detrital form; v) sulphide bound (eg. amorphous sulphides formed in situ or more crystalline forms); vi) matrix bound (eg. bound in lattice positions in aluminosilicates, in resistant oxides or sulphides) (Tessier and Campbell, 1987).

Partitioning among the different sedimentary fractions is important in determining the biological availability of sediment-bound metals and the exchange of metals between sediments and the water column (Luoma and Bryan, 1981). To investigate this the sediment may be fractioned physically, according to grain size or by density gradient separation, and the individual fractions analysed separately. Alternatively, sequential extractions with appropriate reagents can be devised to leach successive fractions of the metals selectively from the sediment samples (Forstner, 1982). The reagents fall naturally into classes of similar chemical behaviour, for example, concentrated inert electrolytes, weak acids, reducing agents, complexing agents, oxidising agents and strong mineral acids. The extractants can be used in sequential fashion. However, there are limitations associated with these methods, ie. the procedures were designed for use with oxic sediments. It would be difficult to select a sequence of reagents that would extract the individual fractions in order without influencing the other sediment constituents. Any sequential extraction procedure will suffer from a certain lack of selectivity, as has been shown theoretically (Sigg et al, 1984) and experimentally (Rapin and Forstner, 1983).

Theoretically, selective extraction requires existence of discrete phases within the sediment that may be dissolved independently. Kheboian and Bauer (1987) argued that many sediments in fact may not contain chemically or physically distinct phases; thus element distribution patterns produced by extraction methods may be artifactual. In addition, even when phases are discrete, there are technical difficulties associated with achieving complete and selective dissolution and recovery of trace metals from those phases.

More than 10 different sequential extraction procedures have been developed. In particular the approach of Tessier et al (1979) has been applied widely and it is on this paper that the methods used in this study are based. Kheboian and Bauer (1987) studied the accuracy of selective extraction procedures for metal speciation (focusing on these methods) in model aquatic sediments. Despite their limitations these methods are applied widely and improvements are being made (Ure et al, 1993). A modified version of the method of Tessier et al (1979) is included in a chemical analysis report for the Commission of the European Community (Ure et al, 1993). Partial extraction of sediments has provided significant insight into the physico-chemical factors influencing the bioavailability of particulate trace metals (Tessier and Campbell, 1987) Many studies have shown that trace metal levels in different organisms are best related not to total metal concentrations in the adjacent sediments, but rather
to relatively easily extracted fractions (Luoma and Bryan, 1978; Diks and Allen, 1983; Tessier et al, 1984a).

Availability of metals in sediment to organisms; with emphasis on copper

Concentration and bioavailability of metals in estuarine sediment to the organisms living in it depend on many different processes and is reviewed in Bryan and Langston (1992). Examples include 1) mobilisation of metals to the interstitial water and their chemical speciation, 2) transformation (e.g. methylation) of metals including As, Hg, Pb and Sn, 3) the control exerted by major sediment components (e.g. oxides of Fe and organics) to which metals are preferentially bound. 4) competition between sediment metals (e.g. Cu and Ag; Zn and Cd) for uptake sites in organisms, and 5) the influence of bioturbation, salinity, redox or pH on these processes.

Under field conditions, identification of dominant processes can be achieved by observing the goodness of fit between metal concentrations in ubiquitous deposit feeding species and levels in various types of sediment extract over a wide spectrum of sediment types. Examples of deleterious effects on benthic organisms that can be attributed to specific metallic pollutants are comparatively rare. Effects are ameliorated by the induction of metal tolerance mechanisms in some species (Bryan and Langston, 1991).

There has been much speculation over the years concerning the biomagnification of metals with increasing trophic levels along food chains. Whilst animals having higher metal concentrations than prey are sometimes found, the only consistent evidence of biomagnification, concerns methylmercury (Bryan and Langston, 1992).

For copper in a dissolved form the free cupric ion (Cu$^{2+}$) is the most readily available and toxic inorganic species of copper, but it only accounts for a small proportion of total dissolved copper in seawater (Wright and Zamuda, 1987). However, the free ion concentration is very sensitive to complexation and its proportion of the total tends to be reduced by the presence of natural organic chelators, or by high salinity when the possibility of inorganic complexation is increased. Wright and Zamuda (1987) noted that even when the cupric ion activity was kept constant the bioavailability of copper to the deposit feeder Mya arenaria increased with decreasing salinity, possibly because competition from Ca and Mg ions for uptake sites was reduced. The bioavailability and toxicity of copper is promoted by some synthetic organic chelators, such as oxine which is a fungicide. These compounds are lipid soluble and the copper complex can rapidly penetrate the cell membrane (Florence and Batley, 1976). It can be concluded that important factors governing the bioavailability of dissolved copper include the cupric ion concentrations, salinity and the presence of organic ligands.
In Restronguet Creek, the adsorption of most of the dissolved Cu by flocculated oxides of iron and associated humic substances during estuarine mixing leads to the very high (3000 µg g⁻¹) sediment concentrations. Interstitial water concentrations in the surface sediments often have concentrations of copper of the order of 100 µg l⁻¹ whereas no more than a few µg l⁻¹ are found in interstitial water from clean sediments (Bryan and Gibbs, 1983; Douglas et al, 1986).

In oxidised surface sediments, Cu is associated both with oxides of iron and organic components such as humics (Luoma and Bryan, 1981). It is not usually clear to what extent Cu availability depends on the level in the interstitial water or that bound to particles. Significant relationships were found between Cu levels in the seaweed Fucus vesiculosus and those in sediments. It was concluded that the weed was able to desorb and accumulate Cu adsorbed on particles of suspended sediment (Luoma et al, 1982).

Among animals, clear relationships with sediment concentrations, ranging from as little as 10 µg g⁻¹ to more than 2000 µg g⁻¹ Cu, were observed in the polychaetes Nereis diversicolor (omnivore) and Nephtys hombergi (carnivore) by Luoma and Bryan (1982), Bryan and Gibbs (1983; 1987). These organisms may therefore be good indicators of availability. It is thought that uptake occurs following close contact between oxidised sediment particles and the body surface, especially at low salinities. In contrast, other worms including Melinna palmata (naturally high in copper) and Tharyx marioni, point to the ability of some species to regulate the body Cu concentration (Bryan and Gibbs, 1987).

The level of iron oxides in the sediment has been shown to determine the availability of sediment bound Cu. The body Cu levels in the freshwater bivalves Elliptio complanata and Anadonta grandis were linearly related to copper/iron ratios in sediment extracts (Tessier et al, 1983). A strong relationship between Cu content in 4 year old M. balthica and sediment bound copper levels in San Francisco Bay was observed by Cain and Luoma (1990).

The aims of this study were to measure the accumulation of copper and survival in individuals from three populations of Corophium volutator and Nereis diversicolor after exposure to three different natural sediments. The test sediments were collected from the same three estuaries as the organisms. These biological responses and physical/chemical analyses of the test sediments were used to investigate intraspecific and interspecific variation in the organisms and the bioavailability of copper in these sediments to both organisms.
MATERIALS AND METHODS

i) TREATMENT OF SEDIMENTS

Three cores (depth 20cm, diameter, 7cm) were collected from each site (see General Methods for further details), and each core was split into quarters with opposite quarters removed for further study. For each study half of each core was frozen for subsequent particle analysis and organic content, the other half for subsequent metal analysis on the different fractions and total sediment. Sediment was also taken from the same three sites to use in the sediment bioassays.

Physical analyses of the sediments were carried out using a Malvern Mastersizer. The mean and median particle size, % clay and silt, % organic carbon, the skew and sorting coefficient were measured.

Chemical analyses of the sediments were carried out on samples that had been prepared using the sequential digestion technique described by Tessier et al (1979). The sediment was dried and crushed to a powder with a pestle and mortar. Approximately 1g of sieved material (<180μm) was weighed and reserved for the extraction procedure, and approximately 1g for the total digest. Blanks were used at each step to test for any digestion related artefacts. Standard reference material was also digested in step 5 (see below) with the total digests of sediments to check quality control. At each step the supernatant was removed and made up to a known volume with deionised water in volumetric flasks.

Sequential extraction procedure

Step 1) Exchangeables

In acid washed glass beakers 8ml 1M MgCl₂ (pH 7 with nitric acid) was added to the sample at room temperature and was continuously agitated for one hour using a magnetic stirrer. The material was then transferred carefully into centrifuge tubes and centrifuged at 3500rpm for 5 minutes. The supernatant was removed by volumetric pipette, acidified with 2.5ml 10% nitric acid, made up to 10 ml with deionised water in a volumetric flask, and stored for later metal analysis. The remaining material was then transferred carefully back into its corresponding beaker removing the last particles with the next steps’ reagents.

Step 2) Carbonates

8ml of 1M Sodium acetate solution (8 ml of 1M; acidified to pH5 with acetic acid (HOAc) was added to the sample and continuously agitated for 5 hours at room temperature. The material was then centrifuged and the supernatant solution and remaining material removed.
as above. However, the supernatant solution was acidified with 1.0ml 10% nitric acid before being made up to 10ml in a volumetric flask with deionised water.

Step 3) Oxides

Hydroxylamine hydrochloride (NH₂-OH HCl: 20ml of 0.04M) in 25%(v/v) acetic acid and heated for 5 hours at approx. 88°C with occasional agitation, a boiling chip was also added. The material was then centrifuged and the supernatant removed as above and acidified with 2.5ml 10% nitric acid, then made upto 25ml in a volumetric flask with deionised water.

Step 4) Organics

Nitric acid (3ml 0.02M) and 5ml 30% H₂O₂ adjusted to pH2 with HNO₃ was added to the sample and heated for 2 hours at approx. 85°C with some agitation. After this period an additional 3ml 30% H₂O₂ (pH 2 with HNO₃) was added and the sample was heated for 3 hours at 85°C with some agitation. The reactants were cooled to room temperature then 5ml 3.2M NH₄ OAc in 20% (V/V) HNO₃ was added and then the sample was diluted to 20ml and continuously agitated for 30 minutes. Centrifugation occurred as before and the supernatant solution was made up to 25ml in a volumetric flask with deionised water.

Step 5) Total metal

Nitric acid (10ml 12-14M) was added to 1g of the original dried sediment in a beaker with a few boiling chips. The reactants were heated to 95°C for 4 hours covered with a watch glass to reflux. To prevent reactants from boiling dry extra acid was added as required. After 4 hours the samples were removed from the heat source and the amount of acid in each container was made up to 10ml, and then made up to 25ml with H₂O whilst contained in a fume cupboard. The liquid was filtered through Whatman No. 1 filter paper into a 50ml volumetric flask and made up to 50ml with deionised water.

ii) SEDIMENT BIOASSAYS

The bioassay method used was a modified version of the procedure described by Swartze et al (1985). *Nereis diversicolor*, *Corophium volutator* and sediment were collected from the three sites at Dulas, Alaw and the Humber. Each of the three populations of *Corophium* and *Nereis* were tested in each of the three sediments. The test procedure for *Corophium* consisted of 42 small glass containers (depth 10cm, diameter 15cm) with a layer of about 4cm of test sediment covered by approximately 4cm of diluted seawater (salinity of 17.5). The test procedure for *Nereis* consisted of 33 large glass beakers (depth, 20cm, diameter, 30cm), with a layer of about 7cm of test sediment covered by approx. 7cm of diluted seawater.

For all test conditions temperature was maintained at 14°C. A 50% water change was performed every 48 hours with minimum disturbance to the sediment. After a two day
acclimation period seven *Corophium* or *Nereis* were introduced to each of the test containers. Each test condition was replicated three times and all organisms quickly burrowed into the test sediment. All tests had an exposure time of 7 days. During this test period the pH, oxygen, temperature and salinity were monitored. Survival of the organisms and avoidance behaviour were determined by daily counts and observations. Pilot studies had shown that these organisms usually avoid the sediment and come to the surface when stressed with unfavourable conditions. If they were dead (immobilised even after probing) it was recorded and the organisms were removed. Control test conditions consisted of organisms in their native sediment. The maximum acceptable control mortality described by Swartze *et al* (1985) was 10%.

After the exposure period, the test animals were sieved from the sediment and dead organisms recorded and removed. Surviving organisms were washed in diluted seawater and frozen for later metal analysis. The guts of the organisms were not evacuated before being frozen so small amounts of the test sediment will be inside the organism. This will therefore have an effect on the 'accumulated' metal levels.

Three choice chambers were made to determine sediment preference of *Corophium* from the three estuaries. Each chamber consisted of a large round glass container (depth 15cm, diameter 30cm) containing four equal amounts of test sediment. Sediments were from Dulas, Alaw, Humber and a mixed sediment (50% Dulas and Alaw sediment). The sediments were initially separated by temporary sliding panels which were removed when the overlying water had been gently added. Diluted seawater (salinity approx. 18) covered the layer of test sediments and a 50% water change was carried out every 48 hours. Fifty *Corophium* from Alaw were introduced into one chamber, 50 from Dulas into another and 50 from the Humber into another. The experiment was carried out in duplicate. After 10 days each sediment was separated using the sliding panels and carefully removed. The sediments were sieved (1mm sieve) and the distribution of *Corophium* determined.

**iii) METAL ANALYSIS**

Organisms were placed in evaporating dishes and dried in an oven (35°C) then stored in a desiccator until cool and weighed. Approximately 3/4 worms and 5/7 amphipods constituted a sample. Samples were digested in boiling tubes with a glass ball on top to minimise evaporation in 1ml of conc. ANALAR nitric acid for three hours at approx. 85-90°C. Blanks and standard reference material were also digested to test for any digestion related artifacts. Samples were made up to 10ml with deionised water in volumetric flasks and stored for later metal analysis.
The sediment, *Nereis* and *Corophium* samples were analysed for copper using an I.C.P.
M.S. (Inductively Coupled Plasma Mass Spectrophotometer). Metal levels are expressed as µg g\(^{-1}\) dry weight. To avoid any matrix effects when analysing the sediment samples all the standards were made with the same matrix that was used in the digestion process of that sample. The matrices used in these methods contained ions that may have potentially competed with the metal ions. Therefore a number of sediment samples were spiked with known amounts of copper were run through first. This tested the recovery of the instrument whilst it was analysing the sediment samples which had been sequentially digested. If instrument drift did occur the machine was re-calibrated.

iv) **STATISTICAL ANALYSIS**

The Spearman rank correlation test (p≤0.05) was carried out on the raw data (using SPSS for Windows) to investigate any correlations between the biological and physico/chemical variables. This non-parametric test analyses the data by replacing the values by ranks and does not assume normal distribution. The data were analysed to establish possible relationships between these variables in, i) all population/all sediment combinations; ii) each separate population/all sediment combinations and iii) all populations in each separate sediment.

A one way ANOVA was carried out on the raw data (p≤0.05) using SPSS for Windows to establish whether significant differences occurred. Post-hoc testing using least significant difference tests was then carried out to determine the actual treatments responsible for any significant differences. Each treatment’s data set had a low variance and transformation was not carried out. The data were analysed to determine i) the statistically significant differences of some of the physical and chemical parameters in the three different sediments. ii) the significant differences of copper levels and survival in the three different populations of *Corophium* and *Nereis* after exposure to all the sediments; and iii) the significant differences of copper levels and survival in both organisms between one sediment exposure to another. The parametric ANOVA test was used instead of the non parametric Krushal Wallis test as post-hoc testing was necessary to establish at which treatment there were significant differences. As there are no similar non-parametric post-hoc tests (like L.S.D.), a parametric test was used.
RESULTS
i) PHYSICAL AND CHEMICAL NATURE OF THE SEDIMENTS

The means and standard errors of the physical properties of the three sediments can be seen in Table 4.0a. The % clay/silt content is also represented graphically in Figure 4.0. Sediment from the Humber site had a statistically significantly higher percentage organic carbon and percentage clay/silt than sediments from the Alaw and Dulas estuaries (Tables 4.1 d and e). Sediment from the Alaw and the Dulas had closer similarity in physical nature with respect to organic carbon, percentage clay/silt, mean particle size, medium particle size, percentage L.O.I. and percentage coal content than sediment from the Humber.

The total copper and the copper in the sequential extracts were significantly higher in Dulas sediment than in both Alaw and Humber sediments (Tables 4.1a, b and c). Alaw sediment had the lowest levels of copper in every fraction (apart from the carbonates), (Table 4.0b, Figures 4.1 and 4.2) compared to both Humber and Dulas sediments. Dulas and Humber sediments had similar levels of copper in the organic fraction (compared with sediment from Alaw). The highest copper content in the Humber sediment were found in the organic fraction whereas the highest copper content in sediment from Dulas was found in the oxide fraction. The least amount of copper in all three sediments was found in the exchangeable fraction.

Spearman’s correlation coefficient showed that the total copper levels in all three sediments were highly correlated with copper in organics, copper in the exchangeable fraction and with the % clay/silt fraction of the sediments (Table 4.2). The % clay/silt content was also correlated with copper in organics, copper in the exchangeables and the % organic carbon content in the sediments.

ii) INTRASPECIFIC VARIATION IN RESPONSES

Sediment Preference of Corophium

The percentages shown in Figure 4.3, 4.4 and 4.5 were calculated from the total number of Corophium found in each of the sediment choices. The tests were carried out in duplicate. Corophium from the Alaw prefered (48%) their own sediment (Fig 4.3) and were found least in the mixture of Alaw and Dulas sediment (9%). Amphipods from the Humber (Figure 4.4) also prefered their own sediment (50%) and were found least in Dulas sediment (17%). Amphipods from Dulas did not have a preference for a particular sediment and were found equally in all sediments (Figure 4.5).
Responses in *Corophium*

Dulas *Corophium* had significantly higher copper levels than organisms from Alaw and Humber after exposure to all three sediments (Tables 4.3a and 4.4a). However, animals from Dulas had higher basal copper levels compared to animals from the Alaw and the Humber (see chapter 1). There was no significant difference between copper in *Corophium* from Alaw and from Humber in all three test sediments. Figure 4.6 and Table 4.3a) indicates that *Corophium* from Alaw and Humber accumulated the highest amount of copper after exposure to Dulas sediment. Copper levels in Alaw *Corophium* were approximately doubled (112.1) after exposure to Dulas sediment compared to both Alaw (53.86) and Humber sediment (52.93).

Table 4.3b and Figure 4.7 shows the means and standard errors of survival of *Corophium* from the three estuaries after exposure to Alaw, Humber and Dulas sediments for seven days. Alaw *Corophium* had significantly lower survival than *Corophium* from Dulas and Humber estuaries (Table 4.4b). However, there were no significant differences between survival of *Corophium* from the Humber and *Corophium* from Dulas after exposure to the three sediments.

The survival (over the seven day test period) of *Corophium* from the Alaw was lower when tested in Dulas sediment compared with Alaw and Humber sediment with most of the mortality occurring in day 1 (Figure 4.8). The survival of *Corophium* from the Humber was similarly lower in the Dulas sediment compared with Alaw and Humber sediment (mostly occurring in days 3-5) (Figure 4.9). The daily survival of *Corophium* from Dulas is not graphically represented due their mortality being so low.

Responses in *Nereis*

Dulas *Nereis* had significantly higher copper levels than Alaw and Humber *Nereis* after exposure to all three sediments (Tables 4.5a and 4.6a). However, animals from Dulas have higher basal copper levels compared to animals from the Alaw and the Humber (see chapter 1). There was no significant difference between copper in *Nereis* from the Alaw and *Nereis* from the Humber in all three sediments (Table 4.6a). Figure 4.10 and Table 4.5a indicates that compared to their basal concentrations *Nereis* from the Alaw and the Humber accumulated the highest amount of copper after exposure to Dulas sediment.

Table 4.5b and Figure 4.11 show the means and standard errors of survival in the three populations of *Nereis* after exposure to the three sediments for seven days. *Nereis* from Dulas had significantly higher survival than *Nereis* from the Alaw after exposure to the different sediments (Table 4.6b). There were no significant differences between *Nereis* from the Alaw and the Humber, and there were no significant differences between *Nereis* from the Humber and Dulas.
*Nereis* from Alaw had the lowest rate of survival in Dulas sediment compared to that in Alaw and Humber sediments, the main mortality occurring between days 2-4 (Figure 4.12). There is no graphical representation of the daily survival in worms from the Humber and Dulas estuaries as their survival was so high.

**Statistical correlations between the biological and physico-chemical parameters**

Table 4.7 shows the correlations using a Spearman test between the biological responses of all three populations of *Nereis* and *Corophium* and the physical and chemical test conditions recorded in all three sediments. A range of correlations were computed but only those that were relevant to this study are mentioned. The variable 'survival' refers to the survival of the animal after exposure to the seven day sediment bioassay and the variable 'LC$_{50}$' refers to the 96 hour LC$_{50}$ value for dissolved copper determined in Chapter One. The data were pooled so that all three populations of *Corophium* and *Nereis* were examined together.

Survival in *Corophium* was significantly (P<0.05) correlated with '% organic carbon', 'total copper levels', and the 'LC$_{50}$ value' for *Corophium*. Copper in *Corophium* was significantly correlated with '% organic carbon' in the sediment and the 'LC$_{50}$ value for *Corophium*'. The survival in *Nereis* was significantly correlated with their 'LC$_{50}$ value' and copper levels. Copper levels in *Nereis* are correlated with their 'LC$_{50}$ values' and their 'survival'.

The data were then separated (Tables 4.8 a, b and c) to examine correlations between the biological responses of each population and the physical/chemical nature of the sediment due to intraspecific differences.

**Corophium**

Copper accumulated in *Corophium* from the Humber (Table 4.8a) was not significantly correlated with any variable. However survival in *Corophium* from the Humber was strongly correlated with '% organic carbon'. *Corophium* from Dulas estuary (Table 4.8b) had copper levels which were significantly correlated with '% organic carbon' content in the test sediments. However, the survival in *Corophium* from Dulas was not correlated with any other variable. Copper levels in *Corophium* from the Alaw estuary (Table 4.8c) were correlated with 'Cu levels in the exchangeable fraction' of the test sediments, the 'total Cu' content in the sediment and 'survival' of *Corophium*. Survival in this Alaw population of *Corophium* was strongly correlated with 'total Cu' in sediment and 'Cu in the exchangeable' fraction of the sediment. Survival was also correlated (to a lesser extent) with 'Cu in the organic fraction', '% organic/carbon' and 'Cu levels in *Corophium*'.

**Nereis**

Copper levels in *Nereis* from the Humber (Table 4.8a) were highly correlated with 'LC₅₀' in *Nereis*, 'total Cu levels' in sediment, 'Cu levels in the exchangeable' fraction and the level of 'copper in the organic' fraction of the test sediments. Survival in this population of *Nereis* was correlated with the levels of Cu accumulated in the worm, total copper in the sediment, Cu in the 'exchangeable' fraction, the '% clay/silt' content and the 'Cu level in the organic' fraction of the sediment.

Worms from Dulas (Table 4.8b) had Cu levels that were correlated to total Cu levels in the test sediment, Cu levels in the exchangeable fraction and levels of Cu in the organic fraction of the sediment. There was no correlation between survival in this worm and any other variable.

Worms from the population sampled at Alaw (Table 4.8c) had levels of Cu which correlated with this worm's survival and the % organic carbon content in the test sediments. Survival in this worm after the seven day bioassay also correlated with the % organic carbon content of the test sediments and the Cu concentration in both organisms.

**iii) BIOLOGICAL EFFECT OF DIFFERENT SEDIMENTS**

**Responses in Corophium**

The means and standard errors of copper accumulated in *Corophium* from the three estuaries after exposure to Alaw, Humber and Dulas sediments can be seen in Table 4.3a and Figure 4.6. All three populations of *Corophium* had significantly higher body copper levels after exposure to Dulas sediment than after exposure to Alaw and Humber sediment (Table 4.9a). There were no significant differences between levels of Cu in *Corophium* after exposure to sediment from the Humber and after exposure to sediment from the Alaw.

Table 4.3b and Figure 4.7 show the means and standard errors of survival in *Corophium* from the three estuaries after exposure to Alaw, Humber and Dulas sediments for seven days. *Corophium* had a significantly reduced survival after exposure to Dulas sediment compared with exposure to Alaw sediment (Table 4.9b). There was no significant difference between survival of *Corophium* in Humber sediment and survival in Alaw and Dulas sediment.

**Responses in Nereis**

Table 4.5a and Figure 4.10 show the means and standard errors of copper accumulated in *Nereis* from the three estuaries after exposure to Alaw, Humber and Dulas sediment. All populations of *Nereis* had a significantly higher body copper level after exposure to Dulas sediment than after exposure to Alaw sediment (Table 4.10a). There were no significant
differences between Cu in *Nereis* after exposure to Humber sediment compared with that after exposure to both Alaw and Dulas sediments.

Table 4.5b and Figure 4.11 show the means and standard errors of survival in the three populations of *Nereis* after exposure to the three sediments for seven days. Table 4.10b shows that there were no significant differences found in survival of *Nereis* after exposure to the different sediments.

Statistical correlations between the biological responses of the organisms with the physical/chemical nature of each sediment

**Corophium**

Table 4.11 shows the correlations between the variables in each sediment. Copper levels in *Corophium* after exposure to sediment from the Humber (Figure 4.11a) were correlated with the ‘LC50 value’ for *Corophium*. Survival in *Corophium* after exposure to Humber sediment was correlated with ‘total Cu’ levels in the sediment, ‘Cu levels in the exchangeable’ and ‘organic fraction’, the ‘% organic/carbon’ and ‘% clay/silt’ content in this sediment.

Copper levels in *Corophium* after exposure to sediment from Dulas (Figure 4.11b) were not correlated with any other variables. Survival in *Corophium* after exposure to this sediment was correlated with the ‘LC50 value’ for *Corophium*, ‘total Cu levels’, ‘Cu levels in the organic’ and ‘exchangeable’ fraction, the ‘% organic/carbon’ and ‘% clay/silt’ content in this sediment.

Copper levels in *Corophium* after exposure to sediment from the Alaw (Figure 4.11c) were only correlated with the ‘LC50 value’ for *Corophium*. Survival in *Corophium* after exposure to this sediment was not correlated with any other variable.

**Nereis**

Cu levels in *Nereis* after exposure to sediment from the Humber (Figure 4.11a) were correlated with the ‘% clay/silt content’ of this sediment. Survival in *Nereis* after exposure to this sediment was not correlated with any other variable.

Cu levels in *Nereis* after exposure to sediment from Dulas (Figure 4.11b) were correlated with the ‘LC50 value’ in *Nereis* and their ‘survival’. Survival in *Nereis* were correlated with their ‘LC50 value’, their ‘Cu levels’, ‘total Cu’ levels in this sediment, Cu levels in the ‘exchangeable’ and ‘organic’ fraction, and the ‘% organic/carbon’ and ‘% clay/silt’ content of this sediment.
Cu levels in *Nereis* after exposure to sediment from the Alaw (Figure 4.11c) were correlated with the ‘survival’ of this organism in this sediment. Survival of *Nereis* after exposure to this sediment was correlated with ‘Cu levels’ in *Nereis*, ‘total Cu levels’, Cu levels in the ‘exchangeable’ and ‘organic’ fraction and the ‘% organic/carbon’ and ‘% clay/silt’ content in this sediment.
DISCUSSION

In this study the physical characteristics of sediments from three different estuaries, their sequential extractable sediment bound copper, the copper accumulated by Corophium and Nereis from three populations and their survival after seven days exposure to these sediments were measured. The results were assessed in two ways; one to assess intraspecific differences in the responses of the three populations of organisms (section (ii) of this discussion) and secondly, to assess how each of the different sediments affected the organisms (section (iii) of this discussion). This allows the role of the bioindicator of available copper in the environment to be discussed with both a biological and environmental bias. The data gained from these investigations were also used in Multivariate and Multiple Regression Statistical analysis (see Chapter 5) to gain further information on how these environmental and biological parameters interact.

i) PHYSICAL AND CHEMICAL NATURE OF THE SEDIMENTS

Sediment from the Dulas estuary had the highest copper levels (approximately 200μg g⁻¹). The Humber sediment had intermediate copper levels (approximately 60μg g⁻¹) and dissolved copper levels in the water column currently exceed the EQS level of 5 μg l⁻¹ (Environment Agency Water Quality Report, 1993). Sediments from the Alaw estuary are 'clean' with very low metal levels (approximately 5μg g⁻¹) and this can be considered the control site. The Dulas estuary receives drainage from past mining activity on Mynydd Parys via the Afon Goch which during estuarine mixing is the source of the very high sediment heavy metal concentrations. This is similar to Restronguet Creek which also receives drainage containing high levels of copper from past mining activity via the Carnon river. In these sediments the adsorption of most of the dissolved Cu by flocculated oxides of iron and associated humic substances during estuarine mixing leads to very high (~3000μg g⁻¹) sediment concentrations (Bryan and Gibbs, 1983). Most of the Cu in Dulas sediment was also found in the oxide fraction.

Metal concentration is usually expressed in terms of the whole dried sediment rather than on a certain fraction of sediment, but particle size distribution, levels of iron oxides and organic matter (Luoma and Bryan, 1981; Tessier et al, 1984a; Langston,1982; and Luoma, 1990) and the particular geochemical fractions with which the trace metals are associated (Luoma and Jenne, 1977) may greatly affect the bioavailable metal concentration in a sediment.
Sequential extraction procedures based on a widely used method proposed by Tessier et al (1979) were used in this study to determine the levels of metal in each fraction. Sediment from the Humber had the highest organic and clay/silt content compared to the other sediments. It was in this organic fraction that most of the copper was bound. Metals adsorb onto small particles and are likely to be associated with the clay/silt fraction (Luoma, 1990). Similar copper levels were also found in the organic fraction in Dulas sediment but the greatest level was in the oxide fraction. Luoma and Bryan, (1981) also found that Cu was associated both with oxides of iron and organic components such as humics in the oxidised sediments. Copper in all the fractions of Alaw sediment were significantly lower than in the other sediments. Copper in the exchangeable fraction which is very loosely bound to sediment particles and is likely to be remobilised to the surrounding water contained the least amount of copper in all three sediments.

ii) INTRASPECIFIC VARIATION IN RESPONSES

In this section the accumulation of copper and the survival of each population of Nereis and Corophium after exposure to the natural sediments is discussed. Earlier studies (see Chapter 1) had shown that the three populations of animals had significantly different copper concentrations and different abilities to tolerate dissolved copper. Organisms from Dulas had significantly the highest body burdens and 96 hour LC50 value compared with organisms from Alaw which had the lowest copper body burdens and LC50 value. Humber organisms had intermediate LC50 values and copper body burdens.

Before considering in detail the ways in which phenotype variations in a species can cause or explain different metal concentrations or survival, it is necessary to define what is meant by the term 'phenotype' in the current context. Krebs (1985) defines phenotype as the observable attributes of whole organisms. However, it may include all biochemical, physiological, morphological and behavioural characteristics of an individual that can (or might in future) be measured. Different phenotypes usually arise due to interactions between different genotypes and environmental factors (Futuyma, 1979).

Depledge and Bjerregaard (1989c) looked at the importance of interactions between physiological state and environmental factors on individual variation in trace metal concentrations in selected marine invertebrates. They found that variability in trace metal levels in the tissues of animals does not simply reflect the bioavailability of metals in the sea. The phenotypic variability in morphology, biochemistry/physiology and behaviour, together with genetic diversity, give rise to much of the variability in trace metal concentrations within populations of animals.
After exposure to all three sediments both *Corophium* and *Nereis* from Dulas had significantly higher copper levels than organisms from both the Alaw and the Humber. However, this would probably be expected as the organisms from Dulas had the highest basal body copper levels anyway. The different accumulation mechanisms of copper in these animals from the different estuaries was discussed in Chapter 2. When the copper levels in the organisms were compared to their control (or basal) levels, (ie., how much they lost or gained depending in which sediment they were tested), *Corophium* from the Alaw accumulated the most copper after exposure to Dulas sediment. Levels of copper decreased in Dulas *Corophium* after exposure to sediment from the Alaw and the Humber. Levels of copper in the Humber *Corophium* did not appear to change very much compared to their control levels, no matter which sediment the organism was exposed to.

*Nereis* from the Alaw and the Humber both had very high levels of copper after exposure to Dulas sediment compared to their control levels. *Nereis* from Dulas appeared to lose copper after exposure to Humber sediments and lost even more after exposure to Alaw sediments. The levels of available copper appeared to be too low in Humber sediment to make much impact on Alaw organisms as their copper levels did not increase after exposure. Aldrich (1986) also found variation in metal concentrations within individuals in populations of marine invertebrates. The biochemical and physiological variability, or ‘differences in physiological state’, were shown to be the major determinants of trace metal uptake, utilisation and/or storage and excretion.

The survival of Dulas and Humber *Corophium* were significantly higher than Alaw *Corophium* in all three sediments. *Nereis* from Dulas were also found to have a significantly higher survival than *Nereis* from Alaw. This would indicate that the available copper in the sediments had a greater toxic effect on organisms from the Alaw and the Humber compared to those from the Dulas.

The reason why Humber *Nereis* were not found to have an overall significantly higher survival than Alaw *Nereis* is probably due to their decreased survival after exposure to the Alaw sediment. Sediment from the Alaw did not affect the survival of any of the other populations of either *Corophium* or *Nereis*. This high mortality in the Humber population of worms could have been caused by fungal infection as there was evidence of fungal attachments on the dead worms that came to the surface.

*Nereis diversicolor* has been well studied and is known to be a good indicator of available Ag, Cd and Cu in marine deposits and a poor indicator of Fe, Mn and Zn (eg. Luoma and Bryan, 1982; Bryan, 1985). Not nearly so many studies have been carried out using *Corophium volutator* which is also a key species in the estuarine environment. However, this species is getting increasingly popular for use in acute and chronic sediment bioassays and is
the species included in the ‘Guideline for conducting 10-day static sediment toxicity tests using marine or estuarine amphipods’ set by the Ministry of Transport and Water Management in Holland (RIKZ).

Measurement of toxicity is one method of assessing the environmental quality of sediments, yet because of the wide range of salinities (in this study intermediate salinities were used) that characterise estuaries few infaunal organisms have both the physiological tolerance and sensitivity to chemical contaminants to serve in estuarine sediment toxicity tests (Dewitt et al, 1989). Sediment bioassays have been well used and are becoming increasingly popular. Hurk et al (1992) also used a sediment bioassay method described by Swartz et al (1985). They found that Corophium volutator was the most sensitive of the three amphipods they tested which would indicate their usefulness in sediment toxicity testing.

Sublethal endpoints have been used such as the inability to reburrow after exposure (emergence), avoidance and immobilisation. Dillon et al (1993) developed a chronic sublethal bioassay for evaluating contaminated sediment with two to three week old post emergent juveniles of the marine polychaete Nereis arenaceodentata using their individual somatic growth rate as the measured test endpoint. Nipper et al (1989) investigated short and long term sediment toxicity test methods with the amphipod Grandidierella japonica. They found that both test methods were sensitive to levels of contamination found in the field, but produced different patterns of effects. Short term mortality was greatest in amphipods exposed to one sediment, while long term exposure produced the greatest reductions in survival and growth in another.

In ‘marginally’ toxic sediments (ie. Humber sediment in this study) it is less common to see exact concurrence among the results of various tests, than when samples are non-toxic or extremely toxic. Mears et al (1986) did an inter laboratory comparison of the sediment toxicity test developed by Swartze et al (1985) using the amphipod Rhepoxynius abronius. They found that sediments that are clearly non-toxic (survival is greater than 87%) and those that are clearly toxic (survival is less than 76%) will be accurately classified whereas those of marginal toxicity (survival is between 76% and 87%) can only be classified based on emergence data.

Correlations were computed to observe any relationships between the biological responses and the physico-chemical characteristics of all three populations in all three of the test sediments. Copper levels in Corophium were found to be correlated with the % organic carbon in the test sediment and the 96 hour LC50 value of that population of Corophium. Whereas copper levels in Nereis were correlated to the LC50 value and their survival. This would indicate that generally, ie. when discussing all populations, the tolerance index (LC50) is
again important in explaining variation in Cu levels in these organisms. The levels of body copper could also explain the variability in the LC50 values in both these organisms.

It is interesting that survival correlated with the copper levels in Nereis but not in Corophium. Presumably, the worm sequesters the copper in a non-toxic form (see Chapter 3) which can also be the case for Corophium too (discussed in Chapter 3), but not to the same extent. If survival does not relate to the organisms' copper levels either positively or negatively it could be due to a number of reasons; it is not copper that is killing the organism, lower levels of copper are exerting a toxic response and killing the organism before it can either excrete it or sequester it in a non-toxic form or there is some ability to regulate this metal and excrete it (see Chapter 2). However, it is more likely to be a mixture of these processes due to the different Corophium phenotypes.

Survival in all populations of Corophium was also correlated with % organic carbon, the organisms LCS0 value and the total copper in the sediment. In Nereis the survival was only correlated with the LC50 value and the copper levels in the organism. It may be expected that the tolerance of an organism (LC50) to copper would relate to the organisms survival in sediments with varying levels of bound copper. However, this is not always the case as found by West et al (1993) who compared the relative sensitivity of three benthic invertebrates to copper-contaminated sediments from the Keweenaw Waterway. They found that although the LC50 values for Lumbriculus variegatus and Hyatella azteca were similar in water only exposures, the survival of L. variegatus was unaffected by copper in the test sediments. This species was able to survive copper contaminated sediments which reduced the survival of other species that had shown similar sensitivities to dissolved copper. Physico-chemical factors, such as particle size could have affected one species but not another, it may be that these environmental factors could affect one phenotype and not another which would agree with this present study.

LC50 values which represent the relative tolerances to copper in both Corophium and Nereis were correlated to organism survival and body copper levels after exposure to each sediment. Indicating in this case that generally, where an organism has a high LC50 value it will have relatively high survival and copper levels. This would only be the case if the organism was able to accumulate copper in a non toxic way. Nereis diversicolor is found in some of the most heavily polluted sediments and is demonstrably far more tolerant of Cu and Zn than the same species from cleaner areas (Bryan and Hummerstone, 1973). Tolerance is due to a lowered permeability to Cu coupled with an increased ability to deposit Cu in tertiary lysosomes (Pirie et al, 1985).

The traditional view of measuring an organisms body burden and using it to indicate levels of pollution is used widely with success (Bryan et al, 1985). However, it may also be
useful to use the presence of tolerance in a population as a direct indication of pollution causation and ecological impact (Hately et al., 1989).

The data in this study was separated to examine the relationships (in each population) between the biological responses and physico-chemical nature of the test sediments. This clearly showed how interactions between responses and sediment features varied intrinsically as well as interspecifically. Correlations involving survival in *Nereis* and *Corophium* from Dulas could not be computed as the survival was high in all three sediments and significantly higher than organisms from both the Alaw and the Humber.

The body copper levels and survival in *Corophium* from the Alaw, relate to copper levels in all of the different fractions of the test sediments. This would indicate that the environmental levels of copper exert a greater impact on Alaw *Corophium* which are the least tolerant population to copper. This was not the case for Humber *Corophium* and survival in Dulas *Corophium* was high in every test sediment. No variable was correlated with the body copper content of the Humber *Corophium* but % organic carbon was correlated with their survival. The % organic carbon was also related to survival in Alaw *Corophium* and body copper levels in Dulas *Corophium* and was probably important in explaining the proportion of copper which was available to these organisms.

Survival and copper levels in Alaw *Corophium* were related, but they were not in the other two populations. The results do indicate that as copper levels increased in Alaw *Corophium* their survival decreased. This suggests that these organisms could not acquire a tolerance within the experimental period and that they cannot store and sequester copper in a non-toxic form. Copper levels in Humber *Corophium* did not relate to their survival and in Dulas *Corophium* their survival was high no matter what their copper levels. This would indicate that there is a lethal level in the body which is low in animals from the Alaw and high in animals from Dulas.

The copper levels and survival in Alaw *Nereis* were not correlated with the concentration of copper in any of the sediment fractions, but there was a correlation with the % of organic carbon in the test sediment, which is probably where a lot of the copper was bound. Physical properties of the sediment (%clay/silt) also related to the survival in Humber *Nereis* which suggests that the physical properties of a sediment are important in explaining the survival in *Nereis*.

The lack of correlation between the survival and copper levels in the Alaw *Nereis* with the copper levels in the sediment may seem surprising. However, this is probably because the Humber sediment did not decrease these worms' survival nor increase these worms copper levels compared to the control situation. This would suggest that the copper in the sediment from the Humber is not that available to worms from the Alaw. It may be that the high organic
carbon in this Humber sediment reduces the bioavailability of the metal to these organisms (Luoma and Bryan, 1982).

The copper levels in the different sediment fractions correlated with the copper levels in both Nereis from the Humber and the Dulas estuaries. The copper levels in Dulas worms decreased in proportion to those in the sediments, whereas the opposite was true for Humber worms. The survival of the Nereis from the Humber also correlated with Cu levels in the sediment fractions, whereas the survival of Nereis from Dulas was always high.

Animals which inhabit sediments can be classified as deposit feeders, filter feeders and burrowers (Rainbow, 1995). Corophium are deposit feeders, and Nereis can adopt a range of feeding mechanisms including deposit feeding and filter feeding using a mucus net. The bioavailable fraction of metals in a sediment may differ depending on this classification, therefore all trophic types should be examined. To gain a complete picture of total heavy metal bioavailability in a marine habitat it is necessary to use a suite of biomonitors, reflecting bioavailibilities from all available sources.

Metal accumulated in organisms is a time integrated measure of metal supply over weeks, months, or even years, according to species analysed (Philips, 1980). Most significantly the metal accumulated is a time integrated measure of the supply of bioavailable metal, as opposed to total metal. However, to determine the specific levels of bioaccumulation causing a particular ecological effect in a given species, it is necessary to distinguish between cause and effect. As the biological consequences of particular levels of bioaccumulation have not been elucidated, there is no way of judging the acceptability of the pre-change (or basal) conditions. Therefore, to be meaningful in measuring effects of marine pollution, the consequences of bioaccumulation are going to have to be stated in terms of demonstrably adverse biological responses to specified levels of bioaccumulation which is likely to vary interspecifically and intraspecifically.

This phenomenon has confounded the interpretation of many biomonitoring studies in which it has often been difficult to distinguish significant changes in metal concentration due to pollution, from natural phenotype variations. However, with a greater understanding of the mechanisms underlying natural variability, it may be possible to distinguish pollutant effects more readily. Depledge (1989c) has suggested that the individuals be grouped together on the basis of similarities in physiological state into so-called 'physiotypes'. Subsequent correlations of susceptibilities to pollutants with various 'physiotypes' may provide a useful tool in predicting the impact of pollutant metals in our naturally varying ecosystems.

It is proposed that 3 different physiotypes (for each animal) have been identified in this study based on low, medium and high basal body copper levels and low, partial and high tolerances to copper in Corophium and Nereis.
Physiotype 1: Alaw *Nereis* - low body copper and low tolerance
Physiotype 2: Humber *Nereis* - moderate body copper and partial tolerance
Physiotype 3: Dulas *Nereis* - high body copper and high tolerance

Physiotype 1: Alaw *Corophium* - low body copper and low tolerance
Physiotype 2: Humber *Corophium* - moderate body copper and partial tolerance
Physiotype 3: Dulas *Corophium* - high body copper and high tolerance

iii) **BIOLOGICAL EFFECT OF DIFFERENT SEDIMENTS**

In this section the biological effects of each of the three different sediments on *Corophium* and *Nereis* are discussed. The copper levels in *Corophium* from all three populations were found to be significantly higher after exposure to sediment from Dulas compared to exposure to Alaw and Humber sediments. Copper levels in *Nereis* were only significantly higher after exposure to Dulas sediments compared with Alaw sediments. This would indicate that the copper in Humber sediments is more available to *Nereis* than to *Corophium* as both are known to be accumulators of this metal.

The results also indicated a significantly lower survival in *Corophium* after exposure to Dulas sediment compared to Humber and Alaw sediments. The Dulas sediment is obviously having the most toxic effect on these organisms. There were no significant differences found in the survival of *Nereis* after exposure to each of the sediments. This may be due to the high level of plasticity found in *Nereis diversicolor* to environmental pollutants (Hately, 1989) where an adaptive ability to phenotypically acquire a tolerance to changing physiological stress is demonstrated. Pesch and Hoffman (1982) observed that sublethal exposure of the nereid polychaete *Nanthes arenaceodenta* to Cu increased the animals tolerance to lethal concentrations of Cu. However, not all animals can adapt, an example being the polychaete *Ophryotrocha diadema* (Parker, 1984). Investigations carried out on *Corophium* and *Nereis* from the Alaw (see Chapter One) indicated that these organisms did not gain an increased tolerance to Cu after a sublethal exposure to it, for the short duration of the experiment.

Over the seven day period, both *Corophium* and *Nereis* from the Alaw showed a reduced survival after one day of exposure to Dulas sediment compared to Alaw and Humber sediment, indicating that this sediment exerted an immediate toxic effect. *Corophium* from the Humber showed immediate mortality when exposed to sediments from both the Alaw and Dulas, indicating that the different physical features of both these sediments initially exerted stress on these organisms. After four days the survival in these organisms decreased further when exposed to Dulas sediment, due to the additional stress of the high metals content.
Partial extractions of sediments have provided significant insight into the physico-chemical factors influencing the bioavailability of particulate trace metals. Many studies have shown that trace metal levels in various different organisms are best related not to metal concentrations in the adjacent sediments, but rather to relatively easily extracted fractions (eg. Tessier et al., 1984a).

When examining all three sediments and all three populations of organisms there are relationships between the physico/chemical nature of the sediment and the Cu levels in the organism. However, when examining each of the sediments separately there are no relationships between copper levels in the organism and any of the physico/chemical sediment parameters, apart from % clay/silt in Humber sediment which was correlated with copper levels in Nereis. It would seem that the natural intra-specific variation in the body copper levels in the organisms outweighs the effect of the sediment and what is accumulated as a result of exposure to it. The natural variation in basal body copper levels is high anyway and during exposure to each of the sediments the intra-specific differences in the ability to accumulate and tolerate copper would cause further variation.

With an increased data set the relationships between copper levels in each sediment and body copper levels would be more likely to be observed, particularly in the more contaminated sediments, despite intra-specific variation. It would be interesting to determine whether organisms with a high body copper level (ie. Dulas organisms) would have an inverse relationship with clean sediment characteristics with an increased duration of exposure and larger sample size. These observations contribute further evidence for the need to account for not only inter-specific differences, but intra-specific differences in managing estuarine and marine environments.

All the sediment characteristics (total Cu, Cu in organics, Cu in exchangeables, % organic carbon and % clay/silt) used in this analysis were found to be correlated to the survival of the Corophium after exposure to Humber and Dulas sediments. Nipper et al. (1989) found that amphipod survival was unaffected by variations in sediment grain size, while this characteristic appeared to have an important effect on growth. This characteristic distinguishes Grandidierella japonica from Rhepoxynius abronius whose sensitivity to fine sediments may result in a significantly reduced survival in the short term tests (Dewitt et al., 1988).

Correlation coefficients could not be computed for the 'survival of Corophium' parameter in Alaw sediments because the values for each population were the same. This was the same for the 'survival of Nereis' parameter in Humber sediments. The same sediment characteristics were found to be correlated to survival of Nereis after exposure to Alaw and Dulas sediments. This would indicate that the physico-chemical features of the sediment were
more important in explaining the variation in the survival of these organisms than the body copper levels after the exposure period.

Wiemen et al. (1992) also found that when investigating metal bioavailability in sediments from differing sources, the chemical and physical characteristics must also be considered. They concluded that the organic content of sediment is likely to be one of the factors controlling the metal bioavailability in sediment, and this present study agrees with this.

The copper levels in Corophium after exposure to sediment from the Alaw and the Humber were correlated with the LC50 value of that population. After exposure to Dulas sediment the variability of copper levels in Corophium were not explained by any of the biological or physico-chemical parameters used. This could indicate that using the LC50 value of an organism as an index of tolerance may not be as significant in explaining variability in copper levels in organisms after exposure to sediments with high levels of copper, compared to sediments with low or moderate levels of available copper.

The LC50 value for Corophium and Nereis were also correlated with their survival after exposure to Dulas sediment only. Therefore, and not surprisingly, indicating that the tolerance index (LC50 value) was found to relate to the ability of that organism to survive in highly metal contaminated sediments.

Copper levels in Nereis were correlated with their survival after exposure to Alaw and Dulas sediments. When exposed to Dulas sediment the LC50 values were correlated to the copper levels in this organism. This could indicate that when an animal is exposed to sediments with increased metals, the tolerant ability of that animal could be used to predict or explain how much metal it will accumulate. Levels of copper in Nereis after exposure to Humber sediment were related only to % clay/silt which was significantly higher in this sediment compared to the others. As metals are adsorbed onto these clay and silt particles this could be an available source of copper to these worms.

Interaction between metals may also affect uptake of some metals by organisms. This could have implications on the uptake of copper by the organisms used in this study, particularly when exposed to Humber and Dulas sediments which contain a mixture of contaminants. It is possible that these metals have a synergistic or antagonistic effect with each other and other factors increasing the toxicity of the sediments. In this study natural sediments were used so it is probable that components other than copper also had an effect on the survival of these organisms. Ahsanullah et al. (1981) reported synergistic accumulation effects of cadmium, copper and zinc on the shrimp Callianassa australiensis. The significant correlation between lead concentration in the tissues of the bivalve Scrobicularia plana and the ratio (Pb)/(Fe) extracted from sediments with 1 N HCL suggested that the presence of iron interfered with the uptake of lead (Luoma and Bryan, 1978).
The ecological and behavioural characteristics of organisms may also affect metal uptake. Biochemical processes involving the acidity of stomach juices and digestive enzyme action may also affect accumulation of metals (Weiman et al., 1992). The internal pH of invertebrates typically ranges from 5 to 8. Therefore only the weak-extractions would contribute to explaining the metals available to these organisms in this way.

The biological significance of sediment bound trace metals is complex and not all that well understood. The accumulation of trace metals by aquatic organisms is influenced by a great number of physico-chemical and biological factors that should be quantitatively characterised. Particulate trace metal partitioning influences the accumulation of the metals in various benthic organisms in laboratory experiments and, in a few cases, in field experiments (eg. Luoma and Bryan, 1982). Although it seems unlikely that chemical extractants can be found which would exactly remove the fraction of metals that are considered 'bioavailable', as there are so many factors that affect metal uptake, the need for legislation to define sediment quality makes the study of the ability of chemical reagents to approximate a bioavailable fraction worth pursuing.

Although empirical studies (eg. using chemical extractants in this study) can give valuable information concerning important sinks in natural sediments, it would also be beneficial to develop theoretical models (Tessier and Campbell, 1987) for describing trace metal partitioning in sediments, and to verify their adequacy in predicting trace metal behaviour under natural conditions. Also the processes that are involved with the scavenging and cycling of metals in sediment/water interactions under changing environmental conditions needs to be understood if environmental impact is going to be assessed and predicted. The problem of assessing the bioavailability of sediment-bound metals is compounded by the fact that metal levels in the organisms may be influenced by physiological factors such as tolerant abilities or sexual condition.

Many of the studies investigating metals in sediments and the responses of organisms either only measure some sort of biological response (eg. West, et al 1993) or investigates the ability of sediment extractants or some physico/chemical feature of the sediment to measure the bioavailability of a metal to an organism (eg. Weimin, et al, 1992). These studies usually ignore the biology of the indicator species used, but reference is normally made to how the organisms ecology or physiology can affect what is available to an organism. Both these areas of research are important but it is perhaps of greater value for the purpose of the biomonitoring of heavy metal availability in the marine environment to combine both areas of work and encourage more collaboration between chemists and biologists.
iv) **SUMMARY**

The main conclusions from the section of the study examining the intraspecific variation in biological responses are:

1) *Corophium* from Dulas and the Humber had a significantly higher survival in all three sediments compared to amphipods from the Alaw.

2) *Nereis* from Dulas only had a significantly higher survival in all three sediments than Alaw *Nereis*.

3) The Cu levels in both *Corophium* and *Nereis* from Dulas were significantly higher than levels found in animals both from the Humber and Alaw after exposure to all sediments. However, the actual amount of copper accumulated in the organisms compared to their basal levels were greatest in *Corophium* and *Nereis* from the Alaw when exposed to Dulas sediment.

4) Survival in *Corophium* from each of the three populations did not correlate with the Cu levels in the organism, but in *Nereis* it did so.

5) Only *Corophium* from the Alaw had body copper levels and survival that correlated with the Cu levels in the test sediment.

6) *Nereis* from the Humber and the Dulas estuaries had body copper levels that correlated with Cu levels in the sediments, but Alaw *Nereis* did not.

7) The level of % organic carbon correlated with the survival and body copper levels in animals from all populations to a greater extent than any of the other physical properties measured in the sediments.

The main conclusions from the section of the study examining the biological effect of the different sediments:

1) Alaw and Humber sediments exerted little effect on the metal levels in the three populations of *Corophium* compared to exposure to Dulas sediment, which caused significantly higher metal levels in the organism and significantly lower survival.

2) There was no difference between metal levels in *Nereis* after exposure to Humber sediment compared to Dulas sediment indicating that the copper in the Humber sediment was more available to *Nereis* than *Corophium*. However, there were no differences in survival in any of the three sediments.

3) Exposure to Humber sediment did not cause a significant difference in survival in *Corophium* and *Nereis* compared to Alaw sediment which was the control site. Most of the copper was bound in the organic fraction of the sediment which generally reduces the bioavailability.
4) There were no significant relationships between the physico/chemical variables of each sediment and the Cu levels in either species. This was probably due to the natural variation in copper levels between the populations.

5) Survival in the organisms did relate to the physico/chemical nature of the test sediment.

6) The LC50 values for Corophium and Nereis only related to survival in organisms after exposure to Dulas sediment.

7) The LC50 value for Corophium can be related to the organisms body copper level after exposure to Alaw and Humber sediments but not to Dulas sediment.
Table 4.0: Mean values (with standard errors) of the physical and chemical variables in three sediments.

a) Mean physical data showing standard errors.

<table>
<thead>
<tr>
<th>Sediment</th>
<th>%org.carb</th>
<th>S.E.</th>
<th>%clay/silt</th>
<th>S.E.</th>
<th>mean(um)</th>
<th>S.E.</th>
<th>med.(um)</th>
<th>S.E.</th>
<th>%L.O.I.</th>
<th>S.E.</th>
<th>sort. coef.</th>
<th>S.E.</th>
<th>skew</th>
<th>S.E.</th>
<th>% coal</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaw</td>
<td>0.98</td>
<td>0.09</td>
<td>16.92</td>
<td>2.69</td>
<td>138.1</td>
<td>4.37</td>
<td>147.4</td>
<td>2.96</td>
<td>1.74</td>
<td>0.41</td>
<td>1.32</td>
<td>0.16</td>
<td>0.612</td>
<td>0.06</td>
<td>1.26</td>
<td>0.32</td>
</tr>
<tr>
<td>Humber</td>
<td>2.82</td>
<td>0.36</td>
<td>86.58</td>
<td>5.27</td>
<td>39.53</td>
<td>21.6</td>
<td>14.73</td>
<td>9.26</td>
<td>3.61</td>
<td>0.45</td>
<td>1.73</td>
<td>0.28</td>
<td>-0.178</td>
<td>0.12</td>
<td>3.51</td>
<td>0.19</td>
</tr>
<tr>
<td>Dulas</td>
<td>0.61</td>
<td>0.09</td>
<td>41.38</td>
<td>4.37</td>
<td>160.7</td>
<td>14.39</td>
<td>116.89</td>
<td>28.5</td>
<td>1.34</td>
<td>0.19</td>
<td>2.16</td>
<td>0.22</td>
<td>0.35</td>
<td>0.11</td>
<td>0.56</td>
<td>0.034</td>
</tr>
</tbody>
</table>

b) Mean chemical data (μg/g Cu) showing standard errors.

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Total Cu</th>
<th>S.E.</th>
<th>Exchangeables</th>
<th>S.E.</th>
<th>Carbonates</th>
<th>S.E.</th>
<th>Oxides</th>
<th>S.E.</th>
<th>Organics</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaw</td>
<td>5.06</td>
<td>0.02</td>
<td>0.57</td>
<td>0.08</td>
<td>0.17</td>
<td>0.03</td>
<td>0</td>
<td>0</td>
<td>0.62</td>
<td>0.005</td>
</tr>
<tr>
<td>Humber</td>
<td>56.75</td>
<td>1.48</td>
<td>1.41</td>
<td>0.1</td>
<td>0.68</td>
<td>0.15</td>
<td>4.03</td>
<td>0.63</td>
<td>17.08</td>
<td>3.1</td>
</tr>
<tr>
<td>Dulas</td>
<td>190.23</td>
<td>31.09</td>
<td>2.53</td>
<td>0.59</td>
<td>28.29</td>
<td>4.2</td>
<td>87.72</td>
<td>7.3</td>
<td>22.5</td>
<td>6.3</td>
</tr>
</tbody>
</table>
Table 4.1: Results of Least Significant Differences tests on the raw data (p<0.05) following a one way analysis of variance

<table>
<thead>
<tr>
<th></th>
<th>D.F.</th>
<th>F</th>
<th>Sig. of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu in organics</td>
<td>2</td>
<td>33.39</td>
<td>0</td>
</tr>
<tr>
<td>Cu in exchangeables</td>
<td>2</td>
<td>18.29</td>
<td>0</td>
</tr>
<tr>
<td>% organic/carbon</td>
<td>2</td>
<td>187</td>
<td>0</td>
</tr>
<tr>
<td>% clay/silt</td>
<td>2</td>
<td>238.45</td>
<td>0</td>
</tr>
<tr>
<td>Total Cu</td>
<td>2</td>
<td>113.07</td>
<td>0</td>
</tr>
</tbody>
</table>

A comparison of the physical and chemical variables in three different sediments

A) Cu in sediment

<table>
<thead>
<tr>
<th></th>
<th>A sed</th>
<th>H sed</th>
<th>D sed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A sed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H sed</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D sed</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B) Cu in organics

<table>
<thead>
<tr>
<th></th>
<th>A sed</th>
<th>H sed</th>
<th>D sed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A sed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H sed</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>D sed</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C) Cu in exchangeables

<table>
<thead>
<tr>
<th></th>
<th>A sed</th>
<th>H sed</th>
<th>D sed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A sed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H sed</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D sed</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D) % organic carbon

<table>
<thead>
<tr>
<th></th>
<th>A sed</th>
<th>H sed</th>
<th>D sed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A sed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H sed</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D sed</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E) % clay/silt

<table>
<thead>
<tr>
<th></th>
<th>A sed</th>
<th>H sed</th>
<th>D sed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A sed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H sed</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D sed</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

KEY

A sed  Sediment from the Alaw  * Significantly different at the 5% level
H sed  Sediment from the Humber
D sed  Sediment from the Dulas
Table 4.2: Spearman Rank Correlations performed on the raw data obtained from the analysis of physical and chemical variables in all three sediments.

| Cu in organics | *** |  |  |  |  |
|---------------|-----|---|---|---|
| Cu in exchangeables | *** | ** |  |  |  |
| % organic carbon | ns  | ns | * |  |  |
| % clay/silt | * | * | * | * |  |

**KEY**

- *: 0.05 > p > 0.01
- **: 0.01 < p < 0.001
- ***: p < 0.001
- ns: not significant
Table 4.3: Mean values (with standard errors) of the biological responses of *Corophium*.

a) Mean copper concentrations (μg/g Cu) showing standard errors in animals after exposure to sediments.

<table>
<thead>
<tr>
<th>Test sediment</th>
<th>Alaw <em>Corophium</em></th>
<th>S.E.</th>
<th>Humber <em>Corophium</em></th>
<th>S.E.</th>
<th>Dulas <em>Corophium</em></th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaw</td>
<td>53.86</td>
<td>2.4</td>
<td>69.3</td>
<td>1.8</td>
<td>111.42</td>
<td>14.2</td>
</tr>
<tr>
<td>Humber</td>
<td>52.93</td>
<td>1.9</td>
<td>63.52</td>
<td>0.23</td>
<td>77.77</td>
<td>1.62</td>
</tr>
<tr>
<td>Dulas</td>
<td>112.1</td>
<td>12.9</td>
<td>72.2</td>
<td>2.48</td>
<td>163.33</td>
<td>22.36</td>
</tr>
</tbody>
</table>

b) Total percentage survival in *Corophium* after exposure to sediments.

<table>
<thead>
<tr>
<th>Test Sediment</th>
<th>Alaw <em>Corophium</em></th>
<th>Humber <em>Corophium</em></th>
<th>Dulas <em>Corophium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaw</td>
<td>95.9</td>
<td>95</td>
<td>95.9</td>
</tr>
<tr>
<td>Humber</td>
<td>91.7</td>
<td>100</td>
<td>95.9</td>
</tr>
<tr>
<td>Dulas</td>
<td>50</td>
<td>87.5</td>
<td>95.9</td>
</tr>
</tbody>
</table>
Table 4.4: Results of Least Significant Differences tests on the raw data (p<0.05) following a One Way ANOVA.

<table>
<thead>
<tr>
<th></th>
<th>D.F.</th>
<th>F</th>
<th>Sig. of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu in Corophium</td>
<td>2</td>
<td>5.83</td>
<td>0.01</td>
</tr>
<tr>
<td>Survival in Corophium</td>
<td>2</td>
<td>4.44</td>
<td>0.02</td>
</tr>
</tbody>
</table>

A comparison of biological responses in three populations of Corophium after exposure to sediments.

a) Cu in Corophium

<table>
<thead>
<tr>
<th></th>
<th>A cor</th>
<th>H cor</th>
<th>D cor</th>
</tr>
</thead>
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<td>A cor</td>
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<tr>
<td>H cor</td>
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</tr>
<tr>
<td>D cor</td>
<td>*</td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

b) Survival in Corophium

<table>
<thead>
<tr>
<th></th>
<th>A cor</th>
<th>H cor</th>
<th>D cor</th>
</tr>
</thead>
<tbody>
<tr>
<td>A cor</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>H cor</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D cor</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

KEY

A cor: Corophium from Alaw
H cor: Corophium from Humber
D cor: Corophium from Dulas

* Significantly different at the 5% level
Table 4.5: Mean values (with standard errors) of the biological responses in *Nereis*.

a) Mean copper concentrations (µg/g Cu) showing standard errors in animals after exposure to sediments.

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Alaw <em>Nereis</em></th>
<th>S.E.</th>
<th>Humb. <em>Nereis</em></th>
<th>S.E.</th>
<th>Dulas <em>Nereis</em></th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaw</td>
<td>67.25</td>
<td>12.7</td>
<td>26.1</td>
<td>0.9</td>
<td>381.3</td>
<td>51.9</td>
</tr>
<tr>
<td>Humber</td>
<td>30.04</td>
<td>7.07</td>
<td>52.54</td>
<td>12.5</td>
<td>633.8</td>
<td>56.2</td>
</tr>
<tr>
<td>Dulas</td>
<td>95.11</td>
<td>9.1</td>
<td>262.09</td>
<td>10.48</td>
<td>832.23</td>
<td>8.7</td>
</tr>
</tbody>
</table>

b) Total percentage survival in *Nereis* after exposure to sediments.

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Alaw <em>Nereis</em></th>
<th>Humb. <em>Nereis</em></th>
<th>Dulas <em>Nereis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaw</td>
<td>95.3</td>
<td>70.8</td>
<td>100</td>
</tr>
<tr>
<td>Humber</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Dulas</td>
<td>58.4</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 4.6: Results of Least Significant Differences tests on the raw data (p<0.05) following a One Way ANOVA.

<table>
<thead>
<tr>
<th></th>
<th>D.F.</th>
<th>F</th>
<th>Sig. of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu in \textit{Nereis}</td>
<td>2</td>
<td>43.26</td>
<td>0</td>
</tr>
<tr>
<td>Survival in \textit{Nereis}</td>
<td>2</td>
<td>2.72</td>
<td>0.085</td>
</tr>
</tbody>
</table>

A comparison of biological responses in three populations of \textit{Nereis} after exposure to sediments.

\textbf{a) Cu in} \textit{Nereis} \hspace{2cm} \textbf{b) Survival in} \textit{Nereis}

\begin{tabular}{ccc}
A ner & H ner & D ner \\
A ner & & \\
H ner & & \\
D ner & * & *
\end{tabular}

\begin{tabular}{ccc}
A ner & H ner & D ner \\
A ner & & \\
H ner & & \\
D ner & * & *
\end{tabular}

\textbf{KEY}\n
\begin{itemize}
\item A ner: \textit{Nereis} from Alaw
\item H ner: \textit{Nereis} from Humber
\item D ner: \textit{Nereis} from Dulas
\end{itemize}

* Significantly different at the 5 % level
Table 4.7: Spearman Rank Correlations performed on the raw data obtained from the biological responses of all the populations of animals and the physical/chemical nature of the sediments.

|                           | LC50 Corophium | Survival Corophium | Cu Nereis | Survival Nereis | LC50 Nereis | Total Cu in sediment | Cu in organics | Cu in exchangeable | % organic carbon | % clay/silt | Cu | LC50 | Survival | Cu | Survival | LC50 | Total Cu | Cu | Cu | %organic |
|---------------------------|----------------|--------------------|-----------|-----------------|-------------|----------------------|----------------|--------------------|-----------------|-------------|-----------------|----------------|----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                           | **             | ns                 | ***       | ns              | ns          | ns                   | ns             | ns                 | ns              | **          | **              | **              | **              | **              | **              | ns             | ns             | ns             | ns             | ns             | ns             | ns             | ns             | ns             | ns             |

**KEY**

* 0.05 > p > 0.01
** 0.01 < p < 0.001
*** p < 0.001
ns not significant
Table 4.8: Spearman Rank Correlations performed on the raw data obtained from the biological responses in animals from each of the populations and the physical/chemical nature of the sediments that they were exposed too.

### a) Humber organisms

<table>
<thead>
<tr>
<th></th>
<th>LC50 Corophium</th>
<th>Survival Corophium</th>
<th>Cu Nereis</th>
<th>LC50 Nereis</th>
<th>Survival Nereis</th>
<th>Total Cu in sediments</th>
<th>Cu in organics</th>
<th>Cu in exchangeables</th>
<th>% organic carbon</th>
<th>% clay/silt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ns</td>
<td></td>
<td>ns</td>
<td>ns</td>
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<td>ns</td>
<td>ns</td>
<td></td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Total Cu in sediment</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<td>ns</td>
<td>ns</td>
<td></td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>% organic carbon</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>% clay/silt</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

**KEY**

- 0.05 > p > 0.01
- 0.01 > p > 0.001
- p < 0.001
- ns not significant
- not computed
Table 4.9: Results of Least Significant Differences tests on the raw data (p<0.05) following a One Way ANOVA.

<table>
<thead>
<tr>
<th></th>
<th>D.F.</th>
<th>F</th>
<th>Sig. of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu in <em>Corophium</em></td>
<td>2</td>
<td>6.59</td>
<td>0.006</td>
</tr>
<tr>
<td>Survival in <em>Corophium</em></td>
<td>2</td>
<td>6.38</td>
<td>0.006</td>
</tr>
</tbody>
</table>

A comparison of the effect of three different sediments on the accumulation of copper and the survival in *Corophium*.

a) Cu in *Corophium*

<table>
<thead>
<tr>
<th>A sed</th>
<th>H sed</th>
<th>D sed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A sed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H sed</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>D sed</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

b) Survival in *Corophium*

<table>
<thead>
<tr>
<th>A sed</th>
<th>H sed</th>
<th>D sed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A sed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H sed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D sed</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

KEY

- A sed: Alaw sediment
- H sed: Humber sediment
- D sed: Dulas sediment
- * Significantly different at the 5% level
Table 4.10: Results of Least Significant Differences tests on the raw data (p<0.05) following a One Way ANOVA.

<table>
<thead>
<tr>
<th></th>
<th>D.F.</th>
<th>F</th>
<th>Sig. of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu in Nereis</td>
<td>2</td>
<td>2.35</td>
<td>0.1</td>
</tr>
<tr>
<td>Survival in Nereis</td>
<td>2</td>
<td>2.38</td>
<td>0.1</td>
</tr>
</tbody>
</table>

A comparison of the effect of three different sediments on the accumulation of copper and the survival in Nereis.

a) Cu in Nereis

<table>
<thead>
<tr>
<th></th>
<th>A sed</th>
<th>H sed</th>
<th>D sed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A sed</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>H sed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D sed</td>
<td></td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

b) Survival in Nereis

<table>
<thead>
<tr>
<th></th>
<th>A sed</th>
<th>H sed</th>
<th>D sed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A sed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H sed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D sed</td>
<td></td>
<td></td>
<td>N.S.</td>
</tr>
</tbody>
</table>

KEY

A sed: Alaw sediment
H sed: Humber sediment
D sed: Dulas sediment
NS: Not significant
* Significantly different at the 5% level
Table 4.11: Spearman Rank Correlations performed on the raw data obtained from the
physical/chemical nature of each of the sediments and the biological responses in
the animals that were exposed to it.

### a) Humber sediment

<table>
<thead>
<tr>
<th></th>
<th>LC50 Corophium</th>
<th>Survival Corophium</th>
<th>Cu Nereis</th>
<th>Survival Nereis</th>
<th>LC50 Nereis</th>
<th>Total Cu in sediment</th>
<th>Cu in organics</th>
<th>Cu in exchangeables</th>
<th>% organic carbon</th>
<th>% clay/silt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
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</tr>
</tbody>
</table>

### b) Dulas sediment

<table>
<thead>
<tr>
<th></th>
<th>LC50 Corophium</th>
<th>Survival Corophium</th>
<th>Cu Nereis</th>
<th>Survival Nereis</th>
<th>LC50 Nereis</th>
<th>Total Cu in sediment</th>
<th>Cu in organics</th>
<th>Cu in exchangeables</th>
<th>% organic carbon</th>
<th>% clay/silt</th>
</tr>
</thead>
<tbody>
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</tr>
</tbody>
</table>

### c) Alaw sediment

<table>
<thead>
<tr>
<th></th>
<th>LC50 Corophium</th>
<th>Survival Corophium</th>
<th>Cu Nereis</th>
<th>Survival Nereis</th>
<th>LC50 Nereis</th>
<th>Total Cu in sediment</th>
<th>Cu in organics</th>
<th>Cu in exchangeables</th>
<th>% organic carbon</th>
<th>% clay/silt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*</td>
<td>_</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<td>ns</td>
</tr>
</tbody>
</table>

**KEY**

- * 0.05<p<0.01
- ** 0.01<p<0.001
- *** p<0.001
- ns not significant
- _ not computed
Figure 4.0: Mean % clay/silt content (showing standard error bars) in three sediments

Sediments

Figure 4.1: Mean total Cu concentrations (showing standard error bars) in three different sediments using sequential digest methods

Sediments

Figure 4.2: Mean Cu concentrations (showing standard error bars) in sequential extracts of three different sediments

Sequential extracts
Figure 4.3: The proportion of Alaw *Corophium* found in different sediments in a choice chamber after 20 days

Figure 4.4: The proportion of Humber *Corophium* found in different sediments.

Figure 4.5: The proportion of Dulas *Corophium* found in different sediments.
Figure 4.6: Mean copper concentrations (showing standard error bars) found in three populations of *Corophium* after exposure to their three different sediments for seven days.
Figure 4.7. Total survival (%) in three populations of *Corophium* after exposure to three different sediments in a seven day bioassay.
Figure 4.8: Percentage survival of Alaw *Corophium* during a seven day bioassay using three sediments.
Figure 4.9. Percentage survival in Humber Corophium during a seven day bioassay using three sediments.
Figure 4.10: Mean copper concentrations (showing standard error bars) in three populations of *Nereis* after exposure to the three different sediments for seven days.
Figure 4.11. Total survival (%) in three populations of *Nereis* after exposure to three different sediments in a seven day bioassay.
Figure 4.12: Percentage survival of Alaw *Nereis* during a seven day bioassay using three different sediments.
CHAPTER FIVE
IMPLICATIONS OF INTRA-SPECIFIC VARIATION AND ITS CONTRIBUTION TO THE MANAGEMENT OF METALS IN ESTUARIES.

INTRODUCTION

Determining the biological significance of trace metal contamination in aquatic environments is a complicated problem. Many aquatic organisms are in contact with both dissolved and particulate trace metals and can in principle accumulate the trace metals either directly from the water or from the solid phases. To determine the specific levels of bioaccumulation causing a particular ecological effect in a given species, it is necessary to distinguish between cause and effect (Peddicord, 1984). Therefore, to be meaningful in measuring effects of marine pollution the consequences of bioaccumulation are going to have to be stated in terms of demonstrably adverse biological responses to specified levels of bioaccumulation which is likely to be different interspecifically and intraspecifically. This variation has confounded the interpretation of many biomonitoring studies in which it has often been difficult to distinguish significant changes in body metal concentration due to pollution, from natural phenotype variations. However, with a greater understanding of the mechanisms underlying natural variability, it may be possible to distinguish pollutant effects more readily.

At present there are numerical models for several disciplines in estuarine and coastal science although there is a longer history of modelling in the physical sciences. There have been recent advances in modelling the biological system at the individual, population, community and ecosystem level (Elliott and Ducrotoy, 1991). However, the value of these models in the management of coastal systems is questionable, especially as the accuracy of prediction will decrease with an increasing complexity of the system being modelled (Elliott and Ducrotoy, 1991). For example, at the ecosystem level, which is probably the ultimate aim of management models, many more fundamental studies (like this study) are required before the functioning of the system is sufficiently well understood to be modelled with a high degree of reliability.

The data gained from the work described in Chapter Four have been subject to Cluster analysis and Multiple Regression analysis respectively to:

i) Explore the patterns and similarities between both the biological and physico/chemical variables and the organism/test sediment combinations.

ii) Describe and interpret patterns and relationships between the variables and create simple predictive models.

The implications of the above will be discussed in the context of estuarine management.
METHODS

The data gained from the work described in Chapter Four have been subject to Cluster analysis and Multiple Regression analysis. The biological parameters are the body copper concentrations in the animal, total % survival in the animal and the LCSO value (for copper) of that animal. The body copper and survival data were gained as a result of the seven day sediment bioassays (see methods in Chapter 4). The LC50 values were determined for copper in solution in an earlier study (see methods in Chapter 1). Organisms used in both studies satisfied the same experimental criteria such as being of similar size and maturity (see General Methods) and were sampled from the same sites.

The physical parameters are the % clay/silt, % organic carbon, % L.O.I. and % coal content in the sediment and the mean, medium, sorting coefficient and skew of the sediment particles. The chemical parameters are copper concentrations in the exchangeable, organic, carbonate, oxide and total fractions of the sediments.

i) CLUSTER ANALYSIS

To examine the relationships between all the biological, physical and chemical parameters simultaneously multivariate statistics were used. The M.V.S.P computer package was used to carry out cluster analysis on the mean data gained from exposing all three populations of *Corophium* and *Nereis* to the different test sediments. Cluster analysis devises a scheme for grouping n samples (each of which has a score on p attributes) into classes. The similar samples are put in the same class. The method is completely numerical and the number of classes is not known. Q and R mode analysis was used on the data. Q mode analysis allows the samples to be clustered based on the attributes. R mode analysis allows the attributes to be clustered based on the samples.

The data were treated:

1) to show the similarities and patterns between the biological and the physico-chemical parameters based on the organism/sediment combinations (R mode);

2) to show the similarities and patterns between the organism / sediment combinations based on the biological and physico-chemical parameters (Q mode).

Cluster analysis is a hypothesis generating technique and allows questions to be asked such as why samples (or biological variables) are similar.

Many algorithms have been proposed for cluster analysis (Manly, 1992). The most commonly used clustering techniques (and the technique used in this study) are the agglomerative hierarchic methods. These take a similarity matrix as their starting point and
successively fuse the samples into groups and the groups into larger clusters, starting with the highest mutual similarities then gradually lowering the similarity level at which groups are formed. The process ends with a single cluster containing all samples (Clarke and Warwick, 1994).

The result of hierarchical clustering is represented by a *dendrogram*, with the x axis representing the full set of samples and the y axis defining a similarity, or in the case of Euclidean distance (the measure used in this study) a dissimilarity level at which two samples or groups are considered to have fused. With quantitative data it is usual to operate with dissimilarities rather than similarities. The standardised Euclidean distance measure was the method used to show the distances between the variables. It is an appropriate measure of dissimilarity between physical/chemical variables (Clarke and Warwick, 1994) and has been used to explore patterns in biological and environmental variables. The Euclidean distance function is also the most frequently used for quantitative variables (Manly, 1992; Digby and Kempton, 1987).

Because of differing magnitude of the different variables, the variables need to be standardised before distances are calculated, so that all p variables are equally important in determining these distances. This would prevent the distance measure being affected by the type and order of magnitude of the variables. To overcome the problem of scaling in this study the distances were calculated on variables standardised to unit variance for the different variables. This meant that the raw data were expressed as standard deviation units (Dunn and Everitt, 1982).

The average linkage clustering method UPGMA (group average or unweighted pair group method) was used to derive the resulting dendrograms as it is the most frequently used agglomerative classification method (Okland, 1990). With group average linkage two groups merge if the average distance between them is small enough (Manly, 1992).

**ii) MULTIPLE REGRESSION ANALYSIS**

Linear Multiple Regression was carried out using the SPSS package to represent mathematically the relationship between the biological responses of the organisms and the physico-chemical nature of the sediments in which they were tested. Linear multiple regressions have been constructed as a first-step towards producing numerical predictive models (Mead, 1971).

The analysis was performed on raw and mean data to describe mathematically this relationship between the chemical, physical and biological variables. The goal of Multiple
Regression analysis is to describe the response variable (dependent variable) as a function of two or more explanatory variables (independent variables).

For each dependent variable Stepwise regression was used to identify the most statistically significant models. This method eliminates non-significant variables by picking the most statistically significant independent variables in a stepwise fashion. However it only picks one model (the statistically 'best' one) and does not identify other good models. Therefore, 'non-Stepwise' regressions were also carried out on other permutations of variables that were thought to be important.

The variables were chosen on the basis of previous work investigating the similarities between the variables and knowledge of the subject. Only models whose independent variables explain a statistically significant proportion of the variation of the dependent variable at the $p \leq 0.05$ level are included in the results. Graphs were constructed to illustrate the performance of the models which show the observed against predicted values for the input data set.
RESULTS

i) CLUSTER ANALYSIS

Similarities and patterns in the physico-chemical parameters in each sediment and the biological parameters in *Corophium* after exposure to the sediments

Figure 5.0 shows the dendrogram produced from cluster analysis on data from all three populations of *Corophium* in all three sediments. Five main groups of variables have been derived, the main division exists between groups 1 and 2 and groups 3, 4 and 5. The level of dissimilarity between each of the groups is quite high compared to the groups indicated in Figures 5.1-5.3, which are created from separate parts of the data to show any intraspecific differences in the patterns of variables giving relatively clear groupings of variables. The distances between variables within groups is broadly similar except for group 2 where the variables ‘survival’ and ‘LC50’ are separated at a higher level. The variable ‘copper in *Corophium*’ is noticeably separated from group 4 which contains the other copper variables. The ‘% organic carbon’ and ‘% L.O.I.’ has a dissimilarity value of zero (this is shown in every dendrogram) which is what would be expected as they measure the same thing.

The dendrogram given in Figure 5.1 shows the patterns in the variables using data from Alaw *Corophium* in all three sediments. The patterns in the variables are similar to those seen in Figure 5.0 except that ‘copper in *Corophium*’ is no longer in a separate group but is now clustered with the other copper related variables in group 4. Distances between groups and variables within groups are generally lower than in Figure 5.0. This would indicate that there is a greater similarity between the variables in a group when the data are separated for each population.

The dendrogram given in Figure 5.2 shows the patterns in the variables using data from Humber *Corophium* in all three sediments. Four groups of variables have been identified with the main division between ‘% clay/silt’, ‘% coal’, ‘% L.O.I.’, ‘% organic carbon’ and the organisms ‘survival’ - group 1, and the remaining variables (groups 2, 3 and 4). The patterns are broadly similar to that for Figure 5.0 with most of the variables falling into the same clusters. The variables ‘copper in organics’, ‘copper oxides’, ‘copper in carbonates’, ‘sorting coefficient’, ‘copper in exchangeables’ and ‘total copper’ in sediment once again group together (group 2) as do the variables ‘% clay’, ‘% L.O.I.’, ‘% coal’, ‘% organic carbon’ (group 1) and ‘skewness’, ‘median’ grain size and ‘mean’ grain size (group 4).

The variables ‘LC50’, ‘survival’ and ‘copper in *Corophium*’ from the Humber, however, do differ from Figure 5.0. Survival now joins group 1 and separates from ‘LC50’ which is in a group of its own (group 3) linked to the predominantly copper related variables in group 2. ‘Copper in *Corophium*’ is no longer separate from the other variables but now shows
similarities with 'mean/median' grain size and 'skewness' in group 4. The distances between
the variables in the groups and the groups themselves are generally lower than for all
populations of *Corophium* combined (Figure 5.0).

The dendrogram given in Figure 5.3 shows the patterns in the variables using data from
Dulas *Corophium* in all three sediments. The variables split in to five groups, the variables in
each group are as given in Figure 5.0. The main division between groups is between group 1
(‘% clay’, ‘% L.O.I’, ‘% coal’, ‘% organic carbon’) and the remaining variables in groups 2,3,4
and 5. The variable ‘copper in *Corophium*’ is in a group of its own linked to the other copper
variables in group 4, this is also a feature of Figure 5.0 (all *Corophium* populations combined)
but not Figures 5.1 and 5.2 (Alaw and Humber *Corophium*) indicating that values for ‘copper in
Dulas *Corophium*’ cause this particular grouping in Figure 5.0. Distances between variables
and groups are once again lower than for the dendrogram obtained for all populations.

**Similarities and patterns in *Corophium* and sediment combinations**

Cluster analysis was used to show the similarities between *Corophium* / sediment
combinations based on the biological responses of the organisms and the physico-chemical
nature of the sediments to which they were exposed. Figure 5.4 shows the dendrogram
obtained using data from all three *Corophium* populations. Three main groups of *Corophium*
population/sediment are evident. The main division is between group 1 (*Corophium*
populations in Dulas sediment) and groups 2 and 3 (*Corophium* populations in Humber and
Alaw sediments respectively).

The patterns in the relationships between the *Corophium* population and sediment
appears to be determined by the physico-chemical data with the main groups divided on the
basis of the type of test sediment. Within each group the similarities between *Corophium*
population become evident. In groups 1 and 2 (Humber and Dulas sediments) Humber and
Dulas populations appear more similar and are separate from Alaw populations. In group 3
(Alaw sediment) Humber and Alaw populations show greater similarities.

Figure 5.5 shows the results of cluster analysis of Alaw *Corophium* only. The
dendrogram shows the main division between Alaw *Corophium* in Dulas sediment and Alaw
*Corophium* in Humber and Alaw sediments. This indicates that on the basis of the biological
and physico-chemical parameters the Alaw *Corophium*/Alaw sediment combination is more
similar to the Alaw *Corophium*/Humber sediment combination than to the Alaw *Corophium*/
Dulas sediment combination, although the level of dissimilarity is very small.
The results of cluster analysis on Humber *Corophium* only are given in Figure 5.6. Here the main division is between Humber *Corophium* in Humber sediments and Humber *Corophium* in Alaw and Dulas sediments.

Figure 5.7 shows the dendrogram based on data using Dulas *Corophium* only. The results show that the Dulas *Corophium* in Humber sediment differ from those in Alaw and Dulas sediments.

**Similarities and patterns in the physico-chemical parameters in each sediment and the biological parameters in *Nereis* after exposure to the sediments**

In Figure 5.8 the results of cluster analysis on the biological and physico-chemical parameters for all three *Nereis* populations in all three sediments are shown. The biological / physico-chemical variables can be split into four main groups. The main division between the groups is between groups 1 and 2 and groups 3 and 4 with distances between groups being higher than dendrograms produced from the separated data (Figures 5.9-5.11). The variables within each group are similar to those for *Corophium* in Figure 5.0 the main difference being that the variable ‘copper in *Nereis*’ is grouped with ‘LC50’ and ‘survival’ in group 4 (with survival strongly separated from the others).

The dendrogram derived from data using Alaw *Nereis* only is given in Figure 5.9. Three main groups of variables are present. Most of the variables are grouped as in the previous dendrogram but the variables relating to *Nereis* i.e. ‘survival’, ‘LC50’ and ‘copper in *Nereis*’ show some differences. ‘Copper in *Nereis*’ and ‘LC50’ are still grouped together (in group 3) but now show similarities with ‘mean/median’ grain size and ‘skewness’, with ‘LC50’ highly separated from the others. ‘Survival’ is not linked with ‘LC50’ as in previous figures but is grouped with ‘% clay’, ‘% L.O.I’, ‘% coal’ and ‘% organic carbon’ in group 1 but is clearly separated from them. The variables ‘copper in organics’, ‘copper oxides’, ‘copper in carbonates’, ‘sorting coefficient’, ‘copper in exchangeables’ and ‘total copper’ in sediment fall into the same group (2) as in previous figures. The main separation between variables is between group 1 and groups 2 and 3, distances between groups/variables are generally lower than for all populations of *Nereis* (Figure 5.8).

Cluster analysis on data using Humber *Nereis* only produces the dendrogram given in Figure 5.10. The variables have been grouped into three main groups, these are similar to those described for Figure 5.9 with the exception that the variables ‘copper in *Nereis*’ and ‘survival’ are now in group 3 as well as the copper related variables and the ‘sorting coefficient’ with ‘survival’ showing similarities to ‘copper in organics’ and ‘copper in *Nereis*’ similar to ‘copper in carbonate’ and ‘copper in oxides’. Distances are generally lower than for Figure 5.8.
The dendrogram for Dulas *Nereis* given in Figure 5.11 shows the same patterns as for Dulas *Corophium* (figure 5.3) with the exception that ‘copper in *Nereis*’ is not highly separated from the other copper related variables in group 4 but displays a high degree of similarity with ‘copper in exchangeables’ and the ‘sorting coefficient’. ‘LC50’ and ‘survival’ show complete similarity in group 3. As in the previous figures ‘% clay’, ‘% L.O.I’, ‘% coal’ and ‘% organic carbon’ group together (group 1) as do ‘mean/median’ grain size and ‘skewness’ in group 2. Distances between groups are high but generally lower than for all populations of *Nereis* (Figure 5.8).

**Similarities and patterns in *Nereis* and sediment combinations**

The dendrograms derived from data on all three populations of *Nereis*/sediment combinations (Figure 5.12) are similar to that for *Corophium* (Figure 5.4) in that three main groups are evident separated on the basis of the sediment type. However, the Humber sediment/worm combinations separate from the Dulas and Alaw sediment/worm combinations. Further groupings of population type are visible within each group. In Dulas sediments the Alaw population differs from Humber and Dulas populations. In Humber and Alaw sediments the Dulas populations were more dissimilar than Alaw and Humber populations.

Figures 5.13 to 5.15 show the dendrograms obtained from cluster analysis using the data for each of the Alaw, Humber and Dulas populations respectively. In each case two main groups are formed with the *Nereis* populations in Dulas sediments differing from those in Humber and Alaw sediments though the distance between groups is not great.

**ii) MULTIPLE REGRESSION ANALYSIS**

Linear Multiple Regressions were performed on the untransformed raw data using LC50 values, body Cu levels and survival in *Corophium* and *Nereis* as dependent variables. The models constructed using the stepwise method are clearly labelled and are always illustrated as model 1 in Tables 5.0-5.5 unless a model could not be created using this method. This method eliminates the poorest variables and only selects the most statistically significant independent variables (at the 5% level) in a stepwise fashion. Other statistically significant models constructed using permutations of variables analysed with the default ‘non-Stepwise’ method are also shown in each table.

All models included in the results section are significant at the $p \leq 0.05$ level (F significance). The independent variables used in each model explained a significant proportion of the variation in the raw data of the dependent variable. No significant models were found...
using the mean data except when the LC50 values for *Corophium* and *Nereis* were used as the dependent variables. The performance of all the models are illustrated graphically showing the observed against predicted values. The perfect model for the observed against predicted values would have a regression line fitted at $b=1$ and all the points should be evenly distributed on the line.

**Cu Levels in *Corophium* and *Nereis***

The amount of variability (as shown by $R^2$) in the levels of copper in each organism after exposure to test sediments for 7 days accounted for by the independent variables ranged from 43% to 48% for *Corophium* (Table 5.0, model 2 and 1) and was 89% for *Nereis* (Table 5.1, model 1). Model 1 is statistically more important in Table 5.0 compared with model 2 as it has the highest correlation coefficient ($R$) and $F$ ratio and accounts for more variability. This model has a very high multiple correlation coefficient ($R$) and $F$ ratio and accounts for nearly all the variability.

All the models' performance are illustrated graphically (Figures 5.16-18) and have points distributed fairly evenly along the fitted lines as there was a wide range of data for copper levels in both organisms. Figure 5.18 has a line fitted nearer to $b=1$ than Figures 5.16 and 5.17 and more of the points lie on the line. These models perform well and the statistically more important models are model 1 (Table 5.1) for predicting copper levels in *Nereis*; and model 1(Table 5.0) for predicting copper in *Corophium*.

**Survival in *Corophium* and *Nereis***

The amount of variability accounted for by the independent variables in the total survival of the organisms after being exposed to test sediment ranged from 52% to 60% in *Corophium* (Table 5.2, model 1 and 2) and from 36% to 37% in *Nereis* (Table 5.3, model 1 and 2). Both models in Table 5.2 for *Corophium* seem statistically valid as the correlation coefficients and the $F$ ratios are high and they account for similar levels of variation. The models in Table 5.3 for *Nereis* are weaker than the models for *Corophium* as the correlation coefficients and $F$ ratios are lower and less variability is accounted for.

The models' performance is represented graphically in Figures 5.19-5.22. These figures display a poor performance as there was insufficient data for survival in these organisms over a wide enough range. The points do not lie on the line and they do not distribute evenly either. An over-all assessment of these statistics would indicate that these models would not perform...
well. However, the models in Table 5.2 are statistically promising and with a larger data set the performance of these models for survival in *Corophium* would improve.

**LC50 value in *Corophium* and *Nereis***

The amount of variability in the LC50 value for the organisms after being exposed to test sediment for seven days accounted for by the independent variables were 60% in both models for *Corophium* (Table 5.4, model 1 and 2) and 88% in both models for *Nereis* (Table 5.5). All models seem statistically valid with high correlation coefficients and F ratios. However, the independent variables 'Survival in *Nereis*' (Model 2, Table 5.5) and 'Cu in sediment' (Model 2, Table 5.4) are not significant (p<0.05) and therefore do not explain a significant proportion of variation in the dependent variables.

The performance of Model 1 (Table 5.4) and Model 1 (Table 5.5) are represented graphically in Figures 5.23 and 5.24. These figures display a poor performance as there were not enough LC50 data over a wide enough range, i.e. only three different LC50 values for each organism entered many times. An over-all assessment of these statistics would indicate that these models would not perform well. However, Model 1 (Table 5.4) and Model 1 (Table 5.5) are statistically promising and with a larger data set with a greater range of values for the dependent variable (LC50) the performance of these models would improve.
DISCUSSION

i) SIMILARITIES AND PATTERNS BETWEEN THE PARAMETERS

The data in this study were examined in two ways to assess; i) the relationships (similarities) shown between the biological and physico/chemical (environmental) variables in one organism/test sediment combination compared with another and; ii) the relationships (similarities) in the different organism/test sediment combinations based on the biological and environmental parameters. M.V.S. techniques are frequently used to link environmental variables to community analysis (Clarke and Warwick, 1994). The variables were standardised before the distances were calculated which meant that the distances were based on the relative magnitude not the absolute values. This enables variables with different units, eg. ‘% survival’ and ‘mean’ particle size to be analysed together. This was done by coding so that all the means were zero and all the variances were one (Manly, 1992).

The dendrograms constructed showing the similarities in the biological responses and physico/chemical nature of the sediments for all and individual populations of *Corophium* /test sediment data show similar patterns. The physical parameters tend to group together or with the mean, median and skew as a separate group. The chemical parameters describing the copper levels in the different fractions group together.

The biological parameters are not grouped together and are found to associate with different physico/chemical parameters in each dendrogram. The parameter 'Cu levels' in all populations of *Corophium* is in a group of its own. This parameter is also on its own in the dendrogram constructed using data from just Dulas *Corophium* /sediment combinations. All the parameters are analysed simultaneously and even though the data have been standardised it seems that the 'Cu levels in *Corophium* ' are so much higher in Dulas animals than the animals from the other estuaries. The other parameters in these two dendrograms group in similar ways.

This is not the case for Alaw and Humber *Corophium*, with the parameter 'Cu levels in *Corophium* ' for Alaw *Corophium* being in the same group as the chemical parameters that described the Cu levels in different fractions of the test sediments. This similarity could be because Alaw *Corophium* are less tolerant to copper and are net accumulators; ie. they seem to accumulate copper more in proportion to the environmental levels compared to *Corophium* from the Humber and Dulas. This can be supported by results found in Chapter Four which show that although there is a statistically significant correlation between 'Cu levels in all populations of *Corophium* ' and 'total Cu in sediment', if the data are split up there is only a statistically significant correlation between 'Cu levels in Alaw *Corophium* ' and the 'total Cu levels' in the sediment in which they were tested.
‘Copper levels in *Corophium*’ from the Humber are in the same group as the mean and median particle size and the skew. Sediment from the Humber has statistically smaller particle size than sediment from the Alaw and Dulas. Therefore these similarities could indicate that the uptake of copper in Humber *Corophium* is more related to physical properties of the sediment than the Cu levels alone. This population of *Corophium* has been used to being exposed to smaller particles in their environment, which is where most of the metal ions are adsorbed.

‘Survival in *Corophium*’ after exposure to sediments and the ‘LC50’ value for copper (or tolerance index) of that population is in the same group in all dendrograms apart from the one constructed using Humber *Corophium*/sediment combination data only. *Corophium* from the Humber are different, with the ‘LC50’ value in a group of its own, and ‘survival’ in a group with parameters describing the physical nature of the sediments. Again these similarities between the biological response in Humber *Corophium* and the physical properties of the test sediment suggest that the physical nature of the test sediment may affect this organism more than the level of copper in the sediment. The Humber population could have an increased 'plasticity' to toxic chemicals due to having to live in an environment with a cocktail of pollutants. The physical and chemical attributes of sediment greatly affect the bioavailability of a metal to an organism (discussed further in Chapter 4).

At a higher level of dissimilarity, all dendrograms apart from the one constructed from Humber *Corophium*/sediment data showed that Cu in *Corophium* is in the same group as all the chemical parameters that described the Cu levels in the different sediment fractions. The parameter 'total copper' is very similar to 'copper in the exchangeable' fraction in each population/sediment combination. The 'sorting coefficient' was always very similar to 'copper in exchangeables. Copper levels in the organic, oxide and carbonate fraction of the sediments were also found to be very similar in all cases.

The dendrograms constructed showing similarities in the biological responses and physico/chemical nature of the sediments for all populations of *Nereis*/sediment combinations indicate four main groups. The biological parameters group together, the physical parameters split up into two groups and the chemical parameters including the sorting coefficient are in the last group. The parameter 'Cu in the exchangeable fraction' can always be found to be very similar to the sorting coefficient in that sediment. These patterns with the physical and chemical parameters can be found in all the *Nereis* dendrograms as was found for *Corophium*. However the biological parameters show different similarities to other parameters in each population of *Nereis*/sediment combination. Copper levels in *Nereis* from Dulas and the Humber were very similar to the chemical parameters describing copper levels in the different
fractions of sediment. However, unlike Alaw Corophium, copper levels in Alaw Nereis were not similar to any environmental parameters describing sediment copper levels.

The dendrograms showing similarities between all populations of Nereis and Corophium/sediment combinations based on the biological, physical and chemical parameters show clear patterns. The biological and environmental variables caused the different populations of organisms in the different sediments to cluster by test sediment not by population. This may be because there are more physical and chemical data describing the sediment than biological data. The same patterns emerge for Corophium and Nereis in most of the dendrograms with Dulas sediment separating out. This could be because the chemical parameters in Dulas sediment are so different from the chemical parameters in the other sediments causing quite different biological responses.

In this study two types of dendrograms have been constructed; one based on the organism/sediment combinations which examined patterns in the biological responses of the organisms and the physico/chemical nature of the test sediment. The second was based on the biological responses and physico-chemical nature of the test sediments which examined patterns in the organism/sediment combinations. Analysis carried out on this first use of the data contributes to the understanding of which biological responses and physico/chemical properties of a sediment are important in affecting populations of Corophium and Nereis. This could be useful in deciding which environmental and/or biological parameters should be measured for biomonitoring purposes or in the construction of predictive models. For example, in this study four chemical parameters were always found to be very similar. It did not therefore seem necessary to include data from all four parameters when performing multiple regression analysis with a view to predictive modelling.

The parameters recommended for use in Multiple regression analysis to potentially describe the variation in the dependent variables are as follows: 'Cu content in the organisms', 'Total % survival', 'LC50 value', 'Total Cu in the sediment', 'Cu in the exchangeables', 'Cu in the organics', '% clay/silt content' and the '% organic carbon content'. These recommendations were based on knowledge of the subject from the literature as well as observations of the patterns and relationships between the variables.

Analysis carried out for the second use of the data produced a clearer picture of how one organism/test sediment combination may differ from another based on the biological responses and nature of the sediment. If an equal number of biological and environmental parameters had been measured then any inter-and/or intra-specific differences will be indicated.

Multivariate statistical techniques are hypothesis generating and have been used in this study as a preliminary means of examining patterns in a number of variables or objects. Cluster analysis has a use in this type of study as it allows a large data set to be broken down into
smaller, easier to detect, groups of information. This could be useful in contributing to the understanding and biomonitoring of bioavailable metals in the marine environment.

ii) CONSTRUCTION OF SIMPLE PREDICTIVE MODELS

Multiple regression analysis is valuable in representing mathematically biological variation based on other parameters (Mead 1971). For example, abundance and production has been modelled in relation to environmental factors (Elliott and Taylor 1989). In this study, the biological variation or dependent variables were copper levels in the organism, survival in the organism and the LC₅₀ value in the organism. The environmental or independent variables were the physico-chemical measurements of the sediments in which the organism was tested. The linear multiple regressions were carried out as a first step towards producing numerical predictive models (Mead 1971).

In this study many regressions were performed on a combination of variables. The inclusion of any variable was not random but based on knowledge of the subject from the literature and personal study. Exploration of the patterns and relationships in all variables simultaneously using multi-variate statistics gave an indication of which variables were similar in different situations.

The stepwise method was also used as it eliminates the poorest variables and only picks the most statistically significant independent variables (at the 5% level) in a stepwise fashion. However, this method only constructs one model and will not identify other good models that are also statistically significant and may use variables that are easier to measure and implement when validating and using the model. Only linear regression was computed and therefore any curvilinear relationships were not explored. It may have been possible to get an improved fit to the linear model by transforming the data. However, it is difficult to determine which transformations are appropriate.

The choice of a parameter to be used in environmental models has management implications. For example, a model that may help predict survival of Corophium in a particular copper contaminated sediment would be practically more useful if one of the significant variables were 'total Cu' in sediment rather than 'Cu in the oxides'. The digests for 'total copper' are more widely used than for 'Cu in oxides'. The nitric or hydrochloric acid based matrix causes less problems in analyses than the matrix used to digest the oxide fraction.

It is important not to rely only on the stepwise regression that constructs one model and try other permutations of variables. It should also be noted that other environmental or biological parameters which have not been measured in this study could also explain the biological variation.
The following equations determined from multiple regression analysis (see Tables 5.0-5.5) are suggested to be suitable for further testing for use in the assessment of the ecological impact of bioavailable copper in sediments using either *Corophium volutator* or *Nereis diversicolor* as indicator species. It is important to clarify that the 'survival in an animal' refers to that animals % survival after the seven day sediment bioassays. Where-as the 'LC50 value' was determined (see Chapter One) from 96 hour acute toxicity tests using dissolved copper.

**Copper levels in *Corophium* and *Nereis***

Copper in *Corophium*  
\[= 42.39(\text{LC50 value}) + 19.29(\text{Cu in exchangeables}) + 2.35 \]
\[= 48.15(\text{LC50 value}) + 0.19(\text{Total Cu}) + 11.22 \]

Copper in *Nereis*  
\[= 17.28(\text{LC50 value}) + 1.2(\text{Total Cu}) - 501.4 \]

The 96 hour LC50 value was very significant in all models predicting copper levels in *Corophium* and *Nereis*. The ability of an organism to tolerate a contaminant is important when choosing organisms to be bioindicators (Rainbow, 1995). Also, when assessing the impact of a metal on a population, if the LC50 value is known then the same model will be more accurate as it would take into account intraspecific differences in the accumulation of copper. The level of total copper in the test sediment is also important in explaining the level of copper in *Corophium* and *Nereis*. Copper in the exchangeable fraction of the test sediment can also explain the variation of copper in *Corophium*. However, the actual level of copper in the organism would also depend on the biology of the organism.

To be more specific, the intraspecific variation in physiological responses to available copper in sediment determines how much copper an organism takes up. ie. this variation could be due to phenotypic or genotypic adaptations to survive in copper contaminated environments. This could be because the more tolerant either *Corophium* or *Nereis* are, the more able they are to accumulate copper in an non toxic form (see Chapter One, Two and Three).

**Survival in *Corophium* and *Nereis***

Survival in *Corophium*  
\[= 20.63(\text{LC50 value}) - 0.1(\text{Total Cu}) + 71.03 \]
\[= 25.11(\text{LC50 value}) - 0.137(\text{Cu in *Corophium*)} - 0.072(\text{Total Cu}) + 75.47 \]

Survival in *Nereis*  
\[= 0.02(\text{Cu in *Nereis*)} + 6.27(\%\text{organic carbon}) + 77.62 \]
\[= 0.02(\text{Cu in *Nereis*)} - 0.066(\text{Total Cu}) + 0.18(\%\text{clay/silt}) + 81.83 \]
The 96 hour LC50 value, total copper in sediment and the copper levels in *Corophium* were found to be significant in explaining the survival of *Corophium* after exposure to test sediments for seven days. The LC50 value for *Nereis* was not significant in explaining the proportion of variation in the survival of *Nereis*. However, the copper levels in this organism, % clay/silt and % organic carbon were found to be significant to this organism's survival.

The results indicate that for *Nereis* the physical nature of the sediment has an effect on the survival of *Nereis*, irrespective of that organism's LC50 value. The tolerance of *Nereis* to copper does not seem to be as important as in *Corophium* in explaining these organisms' survival. This could be due to the plasticity of *Nereis* where as *Corophium* are more sensitive and their survival is more likely to differ from population to population with varying pollution loads.

**LC50 values in *Corophium* and *Nereis***

\[
\text{LC50 value for } \text{Corophium} = 0.006(\text{Cu in Corophium}) + 0.016(\text{Survival in Corophium}) - 0.72
\]

\[
\text{LC50 value for } \text{Nereis} = 0.0005(\text{Cu in Nereis}) - 0.0006(\text{Total Cu}) + 0.3
\]

The 'copper levels in *Corophium*’ and ‘survival’ in *Corophium* after seven days exposure to test sediments is significant in explaining the variability in the ‘LC50 value’ for this species. However, in *Nereis*, it is the body copper levels and the copper levels in the test sediment that are significant in explaining the variation in the ‘LC50 value’. ‘% Survival’ is not significant for *Nereis*, and copper levels in test sediment is not significant in *Corophium*.

**SUMMARY**

Generally, variation in a biological system will be caused by temporal and spatial differences which will affect the different environmental parameters and therefore affect the ecology and physiology of a population of organisms. The ideal model should consider all these parameters and although work carried out in this study did not consider temporal differences, phenotypic and environmental differences due to spatial variation were considered.
These simple models derived from linear multiple regression analyses provide the first step towards the determination of predictive environmental models for use in the assessment of the ecological impact of bioavailable copper in sediments using *Corophium volutator* and *Nereis diversicolor* as indicator species. However, they need to be developed further using an increased data set. There needs to be a greater variability in the raw data set especially for survival data and the 96 hour LC$_{50}$ values. Using more samples of organisms from different populations and more test sediments would increase the data set and the variability in biological responses.

Multiple Regression is a very powerful technique for investigating complex situations. However as done in this study, multiple regression models need to be built up from past knowledge, or from detailed investigation of experimental or observational data (Mead, 1970). The way to build a model of a biological situation is to start with some data, to plot graphs of the relationship between the various variables recorded and to postulate simple models which have a possible biological interpretation. The models derived in this study are simple, and suggest obvious experimental or observational forms of testing which could in turn give rise to more complex models.
KEY FOR DENDROGRAMS

Biological Variables

Cucor       Copper concentration in Corophium
LC50cor     LC$_{50}$ value for copper in Corophium
SurvCor     Survival in Corophium
Cuner       Copper concentration in Nereis
LC50ner     LC$_{50}$ value for copper in Nereis
Survner     Survival in Nereis

Physical Variables

sortcoef    Sorting coefficient
mean(µm)    Mean particle size
medium(µm)  Medium particle size
skew        Skew
%orgcarb    % Organic carbon
%L.O.I.     % Loss of ignition
%clay       % Clay/silt
%coal       % Coal

Chemical Variables

Cuexc       Copper in exchangeables
Cucarb      Copper in carbonates
Cuoxides    Copper in oxides
Cuorg       Copper in organics
TotalCu     Total copper
Figure 5.0: Similarities between the physical, chemical and biological parameters based on data from all three populations of *Corophium* and all three sediments.

Figure 5.1: Similarities between the physical, chemical and biological parameters based on data from *Corophium* from the Alaw population and all three sediments.
Figure 5.2: Similarities between the physical, chemical and biological parameters based on data from *Corophium* from the Humber population and all three sediments.

Figure 5.3: Similarities between the physical, chemical and biological parameters based on data from *Corophium* from the Dulas population.
Figure 5.4: Similarities between all the test sediment and Corophium combinations based on all the data from the physical, chemical and biological parameters.

Figure 5.5: Similarities between the test sediment and Alaw Corophium combinations based on data from the physical, chemical and biological parameters.

Figure 5.6: Similarities between the test sediment and Humber Corophium combinations based on data from the physical, chemical and biological parameters.

Figure 5.7: Similarities between the test sediment and Dulas Corophium combinations based on data from the physical, chemical and biological parameters.
Figure 5.8: Similarities between the physical, chemical and biological parameters based on data from all three populations of *Nereis* and all three sediments.

Figure 5.9: Similarities between the physical, chemical and biological parameters based on data from *Nereis* from the Alaw population and all three sediments.
Figure 5.10: Similarities between the physical, chemical and biological parameters based on data from *Nereis* from the Humber population and all three sediments.

Figure 5.11: Similarities between the physical, chemical and biological parameters based on data from *Nereis* from the Dulas population and all three sediments.
Figure 5.12: Similarities between all the test sediment and *Nereis* combinations based on all the data from the physical, chemical and biological parameters.

Figure 5.13: Similarities between the test sediment and Alaw *Nereis* combinations based on the physical, chemical and biological parameters.

Figure 5.14: Similarities between the test sediment and Humber *Nereis* combinations based on data from the physical, chemical and biological parameters.

Figure 5.15: Similarities between the test sediment and Dulas *Nereis* combinations based on data from the physical, chemical and biological parameters.
Table 5.0: Multiple regressions on RAW DATA

DEPENDENT VARIABLE: Copper in Corophium

<table>
<thead>
<tr>
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<td>B</td>
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<tr>
<td>Cu in Corophium</td>
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<td></td>
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<tr>
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<td>LC50 in Corophium</td>
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<td>Cu in Nereis</td>
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Table 5.1: Multiple Regressions on RAW DATA

DEPENDENT VARIABLE: Copper in Nereis

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<td>Survival in Corophium</td>
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<td>LC50 in Corophium</td>
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<td>Cu in Nereis</td>
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<td>Survival in Nereis</td>
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### Table 5.2: Multiple Regressions on RAW DATA

**DEPENDENT VARIABLE**: Survival in *Corophium*

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### Table 5.3: Multiple Regressions on RAW data

**DEPENDENT VARIABLE**: Survival in *Nereis*

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### Table 5.4: Multiple Regressions on RAW DATA

**DEPENDENT VARIABLE:** LC50 for *Corophium*

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### Table 5.5: Multiple Regressions on RAW DATA

**DEPENDENT VARIABLE:** LC50 for *Nereis*

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<td>LC50 in <em>Corophium</em></td>
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<td>8.95</td>
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<tr>
<td>Cu in <em>Nereis</em></td>
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<td>Survival in <em>Nereis</em></td>
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<tr>
<td>LC50 in <em>Nereis</em></td>
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Fig 5.16: Observed against predicted values using Stepwise Regression
MODEL 1

Figure 5.17: Observed against predicted values
MODEL 2

Figure 5.18: Observed against predicted values
MODEL 1
Fig 5.19: Observed against predicted values using Stepwise Regression
MODEL 1

Figure 5.20: Observed against predicted values
MODEL 2
Figure 5.21: Observed against predicted values using Stepwise Regression MODEL 1

Figure 5.22: Observed against predicted values using Stepwise Regression MODEL 2

Figure 5.23: Observed against predicted values using Stepwise Regression LC50 FOR COROPHIUM

Figure 5.24: Observed against predicted values using Stepwise Regression LC50 FOR NEREIS
CHAPTER SIX
GENERAL DISCUSSION

The main aim of this study was to examine intraspecific variation in *Corophium* and *Nereis* and discuss its importance in the monitoring and management of metals in different estuaries. The ability of an organism to tolerate or not to tolerate metal in its environment is a product of the organism's genotype, physiology and ecology, and will vary spatially depending on the bioavailability of the metal. Recognition of the existence of intraspecific variability is significant in pollution studies. For example, in the interpretation of many biomonitoring studies it has been difficult to distinguish between the effects of pollution and those due to natural variations. This variability in morphology, physiology and behaviour, together with genetic diversity, may give rise to much of the variability in trace metal concentrations within populations of animals. Intraspecific variation in concentrations of copper and the ability to tolerate this metal was found in both *Corophium volutator* and *Nereis diversicolor* in the present study.

In Chapter 1, a high copper tolerance and high body copper levels were found in *Nereis* and *Corophium* from the Dulas estuary. Moderate tolerance and moderate basal copper levels were found in both species from the Humber, compared to animals from the Alaw which showed a very low copper tolerance with very low body copper levels. These copper concentrations and degree of tolerance in *Corophium* and *Nereis* from the three estuaries reflected the levels in the sediments. Tolerance could not be acquired after exposure of juvenile and adult *Nereis* to a range of sublethal copper concentrations over a 30 day period. Tolerance in adult worms from Dulas was not lost after exposure to 'clean' conditions for 30 days supporting the suggestion that it may be genetic (Hateley, 1989). In the Humber estuary there was generally little spatial or temporal variability shown in the metal concentrations in the sediments, *Corophium* and *Nereis*.

The evidence presented in Chapter 2 suggests that as well as interspecific differences in the toxicant uptake and physiological responses to accumulating the metal from solution, there were also intraspecific differences. For example, although *Corophium* from Dulas had the highest total copper concentrations, the actual amounts of copper they accumulated after exposure to external dissolved copper concentrations were significantly lower than in *Corophium* from the Alaw and Humber. The high levels of total body copper suggests that the metal is being sequestered in a non-toxic way, but an exclusion mechanism and/or an excretion mechanism may also be in operation. *Corophium* from the Alaw and the Humber accumulated the highest amounts of copper relative to their basal levels.
The metal accumulation in the tolerant populations of *Nereis* was greater than in the non tolerant organisms. Perhaps due to genotypic or phenotypic adaptations to the high levels of copper in their natural habitat these organisms have an increased ability to sequester the copper in a non toxic form.

The accumulation of copper in the tolerant population of *Nereis diversicolor* was tissue specific (Chapter 3) and the results indicated that tolerance was probably due to increased deposition of Cu in membrane-bound structures located in the cells of the nephridial tubules. Copper was not found in the nephridial area of the non tolerant worms from the Alaw estuary. Pirie *et al* (1985) found copper to be present in the cells of the nephridium and the epithelium of the first segment in metal tolerant *Nereis*.

In Chapter 4 intraspecific variation was found to occur in the survival and body copper concentrations of *Corophium* and *Nereis* after exposure to different natural sediments in experimental situations. The physicochemical nature of each of the sediments affected the bioavailability of copper which caused different responses in *Corophium* and *Nereis*. In Chapter 5 the results gained from experiments described in Chapter 4 were further analysed, and patterns and similarities between the biological responses and physicochemical parameters were examined. Simple predictive models were constructed to explain the variation found in the responses of *Corophium* and *Nereis*. The LC$_{50}$ value was used as an index of tolerance and was found to be important in explaining variation in the survival and copper accumulation in *Corophium* and *Nereis*.

The distribution of tolerance would appear to have enormous potential as an indicator of marine pollution (Klerks and Levinton, 1987; Hately 1989). The presence at a specific location of a population of a species which shows increased tolerance to a toxicant is regarded as evidence of an ecological impact by that contaminant or a close chemical relative (Luoma, 1977). Two of the criteria proposed by Luoma (1977) to validate the presence of tolerance were dealt with in the present study.

In Chapter 1 copper tolerance was shown to accord with Luoma's tolerance criterion iv, "the degree of tolerance is related to the level of exposure to the toxicant". The degree of copper tolerance in *Nereis diversicolor* and *Corophium volutator* constitutes the extent of environmental impact at a site. It is important to emphasise that the influence of factors affecting bioavailability result in the degree of tolerance being related to the level of metal that was available to that organism, and not just total environmental metal levels. It is not possible to precisely state the environmental levels of copper that elicit tolerance. The presence of copper tolerant worms in sediment levels of approximately 700µg g$^{-1}$ copper (Hayle estuary; Bryan, 1976) suggests levels of above approximately 500-600µg g$^{-1}$ are required for tolerance.
However, in the present study copper tolerant *Nereis* and *Corophium* were found in sediments of approximately 200μg g⁻¹ copper.

It is suggested in Chapter 1 (within the limitations of the experiments) that ‘tolerance results from Darwinian selection for tolerant genotypes, it cannot be induced over the lifetime of individuals’ (Luoma criterion i). Copper tolerance was shown not to be induced in *Nereis diversicolor* by exposure to sub-lethal levels of copper for 30 days and neither was it readily lost when tolerant worms were exposed to non-contaminated conditions for a similar period.

Underwood and Peterson (1988) stated that to study the effects of pollution in marine systems three aspects need to be examined. First the relative sensitivities and reliabilities of the methods of detecting pollution (in this case, the degree of tolerance) need to be evaluated. The use of tolerance in *Corophium* and *Nereis* as a biomonitoring tool can be evaluated by criteria that were proposed by Lee *et al* (1980) for use in biological effects monitoring. Those relevant to this study are:

1) Consistent relation of response to individual or species pathology. Tolerance would need to be related to an adverse effect on the growth, reproduction or survival of the individual or the population, and ultimately on the well being of the community/ecosystem. The presence of inherited metal tolerance is direct evidence that metals are having an impact on a particular population, as the contamination must have selected against non-tolerant genotypes.

2) Response to specific or general stressors. It would be necessary to determine whether tolerance was specific in relation to the causative agent. For example, different physiological mechanisms are involved in copper and zinc tolerance and the response to the causative agent is completely specific with respect to these metals.

3) High precision of the method and large response compared with the variance. Can the signal (tolerance) be easily detected above the noise (natural variability)? The LC50 value for the tolerant *Nereis* was approximately 2.5 times greater than that for the non tolerant worms in this study and 1.5 times greater for the tolerant *Corophium* than for the non tolerant animals. As there is no overlap between the 95 % confidence limits for tolerant and non-tolerant animals for both metals (Figures 1.4 and 1.5), the natural variability does not obscure the signal. Highly tolerant or moderately tolerant populations can easily be detected provided sufficient animals are tested, control animals are included, and suitable statistical analyses are applied.

4) Rapid response after contact with stressor. How quickly is there an observable effect? It is difficult to state precisely how quickly measurable levels of tolerance will appear after significant impact by metal contamination. The rate of response will depend on the heritability of tolerance (Falconer, 1960) and also on the rate of contamination (ie. the rate of selection). It would take a few generations for the tolerance to become measurable and therefore the response rate must be measured in years for macrobenthic invertebrates.
5) Applicable to a wide range of phyla and their life histories. Is tolerance specific to particular taxa? Inherited metal tolerance has been identified in bacteria (Timoney and Port, 1982), algae (Russel and Morris, 1970), annelids (Klerks and Levinton, 1987), molluscs and crustacea (Nevo et al, 1984), so that metal tolerance does not appear to be restricted to particular taxa.

8) Ease and cost of method. How expensive is the measurement of tolerance in terms of capital equipment, running costs, training costs and manpower? Acute static toxicity tests to determine LC50 values are quick (96 hours or less), simple and very inexpensive. They also require minimal supervision.

The second aspect that needs to be examined (Underwood and Peterson, 1988) is the problem of interpretation of the pollution, i.e. determining the importance of the observed effects of pollution on the biological system. Species selected for detecting pollution may not provide useful information about the economic effects on exploited parts of natural systems, nor about trophic structure of a community, nor about future sizes of populations of important species. The choice of appropriate species as indicators or detectors of pollution also requires determination of how representative they are of other species likely to be affected by pollution. Evidence that a toxicant has exerted selective pressure on an ecological 'opportunist' like Nereis diversicolor makes it likely that it is having adverse effects on other more specialised species within a community (Luoma, 1977). It is probable that these more sensitive species will be greatly affected by environmental metal levels below those required to elicit tolerance in Nereis diversicolor and Corophium volutator (e.g. Cerastoderma edule in Restronguet Creek; Bryan et al, 1987). Thus, although heavy metal tolerance in Nereis diversicolor is a sensitive method for detecting ecological impact on populations of Nereis diversicolor, it may not be a good indicator of impact on other species. However, non tolerant and partially tolerant Corophium showed a greater sensitivity in sediment bioassays compared to Nereis which may indicate their value for use in sediment toxicity studies.

Finally, Underwood and Peterson (1988) raise the problem of predicting the future consequences of pollution. Some methods used to detect pollutants might be useful as early warnings of future deleterious effects. As an example, biochemical changes at a subcellular level in a target organism precedes cellular and tissue alterations, which in turn should later affect physiological function and ultimately, perhaps, some population parameter. The measurement of the degree of tolerance can only detect the effects of pollutants after sufficient time has elapsed for populations to have changed. However, it can offer a direct measurement of the importance of pollution to the continued functional well-being of the system. A mixture of different types of measures allows the best synthesis of predictive power while providing the most useful information for interpretation of the consequences of pollution to a marine system (Underwood and Peterson, 1988).
It is not usual in pollution monitoring programmes to establish direct identification of the causative agent(s). As ecological effects are non specific, the causative agent must be determined through circumstantial evidence from chemical analyses of the sediment/biota and through bioassays. The specificity of metal tolerance is an invaluable device for determining causation, even when the contamination is a complex mixture of metals causing several tolerant abilities to different contaminants.

It is proposed that divisions of populations on the basis of similarities in the degree of metal tolerance and the level of metal accumulation into 'physiotypes' could reduce the difficulty in distinguishing significant changes in metal concentration due to pollution, from natural variation. Depledge (1989c) has suggested that populations might be divided into different 'physiotypes', or classes of physiological behaviour, and this division might explain some of the individual variability in metal concentrations.

Tolerance distribution and comparison of different 'physiotypes' can be used in the management of marine and estuarine systems in two ways.

1) Tolerance could be used as a direct indicator of the nature and impact of the pollution on the population/community. As it is a result of past and possibly present pollution or contamination, measuring tolerance and comparing metal levels in similar physiotypes has a role in the biological monitoring of pollution.

2) The actual presence of this tolerance (or variability in physiotypes) needs to be taken into account when carrying out Environmental Impact Assessments and determining Environmental Quality Standards. For example, if an impact assessment was carried out on the effect of a pollutant on two physiotypes, the responses (impact) could be quite different due to intraspecific differences and the bioavailability of that pollutant to that organism. Measurable responses in an organism to a metal have been shown in this present study to vary intra- and inter-specifically depending on how tolerant the organism was and what basal metal levels were present.

An example from this study identifying such variation is as follows. After exposure to different copper contaminated sediments the survival of the animal correlated with the copper levels in different populations of *Nereis* but not in *Corophium*. Presumably, the worm sequesters or detoxifies the copper in a non-toxic form (see Chapter 2 and 3). If survival in *Corophium* does not relate to the organisms' copper levels either positively or negatively it could be due to a number of reasons; it is not copper that is killing the organism, lower levels of copper are exerting a toxic response and killing the organism before it can either excrete it or sequester it in a non-toxic form or there is some ability to regulate this metal and excrete it (see Chapter 2). However, it is more likely to be a mixture of these processes due to the different *Corophium* physiotypes. Understanding how this biodiversity in marine and estuarine
communities produces varying responses using laboratory bioassays would contribute to the determination of how populations will respond to a pollutant in their environment.

Figure 6.0 is a summary diagram based on work carried out in this study. It shows the natural variation in three different populations of *Corophium* and *Nereis* and their responses to natural sediments and dissolved copper. The sediment features (A) causing the different bioavailibilities of copper (in section B) were based on the three sediments used in this study. These parameters were found to be important in explaining the variation in biological responses of the animals.

The ability to tolerate copper and the amount of copper accumulated in three different populations of *Corophium* and *Nereis* are also shown in B. These responses are a result of long term exposure to the bioavailable copper levels in the sediment in that animal's environment. The degree of copper tolerance and the concentration of accumulated copper were used as a basis to separate each population into different physiotypes. Variation in copper tolerance and the comparison of similar physiotypes for biomonitoring purposes have management implications which are identified in section B of the diagram.

The responses (accumulation of copper, and survival) of the three different physiotypes of *Corophium* and *Nereis* after exposure to the three different sediments are shown in C. The large arrows represent each sediment, which point to the small arrows which represent each of the physiotypes. These smaller arrows point to the responses of the animal (shown in the boxes) after exposure to a certain sediment. The broken arrows represent the *Corophium* physiotypes and the solid arrows represent the *Nereis* physiotypes.

The blocks of colour extending from section B (behind the boxes in section C) to section D represent the different physiotypes of *Corophium* and *Nereis*. The small arrows in section D that extend from these blocks of colour also represent the different physiotypes of *Corophium* and *Nereis* and point to the responses of these animals to sublethal levels of dissolved copper. The management implications of the information shown in C and D have been identified and highlighted.

As metal contamination will vary temporally and spatially between estuaries as well as within an estuary, it is important to include tolerance studies to identify such intraspecific variation when setting water and sediment quality standards and to take into account the exposure history of different populations prior to their use in toxicity bioassays. As there are at present no standards set for copper in sediment or biota it is proposed that studies investigating the different physiotypes be carried out to help determine the appropriate EQS at different sites. This would produce sensitive and responsive management tools for estuaries rather than a blanket approach. This may be more appropriate in some situations, particularly where certain metals are problematical as is the case of copper in the Humber estuary.
Figure 6.0. A diagram to summarise the relationships between copper in the sediment and animals and the responses of the latter, including management implications.

**KEY**

- Impact of low copper bioavailability
- Impact of moderate copper bioavailability
- Impact of high copper bioavailability

**Responses of...**

- Responses of a low tolerant *Nereis* (physiotype 1)
- Responses of a moderately tolerant *Nereis* (physiotype 2)
- Responses of a highly tolerant *Nereis* (physiotype 3)

- Responses of a low tolerant *Corophium* (physiotype 1)
- Responses of a moderately tolerant *Corophium* (physiotype 2)
- Responses of a highly tolerant *Corophium* (physiotype 3)
SEDIMENT FEATURES

A

LOW COPPER BIOAVAILABILITY IN SEDIMENT

MODERATE COPPER BIOAVAILABILITY IN SEDIMENT

HIGH COPPER BIOAVAILABILITY IN SEDIMENT

B

NERES PHYSIOTYPE 1

LOW TOTAL Cu

LOW TOLLERANT NERES

COROPHIA PHYSIOTYPE 1

LOW BODY Cu

LOW TOLLERANT COROPHIA

NERES PHYSIOTYPE 2

MEDIUM Cu

MODERATELY TOLERANT NERES

MEDIUM Cu

MODERATELY TOLERANT COROPHIA

NERES PHYSIOTYPE 3

HIGH Cu

HIGHLY TOLERANT NERES

HIGH Cu

HIGHLY TOLERANT COROPHIA

C

INCREASED TOTAL BODY COPPER

NO CHANGE IN TOTAL BODY COPPER

REDUCED TOTAL BODY COPPER

REDUCED SURVIVAL

NO CHANGE IN SURVIVAL

LOW ACUMULATION

HIGH ACUMULATION

D

RESPONSES TO NATURAL SEDIMENTS

RESPONSES TO D Dissolved COPPER

ABILITY TO REGULATE COPPER

CANNOT REGULATE COPPER

PARTIALABILITY TO REGULATE COPPER

MANAGEMENT IMPLICATIONS FOR BIOLOGICAL MONITORING.
1) The presence of metal tolerant animals at a site is strong evidence that ecological impact has occurred.
2) Divisions of populations on the basis of similarities in the levels of copper tolerance and copper accumulation into ‘physiotypes’ could reduce the difficulty in distinguishing significant change due to pollution, from natural phenotype variations.

MANAGEMENT IMPLICATIONS FOR ENVIRONMENTAL IMPACT ASSESSMENT AND DETERMINATION OF ENVIRONMENTAL QUALITY STANDARDS.
Knowledge of how biological responses to pollutants may differ between various ‘physiotypes’ could prove useful in predicting the impact of pollutant metals in our naturally varying marine and estuarine ecosystems. This would produce a more sensitive approach for determination of Environmental Quality Standards.
REFERENCES


Fraser, J., (1980). Acclimation to lead in the freshwater isopod Asellus aquaticus. Oceanologia. 45.419-420


National Institute for Coastal and Marine Management (RIKZ). Guideline fo conducting 10 day static sediment toxicity tests using marine or estuarine anthropods. Ministry of Transport and Water Management.


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