A study of an estuarine benthic community subjected to petrochemical effluents

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A thesis presented for the degree of
Doctor of Philosophy

December 2001

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Helen Davis

ABSTRACT

This study has assessed the impact of a petrochemical complex, which discharges its effluents onto an intertidal mudflat. The Grangemouth petrochemical complex on the Forth Estuary, Scotland discharges two effluents on to the Kinneil intertidal area. The results of a 24-year monitoring programme of the Kinneil intertidal area, carried out between 1976 to 1999, are analysed. The relative impact of the effluents on the macrobenthic community is considered along with other potential pollution sources and climatic factors. During the study period a clear increase in the diversity, evenness and species richness was observed over the whole area. This is attributed to the increased quality of the refinery effluent, the chemical effluent and the River Avon, which also crosses the area. The group analysis showed that although all areas have shown an increase in diversity there are still three areas that can be considered impacted (Groups 1, 2 and 4). Two major changes in the species composition were seen in 1979 when *Manayunkia aestuarina* was first found and in 1994 when *Streblospio shrubsolii* was first recorded.

The impact of the recent movement of the chemical outfall from a hightide position to a lower shore site is also considered. A detailed survey of the areas around the new lower shore and old upper shore outfalls indicated that there was a spatial difference in the species distribution, which can be explained by the distance from the refinery outfall, the hydrocarbon concentration of the sediments and/or the station height. No change in the community composition was detected after the movement of the chemical outfall in January 1998, although seasonal changes were seen.

CONTENTS

CD-ROM containing data collected for long-term and short-term surveys.

ACKNOWLEDGEMENTS

I would like to thank Dr. Donald McLusky and Prof. David Bryant for their advice and support throughout my research and for their guidance during the preparation of this thesis. I would also like to thank NERC and British Petroleum for their financial assistance through the allocation of a Case studentship. I am also grateful to British petroleum for their assistance with the sediment hydrocarbon analysis which they funded and for the effluent information. I would also like to thank the Scottish environment protection agency for the use of their boat and for the water quality information. I am very grateful to Alasdair Sherman for all the help with sampling and to Catherine Howie and Tim Benton for their statistical advice and lastly to all who helped in the collection of the long-term data.

ABBREVIATIONS

ABC – Abundance biomass curve

ARM – Artificial refinery mixture

BOD – Biological oxygen demand

COD – Chemical oxygen demand

DO – Dissolved oxygen

EC – Electrical conductivity

NAO – North Atlantic Oscillation

PAH – Polynuclear aromatic hydrocarbons

SAB – Species abundance biomass

TAH – Total aliphatic hydrocarbons

UCM – Unresolved complex mixture

1. INTRODUCTION

1.1 ESTUARIES

The land surrounding an estuary is often highly industrial and many of these industries have an impact on the estuarine environment. Some like oil refineries and chemical plants discharge effluents into the estuary which contain many different chemicals (Cote, 1976). Whilst others like power stations remove water and its associated flora and fauna, particularly the potentially valuable fish stocks (Kennish, 1992). Estuaries are also subject to land reclamation for industrial or agricultural purposes and it has been suggested that in some estuaries land claim has had more of an impact on the invertebrate, fish and shore bird populations than pollution (McLusky *et al*, 1992). The estuarine environment is therefore controlled not only by natural processes but also by anthropogenic factors. Firstly the natural estuarine environment will be considered followed by how it could be affected by pollution and specifically by oil refinery effluents.

1.1.1 Physical environment

The estuarine environment is very variable with respect to physical conditions like salinity, temperature and oxygen concentration (Wolff, 1983). The main variability is in the salinity, which alters along a gradient from seawater (35psu) to freshwater (<0.5psu) (McLusky, 1989). Water temperature, although relatively stable in most marine environments, can be very variable in the estuarine environment due to the small volume of water and the large surface area (Nybakken, 1993). The oxygen content of the water also varies, it can become low in oxygen during the summer months, whilst the sediment is largely depleted of oxygen all year and is usually anoxic below a few centimetres (Hunter, 1981; Nybakken, 1993). The sediment load of an estuary is always constantly changing with respect to the tidal state, currents and wave conditions (McLusky, 1989). It is the variability of these conditions that can make it a difficult environment to inhabit. It does however have some advantages for those animals that can overcome these obstacles. Estuaries are relatively sheltered and highly productive all year, providing a rich and easily available food source for the inhabitants (McLusky, 1989).

1.1.2 Benthos

Only those organisms that have a high tolerance to the variable physical conditions are able to survive in estuaries. Typically, therefore, many of these species are opportunistic, and are capable of rapidly colonising areas after sudden environmental change (Newell *et al.*, 1998). As estuarine species are already adapted for living under stressful conditions they are also probably more tolerant to pollution than species living in more stable environments, due to their low degree of specialisation and high genetic diversity (Wolff, 1983). As estuaries are areas of high physiological stress, the stability-time hypothesis predicts that the community will be controlled by physical factors rather than biological factors (Sanders, 1963). The diversity in a stressful environment, such as an estuary, is often lower due to fewer species having the physiological adaptations, behavioural characteristics or spatiotemporal recruitment patterns necessary to survive in the stressful conditions (Dauer & Ranasingle, 1992), although they tend to have an increased abundance (Nelson-Smith, 1970).

The type of sediment can also have an effect on the distribution of species. Larvae actively select a suitable substratum on which to settle and establish a population (Gray, 1974). It is not known, however, whether species are limited to a certain type or to the water currents that cause the formation of certain sediments (Wolff, 1983). There is some evidence however to suggest that it is not usually the sediment properties alone that determines species distributions (Snelgrove & Butman, 1994). Once a population is established the benthos interacts with the sediment and consequently changes its properties (Wolff, 1980).

The climatic conditions also vary temporally and can cause fluctuations in community structure between and within years. Often changes in the climate can be seen to effect the community composition (Carpentier *et al.*,1997; McCroy, 1987). As benthic communities are mainly composed of sessile or relatively immobile infaunal species, recruitment of larvae and the mortality of the adults are the major causes of change to the community structure (Pearson and Rosenberg, 1978). Recruitment for most species occurs in the spring and therefore spring is a critical period for the development of distribution patterns both regionally and in the long

term (Holland, 1987). Many opportunistic species have a short life cycle and tend to breed throughout the year enabling them to colonise areas during the whole year (Pearson & Rosenberg, 1978). Consequently opportunistic species are usually the first colonisers of any habitat that becomes vacant, whether due to physical / biological disturbance or pollution and are common in the stressful estuarine environment.

1.1.3 Mudflats

Many estuaries have associated intertidal mudflats, which are created by the deposition of fine sediment in areas where currents are slow. Mudflats are characterised by having high organic content and low redox potentials. The oxygen levels within the sediment decrease with depth and often become anoxic within a few centimetres of the surface. These anoxic areas cannot be inhabited by the majority of the estuarine benthos which require oxygen for survival (Wolff, 1983). Consequently the majority of the estuarine benthos is found within the upper 5 cm of the sediment and those that are below this depth usually have contact with the overlying water column. Ratcliffe *et al.* (1981) found that for *Macoma balthica* smaller animals were found near the surface and larger animals progressively deeper. Therefore the depth of sampling may effect the size distributions found. Typically the benthos has an aggregated pattern of distribution within the sediment (Thrush, 1991), therefore instead of each species being evenly distributed throughout the sediment they are often found to have a clumped distribution. The spatial distribution patterns are created during settlement (Lewin, 1986; Holland, 1987; David *et al.* 1997) and may be related to physical factors such as depth, hydrodynamics or sediment type (Barnes, 1981; Udalov *et al.*, 2000) and can also be affected by inter and intra specific competition (Moore *et al.*, 1987; Flach & de Bruin, 1993; Jensen & Andre, 1993; Flach, 1996; Lawrie *et al.* 2000), predation (Ambrose, 1991; Wilson, 1991), post-settlement dispersal (Beukema, 1993; Norkko *et al.*, 2001) or disturbance (Connell, 1978, Pearson & Rosenberg, 1978).

1.2 POLLUTION

The estuarine environment receives pollution in many different forms including sewage, heavy metals, pesticides and oil. These different pollutants can have various effects on the environment. There are two main types of effect, organic enrichment and toxicity (Spies *et al.*, 1988).

1.2.1 Sewage pollution

An organic enrichment effect would predict that the area around the source of pollution would have a reduced species richness and diversity (Mucha & Costa, 1999) and possibly be deviod of fauna (Pearson & Rosenberg, 1978). With increasing distance the abundance and number of species would increase to a peak of opportunists. This peak would then decline and the endemic species numbers would increase. Finally the abundance and species number would fall to the normal levels for the area with no pollution. This pattern is called the species abundance biomass (SAB) relationship (Pearson and Rosenberg, 1978). The areas around sewage discharges often show this typical SAB relationship (Dauer & Conner, 1980; Moore & Rodger, 1991; Simboura *et al.*, 1995).

1.2.2 Heavy metal pollution

With a lethal toxic effect it would be expected that with decreasing toxicity there would be an increase in survival, reproductive success or growth, but no enhancement of the community. No peak of opportunist or enrichment of the endemic fauna is expected. Toxicity would cause the total number of species and individuals to decrease but there may not be a change in the values of the diversity indices (Olsgard & Gray, 1995). Heavy metals are considered to be toxic to many marine and estuarine organisms. Different metals have varying toxicity and some species are more sensitive than others (Mance, 1987). The effects of heavy metals also vary with temperature, salinity, pH and valence and can act synergistically with one another (Cote, 1976; Mance, 1987). Some animals can become tolerant to certain heavy metals after acclimation (Stubblefield, 1989). Some animals, especially molluscs, also accumulate metals within their tissues (Pringle *et al.*, 1967; Frithsen, 1984). Toxicity may not be lethal but may cause sublethal effects on

growth (Conradi & Depledge, 1998; Conradi & Depledge, 1999) and reproduction (Krause, 1994; Conradi & Depledge, 1998; Conradi & Depledge, 1999). It can also effect the immune system causing increased susceptibility to disease (Galloway & Depledge, 2001).

1.2.3 Oil pollution

Oil enters the marine environment from many different sources including drill cuttings from oil platforms, spills from oil tankers, urban runoff, ballast water, fixed installations, natural seeps, atmospheric fallout, marine phytoplankton and oil refinery effluent (Johnston, 1984). The specific composition of oil is variable and dependent on its source. However all oil has five components, Saturated non-cyclic hydrocarbons (paraffins), Cyclic hydrocarbons (cycloalkanes), Olefinic hydrocarbons (alkenes), aromatics and non-hydrocarbons (sulphur compounds, nitrogen-oxygen compounds and heavy metals)(Cote, 1976).

When oil enters the marine environment it is subjected to a number of different processes including evaporation, dissolution, advection, dispersion, photochemical oxidation, emulsification, adsorption onto suspended particulate material, biodegradation and sedimentation (Carlberg, 1980; Harrison, 1996). The different components of oil undergo these processes at different rates due to their different physico-chemical properties (Readman *et al.*, 1992). The weathering of the oil is a very important factor controlling the subsequent distribution of the hydrocarbons within the sediments (Mayo *et al.*, 1978).

Oil is known to be toxic to many marine organisms although not all of the individual components are toxic and some have been found to be more toxic than others (Van Gelder-Ottway, 1976). The mono aromatics are the least toxic with the toxicity of the other aromatics increasing with increasing molecular size or increasing degree of alkylation of the nucleus (Anderson, 1979). Anderson *et al.* (1974a) and Tatem *et al.* (1978) both found that naphthalenes were highly toxic and that the dimethylnaphthalenes had the highest toxicity. The actual impact of the oil is however dependent on its concentration, residence time and temperature (Oviatt *et al.*, 1982). Oil can affect marine organisms in a number of different ways. It can

kill them directly through coating and the resultant asphyxiation, by contact poisoning, or through exposure to water-soluble components. Lastly, oil is capable of causing sublethal and stress effects, carcinogenic and mutagenic effects and can affect the behaviour of individuals (Cote, 1976). Different marine organisms have varying sensitivities to the toxic components of oil. Crustaceans and notably Amphipods have been identified as especially sensitive (Straughan, 1977; Oviatt *et al.*, 1982; Suchanek, 1993). Anderson *et al.* (1974b) also discovered that refined oils were considerably more toxic than crude oil to all the animals that were tested. Shaw *et al.* (1976) suggested that mortality of *M. balthica* could be used as an indicator of oil contamination. Hydrocarbons are also readily accumulated within many organisms, but the rate and patterns of uptake are usually species-specific (Anderson *et al.*, 1974a). Crustaceans (Anderson *et al.*, 1974a) and bivalve molluscs (Anderson, 1979) have been found to readily accumulate petroleum hydrocarbons because they have an inefficient or absent Mixed-function oxygenase (MFO) system (Kennish, 1991). Once hydrocarbons have been incorporated into animal tissue they have varying residence times. In general heavier molecular weight aromatic have a longer residence time than lower molecular weight aromatics (Teal *et al.*, 1978).

Oil has also been seen to have an organic enrichment effect, as it is a source of additional organic carbon. Nance (1991) found that near an oil / gas field in New Bayou there was an enhancement of the benthic fauna. In fact the calculated benthic gain from the enrichment area was twice that of the calculated benthic loss due to toxicity. Algal growth has also been observed to increase in areas with high polynuclear aromatic hydrocarbon (PAH) concentrations (Carman *et al.*, 1995). The organic enrichment effect has also been found to occur at natural oil seeps. The oil in these areas seems to increase the abundance and biomass of the benthic organisms (Davis & Spies, 1980; Agard *et al.*, 1993; Steichen *et al.*, 1996). In only one of these investigations (Streichen *et al.*, 1996) was there found to be a suppression in the fauna at any point that was related to the oil concentration and not to other physical factors like oxygen concentration. In fact Agard *et al.* (1993) and Straughan (1977) found that the benthic organisms found at natural seeps were not stressed and they were able to live and breed in these areas with chronic hydrocarbon inputs. It has been suggested that benthic fauna that have been exposed to chronic inputs have evolved higher tolerances to the toxic effects of

hydrocarbons. However Straughan (1977) argues that this is unlikely as most of the benthic organisms produce pelagic larvae and therefore the juveniles are recruited from areas away from the natural seeps. Straughan (1977) suggests that it is however possible that there is a selection process that only allows those juveniles, which have higher tolerances, to survive in these areas. If this is true then it would be expected that this could also be the case in other areas with chronic hydrocarbon contamination like at oil refinery effluent discharges.

It has yet to be proven whether oil has a purely toxic effect or acts as an organic enrichment, or whether it is a trade off between these two effects. It is thought that under spill conditions there is an initial toxic response. However under chronic conditions it is thought that the changes in the abundance and diversity that are seen may be due to organic enrichment, and the role of toxicity is unclear. Spies *et al.* (1988) investigated the roles of toxicity and organic enrichment by comparing the responses of benthic invertebrates to crude oil and kelp. It was found that the response of the benthic fauna to crude oil was similar to that of kelp and other organic matter, and that the toxicity may have made a small contribution to the outcome in reducing some of the groups of organisms. It is possible that it is the toxicity that determines which species are able to survive, but the level of organic enrichment determines the abundance of these species and may cause the exclusion of further species. Olsgard & Gray (1995) suggest that it is possible to separate the impacts resulting from organic enrichment and toxicity. They state that when toxic effects occur, usually the number of species and individuals decrease and as a consequence the diversity index does not change, whereas organic enrichment is often seen by a decrease to the diversity index.

Some studies have shown oil to have relatively little or no effect on benthic communities. Anderson *et al.* (1978) found that the abundance of organisms in oiled and clean sediments were not significantly different and that there was only a minimal impact of the oil on recruitment of larvae. It may be that there is a minimum concentration before any effects can be detected. Nance (1991) found that a sediment hydrocarbon concentration of 2.5 mg g^{-1} dry sediment was the average value needed to depress the population abundance. Therefore although oil is known to cause toxic and enrichment effects, the extent of the effects will depend on the

concentrations found in the environment, the physical conditions, the species involved and the type of oil. If input of oil is reduced to a low enough level, then there is the potential for the effects to be reduced and maybe even stopped altogether.

1.2.4 Role of the sediment

The sediments are an important sink for hydrocarbons and other compounds within an estuarine system (Knap *et al.*, 1982). Oil is capable of remaining within the sediment for many years, as it is not readily degraded under low oxygen conditions. Oviatt *et al.* (1982) found that 10 to 20 percent of the oil that ended up in the sediment still remained after one year, and intertidal sediments in Nova Scotia retained oil for at least six years (Southward, 1982). Pollutants can however be resuspended into the water column by the actions of the benthic organisms (Landrum & Robbins, 1990). Once in the water column they are again able to affect the benthic communities. Even if the contaminants are not resuspended and remain in the sediment they are capable of affecting certain organisms like deposit feeders, such as *Macoma balthica* and *Pygospio elegans* which ingest sediment particles and therefore the pollutants, such as hydrocarbons, along with them. The ability of many polychaete worms to accumulate and metabolise PAHs allows them to be passed on through the food chain to crustaceans, birds and fish and ultimately to humans (Driscoll & McElroy, 1996). Sediment properties like organic carbon content, particle size, clay type and content and pH all affect the bioavailability and accumulation of contaminants (Landrum & Robbins, 1990). Therefore the type of sediment and the types of species living within the sediment will help to determine what effects pollutants may have.

1.2.5 Recovery

After pollution abatement there is usually a period of recovery. Recovery is generally defined as "the establishment of a successional community of species which progresses towards a community that is similar in species composition, population density and biomass to that previously present, or at non-impacted reference sites" (Newell *et al.*, 1998). The changes that take place temporally also

often conform to the SAB relationship seen spatially around point sources of pollution (Pearson & Rosenberg, 1978). Recolonisation usually starts with opportunistic species, usually polychaetes, which reach high abundance levels. After this the biomass and species composition will undergo a series of fluctuations of declining amplitude for several years until stability is regained (Southward, 1982). Dauer & Simon (1976) noticed that the repopulation of the higher tide levels occurred more rapidly than lower tidal levels. The time recovery may take depends on many different factors including the complexity of the original community, the type of impact and its extent, habitat type and the weather (Suchanek, 1993).

1.3 OIL REFINERY EFFLUENTS

Petroleum refinery wastewaters are made up of many different chemicals which include oil and greases, phenols (creosols and xylenols), sulphides, ammonia, suspended solids, cyanides, nitrogen compounds and heavy metals like chromium, iron, nickel, copper, molybdenum, selenium, vanadium and zinc (Cote, 1976). Refinery effluents tend to have fewer of the lighter hydrocarbons than crude oil but more polycyclic aromatics which tend to be more toxic and more persistent in the environment (Tatem *et al.*, 1978). From 1969 to 1997 the amount of oil that is discharged in the effluent of European refineries has decreased by 98.6% (Figure 1.1). The discharge levels of ammonia, sulphides and phenols have also reduced by 23%, 49% and 21% respectively from 1993 to 1997. Burks (1982) noted that the number of components within a wastewater is determined by the number of components in the original crude oil stock, and the resultants from the fractionation process, plus any addition of chemical additives within the refinery operations. This means that each effluent is unique and can vary on a daily basis depending on which units within the refinery are in operation. This makes it hard to generalise on the effects of oil refinery effluent. Refinery effluents are generally considered to be toxic but because they also contain oil and ammonia, they also have the potential to have an enrichment effect.

The total quantity of aqueous effluent that is being discharged by oil refineries has also decreased over the years, for example European refineries discharged 3119 x 10^6 t/yr from 80 refineries in 1969 to 2942 x 10^6 t/yr from 105 refineries in 1997

(Table 1.1). The decrease between 1974 and 1978 is thought to be due to more refineries using air cooling and recirculating cooling water systems. There are around 113 refineries in Europe (Concawe, 1998) which can be categorised into four different types depending on their complexity (Table 1.2). Over the years the complexity of refineries has increased and since 1969 there has been the introduction of more effective treatment systems. The three main treatment processes for effluent before its discharge are gravity separation (API separators, tank separation), advanced treatment (flocculation, sedimentation, filtration) and biological treatment (biofilters, activated sludge, aerated ponds)(Concawe, 1998). The percentage of refineries that have all three treatment processes has increased over the years from only 23% in 1969 to 88% in 1997 (Table 1.3). As not all refineries have the same processes, the effluents that are produced will have different chemical compositions depending on the type of treatment it receives (Lehtinen, 1986). Therefore each effluent could potentially have a different effect due to its different composition. As the number of refineries that have treatment systems increases the impact from them should be decreasing.

1.3.1 Fate of the effluent

The fate of an effluent once it is discharged into the environment depends on the conditions and hydrodynamics of the receiving water (Riddle, 1997). The effluent is inevitably diluted within the receiving water but to what extent depends on the size of the recipient water body and where the outfall is located, whether it is intertidal or subtidal. Grahl-Nielson (1987) dyed the discharge water from an offshore operation and found that the discharge was unevenly distributed in the recipient waters.

Most studies on the fate of refinery wastes just consider the hydrocarbons within the effluent. The volatile compounds are lost from the water column through weathering (Cranthorne *et al.*, 1989). The remaining compounds undergo sedimentation and biodegradation. Knap & Williams (1982) found that the most important removal mechanism was sedimentation and that in Southampton Water 70% of the hydrocarbons were found in the sediments after one hour. Compounds with high water solubility such as aromatics were absorbed slower than non-polar

compounds like aliphatics. In Southampton Water biodegradation occured rapidly, hydrocarbon concentrations were reduced by 70% after 40 days, much faster than in other areas (Knap & Williams, 1982). The increased speed of biodegradation was attributed to the substantial population of oil degrading microbes in the area that had accumulated over the 50 years of chronic discharge. Most of the hydrocarbons that are degraded are lower molecular weight aliphatic fractions. This means that over time hydrocarbon concentrations do decrease but due to the constant effluent discharge they are always being replenished. Therefore if the discharges were to cease or the hydrocarbon concentration within effluents were to be reduced then there is the potential for the hydrocarbon concentrations to decrease to lower levels within the sediment.

Le Dreau *et al.* (1997) observed that around a petroleum refinery in the Gulf of Fos (South France) there were 3 zones of contamination of the sediment. Firstly a highly contaminated zone near the refinery (50 g kg^{-1} sediment dry weight), followed by a less contaminated zone in the deep creek (~3 g kg^{-1} sediment dry weight), with a final slightly contaminated zone in the open sea (~0.1 g kg^{-1} sediment dry weight). Other studies have also shown that the area of high contamination is often localised to the vicinity of the outfall and decreases with distance (Knap *et al.*, 1982; Armannsson *et al.*, 1985; Moore *et al.*, 1987; Talsi, 1987). The hydrocarbons seem to sediment out near to the discharge point.

There also seems to be a pattern of hydrocarbon distribution with depth but this varies depending on the history of the discharge and sedimentation rates of the area concerned (Huntley *et al.*, 1993). Talsi (1987) observed that around the Neste Oy's oil refinery in Finland the maximum concentration of oil was at 4-14cm and that there seemed to be no further degradation at this depth. At Rhode Island Sound, USA, it was discovered that the hydrocarbon concentration decreased with depth and that with increasing depth a greater percentage of the oil was of biogenic origin (Van Vleet & Quinn, 1978). This would suggest that in this area degradation of the light fractions was occurring within the sediment leaving the heavier biogenic hydrocarbons, which could be due to a slow sedimentation rate. The pattern of the concentration of contaminants with depth of the sediment can also be linked to the history of the inputs to the area. Cranthorne *et al.* (1989) found that at Kinneil in the

Forth Estuary the aliphatic concentration increased with depth, which could be a reflection of the reduced hydrocarbon content of the effluent over the years. Knap *et al.* (1982) observed that in Southampton water there was a distinct oil horizon within a core at 90-100cm depth, which they attributed to the expansion of the oil refinery in this area around 1950 and a subsequent reduction in discharges. These studies show that few generalisations can be made between different areas as to the fate of the components in the effluent.

1.3.2 Toxicity tests

There are many different ways of testing the toxicity of different compounds but these fall into two main categories. Firstly the acute lethal test which usually lasts 96 hours. The aim of this type of test is to find out the lethal concentration of a substance. Secondly there are sublethal tests. These can take many forms but test for any sublethal reactions that a substance has that could cause a problem for the individual and/or the population over a long period of exposure. Measurements of sublethal effects may be on respiration rates, growth rates, reproductive success and behavioural changes. Acute tests are the most common but sublethal tests are also important especially when looking at the impact of a chronic problem like refinery effluents. Many different species have been used to look at the toxicity of oil refinery effluents including species of fish, crustaceans, plankton and algae.

The toxicity of oil refinery effluent is dependent on a number of factors. These include the volume, quality, salinity and variability of the discharge, the siting of the outfall, the physical and chemical conditions of the discharge area, the proximity of other effluents and pollutants and the biological condition of the discharge area (Concawe, 1979). The different components of the refinery effluent can have varying effects and toxicities (Smith, 1974). The oil in the refinery effluent can affect marine organisms in a number of different ways. It can kill them directly through coating and asphyxiation, contact poisoning, or through exposure to water-soluble components. It can also cause the destruction of more sensitive juveniles or of the food organisms therefore wiping out a population. Lastly oil is capable of causing sublethal and stress effects, carcinogenic and mutagenic effects and can affect the behaviour of individuals (Cote, 1976). The toxicity of ammonia is

dependent on pH, oxygen concentration and temperature (Cote, 1976). With increasing pH (Burks, 1982) and decreasing O_2 (Cote, 1976), ammonia becomes more toxic. Ammonia is removed by bacteria in well oxygenated areas and is therefore not likely to be accumulated by marine organisms (Concawe, 1979). Sulphides on the other hand are also removed by bacteria (Concawe, 1979) but have the opposite relationship with pH. The toxicity of sulphides increases with decreasing pH. Cyanides are also very toxic to marine organisms and its toxicity is affected by synergism with other chemicals like Ammonia and Zinc. Cyanide affects the transport of oxygen from the blood to the tissues (Cote, 1976). Phenols on the other hand are less toxic and are readily biodegraded by bacteria within 200 minutes given the right conditions (Cote, 1976). Lastly, heavy metals can have toxic effects. The different metals have varying effects which also vary with temperature, salinity, pH and valencey and can act synergistically with one another (McLusky *et al* , 1986). The exact effects of refinery effluent and its constituents thus can and do vary from species to species.

1.3.2.1 Phytoplankton and algae

There are very few studies that look at the effects of refinery effluent or its components on algae. Refinery effluent has been shown to reduce the abundance and growth of phytoplantkon and algae (Saha & Konar, 1985; Sherry *et al.*, 1994). More studies are needed on the effects on algae as they are an important link in the food chain. Reduced productivity of phytoplankton and/or algae will have a knock on effect to the other organisms in the environment, such as crustaceans and fish.

1.3.2.2 Invertebrates

Many studies have used freshwater and marine invertebrates as test organisms to observe the effects of refinery effluent and its individual components. Crustaceans seem to be more sensitive than other aquatic organisms (Smith, 1987). Other studies have found marine/estuarine species to be more sensitive than fresh water species (Scheier *et al.*, 1979; Bleckmann *et al.*, 1995). *Nereis diversicolor* and *Macoma balthica* have been found to be species that are particularly resistant to the effects of refinery effluent (Cote, 1976; Leppakoski & Lindstrom, 1978).

The conditions of the toxicity tests are also very important. Using sediment within a toxicity experiment has varied effects. Smith (1987) found that during acute toxicity tests the presence of a substrate caused enhanced survival. Whilst Scheier *et al.* (1979) found that the addition of sediment increased the toxicity. The toxicity of the effluent may also change with storage. There was a significant loss in toxicity when the effluent was stored for 24 hours before use in an experiment (Bleckmann *et al.*, 1995). The effects of the two effluents that are discharged from BP Grangemouth on four marine invertebrates have been compared. It was found that the petrochemical effluent was more toxic than the oil refinery effluent (Smith, 1987) This suggests that it is not necessarily the oil, but may be some of the other chemicals in the petrochemical waste that have the greatest toxic effects.

Some studies have tried to identify the relative toxicity of individual components so that the chemical or group of chemicals that cause the toxic effects can be determined. Hall *et al.* (1978) investigated the toxicity of six components of refinery effluents. The order of toxicity was determined starting with the most toxic. No 2 fuel oil > sulphide > ammonia > phenol > chromium > kalinite. Fuel oil was also found to be the most toxic component of an artificial refinery mixture (Buikema *et al.*, 1981). Storey (2000) also observed that ammonia was more toxic than phenol to *Corophium volutator*, whereas oil was found to have no acute toxic effect. Reece & Burks (1985) tried to isolate the fractions of refinery wastewaters that were lethal to *Daphnia magna* using stepwise treatments and toxicity teats. The components which were found to be most toxic were the steam volatile, base neutral, aromatic compounds. Eleven PAHs (Polyaromatic Hydrocarbons) were identified (Table 5) but it was noted that although all these compounds were toxic they must be working in an additive or synergistic manner to produce the toxic effects shown in the experiments. The test conditions also affect the toxicity of the individual components. Changes in the salinity and temperture have been shown to affect the toxicity (Storey, 2000; Hall *et al.*, 1978) Animals from different locations and different genera showed the same effects, but larvae were more sensitive than adults (Hall *et al.*, 1978).

Sublethal effects of effluent components on reproductive success have also been considered. Buikemia *et al.* (1981) looked at the effects of ammonia, phenol, chromate and fuel oil on the reproduction and growth of *M. bahia*. No animals that were exposed to ammonia survived to reproductive maturity. Those animals exposed to phenol, chromate and fuel oil experienced reproductive impairment. Phenol also caused growth inhibition whereas chromate caused the animals to swim in spirals. Changes in behaviour have also been noticed in other studies. During 96 hour tests, zooplankton (*Daphnia magna*) became erratic and uncoordinated in the water column when exposed to *n*-heptane, cyclohexane, benzene, diesel oil, mobil oil and oil refinery effluent (Das & Konar, 1988). Some species of invertebrates have been noted to have certain behavioural responses, limpets have a drop-off reaction, winkles retract into their shells and ragworms are seen to escape from their burrows (Baker, 1976a). There are therefore many ways in which the refinery effluent can effect invertebrates, all of which will have an effect on the population structure of these species and their spatial distributions. Sublethal effects can be very important as changes in behaviour can also lead to mortality (Dicks, 1976b).

1.3.2.3 Fish

Fish have been used for the toxicity testing of oil refinery effluent in many different studies, most of which have looked at sublethal effects. Many different species of fish have been tested over the years (Clemens & Summers, 1953; Irwin, 1965), with much variation within and between species. Pessah *et al.* (1973) found that the toxicity of refinery effluent to rainbow trout (*Salma gairdneri*) and fathead minnows (*Pimephales promelas*) decreased with increasing wastewater treatment. Two experiments have looked at the effects of Haldia refinery effluent on *Tilapia mossambica* using 96 hour toxicity tests. The LC50 (median lethal concentration) value for the refinery effluent was 54%. At 80-100% refinery effluent the fish usually died within 24 hours showing signs of respiratory distress, surfacing and secretion of mucus. (Saha & Konar, 1984a). Saha & Konar (1984b) observed the respiratory and feeding rates of *T. mossambica* exposed to different concentrations of effluent. At 2.1% and 5.84% of refinery effluent there was an increase in respiratory rate but no effect on feeding rate.

Saha & Konar (1984b) used longer 90 day toxicity tests to look at several sublethal effects on *T. mossambica*. None of the fish died over the 90 day experiments. At 2.10% refinery effluent the fish yield was significantly reduced, the fish showed signs of respiratory distress and hampered growth. At 0.58% and 5.84% refinery effluent the maturity index for females varied significantly from the controls. Fecundity of the fish in contact to refinery effluent was discovered to decrease but not significantly. Rowe *et al.* (1983a) also found that fecundity was affected by refinery wastewater in the flagfish, *Jordanella floridae*. They also showed that the 1st and 2nd generations were smaller and that spinal curvature was present in the 2nd generation and all fish showed haemorrhaging of the fins. Rainbow trout (*Salmo gairdneri*) have been observed to have erosion of the caudal fins when in contact with 31% refinery effluent (Rowe *et al.* 1983b). The growth of rainbow trout in 30% effluent is severely reduced and is still reduced at 10% refinery effluent. Stubblefield (1989) looked at the effects of pre exposure to refinery effluent on rainbow trout. There was no increase in tolerance, in fact pre-exposure caused the fish to become more sensitive to the effluent at lethal concentrations. Graham & Dorris (1968) observed the behavioural effects of refinery effluent on fathead minnows. When in contact with the effluent the fish showed signs of distress, they did not school and had a sluggish or nil response to disturbance. Erratic swimming, darkening of the integument, paralytic spasms and periods of immobility indicated severe stress, after which death usually followed within a few hours.

The impact of the components of refinery wastes on fish has been determined by Pickering & Henderson (1966) who recorded the acute toxicity of several petrochemical compounds to four species of fish. Of the compounds that were tested O-chlorophenol and O-cresol were the most toxic and methyl methacrylate and isoprene were the least toxic. Three of the petrochemical toxicities were affected by water quality. Soft water increased the toxicity of methyl methacrylate, styrene and vinyl acetate. Tests using fathead minnow fry and adults showed that the fry were more tolerant to methyl acethacrylate and less tolerant to vinyl acetate than the adults. Stubblefield (1989) used rainbow trout to determine the effects of acclimation on the toxicity of Zinc, Cadmium and Phenol. With both the heavy metals, an increase in tolerance and resistance after pre exposure was seen in adults and juveniles. The adults were more sensitive to the toxic effects of the heavy

metals than the juveniles. However there was no change in the tolerance of the fish to phenol with pre-exposure.

It can be seen that different species of fish just like invertebrates have different sensitivity to refinery effluents and to the different components. Many are killed or show sublethal effects when in contact with refinery effluents. Fish however are more able to avoid refinery effluents by swimming away, whereas sessile invertebrates can not do this.

1.3.3 Field surveys

Many ecological monitoring programmes have been undertaken in areas near to oil refineries to assess the impact they have on the environment. The majority of the surveys have looked at the impact on the estuarine or marine environment especially refineries that discharge onto intertidal areas. Most of these intertidal areas are mudflats or soft bottomed sandy areas although rocky shores and saltmarshes are also involved. The community that is studied in these surveys is commonly that of the macrobenthos, as it is relatively easy to sample.

1.3.3.1 The effect on the environment

The areas around oil refinery outfalls all show a similar response to the refinery effluent, whether it is a rocky shore, soft sediment or the water column. The area around the discharge is often found to have a low diversity and abundance of fauna due to the inability of many species to survive in such close proximity to the effluent (Wharfe, 1975; Petpiroon & Dicks, 1982; Saha & Konar, 1984a; Talsi, 1987; Dicks & Levell, 1989; Panigrahi & Konar, 1989; Gibbons, 1991). In some cases the area adjacent to the outfall can be completely absent of any fauna, such as in the Hooghly Estuary, India (Saha & Konar, 1984a), or the Rio Ojailen tributary, Spain (Meynell, 1973). Often the impacted area is limited to a specific distance from the discharge point. This distance varies depending on the site and the effluent type. In Milford Haven the impacted area was limited to 200 metres from the outfall (Petiproon & Dicks, 1982), whereas in the Hoogly Estuary it extended to 700m (Saha & Konar, 1984a). Wharfe (1975) noted that the impacted area in the Medway

Estuary was limited to an area of 1.5 km around the outfall. In Southampton Water two distinct groups could be defined based on the level of impact. Group 1, the area of gross pollution, included the stations around the discharge that had elevated hydrocarbon and trace metals. This group was dominated by the polychaetes *Hediste diversicolor*, *Capitella capitata*, *Polydora sp.* Group 2 which was situated above and below the affected zone had more diverse fauna. The larvae of the species that were found only in group 2 were not able to survive settlement at group 1 sites, possibly due to a toxicity effect of the sediment in that area (Houston *et al.*, 1983). Effects on the flora have also been seen. In both the Medway Estuary and Milford Haven, algal growth has been seen to increase near the effluent, algae are notably abundant around the outfalls in these areas (Baker, 1976b; Petipiroon & Dicks, 1982).

Often oil is thought to be the main component of the effluent to cause the adverse effects as it is thought to be toxic. However, some field studies suggest that it may be other components within the effluent that could be causing the effects. Wharfe (1975) found that the species richness was negatively correlated with the oil concentration of the sediment but *Nereis diversicolor* was present in areas contaminated with oil. Therefore it was concluded that oil alone could not be responsible for the effects seen in the area around BP Colemouth Creek. Also the oil content of the refinery effluent at Milford Haven was reduced but no reduction in the area of impact was seen. It was considered that the low salinity of the effluent might be an important factor causing the impact to the area rather than the oil itself (Petiroon & Dicks, 1982).

Field studies have shown that although oil refinery effluents have varying compositions they all tend to have a similar impact on the community, which decreases with distance from the source. It is not known what components of the effluents are the major causes of the effects or if in fact it is just the salinity of the effluent.

1.3.3.2 Recovery

It can be seen that if the toxicity of an effluent is reduced or the effluent is stopped completely, the area of impact is able to recover. The time it takes for the area to recover varies and depends on the area and the type of organisms involved. In Porvoo, Finland, the subtidal area was monitored to observe the effects of the addition of a new treatment plant to the refinery in 1973 (Leppakoski & Lindstrom, 1978). An improvement in the macrofauna was seen in 1974 and 1975 with an increase in the number of species and diversity. The species that were found to recolonise most successfully included the amphipods *Pontoporeia affinis* and *Corophium volutator*, the oligochaete *Tubifex costatus*, the polychaetes *Harmathoe sarsi* and *Polydora redeki* and the bivalve *Cerastoderma edule*.

The improvement in the quality of the effluent at Southampton Water in 1971 produced a dramatic improvement in the condition of the nearby saltmarsh (Dicks & Levell, 1989). Between 1972 and 1974 the beginnings of recolonisation of the denuded area of the saltmarsh could be seen (Dicks, 1976a) and by 1981 much of the impacted area had been recolonised by the main plant species in the area (Dicks & Levell, 1989). Baker (1971) deduced that the reason for the death of *Spartina* and the appearance of bare patches of mud was repeated light oiling of the *Spartina* shoots. The oil content of the soil, the pH of the water and soil, and the sulphide concentration and temperature of the effluent, did not seem to have an effect and *Spartina* was found to grow in jars of outfall water and pots of soil from the denuded area.

The refinery at Milford Haven closed in March 1983 and monitoring of the rocky shore area was carried out to see if there was any change (Dicks & Levell, 1989). The year 1984 saw increased recruitment of juvenile limpets, *Patella vulgata*, along the shore but especially near the outfall. During the following years further recruitment was noted but the average limpet became smaller and occurred at increased densities. The barnacle population showed a different pattern. In 1984 there was an increase in the numbers of juvenile and adult barnacles but not near the outfall where there were fewer still. In 1985 a distinct gradient of density could be seen with increased densities going away from the outfall, however in 1986 this

gradient was less pronounced and only one station near to the old outfall had reduced numbers of barnacles. Therefore it was concluded that the effluent had been the main factor causing the exclusion of limpet and barnacles from the area around the outfall.

1.3 FORTH ESTUARY

The Forth estuary (Figure 1.2) is approximately 48 km in length from Stirling in the west to Queensferry in the east (McLusky, 1987). The estuary varies between the partially mixed state and the well-mixed state, this is dependent on the freshwater flow, tidal range and tidal state (Webb & Metcalfe, 1987). From Grangemouth and further upstream there is a double high and low tide. The estuary and the Firth of Forth receives domestic and industrial aqueous wastes from around a quarter of the population of Scotland (Leatherland, 1987). There is therefore the potential for undesirable impacts to the estuarine environment if the discharges are not monitored and controlled.

The main inputs into the estuary have been from sewage works and a brewery and distillery in the Alloa area, heated cooling water from Kincardine and Longannet power stations and petro-chemical effluent from the Grangemouth petrochemical complex (McLusky, 1982a). The Forth has also been subjected to industrial discharges containing mercury and other heavy metals, including chromium and cadmium (Davies, 1987).

Certain areas within the Forth are designated Sites of Special Scientific Interest (SSSI) for their botanical, ornithological and geological attributes (Leatherland, 1987). Not only is the estuary an important area for wintering birds (Bryant, 1987) but it also sustains a fishery and has a recreational value. It is also important for shipping with a major port at Grangemouth (Letherland, 1987).

The hydrodynamics of the estuary cause the deposition of sediments in the shallow waters, mostly in the upper estuary between Stirling and Grangemouth (Clarke & Elliott, 1998), this causing the development of extensive mudflats. The intertidal areas of the Forth estuary are approximately 22.6 km^2, of which Kinneil (See 1.6),

Skinflats and Torry Bay are the largest (McLusky, 1982a). These habitats are dominated by fine-grained muds which have a salinity range of 0-32 psu across the length of the estuary (McLusky, 1987). Cranthorne *et al.* (1989) studied the hydrocarbon content in the sediments of the Forth and found that the majority were aliphatic and aromatic hydrocarbons which is indicative of a petrogenic source. Davies (1987) also found that the intertidal sediments contained heavy metal contaminants, with the upper zone of sediment (0-20 cm) having higher concentrations of these metals than the deeper zone (30-50 cm). The gradient within the sediment can be seen along the estuary with sediments at Lonagannet being relatively non-polluted, to the sediments at Grangemouth being heavily polluted (Ajayi & Poxton, 1987). Land reclamation has substantially reduced the size of the intertidal area of the estuary by around 50% over the last 200 years. This has had an impact on the invertebrate biomass and production, which has shown a decrease of around 55% during this period (McLusky *et al.*, 1992). This will have had an effect on the fishery and the bird populations, which feed on the invertebrates, within this area.

McLusky (1987) examined the intertidal habitats of the Forth estuary. The fauna of the upper estuary (Stirling to Alloa) was nearly exclusively oligochaete worms, which were very abundant due to the organic enrichment from domestic waste in this area. The middle estuary (Alloa to Bo'ness) had the greatest biomass and production of macrobenthos for the whole estuary. The lower estuary, (Bo'ness to Queensferry) had a lower biomass and abundance of macrofauna but had the highest diversity. The production / biomass ratio of the Forth in general is lower than that found for similar estuaries, and it thought to be due to pollution rather than natural environmental factors (McLusky, 1987).

1.5 BP GRANGEMOUTH

The petrochemical complex at Grangemouth is one of BPs largest industrial sites with coverage of 700 hectares. The complex is made up of an oil refinery and a petrochemical plant. The oil refinery started its operations in 1924 with a throughput of 360,000 tons per year, now the refinery has an annual capacity of 10 million tonnes (BP, 2001). The oil mainly comes from the North Sea, through the

Forties pipeline system, direct to Grangemouth. Three processes take place at the oil refinery, distillation, conversion and upgrading and purification, this produces seven main commercial products, which are then exported (BP, 2001).

The petrochemical plant was commissioned in 1951 and was the first in Europe. The plant uses two basic feedstocks, gases and light distillate and produces over 1.8 million tonnes of petrochemical products each year (BP, 2001). The main process within the site is the cracking of the feedstocks to provide alkenes, this is done within the ethylene crackers. The alkenes are then used in other processes on site, which convert them into many different commercial products (BP, 2001).

1.5.1 Aqueous wastes

Both the refinery and the petrochemical plant produce aqueous wastes that are discharged separately from two outfalls onto the Kinneil mudflat. Figure 1.3 shows the location of the outfalls. The refinery outfall (C) has always been situated in the same position but the chemicals outfall has moved position twice over the years in bids to reduce its impact. Firstly it was moved in February 1979 from its original position (A) to a position (B) further away from the river Avon. This was to help the cleaning up of the Avon that was being undertaken at this time. It was moved again in January 1999 this time to a lower shore site (D), so that the effluent would be further diluted and would as a result have a reduced impact. The refinery effluent is actually made up of two separate effluents, the refinery process water and ballast water, which are discharged together.

Over the years BP has attempted to reduce the toxicity and volume of both effluents. The toxicity of the chemicals effluent has gradually been reduced by the closing down of old plants and the opening of more efficient newer plants. In 1985 the phenol plant was closed which reduced the phenol emissions from 58 t yr^{-1} to 7 t yr^{-1} the following year. In 1990 the acrylonitrile plant was closed which also reduced aqueous emissions of acetonitrile, acrylonitrile, ammonia and cyanide compounds (McLusky & Martins, 1998). The refinery effluent has been improved by the addition of a biological treatment plant in 1994. This substantially reduced the toxicity of this effluent.

1.6 KINNEIL

Kinneil is the largest of the three main mudflats on the Forth estuary at 5.71 km^2 at low water on neap tides and 6.42 km^2 at spring tides (McLusky, 1982a). The median particle diameter of the sediment is around 0.034 mm (McLusky, 1987) and 85% is in the silt or clay fraction (McLusky & McCroy, 1989). The water temperature at Grangemouth varies seasonally with temperatures around 17°C in July to 4°C in January (Webb & Metcalfe, 1987). Davies (1987) analysed the sediment for the metal contaminant concentrations and found that they were higher in the south-western corner and decreased outwards from there with a slight increase at the edge of the tidal flat. The benthic macrofauna at Kinneil are mainly species that are often associated with organically enriched sediments (Pearson & Rosenberg, 1978) and the majority are considered to be opportunistic. The community is comprised of around 18 species including polychaetes, oligochaetes, molluscs and crustaceans.

Kinneil has several sources of pollution as well as the effluents from BPs petrochemical complex. The Kinneil sewage works discharges treated domestic effluent onto the intertidal area and contaminants are also able to leach out of a refuse site. The rivers in the area are also a source of contaminated water. The river Avon which cuts directly across Kinneil being the largest, with the smaller Grange burn at the western end. The river Carron enters the estuary west of Grangemouth Docks, which forms the western boundary of Kinneil.

1.7 AIMS

The Kinneil mudflat benthic community has been studied to try to determine what impact if any the petrochemicals complex at Grangemouth has had on this community. The hypotheses tested in the present study are: -

- There has been an increase in diversity and species richness of the benthic macroinvertebrates at Kinneil, over the period 1976 to 1999, which has been caused by changes in the refinery and/or chemical effluents.

- Different areas of the mudflat have shown different changes in the community composition, over the period 1976 to 1999.
- The movement of the chemical outfall in January 1999, to a lower shore site, did not cause a change in the abundance or biomass of the community, or the sediment properties, around the new lower shore outfall or the old upper shore outfall.
- There is no difference in the size of the organisms found at the upper shore and lower shore sites.

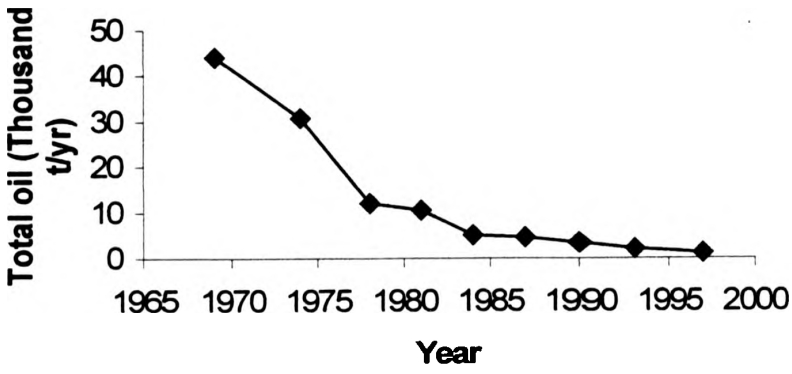


Figure 1.1. Total oil content of the effluent from refineries in Europe (From Concawe, 1998).

Table 1.1. Aqueous effluent discharge data for European refineries from 1969 to 1997 (From Concawe, 1998).

Year of survey	1969	1974	1978	1981	1984	1987	1990	1993	1997
Number of refineries reporting these data	80	108	111	104	85	89	95	95	105
Total aqueous effluent (10^6t/yr)	3119	3460	2938	2395	1934	1750	1782	2670	2942
Aqueous effluent (t/t capacity)	8	4.9	3.9	3.4	3.2	3	3	4.3	4.4
Aqueous effluent (t/t oil processed)	nd	nd	5.4	5.4	4.6	3.9	3.5	4.8	4.7

Table 1.2 Classification of refineries (From Concawe, 1998).

Type I	Simple (non-conversion) refinery: composed of crude oil distillation; reforming; treatment of distillate products, including desulphurisation and/or other quality improvement processes (i.e. isomerisation or speciality manufacturing).
Type II	Type I plus catalytic cracking and/or thermal cracking and/or hydrocracking.
Type III	Type II plus steam cracking and/or lubricant production within the refinery fence.
Type IV	Refineries not in above categories, e.g. those producing only bitumen, lubes, etc. Which import their feedstocks from other sources.

Table 1.3 Waste water treatment systems in oil refineries in Europe (From Concawe, 1998).

Year of survey	Number of refineries reporting these data	Refineries equipped with:					
		Gravity separation		Gravity separation and advanced treatment		Gravity separation, advanced treatment and biological treatment	
		No.	%	No.	%	No.	%
1969	82	51	62	12	15	19	23
1974	112	47	42	21	19	44	39
1978	109	40	37	15	14	54	49
1981	105	31	30	19	18	55	52
1984	85	15	18	8	9	62	73
1987	89	13	15	10	11	66	74
1990	95	7	7	12	13	76	80
1993	95	6	6	8	8	81	85
1997	105	6	6	8	8	92	88

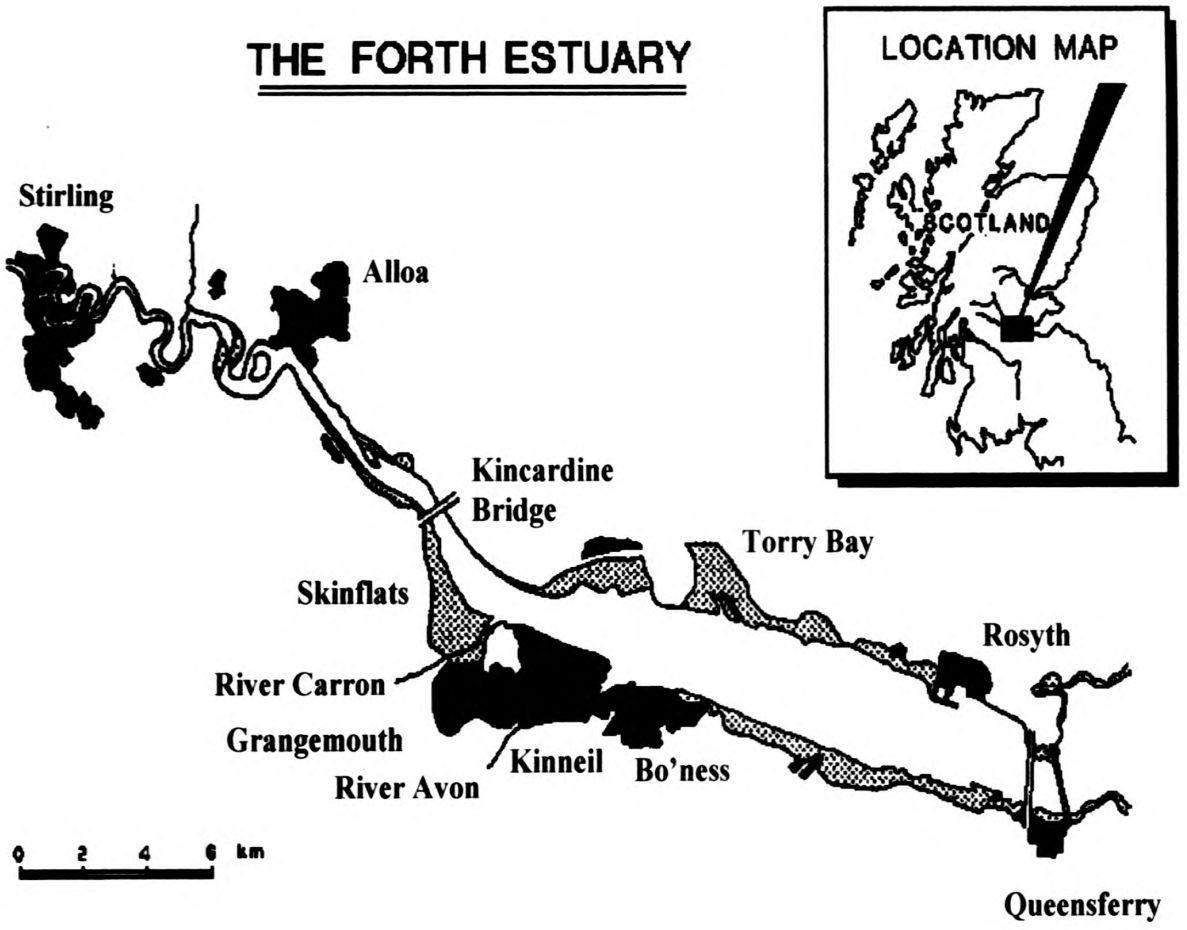


Figure 1.2. Map showing the location of the Forth Estuary. Black – Towns, Shaded – Intertidal mudflats, Blue – Kinneil.

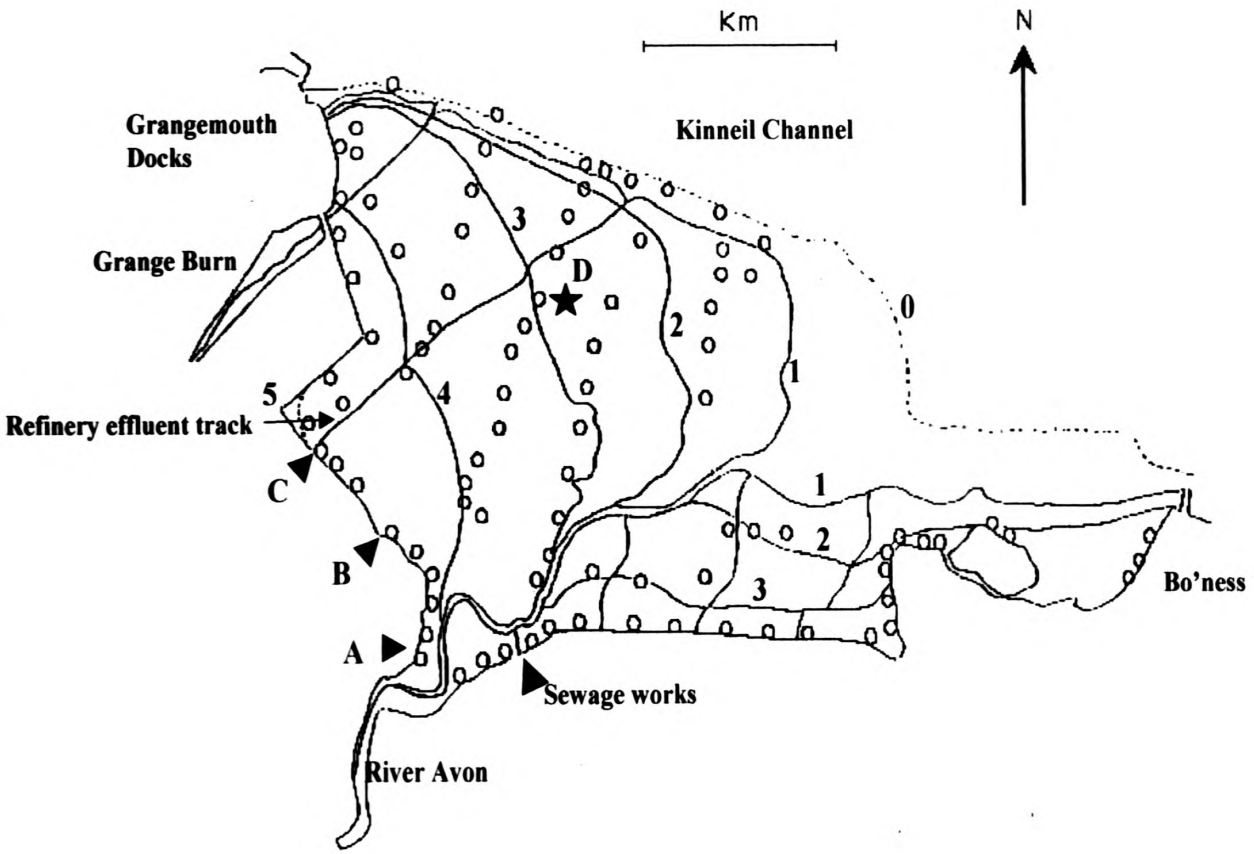


Figure 1.3. Map showing the sites of the effluents outfalls. A – Chemicals outfall until 1979 (Site 1), B – Chemicals outfall 1979-1999 (Site2), C – Refinery + Ballast water outfall, D – Offshore chemicals outfall from 1999 (Site 3) and the height contours from 0-5.

2. METHODS

2.1 SAMPLING METHODS

The methods for the collection of the samples and their subsequent analysis for both the long term and field survey data are outlined.

2.1.1 Long term data

The Kinneil mudflat has been surveyed yearly since 1976 (McLusky, 1976). Every year during July/August samples were collected along a series of parallel transects (A-J) (Figure 2.1). The transects are approximately half a kilometre apart and the stations are at approximately 100 metre intervals. There are 90 stations although in some years certain stations were not sampled. From 1976 till 1995 the stations were located using compass bearings from conspicuous objects, mainly navigation markers and land features. In 1996 the stations were located using the same method but the GPS fixes were noted for each station (Table 2.1) (McLusky, 1996). The GPS fixes were then used to locate the stations in the following years.

The upper shore stations were sampled by walking out onto to the mud. From 1976 till 1982 two 5 x 5 x 5cm cores and one 10 x 10 x 10 cm core were taken at each station (McLusky, 1976; McLusky, 1982b). The lower shore stations were sampled from a boat using a hand-held Van Veen grab. The grab collected a sample 10 x 10cm in surface area and to a depth of 8cm. The three cores were then taken by sub-sampling the grab samples. The larger cores were used to assess the abundance of *Macoma balthica* and *Cerastoderma edule* and the smaller cores were used to assess the abundance of all other species. From 1983 onwards (McLusky, 1983) only two 5 x 5 x 5 cm cores were taken at each station, which were used to assess the abundance of all species. Each year the individual samples were placed into randomly labelled plastic bags.

In the laboratory each sample was sieved using a 250μm sieve. The residue was placed into a labelled petri dish with 4% Formalin stained with Rose Bengal stain.

The samples were then examined under a binocular microscope. The macroinvertebrates were identified and counted. All the macroinvertebrates were identified to species level except for the Oligochaetes, which were grouped together as Oligochaetes.

2.1.2 Field survey sampling

The 13 upper shore stations and 14 lower shore stations around the new chemicals outfall were sampled four times a year in November, February/March, May and July, from November 1998 to November 2000 (Table 2.2). The stations were located using a hand-held GPS compass using Global Positioning Satellite fixes (Table 2.3). The upper shore stations and lower shore stations were sampled as in the long-term data, except that three 5 x 5 x 5 cm cores were taken. The same stations and methods as the long-term data were used, so that the results would be able to be compared. This would be useful as the stations could only be sampled once before the movement of the outfall. The extra core was taken so that sediment analysis could be undertaken which was not done in the long-term monitoring.

2.1.2.1 Faunal analysis

The same method of sorting and fixation were used as in the long-term data, again for comparability. The macroinvertebrates were identified and counted, except this time the Oligochaetes were also identified to species level. This was previously not done in the long-term monitoring because of time restrictions. It is known that several species make up the oligochaetes at Kinneil (Bagheri & McLusky, 1982) which have different life cycles and abundances. The movement of the outfall could potentially effect the spatial distributions and temporal abundance of these species, which would not be detected if they were not identified to species level. The oligochaetes were subsampled for identification to species level. This would allow a measure of the abundance of the individual species without overly increasing the time spent on identification. Ten percent of the total number of Oligochaetes, or at least 10 were randomly removed from a sample and placed on a microscope slide. A drop of Lactophenol was placed on top of the specimens followed by a coverslip.

The slides were left for at least 24 hours and then examined under a compound microscope. The rest of the sample was preserved in 70% ethanol.

The sizes of *Nereis diversicolor*, *Nephtys hombergii*, *Eteone longa*, *Macoma balthica*, *Cerastoderma edule* and *Hydrobia ulvae* were measured. For *N. diversicolor*, *N. hombergii* and *E. longa* the body width was measured shoulder to shoulder as the widest part of the body, excluding parapodia (Olive, 1977), and for those specimens that were intact the length was also measured (Figure 2.2). A regression analysis was then used to calculate an estimate of the length for the remaining specimens (See 7.2.1). Both the shell length and height was measured for *M. balthica* and *C. edule* and the shell height for *H. ulvae* (Figure 2.2).

The biomass for each species was determined using the preserved samples. A number of individual animals were used to determine the ash-free dry weight *of N. diversicolor* (20 individuals), *N. hombergii* (20 individuals), *E. longa* (5 individuals), *M. balthica* (30 individuals), *C. edule* (20 individuals), *H. ulvae* (30 individuals), *Corophium volutator* (10 individuals), and *Mytilus edulis* (15 individuals). Known sized animals were used for the first six species. For the smaller species individual animals were grouped together. Ten individuals were grouped together for Oligochaetes, *Streblospio shrubsolii* and *Pygospio elegans* with 3, 3 and 2 replicates respectively, and 20 individuals with 3 replicates for *Manayunkia aestuarina*. The biomass of the species at Kinneil was previously measured by Yule (1996). The same method was used in this study so that the results could be compared. The animals were put into crucibles and the samples were placed in a drying oven at 60°C for 24 hours. The samples were then weighed on a five-figure balance and put into a furnace at 450°C for 6 hours. They were then re-weighed and the ash-free dry weight was calculated.

2.1.2.2 Sediment analysis

One sample from each station was used for analysis of organic matter content and sediment particle size. Many different methods are used for the measurement of organic matter content. Parker (1983) notes that the different methods including loss

on ignition and hydrogen peroxide, all provide adequate assessments of the content of sedimentary organic matter, although they can not be compared. As the samples were not being compared with any others only themselves, the simpler loss on ignition method (Luczak *et al.*, 1997) was chosen. The sediment samples were put into foil containers and placed in a drying oven at 105°C for 24 hours. They were then removed from the oven and the individual sediment particles were separated using a rubber pestle and mortar. A weighed amount of the sediment was placed in a crucible and placed into the furnace at 500°C for 6 hours and subsequently weighed again to calculate the loss of organic matter. For the particle size analysis the same sample, that had undergone the combustion process, was used. The individual particles of the sample were separated again as before. These samples were then put through a 250μm sieve and the fraction >250 μm was weighed. The <250 μm fraction was placed in a container with water and 10ml of Calgon. The solution was then mixed using a magnetic stirrer and analysed for particle size distribution using a Coulter Counter.

2.1.2.3 Hydrocarbon analysis

An additional sediment sample was collected at each station in May 2000. Fourteen of the sediment samples were sent of to M-Scan to be analysed for their hydrocarbon content (See appendix 1 for methods). Not all of the 27 stations could be analysed due to budget restrictions therefore a sub set of stations was chosen. The stations closest and furthest from the potential hydrocarbon source, the refinery outfall and track, along each transect were chosen so that any differences would be detected. The stations that were sampled for their sediment hydrocarbon content were A1, A2b, A3, A4, B2, C2, C5, B5, B7, C13, C16, C18, D11 and D14.

2.2 ENVIRONMENTAL DATA COLLECTION

Data on the changing environmental conditions and the different pollution sources in the Kinneil area have been collected from a variety of sources. Data for both the long-term analysis and the survey analysis were obtained.

2.2.3 Long term data

Changes in climate are known to effect the survival of some species, especially winter temperatures (De Jong *et al.*, 1999). Two measures of the climate are used to see if they are important in explaining the changes in the community composition, the local air temperature (Stirling) and the North Atlantic Oscilation (NAO). The NAO is the difference between the normalised pressure anomalies in winter in the Azores and in Iceland. A NAO index >1 indicates a low pressure field over the North Sea, strong westerly winds, warm temperatures and high precipitation, high river run-off and high nutrient input to coastal areas, whilst a value <1 indicates the opposite (De Jong *et al.*, 1999). Therefore the NAO may influence the community structure in areas in and close to the North Sea.

The maximum and minimum air temperature for each month was acquired from Stirling University's environmental science department, which measures air temperature within the University grounds. The maximum and minimum value for each year was determined. A year was considered to be the time in-between sampling trips (Table 2.5), which was usually August to August and not January to December.

Monthly averages of the North Atlantic Oscillation (NAO) were gained from the web site http://www.cpc.ncep.noaa.gov/data/teledoc/nao.html and were again averaged for each year (August to August).

Pollution may also effect the distribution of species both spatial and temporally (See section 1.2). The main potential sources of pollution at Kinneil are the two effluents (Mohd-Long, 1987; McLusky & Martins, 1998), the two rivers (Latimer & Quinn, 1998), the Kinneil channel (Griffiths, 1997) and the Kinneil sewage works (Dauer & Conner, 1980; Moore & Rodger, 1991; Simboura *et al.*, 1995). Water quality data was obtained from the Scottish Environmental Protection Agency (SEPA) from sampling stations situated outside the Kinneil sewage works, at Jinkaboot bridge over the river Avon, in Grange Burn and in the main channel of the Forth Estuary just of the Kinneil mudflats (Figure 2.3). The sampling stations measured a selection of water quality variables, which differ for the four different areas, these

can be seen in Table 2.4. Effluent data were acquired from BP Grangemouth on the composition of the refinery, chemicals and ballast water effluents. Again different aspects of the three effluents were measured as can be seen in Table 2.4. The water quality and effluent data were, like the climate data averaged for each year.

Relevant information could not always be gained for all 24 years (Figure 2.4). Additional data for the chemical effluent and River Avon were obtained from McLusky & Martins (1998) for the period 1976 to 1997. The missing 1998 and 1999 values were added and this was used as an additional data set that was named Chemical/Avon. This data set included the biological oxygen demand (BOD) for the chemicals effluent and the BOD, suspended solids (SS) and ammonia concentrations for the River Avon.

2.2.2 Survey data

The environmental data used for the spatial analysis can be seen in Table 2.6. Water quality data for the Avon, Kinneil channel and sewage works, along with the Temperature and NAO data and the chemical and refinery effluent data were collected for the period July 1998 to November 2000 from the same sources as the long-term data (Table 2.7). The ballast water data could not be used due to the lack of data for long time periods during the two year period and the Grange burn data was not available. This time an average for the months between each sampling event (see Table 2.2) was obtained, starting in August 1998 for the November 1998 sample. The upper shore and lower shore environmental data were calculated separately as they have slightly different sampling times.

The salinity of the surface water at the shore stations was also measured using a refactometer. The salinity was only measured during two of the sampling times in February 1999 and July 1999. This data was used in the spatial analysis.

2.3 STATISTICAL METHODS

The manipulation of the raw long term and survey data is described and the statistical techniques used in the analysis of the data are explained. For both the

long-term and the survey data univariate and multivariate techniques have been used. The two techniques could potentially give similar results and therefore corroborate any findings. However they could also potentially provide different information which will aid interpretation (Gray *et al.*, 1988; Clarke & Warwick, 1994).

2.3.1 Long term data

The abundance of each species, at each station, for each year was obtained by aggregation of the two 5 x 5 x 5cm cores. As larger 10 x 10 x 10 cm cores were used before 1983, a conversion factor that was derived by McLusky (1983) by duplicate sampling was used, which adjusted the abundance value for the different core size. The data was then used in the different multivariate analyses. The Shannon-Wiener diversity index, evenness, richness and number of individuals per station (See 2.3.3) were also calculated for each station, for each year.

Firstly the changes in diversity and species composition for the whole areas were analysed. The means for each species for all 90 stations and the mean diversity for all stations for each year were calculated. The data were then used in the mean analysis (See Chapter 3). To examine different areas of the mudflat the stations were sub-divided using Cluster analysis (See 4.2.1) on the randomly chosen year 1994, which created 5 groups (See 4.2.1). It is recognised that the choice of 1994 is arbitrary, but the purpose was to create groups of stations that could be subjected to further detailed analysis and only one year could be used. The five groups were considered to be too large and encompassed too great an area and range of stations (e.g. low tide and high tide). To produce groups that would be representative of a small area of the mudflat the five groups were further divided into east and west of the Avon and high shore and low shore stations. This created 10 groups (See 4.2.1) which were used in the group analysis (See Chapter 4). The mean of all the stations within each group was calculated and this was used in the subsequent analyses.

2.3.2 Survey data

The abundance data were handled in the same way as for the long-term data (See 2.2.1). The biomass for each species, in each sample, was determined using the ash free dry weights for each species and their abundance per sample. For those species that were measured a linear least squares regression was calculated which was used to determine the biomass for each individual in each sample (See 6.2.1).

2.3.3 Univariate analysis

Univariate methods condense down community data into a single value for each sample, such as a diversity index. Univariate tests are usually straightforward and well known and understood. They also have specific significance tests so that the significance of any result can be tested. In reducing the species matrix down to just a single value there is however the potential for information to be lost. They do however unlike the multivariate methods give an indication of the direction of change, for example whether it is deleterious (Clarke & Warwick, 1994). Many studies on the effects of pollution and organic enrichment have found that univariate measures such as diversity indices are valid methods for detecting disturbance effects (Mucha & Costa, 1999; Estacio *et al.*, 1997).

2.3.3.1 Diversity, evenness, species richness and number of individuals

The Shannon-Wiener diversity index (H') is the most commonly used diversity measure. Its equation can be seen below where p is the proportion of the total count arising from the ith species.

$$H' = - \Sigma_i p_i (\log_2 p_i)$$

This is a measure of both the species richness and the equitability. A low diversity is often an indication of stress on the community (Dauer & Ranasingle, 1992).

Pielou's evenness index (J') is a measure of the equitability of species abundances.

$J' = H'(observed) / H'_{max}$

H'_{max} is the maximum possible diversity if all species were equally abundant. Evenness is therefore a measure of the relative abundance of each species within the community, where J' = 1 then all the species have the same abundance. A low evenness indicates that the community is dominated by one or two species.

The species richness and the number of individuals per station were also calculated. These univariate measures were used in further analyses.

2.3.3.2 Stepwise Multiple Linear Regression

Stepwise multiple regression analysis (MRA) has been used here to compare species diversity, evenness, species richness, number of individuals and total biomass with environmental variables such as the effluent composition and climate changes. A forward multistep procedure is used, with 0.05 as the probability of F to enter. The stepwise MRA is used to identify a set of best predictors from the environmental variables that model the response variable; for example the temporal change in diversity. The statistical validity of the fitted model was tested using F and t tests, whilst diagnostic checks on the residuals were also completed to assess if the model adhered to the inherent assumptions (Gardiner, 1997). The goodness-of-fit of the model is assessed using the coefficient of determination which has been adjusted for the number of variables within the model, Rsq(adj). The higher the Rsq(adj) percentage the greater the fit of the model (Gardiner, 1997). The stepwise regression analysis was performed in MINTAB v13. MRA has been shown to produce useful models that can be used as a tool to evaluate further changes (Gonzalez-Oreja & Saiz-Salinas, 1999).

2.3.3.3 k-dominance curves and ABC plots

K-dominance curves are just graphical plots of cumulative % dominance against species rank. It has been noted that polluted areas are often characterised as being dominated by one or two species. Dominance plots are therefore a way to tell how

impacted a site is. A polluted site will have an elevated k-dominance curve compared to an unpolluted site. By comparing a number of k-dominance curves the relative impact can be determined.

ABC (Abundance/ biomass comparison) plots are k-dominance plots that contain both the species abundance and species biomass curves. By comparing the position of the two curves an assessment of the level of disturbance can be made (Warwick, 1986; Warwick *et al.*, 1987; Clarke & Warwick, 1994). Under stable conditions the dominants of a macrobenthic community are species with a long life span and large body size. When a disturbance occurs the opportunistic species tend to become dominant which are usually short lived, small bodied animals. It is this change in the difference between the abundance and biomass dominance of a community that ABC plots are based on. There are three states that can be recognised by the ABC plots, undisturbed, where the biomass curve will be above the abundance curve. Moderately disturbed, where the abundance and biomass curves will be close and may cross over. Lastly grossly disturbed, where the abundance curve will be above the biomass curve. These three states can all be recognised without the need for a control site as the two curves act as controls against each other. The W-statistic is a measure of the difference between the two curves. The equation used can be seen below.

$$W = \sum_{i=1}^{s} (B_i - A_i)/[50(S-1)]$$

W values that tend to +1 indicate an even abundance across species but biomass dominated by a single species. The opposite is true for values that tend to –1. Although k- dominance and ABC curves have been shown to be useful in indicating disturbance effects (Gray *et al.*, 1988; Anderlini & Wear, 1992; Estacio *et al.*, 1997) there is evidence to suggest that ABC curves may not be as valid in estuarine environments (Beukema, 1988; Dauer *et al.*, 1993; Warwick & Clarke, 1994; Mucha & Costa, 1999). ABC curves have been used in this study to determine if they may be a useful tool in determining the level of disturbance at the study site in subsequent surveys.

2.3.3.4 General linear models (GLMs)

A general linear model (GLM) is the equivalent of a balanced analysis of variance (ANOVA) but it can cope with unbalanced data, such as the size data from the two-year survey (Minitab, 1999). The GLM assesses the level to which each variable explains the variations in the response and tests the significance. This technique has been used for the size data to determine if the site (upper or lower shore) or the sampling time explained the difference in the population size distributions of six species. It should be noted that the data for the individual stations was combined for the two areas due to the lack of data, therefore assuming that there was no variation between stations within site. Within the model both the main effects and the interactions can be considered. The significance of each term within the model is assesses by an F test and the p-value. If $p > 0.05$ then the term is highly significant or if $p < 0.05$ then the term is not significantly and does not explain the variation in the response. The interactions can only be assessed if there is enough data for each term (full rank) which is not always the case for the size data, as some species had no individuals during certain time periods and in these cases only the main effects could be considered. The pairwise comparisons were assessed for the main effects and interaction terms to provide an indication of where exactly the differences occurred. Again this used an F test and p-value where if $p > 0.05$ then the difference was highly significant, if $p > 0.10$ then the difference was slightly significant, if $p < 0.10$ then the difference was not significant. The main effect plot and the interaction plots are graphical representations of the relationships between and within the terms in the model.

2.3.4 Multivariate analysis

Multivariate analyses uses the community data as the basis for the subsequent calculations. Multivariate tests are very powerful and can often pick up more subtle changes that can be lost using univariate techniques (Clarke & Warwick, 1994; Cao *et al.*, 1996; Coimbra *et al.*, 1996). All of the multivariate techniques were performed using PRIMER v5.

2.3.4.1 Similarity/Dissimilarity matrix

The similarity of a pair of samples is expressed using a similarity coefficient. There are several different similarity coefficients that can be used, including the Bray-Curtis, Canberra and Correlation coefficients. The Bray-Curtis coefficient is commonly used in ecological studies. The equation of the similarity between samples *j* and *k* can be seen below (S_{jk}), where *yij* represents the abundance (*y*) of species *i* in sample *j*.

$$S_{jk} = 100\left(1 - \frac{\sum_{i=1}^{p} |yij - yik|}{\sum_{i=1}^{p} (yij + yik)}\right)$$

This coefficient is not affected by joint absences or scale changes in the measurements. It also holds that when two samples have no species in common S = 0 and when they are identical S = 100. It is often necessary to transform the data before using the Bray-Curtis coefficient to downweight the importance of highly abundant species and thereby allow the rarer species to influence the result as it gives more weight to the abundant species than to the rare ones (Field *et al.*, 1982).

The Canberra coefficient automatically adjusts the weighting of each species. This coefficient is similar to the Bray-Curtis and it is also not affected by joint absences. The equation for the similarity can be seen below, where p is the number of species present in one or other sample.

$$S_{jk} = 100\left(1 - p^{-1}\sum_{i=1}^{p} \frac{|yij - yik|}{(yij + yik)}\right)$$

The way this coefficient automatically weights the species by separate scaling constraints for each species means that it has a tendency to cause overdomination by rare species, which is not usually desirable (*Field et al.*, 1982).

The Correlation coefficient is the standard statistical Pearsons correlation coefficient (r). Pearson correlation coefficient produces a value in the range of 1, -1 which is

not that of a similarity coefficient which should be 0, 100, therefore r has to be converted. This coefficient is not often used for community data as this type of data usually has many zero values and the correlation coefficient is dependent on joint absences. Hence, when two samples have zeros for the same species this will increase their similarity. The equation for the correlation coefficient (r_{jk}) can be seen below, where $y_{.j}$ represents the mean count over all species for sample j.

$$R_{jk} = \frac{\Sigma_i (y_{ij} - \overline{y}_{.k})}{\sqrt{[\Sigma_i (y_{ij} - \overline{y}_{.j})^2 . \Sigma\Sigma (y_{ik} - \overline{y}_{.k})^2]}}$$

The equation for converting r into a similarity measure is:

$$S_{jk} = 50\ (1 + r_{jk})$$

A dissimilarity matrix is formed in the same way as the similarity matrix except that a dissimilarity coefficient is used. The dissimilarity coefficient measures how different two samples are. The most commonly used dissimilarity coefficient is Euclidean distance. The Euclidean distance is the distance between two points in space. The equation for this is

$$D_{jk} = \sqrt{[\Sigma_{i=1}^{p} y_{ij} - y_{ik})^2]}$$

This means that each sample in the species matrix is represented by a point in high-dimensional species space, which can be related to all the other samples (Clarke & Warwick, 1994).

For the long-term data analysis the Euclidean distance coefficient was used. Although it is not commonly used for species data it was used because unlike the Bray-Curtis coefficient it gives equal weighting to all species. This is important for the long-term data for several reasons. Not all of the fauna were identified to the same level, the Oligochaetes were not identified down the species level, which

caused this one group to have a high abundance, compared to most other species. The species *Manayunkia aestuarina* also had a very high abundance compared to the other species. Although the remaining species have a low abundance they can not be considered rare species, they are in fact common species which are just found at lower levels. It was therefore considered important that these low abundant species were taken into account and that the two abundant species should not dominate the analysis. The fact that the Bray-Curtis coefficient also does not take into consideration joint absences which is usually considered to be a good thing was not in this case. The area under observation is very small and most species are found over the entire area. This means that joint absences may be important, it may be not what species are found but what species are missing from certain areas that should be considered.

For the survey data the Bray-Curtis coefficient was used. This data was completely identified to species level and therefore the high abundance of some species can be compared at the same level and should be taken into consideration. During the survey several new species were identified which were only occasionally found at a few select sites. These species can therefore be considered as rare species. The Bray-Curtis coefficient unlike like the Euclidean distance will not over estimate the importance of these rare species.

2.3.4.2 Transformations

Before the abundance and biomass data is used in any multivariate analysis a transformation is often needed to obtain the desired weighting of common and rare species. There are many different transformations that can be used, the most common are square root, 4^{th} root and the log transformation. Without any transformation, only the most abundant species normally contribute to the result. The square root transformation allows the intermediate abundance species to play a larger role. The 4^{th} root and log transformations have similar effects and allow the rarer species to be considered (Field *et al.*, 1982). If a log transformation is to be used it has to be log(y+1) as community data often contains high numbers of zero

values. The most severe transformation is to reduce the abundance data to presence/absence.

As the benthic community in the long-term data analysis was composed of relatively few species and the majority of those were at low numbers a 4^{th} root transformation was chosen. This allowed the analysis to take into consideration the "rare" species, as in this instance they were not just chance species, but widespread members of the community with a low abundance. This transformation also helped to decrease the error in the sampling due to the animals living in aggregations, as it plays down the dominant species (Clarke & Warwick, 1994).

For the survey data the less server square root transformation was used. This data unlike the long-term data does include species that can be considered rare species. Therefore the square root transformation was used rather than the 4^{th} root so that these would not influence the results to heavily. This also allowed any error in sampling due to the aggregated abundance to be taken into account.

2.3.4.3 Cluster analysis

Hierarchical cluster analysis is used to group stations with similar community compositions. It is widely used in benthic community studies where pollution is involved (Anderlini & Wear, 1992; Estacio *et al.*, 1997). The stations that have similar taxa with similar distributions are located together in a hierarchical fashion within a dendrogram (Norris & Georges, 1993). This is done by firstly producing a similarity/dissimilarity matrix for each sample (See 2.3.2), which are then compared. If a similarity matrix is used then samples with the highest similarity are fused into groups. If a dissimilarity matrix is used however then the samples which have the lowest dissimilarity are grouped together. The groups are successively grouped into larger and larger groups until they form one large group. There are three different linkage options that can be used, Single, Complete and group-average linkage. Single Linkage uses that minimum distance apart (nearest neighbour) to group the samples. Complete linkage is the opposite and uses the furthest distance apart (furthest neighbour) to group the points and group-average linkage uses the average distance. Single linkage has a tendency to produce chains

of linked samples starting with one large group and then adding on further samples one by one. Complete linkage also produces chains but starting with small clusters. Group-average linkage however usually produces a moderate number of medium clusters which is usually more helpful, this was therefore chosen as the preferred method.

Before the cluster analysis can be undertaken the data often needs to be transformed (See 2.3.4.2) and the most suitable similarity or dissimilarity matrix has to be chosen (See 2.3.4.1). If the results from the cluster analysis are to be used to create an MDS plot (See 2.3.4.4) then the rank similarity or dissimilarity matrix should be used so that the results can be compared.

2.3.4.4 Multi dimentional Scaling (MDS)

MDS is a non-metric ordination technique. Unlike Principal components analysis (PCA) MDS is very flexible in that it can use other dissimilarity measures other than Euclidean distance, like Bray-Curtis, which is used in Chapters 6 and 7. MDS also has better distance preserving properties than PCA, which means that the 2-dimensional ordinations will be more accurate (Warwick *et al.*, 1988). Due to the lack of model assumptions, the ordinations represent the original data well and are usually easy to explain (Clarke & Warwick, 1994). Many studies on marine benthos have used this method and found it to be very useful (Gray *et al.*, 1988; Craig *et al.*, 1993; Estacio *et al.*, 1997; Jackson & Jones, 1999). A similarity or dissimilarity matrix is used to construct a "map" of the relative similarity or dissimilarity of the samples in a specific number of dimensions. The closer the points are on the ordination the more similar they are taken to be. The ordination produces a stress value which is a measure of the distortion of the distance rankings of the ordination from the original similarity or dissimilarity rankings. The stress equation can be seen below:

$$\text{Stress} = \Sigma_j \Sigma_k (d_{jk} - \hat{d}_{jk})^2 / \Sigma_j \Sigma_k d_{jk}^2$$

If there is no distortion then the stress will equal zero but this is rare. The greater the dimension of the plot the lower the stress will be as it becomes harder to accurately

plot the data at lower dimensions. The number of samples in the plot also affects the stress. The greater the number of samples the higher the stress as this also makes it harder to plot accurately. An MDS algorithm is used to find the best ordination of the samples i.e. the plot with the lowest stress value. The stress value of the ordination can therefore be used to assess the usefulness of the ordination (Clarke, 1993). If the stress is <0.05 then the ordination is an excellent representation. If the stress is <0.1 it is a good ordination although the fine structure could need closer examination. If the stress is <0.2 then the ordination is still potentially useful although the specific details should not be relied upon. To check the ordination the cluster results can be superimposed on the MDS plot to test fit. If the stress is >0.3 then the ordination should be discarded and a higher dimensional ordination would probably be more realistic.

2.3.4.5 BIO-ENV

BIO-ENV is a procedure within PRIMER that is a multivariate method of linking community patterns to environmental data (Clarke, 1993; Clarke & Anisworth, 1993) and has been used in marine benthic studies (Estacio et al., 1997; Jackson & Jones, 1999). As with Cluster analysis and MDS it uses similarity/dissimilarity matrices as the basis of the calculation. Before the BIO-ENV procedure can be used the data first have to be analysed to see if any transformation is needed. Draftmans plots (Clarke & Ainsworth, 1993) for all the different environmental variable combinations are examined to see if any are highly skewed, those that are should be transformed to remove skewness. The collinearity of the variables also needs to be tested. Those variables that have correlations of higher than 0.95 should be reduced to a single variable. Then the matrices from the community data and the environmental data can be compared. The community matrix is compared to all the different combinations of the environmental variables and the Spearmans rank correlation coefficient (ρ_s) is calculated for each combination. The equation which is used can be seen below, where N = n(n-1)/2 and s_i and r_i are the values from the two matrices.

$$p_s = 1 - \frac{6}{N(N^2-1)} \sum_{i=1}^{N} (r_i - s_i)^2$$

The correlation coefficient acts as a measure of the agreement of the two matrices, where a value of zero means there is no match between the two and values of 1 or -1 meaning that there is a perfect match. The best subset of environmental variables is therefore the combination that generates the highest p_s value. Only those variables that generate p_s values greater than 0.75 are in this case considered to be important in explaining the changes in community structure.

2.3.4.6 SIMPER

SIMPER is a program within PRIMER that calculates the average dissimilarity (δ) between two groups (Clarke, 1993). This is then broken down into the individual contributions of each species to δ. The equation that is used to calculate this is based on the Bray-Curtis dissimilarity, where two samples j and k are being compared. The ith species $\delta_{jk}(i)$ can be defined as the ith term in an summation equation.

$$\delta_{jk}(i) = 100. | y_{ij} - y_{ik} | / \Sigma_{i=1}^{p} (y_{ij} + y_{ik})$$

$\delta_{jk}(i)$ is then averaged for all pairs of samples to give the average contribution of that species (δi), to the average dissimilarity between the two groups.

It is also possible to determine which species are good discriminating species between the two groups. These are the species that contribute consistently to the average dissimilarity across all pairs of samples. This is calculated using the ratio δi / SD(δi) where SD(δi) is the standard deviation of $\delta_{jk}(i)$. Therefore if a species has a large contribution to the average dissimilarity (δi) and a small standard deviation (SD(δi)) the ratio will be large and the species consistently contributes over all pairs of samples, to the dissimilarity between the two groups and is considered to be a good discriminating species for those groups (Clarke, 1993). SIMPER has been shown to be helpful in identifying the species that are responsible for the grouping of stations (Jackson & Jones, 1999).

2.3.4.7 ANOSIM

ANOSIM stands for the analysis of similarities and is a statistical test found in PRIMER. It is used to determine the differences between sites contrasted with the differences among replicates within sites. The test uses the rank similarities between samples from the similarity matrix. The equation that is used can be seen below where r_w is the average of all rank similarities within sites and r_s is the average of rank similarities between sites.

$$R = (r_s - r_w)/(M/2)$$

Where $M = n(n-1)/2$ and n is the number of samples. R can never be outside the range –1 to 1. R=1 when all replicates within sites are more similar that any replicates from different sites, and R=0 when the null hypothesis is true and therefore the similarities within and between sites are on average the same. An R value below zero is unlikely as this means that the sites are more similar than the replicates within sites (Chapman & Underwood, 1999). A global significance test based on the number of samples is used to determine whether the calculated R value is high enough so that the null hypothesis can be rejected. If $p < 0.1\%$ then the null hypothesis can be reject and a difference between sites can be detected.

2.3.4.8 Index of Multivariate Dispersion (IMD) and relative dispersion values

This test statistic measures the relative variability between two groups such as control and impacted sites. It is based on the ecological fact that the variability among samples from impacted sites is often greater than that from control sites (Clarke & Warwick, 1994). The IMD is calculated by again using the similarity matrix using the equation below, where r_t is the average rank among impacted samples and r_c is the average rank among control samples.

$$IMD = 2(r_t - r_c) / (N_t = N_c)$$

$$N_c = n_c(n_c - 1)/2 \text{ and } N_t = n_t(n_t - 1)/2$$

where n_c and n_t are the number of samples in the control and treatment groups. The IMD will be equal to +1 when all similarities among impacted samples are lower that any similarities among control samples. The opposite is true if IMD is equal to −1. The relative dispersion value is just a measure of the variability within a group and is complementary to the IMD information. The higher the relative dispersion value is the more variability there is within the group. The greater the difference between the relative dispersion values between two groups the larger the IMD value will be (Clarke & Warwick, 1994).

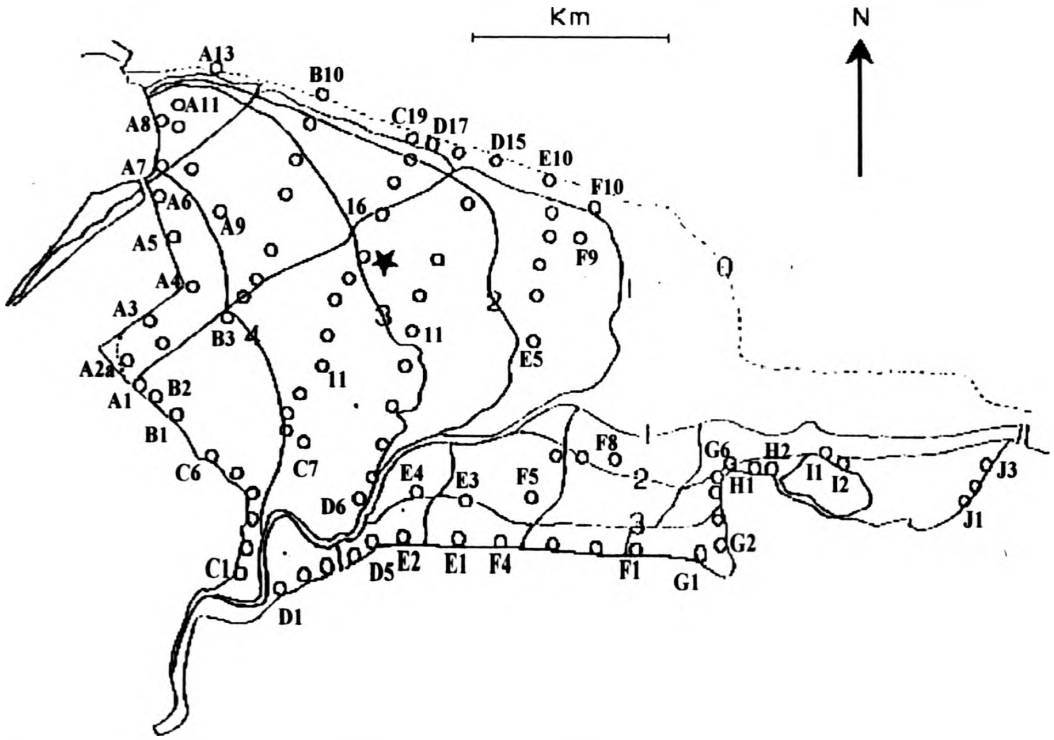


Figure 2.1. Map of Kinneil showing the numbering of the stations along transects A-J.

Table 2.1 GPS fixes for all 90 stations sampled in the long term monitoring survey.

Station	OS Grid East	OS Grid North
A1	29531	68224
A2	29527	68231
A3	29536	68260
A4	29555	68272
A5	29546	68300
A6	29542	68319
A7	29542	68330
A8	29541	68343
A9	29573	68306
A10	29557	68328
A11	29551	68349
A12	29551	68362
A13	29571	68381
B1	29550	68207
B2	29541	68215
B3	29577	68252
B4	29585	68263
B5	29592	68275
B6	29599	68291
B7	29604	68313
B8	29608	68335
B9	29616	68352
B10	29620	68376
C1	29584	68135
C2	29588	68146
C3	29590	68157
C4	29590	68169
C5	29583	68180
C6	29570	68185
C7	29618	68194
C8	29609	68198
C9	29610	68206
C10	29616	68217
C11	29623	68230
C12	29627	68245
C13	29632	68263
C14	29638	68275
C15	29648	68290
C16	29655	68306
C17	29660	68322
C18	29668	68337
C19	29668	68349
D1	29606	68123
D2	29617	68131
D3	29629	68124
D4	29641	68139
D5	29652	68144
D6	29645	68167
D7	29650	68177
D8	29654	68192
D9	29659	68211
D10	29668	68232
D11	29671	68248
D12	29677	68268
D13	29684	68286
D14	29700	68311
D15	29710	68333
D16	29694	68336
D17	29676	68343
E1	29697	68144
E2	29665	68146
E3	29700	68166
E4	29675	68163
E5	29731	68245
E6	29733	68268
E7	29736	68283
E8	29741	68299
E9	29740	68306
E10	29740	68323
F1	29784	68140
F2	29765	68145
F3	29742	68144
F4	29717	68144
F5	29720	68166
F6	29745	68184
F7	29757	68184
F8	29774	68184
G1	29821	68142
G2	29836	68141
G3	29833	68153
G4	29829	68168
G5	29829	68173
G6	29835	68181
H1	29845	68179
H2	29854	68179
I1	29884	68192
I2	29889	68182
J1	29956	68164
J2	29960	68171
J3	29970	68175

Table 2.2. Boat and Shore sampling dates for the field survey.

Boat	Shore
2nd November 1998	10th Novermber 1998
16th March 1999	17th Febuary 1999
12th May 1999	5th May 1999
9th & 12th July 1999	5th July 1999
5th Novermber 1999	9th November 1999
3rd March 2000	22nd Febuary 2000
17th May 2000	5th May 2000
28th July 2000	19th July 2000
9th Novermber 2000	14th November 2000

Table 2.3. GPS fixes for the 27 stations sampled in the field survey.

Station	OS Grid East	OS Grid North
A1	29531	68224
A2a	29527	68231
A2b	29536	68233
A3	29536	68260
A4	29555	68272
B1	29550	68207
B2	29541	68215
B5	29592	68275
B6	29599	68291
B7	29604	68313
C1	29584	68135
C2	29588	68146
C3	29590	68157
C4	29590	68169
C5	29583	68180
C6	29570	68185
C12	29627	68245
C13	29632	68263
C14	29638	68275
C15	29648	68290
C16	29655	68306
C17	29660	68322
C18	29668	68337
D11	29671	68248
D12	29677	68268
D13	29684	68286
D14	29700	68311

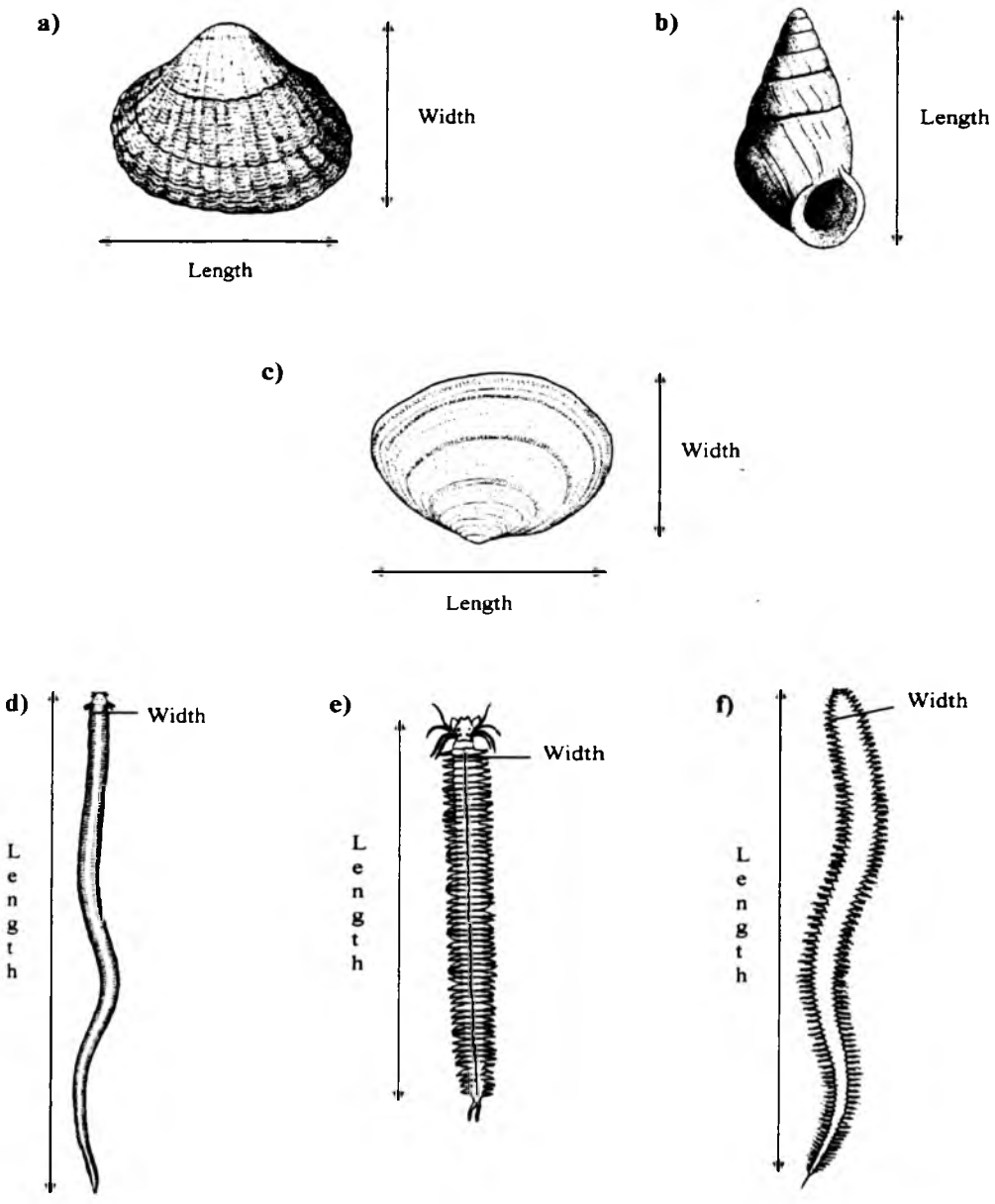


Figure 2.2 The measurements for the size of a) *Cerastoderma edule*, b) *Hydrobia ulvae*, c) *Macoma balthica*, d) *Eteone longa*, e) *Nereis diversicolor* and f) *Nephtys hombergii* (From Day, 1967; Hayward & Ryland, 1990; Barnes *et al.*, 1993).

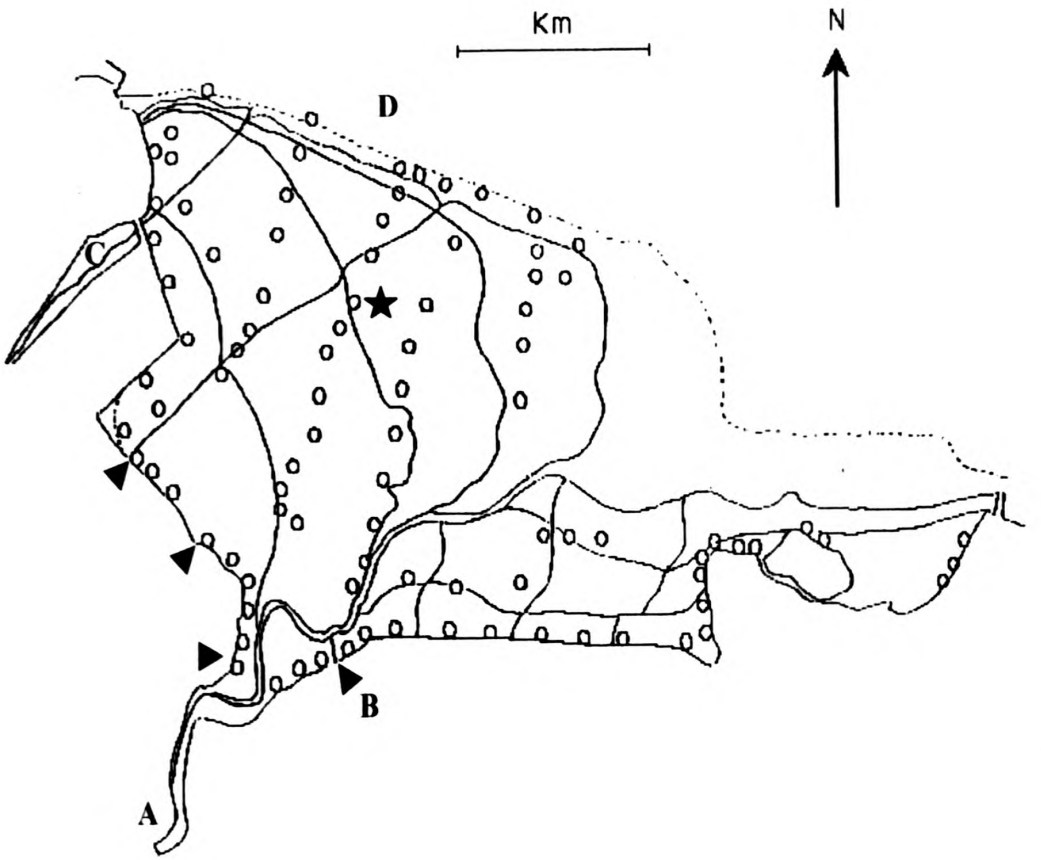


Figure 2.3. Map showing the sampling stations for the water quality data. A – Jinkaboot bridge (Avon), B – Sewage Works, C – Grangeburn, D – Kinneil channel.

Table 2.4. Variables measured in the water quality and effluent data sets for the long-term analysis, where SS – Suspended solids, BOD – Biological oxygen demand, COD – Chemical oxygen demand, EC – Electrical conductivity, TSS – Total suspended solids.

Kinneil Channel	Sewage works	Avon	Grangeburn	Refinery effluent	Chemcial effluent	Ballast water	Chemical/Avon
Temperature °C	BOD+ATU (mg/l)	BOD (mg/l)	BOD (mg/l)	Flow (Ml/day)	Ammonia (mg/l)	Flow (Ml/day)	Avon BOD (mg/l)
Salinity (ppt)	Kjeldahl (mg/ll)	BOD+ATU (mg/l)	DO (mg/l)	Temperature °C	Phenol (mg/l)	pH	Avon SS (mg/l)
Ammonia (mg/l)	Ammonia (mg/l)	COD (mg/l)	EC (us/cm)	pH	Benzene (mg/l)	EC (us/cm)	Avon Ammonia (mg/l)
Phosphate (mg/l)	pH	DO (mg/l)	Ammonia (mg/l)	EC (us/cm)	BOD (tc/d)	TSS (mg/l)	Chemcial BOD (tc/d)
	SS (mg/l)	DOsat (%)	pH	TSS (mg/l)	COD (tc/d)	BOD (mg/l)	
		EC (us/cm)	SS (mg/l)	BOD (mg/l)	Floride (mg/l)	COD (mg/l)	
		Kjeldahl (mg/ll)		COD (mg/l)	Acetonitrile (mg/l)	Ammonia (mg/l)	
		Alkalinty (mg/l)		Ammonia (mg/l)	Sulphide (mg/l)	Sulphide (mg/l)	
		Ammonia (mg/l)		Lead (mg/l)	Hydrocarbon (mg/l)	Phenol (mg/l)	
		Chloride (mg/l)		Chromium (mg/l)	SS (mg/l)	Hydrocarbon (mg/l)	
		Hardness (mg/l)		Copper (mg/l)	Chromium (mg/l)		
		Iron (mg/l)		Nickel (mg/l)	Copper (mg/l)		
		Nitrate (mg/l)		Zinc (mg/l)	Nickel (mg/l)		
		Sulphate (mg/l)		Hydrocarbon (mg/l)	Zinc (mg/l)		
		pH					
		Silicate (mg/l)					
		Phosphate (mg/l)					
		SS (mg/l)					

Table 2.5. Sampling dates for each year for the long-term survey.

Year	Sampling time
1976	August
1977	August
1978	August
1979	3 – 13 August
1980	4 – 26 August
1981	11 – 27 August
1982	3 – 12 August
1983	9 – 24 August
1984	8 – 14 August
1985	22 – 28 August
1986	18 – 22 August
1987	24 – 30 August
1988	8 – 15 August
1989	31 July – 2 August
1990	30 July – 3 August
1991	29 July – 2 August
1992	4 – 13 August
1993	26 July – 5 August
1994	4 – 12 August
1995	1 – 8 August
1996	5 – 13 August
1997	23 June – 1 July
1998	27 July – 5 August
1999	5 – 9 July

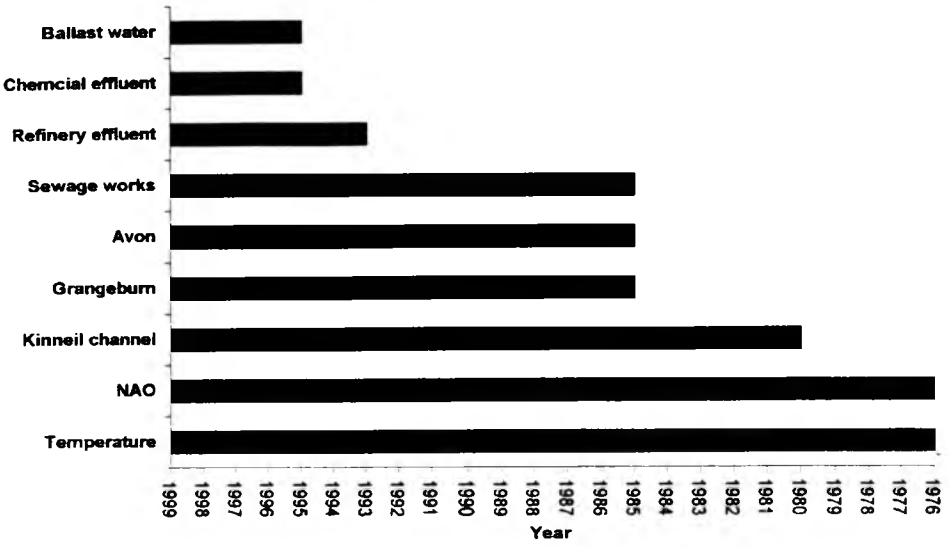


Figure 2.4. The availability of the different sources of water quality, climate and effluent data.

Spatial variables
Distance from refinery outfall
Distance from chemical 2 outfall
Distance from chemical 3 outfall
Distance from Grange Burn
Distance from River Avon
Distance from Kinneil Sewage works
Distance from the refinery track
Distance from the refinery track end
Distance from the mian estuary channel
Shore height
Mean particle size
% Clay
% Silt
% Sand
% Organic matter

Table 2.6. Variables used in the spatial environmental analysis.

Table 2.7. Variables measured in the water quality and effluent data sets for the Survey analysis, where SS – suspended solids, BOD – Biological oxygen demand, COD – Chemical oxygen demand, TSS – Total suspended solids, EC – Electrical conductivity, NAO – North Atlantic oscillation.

Kinneil Channel	Sewage works	Avon	Avon cont...	Refinery effluent	Chemcial effluent	Temperature/NAO
Temperature °C	BOD (mg/l)	BOD (mg/l)	Total P (mg/l)	Flow (Ml/day)	Ammonia (mg/l)	Minimum Temp (°C)
Salinity (ppt)	Kjeldahl (mg/ll)	BOD+ATU (mg/l)	Sulphate (mg/l)	pH	Phenol (mg/l)	Maximum Temp (°C)
Ammonia (µM)	Ammonia (mg/l)	COD (mg/l)	Silicate (mg/l)	EC (us/cm)	Cadmium (ug/l)	Rainfall
Phosphate (µM)	pH	DO (mg/l)	Calcium (mg/l)	TSS (mg/l)	BOD (tc/d)	NAO
pH	SS (mg/l)	DOsat (%)	Magnesium (mg/l)	BOD (mg/l)	COD (tc/d)	
Turbidity (NTUs)	Flow (l/sec)	EC (us/cm)	Iron (mg/l)	COD (mg/l)	Flouride (mg/l)	
Nitrite (µM)	EC (us/cm)	Kjeldahl (mg/ll)	Cadmium (ug/l)	Ammonia (mg/l)	Sulphide (mg/l)	
	TON (mg/l)	Alkalinty (mg/l)	Lead (ug/l)	Lead (ug/l)	Hydrocarbon (mg/l)	
	Phosphate (mg/l)	Ammonia (mg/l)	Chromium (ug/l)	Chromium (ug/l)	TSS (mg/l)	
	Cadmium (ug/l)	Chloride (mg/l)	Copper (ug/l)	Copper (ug/l)	Chromium (ug/l)	
	Lead (ug/l)	Hardness (mg/l)	Nickel (ug/l)	Nickel (ug/l)	Copper (ug/l)	
	Chromium (ug/l)	Flow (l/sec)	Zinc (ug/l)	Zinc (ug/l)	Nickel (ug/l)	
	Copper (ug/l)	pH	Mercury (ug/l)	Hydrocarbon (mg/l)	Zinc (ug/l)	
	Nickel (ug/l)	SS (mg/l)			Flow (ml/day)	
	Zinc (ug/l)	TON (mg/l)			pH	
	Mercury (ug/l)	Nitrate (mg/l)			EC (us/cm)	
		Nitrite (mg/l)				
		Phosphate (mg/l)				

3. LONG TERM DATA – TEMPORAL ANALYSIS

3.1 INTRODUCTION

3.1.1 Temporal effects of refinery effluents

Few studies have looked at the temporal changes that occur in communities affected by refinery effluents. Those that have, are concerned with areas where the refinery effluent has stopped (Dicks & Levell, 1989) or had its toxicity reduced (Dicks, 1976b; Leppakoski & Lindsrom, 1978). These studies have all shown that improving the quality or stopping the effluent usually results in an increase in the diversity and species richness of the area, which is seen as an indication of recovery. Most of these surveys only lasted a few years and therefore may not have shown the full extent of any changes in the community. The Kinneil data set comprises a much longer time series, spanning 24 years, and is therefore better able to demonstrate any long term effects. The Kinneil area is different from most areas that have been studied, in that two different effluents are discharged, the refinery and chemical effluents. The qualities of the two effluents have both changed over the survey period but also the chemical outfall has been moved twice. In addition, there are other possible pollution sources that may affect the area, namely the river Avon and Grange burn, the Kinneil sewage works and the main estuary channel, which have experienced changes in their water quality over the monitoring period.

3.1.2 Previous temporal analysis of the Kinneil long-term data

The Kinneil area has been monitored annually since 1976 to assess the impact of the refinery and chemicals effluents on the benthic community (McLusky & Martins, 1998). The long-term data set, examined in terms of yearly mean abundances for the whole area, have been analysed previously (McLusky & McCrory, 1989; McLusky & Martins, 1998) to determine if there had been any temporal trends in the community structure at Kinneil. This showed that there was an increase in the mean

species diversity, evenness and number of species over time. Increases in the abundance of *Corophium volutator*, *Macoma balthica* and *Eteone longa* were also noticed (McLusky & Martins, 1998). McLusky & McCrory (1989) identified a substantial change in the similarity of the benthic community between 1978 and 1979. It was noted that the mean abundance of organisms at Kinneil was stable between 1976 to 1978 and more variable after 1979. The two studies tried to relate the increase in the diversity to changes in environmental factors. McLusky & McCrory (1989) found that there was no specific linear relationship between temperature and species diversity. Whilst McLusky & Martins (1998) showed that the refinery COD and the BP chemicals score accounted for 83.3% of the change in Shannon-Wiener diversity. Analysis for individual species found that the refinery COD, Chemicals BOD, Chemicals score and Ammonia levels in the Avon accounted for changes in mean abundance at different levels in the different species. Only *Macoma balthica*, *Hydrobia ulvae*, *Eteone longa* and *Cerastoderma edule* had no significant relationship with any of the environmental variables utilised (McLusky & Martins, 1998).

3.1.3 Requirement for further analysis of the annual mean abundance data

Although the annual mean abundance data has been analysed before, there are aspects that have not been explained. The analyses on the species changes within the community were assessed for the early years of the monitoring period. Other than for general trends in changing diversity and evenness, the years after 1986 have not been examined in detail. There are a number of different multivariate techniques that can be applied to the data such as MDS and SIMPLER (See 2.3.4) that have not been utilised in previous studies. These may add to the existing knowledge on the changes in the community structure of this area. An attempt has also been made to relate any community changes to environmental factors. Only limited environmental data have been used in previous studies. The impact from pollution sources other than the petrochemicals complex, such as the sewage works, the rivers and the individual chemical components of the effluents has not been considered in detail before. In previous studies linear regression analysis of univariate measures such as species diversity were used to identify possible influential environmental factors.

There are however other multivariate techniques that take into consideration the whole community and also include non-linear relationships.

3.1.4 Aims

The yearly mean abundance and species composition for the whole area for the monitoring period 1976 to 1999 have therefore been reanalysed, using both multivariate and univariate measures, with the following aims: -

- To determine if the increase in the species diversity, evenness and number of species previously observed continued until 1999.
- To determine if any other changes in the community have occurred other than the one previously detected in 1979.
- To determine which species have been responsible for the increased diversity and the changes in community composition.
- To determine which environmental factors may have been responsible for the observed changes in the benthic community.

3.2 RESULTS

3.2.1 Temporal changes in the benthic community

The changes in mean diversity, evenness, number of species and individuals per station are seen in Figure 3.1. There has been a gradual overall increase in the mean diversity from around 0.75 to 1.75 with a large increase in diversity between 1994 and 1995. The mean evenness has also changed, with a gradual increase between 1976 and 1994 (0.45 to 0.55), then between 1994 and 1996 there was a further increase from around 0.55 to 0.65, with a subsequent levelling off. The mean number of species per station shows a gradual increase over time, from around 3 to around 7 species per station. The regression analysis indicates that the diversity, species richness and evenness all show a significant increase over time (Table 3.1). Although there has been a general increase in diversity, evenness and species richness yearly variations are clearly evident.

The mean number of individuals (total abundance) shows a large peak of 1000 individuals per $50cm^{-2}$ in 1980 and a smaller peak of 750 individuals per $50cm^{-2}$ in 1986. The mean abundance of individual species (Figures 3.2 to 3.5) show that *Cerastoderma edule*, *Hydrobia ulvae* and *Nereis diversicolor* all show fluctuations in abundance over time but with no clear trend. Some species, however, do show trends. *Macoma balthica*, *Corophium volutator* and *Eteone longa* all show a significant increase in abundance over time (Table 3.1). The graphs for these species suggest that the increase did not start until 1985. It can also be seen that *Streblospio shrubsolii* was not found until 1994 after which its numbers have increased steadily. Other species show peak years when abundances were extremely high compared to other years. *Manayunkia aestuarina* shows a peak abundance in 1980. Oligochaetes, *Pygospio elegans*, *N. hombergii* and *M. aestuarina* all show a peak in abundance in 1985 or 1986. In 1992, *N. hombergii* and in 1995/96, *P. elegans*, *S. shrubsolii* and Oligochaetes show peaks in their abundance. *M. aestuarina* was first found in 1979 and although it shows several peaks of abundance it appears to decline after 1986 until by 1994 it is at very low levels. The distribution of *M. aestuarina* in the year 1980 can be seen in Figure 3.6. The highest abundances were in the area where the chemicals effluent flows across the mudflat but at some distance from the outfall, whilst the abundance is reduced near to the outfall itself.

The MDS plot in Figure 3.7 shows that there was a substantial change in the community composition between the years 1978 and 1979 and again between 1993 and 1994. The results from the SIMPER analysis (Table 3.2) indicate that *Manayunkia aestuarina*, Oligochaeta and *Hydrobia ulvae* are responsible for 92% of the average dissimilarity (52.87) between group 1 (1976 to 1978) and group 2 (1979 to 1993). *M. aestuarina* and Oligochaeta spp can be considered as good discriminating species between these two groups, because both show an increase in abundance over this period. For groups 2 (1979 to 1993) and 3 (1994 to 1999) the average dissimilarity is 47.10. The species *M. aestuarina*, Oligochaeta, *Streblospio shrubsolii*, *H. ulvae* and *Macoma balthica* contribute 91% to the average dissimilarity. Of these species *M. aestuarina*, *S. shrubsolii and M. balthica* can be considered as good discriminating species. *M. aestuarina* shows a decrease in

abundance whilst *S. shrubsolii* and *M. balthica* both exhibit an increase in abundance over this period.

3.2.2 Environmental changes affecting Kinneil

Changes in pollution levels and the natural environment are considered.

3.2.2.1 Climatic data

The climatic data (Figure 3.8a) measured as the air temperature (maximum and minimum) and the North Atlantic Oscillation (NAO) have shown little change between 1976 and 1999 with only slight fluctuations being recorded.

3.2.2.2 Refinery effluent

The variables measured within the refinery effluent for the period 1993 to 1999 (Figure 3.8b-d) show a decrease between 1993 and 1995 for all variables except the flow, temperature, total suspended solids (TSS) and pH. There is a notable drop in the hydrocarbon content from 1994 to 1995. The increase in the quality of the effluent, which is seen by a decrease in the biological oxygen demand and the concentration of many heavy metals and hydrocarbons, is likely to be due to the installation of the biological treatment plant in 1994.

3.2.2.3 Ballast water

The data for the variables in the ballast water (Figure 3.9) indicate that for the period 1995 to 1999, there has been little change in all variables except the electrical conductivity (EC) and the hydrocarbon concentration. The EC increased dramatically between 1995 and 1997, whilst the hydrocarbon levels peak in 1995 and 1999, although these levels are still low compared to those found in the refinery effluent.

3.2.2.4 Chemical effluent

The data for the variables in the chemical effluent (Figure 3.10a-c) for the period 1995 to 1999 indicate that most variables showed little change. There has however been an overall increase in the concentration of acetonitrile and a decrease in sulphides. The BOD for the chemical effluent from 1976 to 1999 (3.10d) has shown

a gradual decrease, which indicates that the effluent has gradually been improving in quality.

3.2.2.5 Avon water quality

The water quality data for the Avon (Figure 3.11) indicates that many of the variables have remained fairly constant over the period 1985 to 1999. Some, however, have shown decreases in their levels, these include the suspended solids (SS), nitrogen (kjeldahl or KJ) and the biological oxygen demand from the carbonaceous component (BOD+ATU). The largest decrease for these three variables took place in the late 1980s after which they show little variation. The data on the Avon BOD and ammonia (NH_3) for the period 1976 to 1999 can be seen in Figure 3.10d. A decrease took place between 1976 and 1979, after which there was a continuing gradual decrease in the level of ammonia.

3.2.2.6 Grange burn water quality

The water quality data taken from Grange burn (Figure 3.12) for the period 1985 to 1999 indicates that most variables have remained fairly constant including the biological oxygen demand (BOD+ATU), dissolved oxygen (DO), pH and electrical conductivity (EC). The level of ammonia and of suspended solids (SS) have shown fluctuations in their concentrations with ammonia levels peaking in 1987 and 1991, and SS in 1992 and 1994.

3.2.2.7 Kinneil channel water quality

The change over time from 1980 to 1999 for the water quality data from the Kinneil channel (Figure 3.12) indicates that the temperature, salinity, dissolved oxygen (DO) and the phosphate levels have all remained fairly constant. The level of ammonia has however shown a steady decrease over the period.

3.2.2.8 Sewage works

The sewage works data (Figure 3.12) from 1985 to 1999 shows that most variables have shown little change but that the biological oxygen demand (BOD+ATU) has steadily decreased.

3.2.3 Community change in relation to environmental factors

The changes in the species diversity have been compared to the changes in the different sources of pollution and climate by using two methods, stepwise multiple regression analysis (See 2.3.3.3) and the BIO-ENV procedure (See 2.3.4.5), to determine what has been the most likely cause of the changes in the community composition (Table 3.3).

The multiple regression and BIO-ENV analyses indicate which of the pollution or environmental sources best explain the change in diversity or community composition at the different time periods. Although many of the pollution sources produced significant regression models many had a low Rsq(adj) value, indicating that they only explain a small percentage of the variation in the species diversity over the specified time period. Only the chemical BOD (1976 to 1999), Kinneil Channel (1980 and 1985 to 1999), refinery effluent (1993 and 1995 to 1999) and the Avon (1995 to 1999) produced significant models with a high Rsq(Adj) value indicating that they explain a large percentage of the variation in the species diversity over the specified time periods. The regression models for these can be seen in Table 3.4. The BIO-ENV produced similar results with the highest correlations being the Chemical/Avon (1976 to 1999), the Kinneil channel (1980 to 1999), the sewage works (1985 to 1999), the Avon (1993 and 1995 to 1999), the refinery effluent (1993 and 1995 to 1999) and finally the chemical effluent and ballast water (1995 to 1999). The identified variables for each of these results can be seen in Table 3.5.

3.3 DISCUSSION

3.3.1 The state of the benthic community

Dauer (1997) notes that a healthy benthic community is one that has a high biomass and species richness and that an increase in these measures indicates an improvement in the health of an area. Many areas that have been subject to heavy

pollution show an increase in diversity and species richness if the level of pollution is reduced or abated (Cato *et al.*, 1980). This change in the diversity and species richness has been observed with several different communities around oil refineries. The subtidal benthic community in Porvoo, Finland showed an increase in diversity and the number of species when a new treatment plant was added (Leppakoski & Lindstrom, 1978). A long-term survey of a saltmarsh showed that after the water quality of the effluent was improved (Dicks & Levell, 1989) the previously denuded areas of the saltmarsh underwent recolonisation (Dicks, 1976a). Kinneil has shown a clear increase in diversity and species richness over the 24 year survey period. *Macoma balthica*, *Corophium volutator* and *Eteone longa* show a significant increase in their abundance over time. The increase in the abundance of these three species can be considered as an improvement as they are not regarded as opportunistic species (Pearson & Rosenberg, 1978; Dauer, 1997). At the same time the opportunistic species *Manayunkia aestuarina* has declined in abundance. All this confirms that there has been an improvement in the health of the Kinneil intertidal area over the survey period, which is consistent with that found by previous studies of the Kinneil data, and other similar areas.

3.3.2 Succession

Although an increase in the number of species and diversity is seen with time, recovery usually involves the succession of species within the community. Pearson & Rosenberg (1978) described a general overview of the successional process for macrobenthic communities. The succession process involves four main phases; firstly after the initial defaunation of an area it is recolonised by small opportunistic species. Within a short time the abundance of these will increase to extremely high levels, the so-called peak of opportunists. Following this peak the numbers will reduce and new species will invade the area, this is called the ecotone phase, which develops into a transitory community including more species and larger individuals. Finally the community will develop further into a 'normal' mature community. This pattern of succession has been observed in benthic communities that have been affected by many different pollution and disturbance events, such as oil spills (Southward, 1982) and organic enrichment (Pearson & Rosenberg, 1978; Ueda *et*

al., 1994). At Kinneil several of the phases of succession have been observed over the 24 year period. The changes that were indicated by the MDS analysis that occurred around 1979 and 1994 were due to the introduction of new species. In 1979 this was of the opportunistic species *Manayunkia aestuarina*, a small polychaete, whose abundance rapidly increased to peak levels in 1980, creating a peak of opportunists. The following year the abundance of *M. aestuarina* dramatically decreased and over the following years many of the larger individuals such as *Macoma balthica*, *Corophium volutator*, *Eteone longa* and *Nephtys hombergii* show an increase in abundance. The introduction of *Streblospio shrubsolii* in 1994 did not produce a peak of opportunists, instead only continuing increases in the abundance of the larger species like *M. balthica*. This indicates that at this time a change occurred which allowed the further progression of the successional process. It can not be determined whether the community has yet reached the 'normal' mature community state. It can be concluded that the changes that have occurred at Kinneil support the successional pattern that was proposed by Pearson & Rosenberg (1978).

3.3.3 Factors causing the changes in the benthic community

Changes in the benthic community have therefore been observed at Kinneil that indicate that there has been an improvement in the health of the community that follows the typical pattern of succession. There are however several possible reasons for these changes, including disturbance events, pollution and climate change. The possible reasons that have been investigated are climate changes, changes in the petro-chemical effluents and the other potential pollution sources to the area. The relative importance of these factors in causing the changes in the benthic community at Kinneil is discussed.

3.3.3.1 Climate

Temperature is one of the key factors structuring marine communities and can limit the geographical distribution of species (De Jong, 1999). Different species have different temperature ranges in which they are able to survive, changes in the temperature can therefore cause mortality. Certain species are known to be sensitive

to winter temperatures, such as *Nephtys hombergii* (Beukema, 1991; Beukema *et al.*, 2000) and *Cerastoderma edule* (Jensen, 1992b). On the other hand, *Macoma balthica* and *Mytilus edulis* are both resistant to low winter temperatures (De Jong, 1999), *M. balthica* can also withstand high temperatures (Ratcliffe *et al.*, 1981). Temperature can also affect the community through recruitment patterns. The reproduction of many macrozoobenthic species is triggered by water temperature (De Jong, 1999). The recruitment of *Pygospio elegans* has been shown to be determined by temperature and salinity (Bolan & Fernandes, *Pers. comm.*). The density of recruits in some species, particularly bivalves, is related to the foregoing winter. After a serve winter, recruitment tends to be high, whilst after a mild winter recruitment tends to be low (Beukema *et al.*, 2001). Temperature can therefore directly increase or decrease the abundance of macrobenthic species. It can also have indirect effects by increasing or decreasing the abundance of prey and of predators. During the 24 years that the survey was undertaken at Kinneil the air temperature and the North Atlantic Oscillation (NAO) have shown little change. It is therefore perhaps not surprising that neither the multiple regression nor the BIO-ENV analysis found the temperature or the NAO to be important in explaining the change in the community structure during any time period. This agrees with the previous findings from McLusky & McCrory (1989) that also found no relationship between temperature and species diversity. It can be concluded that the overall increase in the species diversity and richness, or the introduction of the two species, has not been caused by changes in the air temperature or NAO. Although the climate did not cause the major changes in the community structure, it still may play a role in determining the yearly variations in abundance.

3.3.3.2 Improved quality of the effluents

3.3.3.2.1 Refinery effluent

Refinery effluents constitute a mixture of different chemicals, the exact composition of an effluent is dependent on the processing plant and can vary on a daily basis depending on the units in operation (Burks, 1982). It is therefore hard to generalise their effects. Refinery effluents and their components have been found to have both lethal (Hall *et al.*, 1978; Smith, 1987) and sublethal (Buikema *et al.*, 1981; Das &

Konar, 1988) effects on marine invertebrates. Not all species show the same responses, both *Nereis diversicolor* and *Macoma balthica* have been shown to be particularly resistant to refinery effluents (Cote, 1976; Leppakoski & Lindstrom, 1978). On the other hand crustaceans tend to be more sensitive than other aquatic organisms (Smith, 1987). The toxicity of the refinery effluent from Kinneil has been tested several times over the years using several species that are found at Kinneil. Smith (1987) first tested the toxicity of the effluent to *Praunus flexuosus*, *Corophium volutator*, *Macoma balthica* and *Hydrobia ulvae*. The refinery effluent at that time was found to be lethal to all four species, with lethal concentrations (LC_{50}) of 2.55% (*P. flexuosus*), 16% (*C. volutator*), 35% (*M. balthica*) and 100% (*H. ulvae*) refinery effluent in seawater. Since then the refinery effluent has had a biological treatment plant installed in 1994, which increases the quality of the effluent before it is discharged. Toxicity testing in 1995 using *C. volutator*, *M. balthica* and *Nereis diversicolor* showed that although the refinery effluent was still toxic at 100% concentration, the toxic effects were largely removed after the addition of 10% seawater (McLusky & Colbourne, 1995). This indicates that the toxicity of the effluent had been reduced. The toxicity has continued to reduce further, because when tested in 2000, even 100% effluent did not cause 50% mortality after 96 hours (Storey, 2000). The different variables that are measured in the refinery effluent indicate that it was the biological treatment plant that caused a reduction in the concentration of many of the chemicals within the effluent after 1994 and they have continued to decrease until the end of the survey in 1999. McLusky (1982a) found in 1980 there was a defaunated area around the refinery outfall, which was attributed to the toxic effects of the refinery effluent. A reduction in the toxicity of a refinery effluent was found to increase the diversity and species richness of the benthic community in Porvoo, Finland (Leppakoski & Lindstrom, 1978), and therefore it is likely that it will have affected the benthos at Kinneil. It has been seen that there was a clear change in the community composition between 1993 and 1994, marked by the introduction of *Streblospio shrubsolii*. The regression and BIO-ENV analysis both produced highly significant models for the period 1993 to 1999 and 1995 to 1999 using the environmental variables measure in the refinery effluent. It seems probable that the reduced toxicity in 1994 allowed new species to colonise, whilst the continued reduction in toxicity after this time has allowed further succession of the benthic community. It therefore seems that the

refinery effluent has been an important factor controlling the benthic community at Kinneil. This is consistent with the findings of other studies that have looked at the impacts of refinery effluents (Dicks, 1976a; Leppakoski & Lindstrom, 1978; Dicks & Levell, 1989).

3.3.3.2.2 Ballast water

The ballast water is discharged along with the refinery effluent, making it difficult to determine its individual effects. Data on the ballast water is only available for the period 1995 to 1999, making it hard to discern any long-term effects it may have had. For this period none of the variables that were measured have shown any specific trends, only yearly fluctuations. The fluctuation in the COD was found to be correlated to that of the community using the BIO-ENV analysis. This is likely to be a coincidence due to the limited amount of data and the fact that the community data does not show any clear trend over this period. This study has therefore not been able to determine the importance of the ballast water in effecting the change to the benthic community during the monitoring period. It is likely that the effect of the ballast water is linked to those of the refinery effluent as they are mixed together when discharged.

3.3.3.2.3 Chemical effluent

The chemical effluent consists of many different chemicals many of which like ammonia, phenol and oil are known to be toxic to invertebrates (Storey, 2000). Smith (1987) tested the toxicity of the chemical effluent and found that it was more toxic than the refinery effluent although it had a lesser volume. It seems likely therefore that the chemical effluent would also have had an effect on the benthic community. McLusky (1982a) found that the benthic community around the chemical outfall in 1980 was reduced, in that there were few species at a low abundance. The BOD of the chemical effluent has shown a gradual decrease over the survey period. It is known that BP have been actively trying to reduce the toxicity of this effluent and that the closure of the phenol plant in 1985 and the acetonitrile plant in 1990 (McLusky & Martins, 1998) are likely to have reduced the concentration of certain chemicals within the effluent. The lack of detailed data for the chemical effluent discharge however makes it hard to determine whether these changes are responsible for the change in the benthic community. The multiple

regression analysis and BIO-ENV both produced significant models, of the change in diversity, for the period 1976 to 1999, using the chemical BOD. The gradual increase in the quality of the chemical effluent is therefore probably responsible, at least in part, for the increase in the diversity, evenness and species richness over the survey period. It can therefore be concluded that the chemical discharge is probably just as important in affecting benthic community structure as the refinery effluent.

3.3.3.3 Movement of the chemical outfall

It has already been shown that the chemical effluent was having an impact on the environment especially at the beginning of the survey period. It is therefore likely that the movement of the chemical outfall in 1979 from the River Avon to a site closer to the refinery outfall would have caused a change to the benthic community at both the old and new outfall sites. McLusky (1982a) showed that there was an increase in the diversity and species richness in the area around the old outfall. There was also a change in the species composition around the new outfall site. At the new site the species *Macoma balthica* and *Nereis diversicolor* disappeared and *Manayunkia aestuarina* appeared. McLusky & McCrory (1989) found by clustan analysis that there was a change in the similarity of the community between 1978 and 1979, which they attributed to the introduction of the species *Manayunkia aestuarina*. The MDS plot from the present study also confirmed that there was a change in the community at this time and the SIMPER analysis showed that this was primarily due to the species *M. aestuarina*. This analysis therefore has confirmed by further statistical testing the hypotheses of the previous study. No test could be performed to test the hypothesis that it was the movement of the outfall that caused the change in the species composition, but it is highly coincidental and seems the most logical explanation. The effects of the second movement of the chemical outfall in 1999 are considered later (Chapters 5, 6 and 7).

3.3.3.4 Other pollution sources

As well as the petro-chemical effluents, Kinneil is subjected to potential pollution from four other sources, which have previously not been considered. The impacts from these alternative pollution sources are discussed.

3.3.3.4.1 River Avon

The river Avon receives effluents from a paper mill and well as several sewage works in land (FRPB, 1989). The river flows across the Kinneil mudflat to the main channel and there is therefore the potential that the pollutants within the river water may effect the benthic community at Kinneil. There has been a clear decrease in the BOD and ammonia concentration of the Avon over the 24 year monitoring period. A decrease in the suspended solids, nitrogen (kjeldahl) and BOD+ATU was seen between 1985 and 1999. This indicates that the water quality of the Avon has improved over the study period, which may have affected the benthic community. The multiple regression and BIO-ENV analysis produced significant models from the Avon water quality data for the period 1995 and 1993 to 1999 but not for any of the longer time periods. This suggests that the Avon has not been an important factor structuring the Kinneil mudflat benthic community in the long term, although it may have a localised or short term effect.

3.3.3.4.2 Grange burn

The Grange burn is a smaller river than the Avon and joins the Kinneil mudflat to the west of Grangemouth Docks. The river receives urban runoff from the surrounding areas and there have been small pollution incidents such as oil spills (FRPB, 1989). None of the water quality variables that have been measured have shown any specific trends during the period 1985 to 1999. It is therefore not surprising that the regression and BIO-ENV analysis did not produce any significant models from the Grange burn water quality data. The water quality of the Grange burn has not had a detectable effect on the Kinneil benthic community.

3.3.3.4.3 Kinneil channel

The main channel of the estuary carries pollutants to Kinneil that have entered further up the estuary. The main sources of pollutants to the estuary over the 24 years have been from several sewage works, a brewery and distillery, Grangemouth docks, the Carron River and an effluent from a chemical plant (Zeneca) at Skinflats (McLusky, 1982a; Davies, 1987). Over the years there have been clear improvements to the overall quality of the Forth estuary. There has been a reduction in the inputs of organic wastes by municipal and industrial sites, and of other

hazardous substances (Griffiths, 1997). It should be noted that the Kinneil channel data measurements are taken in the main channel just off Kinneil and therefore not only take into account the pollutants from up stream but also those from Kinneil itself, such as the refinery and chemical effluents. The water quality data for the Kinneil channel indicates that there has been a decrease in ammonia concentrations since 1980. Toxicity tests have found ammonia to be toxic to benthic invertebrates at fairly low levels, with an LC_{50} of $18mgl^{-1}$ (Storey, 2000). Levels of ammonia in the Kinneil channel were around this level during the early 1980s, only after 1987 did the levels start to drop below this level. The multiple regression analysis indicates that the reduction in ammonia may have caused the increase in diversity. Although the change in diversity can be related to the ammonia levels the exact reasons for the decrease in ammonia can not be determined, as there are several possible reasons. It could be due to the sewage works upstream, or to the effluents at Skinflats or Kinneil, although it is likely to have been a combination of them all. This however indicates that the general increase in the water quality of the Kinneil channel may have affected the invertebrate community at Kinneil, a factor which has not been taken into consideration before.

3.3.3.4.4 Kinneil sewage works

The wastewater from sewage works has been shown to effect marine benthic communities, through organic enrichment (Dauer & Conner, 1980; Anderlini & Wear, 1992; Simboura *et al.*, 1995). A change in the quality of the sewage effluent can result in a change in the benthic community composition (Moore & Rodger, 1991). The data on the variables measured in the sewage effluent indicates that most have shown little change except for the BOD which has decreased since 1985. The multiple regression and BIO-ENV analyses indicated that the sewage works was probably not an important factor governing the overall community structure at Kinneil.

3.4 CONCLUSIONS

There has been a general improvement in the condition of the benthic community and there have been two periods when there has been a change in the community

composition via the addition of new species. These changes can be explained by the succession phases described by Pearson & Rosenberg (1978). A peak of opportunists was seen in 1980 after which the abundance of the opportunists decreases and the abundance of the larger species increases, which is consistent with the ecotone phase. The introduction of a new species and the further increase in abundance of the existing species after 1994 is indicative further succession possibly resulting in a transitory phase.

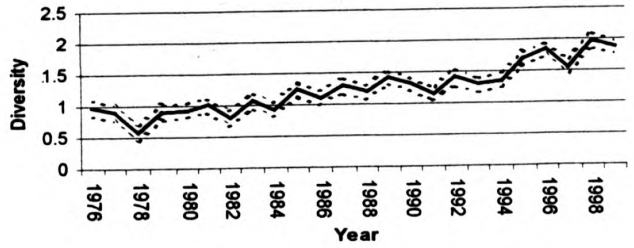
The analysis of the yearly average data for the whole mudflat suggests that pollution is most likely to be the cause of the changes in the community rather than changes in the climate. Of the pollution sources that have been considered, the changes in the refinery and chemicals effluents best explain the change in the community.

It is hypothesised that the effluents have had toxic and enrichment effects, however the enrichment effect can only be seen when toxicity is low. The movement of the chemicals outfall in 1979 caused the area around the new discharge point to become impacted. A trade off in the toxic and enrichment effects was seen in the area affected by the discharge. Close to the outfall the effluent had a toxic effect, however with increasing distance the toxicity was reduced and an enrichment effect was seen as a peak of opportunists in 1980. The reduction of the toxicity of the chemicals effluent in the 1980s caused the increase in the diversity, evenness and species richness to a certain level but any further increase was inhibited by the toxicity of the refinery effluent. The reduction of the toxicity of the refinery effluent in 1994 allowed a further increase in the diversity, evenness and species number, over the whole area. The refinery effluent was probably also having an enrichment effect as the addition of the biological treatment plant in 1994 also caused an apparent decline in the number of opportunists.

This chapter has only considered the change in the overall mean abundance for the entire area. Different areas of the mudflat are likely to have been affected differently. It may be for example, that only those areas close to the outfalls have shown the increase in diversity and the rest of the mudflat remained the same throughout. On the other hand the whole area might have shown an improvement.

The only way to determine exactly what happened is to look in more detail at smaller areas of the mudflat. This is addressed in Chapter 4.

a)



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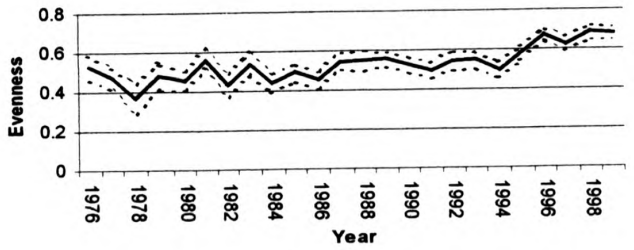

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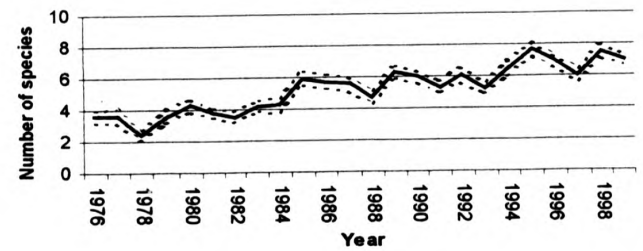


d)

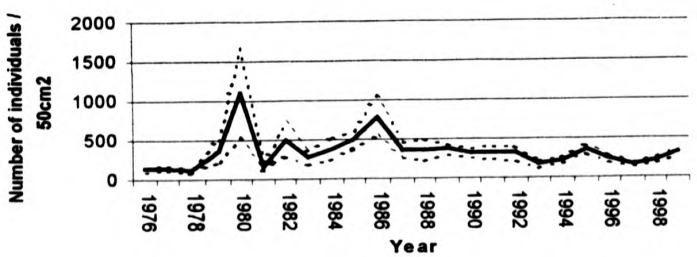


Figure 3.1 Graphs showing the change over time of the mean (solid line) a) Diversity, b) Evenness, c) Number of species, d) Number of individuals, with the 95% confidence limits (dashed line).

Table 3.1 Regression analysis results for the mean diversity, evenness and species richness over time, along with the mean abundance for the species *Macoma balthica*, *Corophium volutator* and *Eteone longa*.

Regression equation	**p**	**F**	**Rsq**
Diversity = -89.5 + 0.0457 Year	0.000	97.72	81.6%
Evenness = -16.4 + 0.00853 Year	0.000	28.43	56.4%
No Species = -353 + 0.180 Year	0.000	88.82	80.1%
Macoma abundance = -1699 + 0.861 Year	0.000	23.33	51.5%
Corophium abundance = -874 + 0.442 Year	0.000	17.60	44.4%
Eteone abundance = -311 + 0.157 Year	0.000	19.08	16.4%

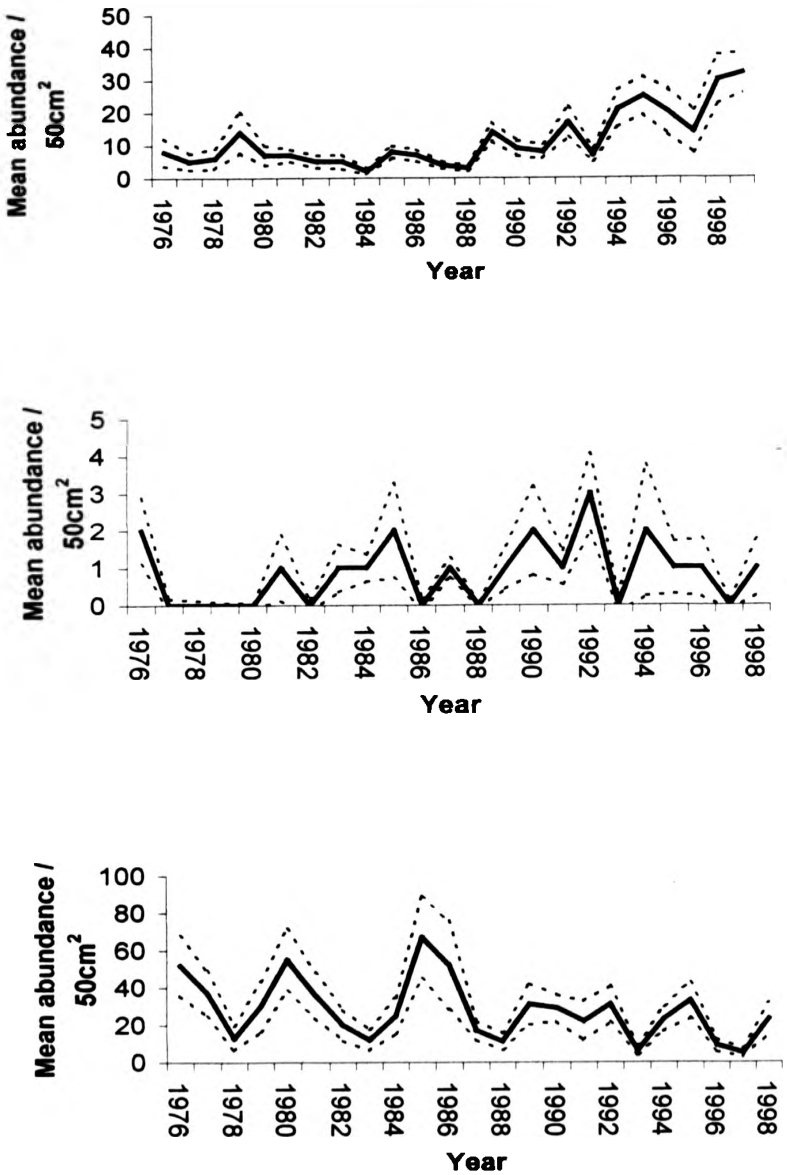


Figure 3.2 Change over time in the mean abundance (solid line) of *Macoma balthica* (Top), *Cerastoderma edule* (Middle) and *Hydrobia ulvae* (Bottom) with 95% confidence limits (dashed lines).

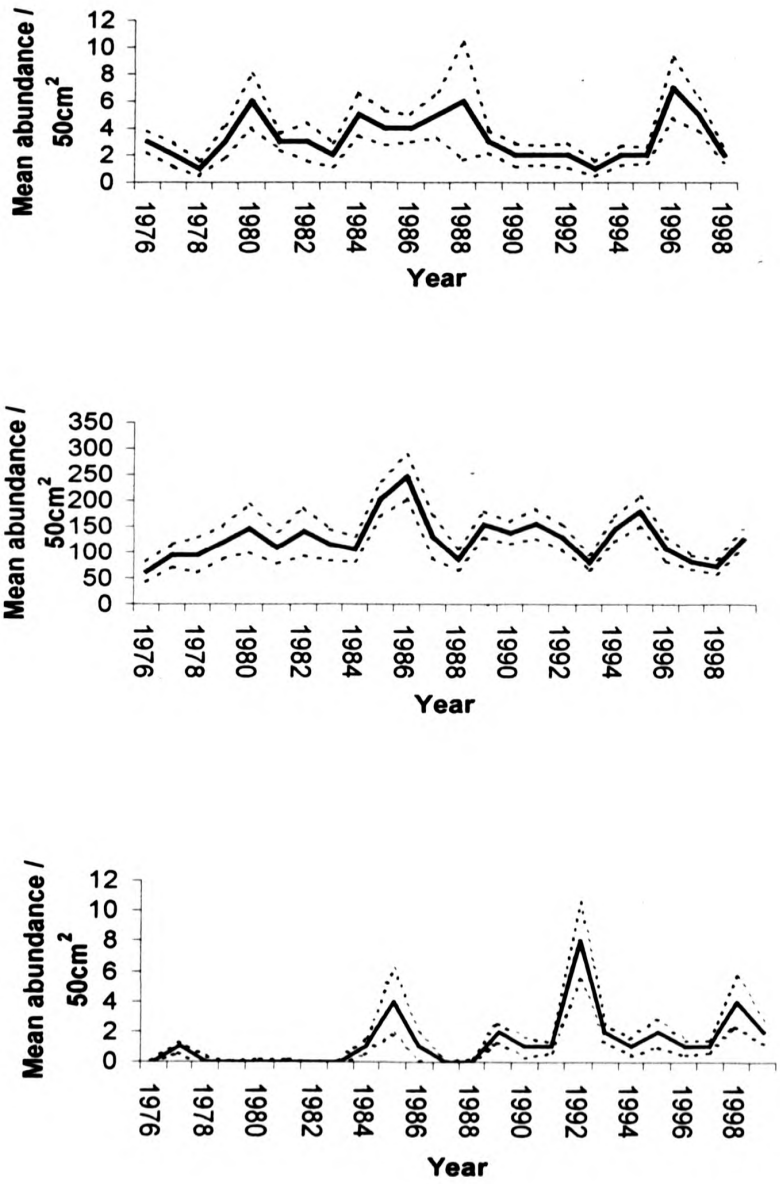


Figure 3.3 Change over time in the mean abundance (solid line) of *Nereis diversicolor* (Top), Oligochaetes (Middle) and *Nephtys hombergii* (Bottom) with 95% confidence limits (dashed lines).

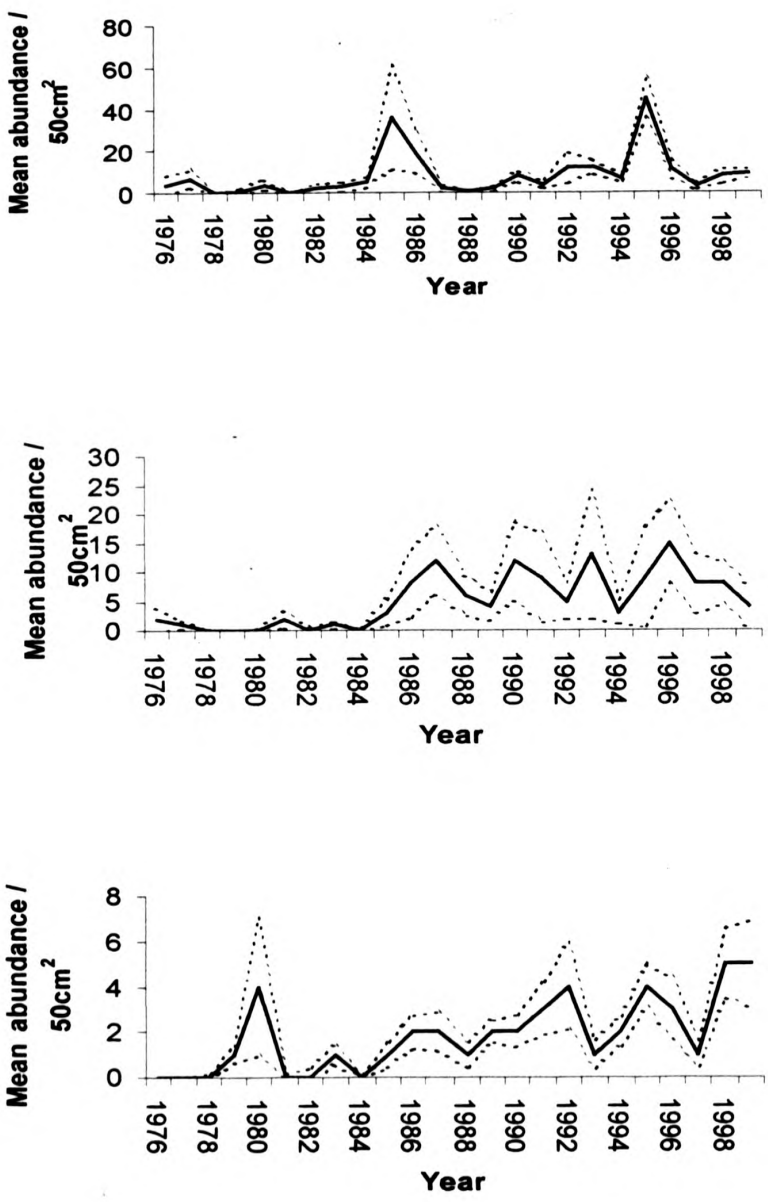


Figure 3.4 Change over time in the mean abundance (solid line) of *Pygospio elegans* (Top), *Corophium volutator* (Middle) and *Eteone longa* (Bottom) with 95% confidence limits (dashed lines).

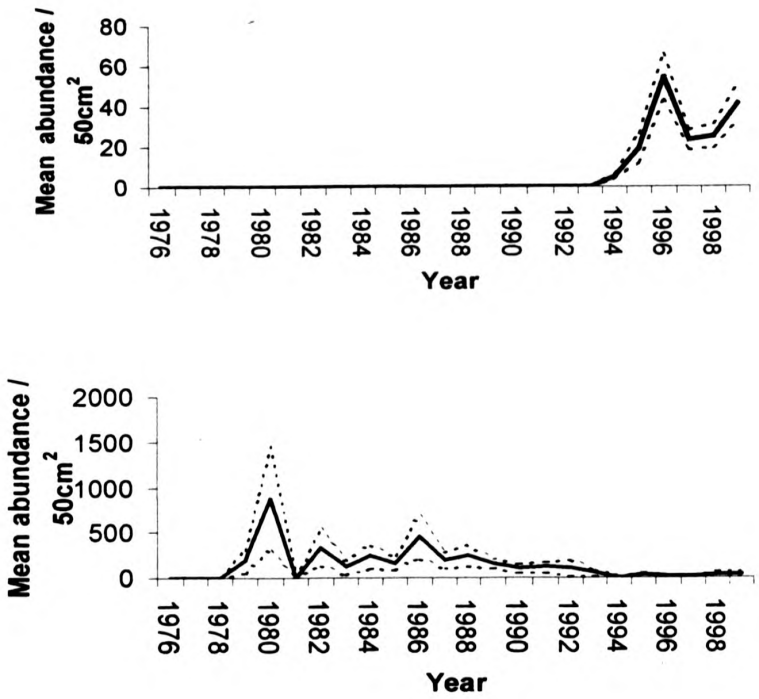


Figure 3.5 Change over time in the mean abundance (solid line) of *Streblospio shrubsolii* (Top) and *Manayunkia aestuarina* (Bottom) with 95% confidence limits (dashed lines).

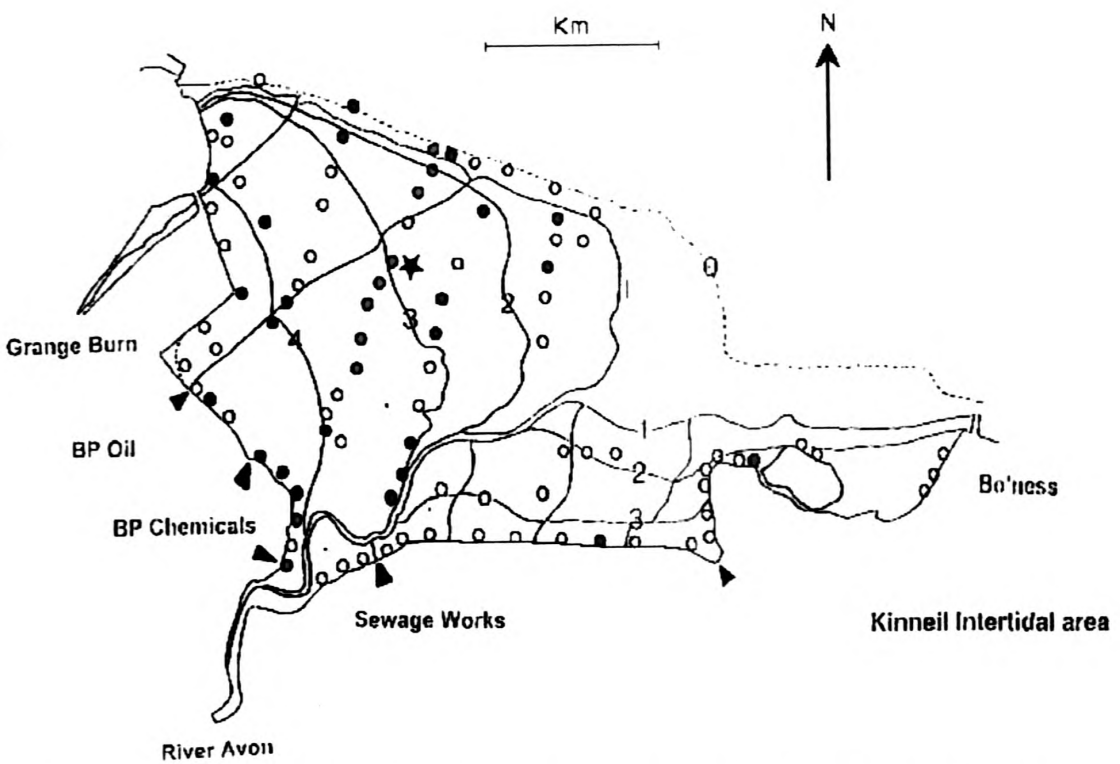


Figure 3.6. Map of Kinneil showing the relative abundance of *Manayunkia aestuarina* in 1980. The different colour circles indicate the difference abundance levels, white - none, Green – 1-100, Yellow – 100-1000, Red - >1000 individuals/50cm^2.

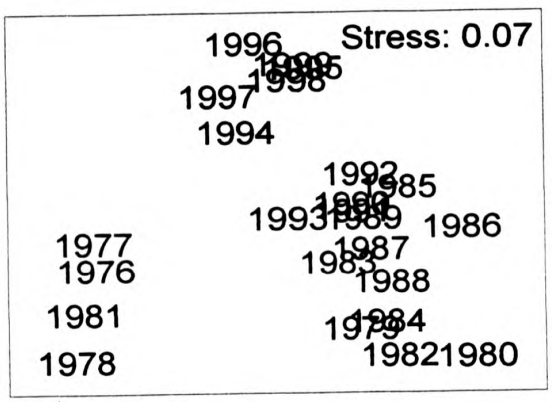


Figure 3.7. MDS plot showing the change in the mean community composition from 1976 to 1999.

Table 3.2. SIMPER results for the mean analysis showing the differences between the years 1976-1978, 1979-1993 and 1994-1999 (* = Good discriminating species).

Average dissimilarity = 52.87					
Species	**1976-1978 Average abundance**	**1979-1993 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	0	221.67	2.06*	65.91	65.91
Oligochaete	83.67	137.2	1.73*	18.75	84.66
Hydrobia	34	29.6	1.28	7.6	92.26
Average dissimilarity = 47.1					
Species	**1979-1993 Average abundance**	**1994-1999 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	221.67	22.5	1.7*	55.83	55.83
Oligochaete	137.2	118.83	1.4	14.88	70.7
Streblospio	0	27.83	1.56*	9.75	80.45
Hydrobia	29.6	22.67	1.3	5.78	86.24
Macoma	7.8	23.67	1.93*	5.47	91.71

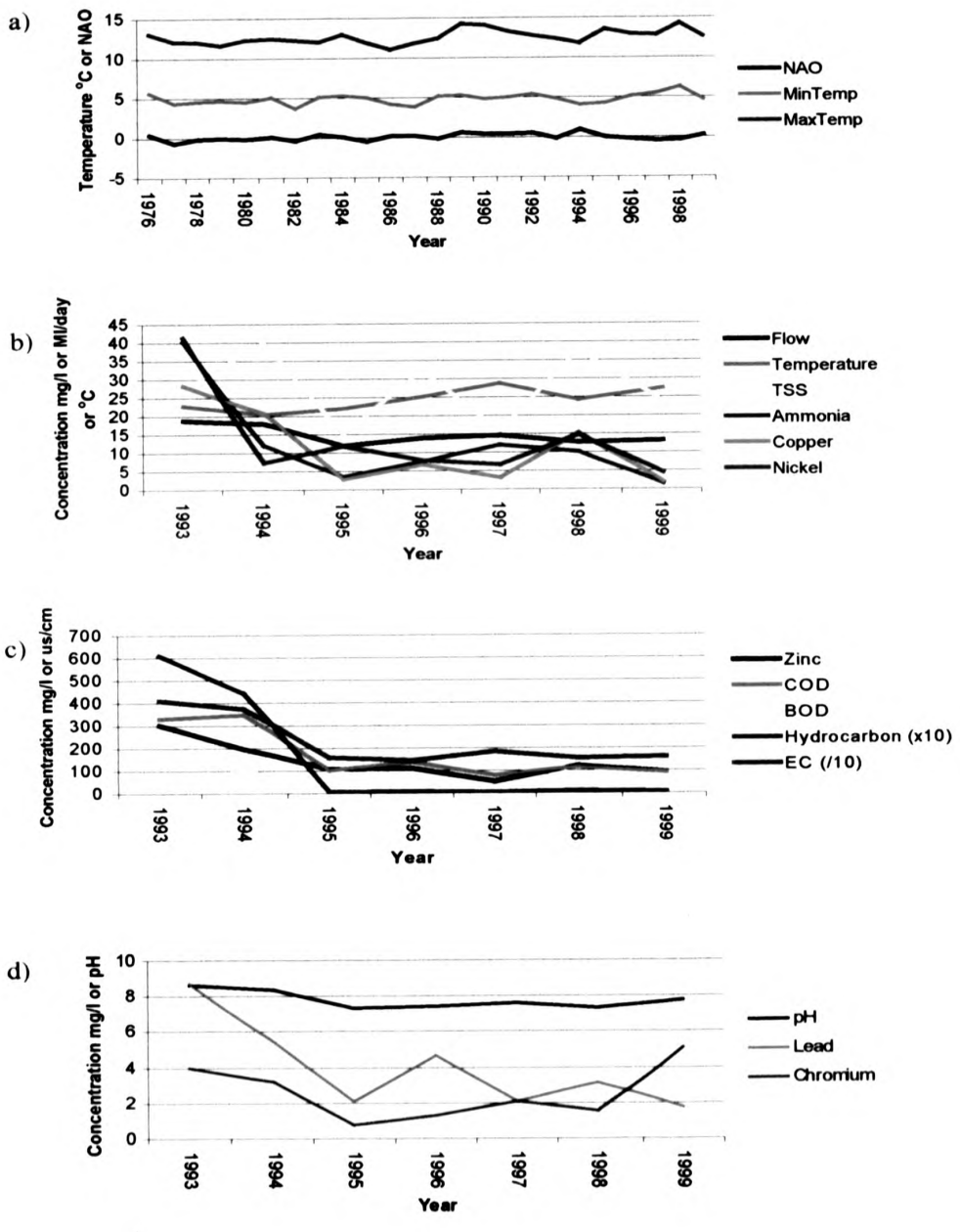


Figure 3.8. Change over time in the variables measured for the period 1976 to 1999 for the climate (a) and for the period 1993 to 1999 for the refinery effluent (b, c, d). All measured in mg/l except Flow (Ml/day), Temperature (°C), EC (us/cm), NAO and pH.

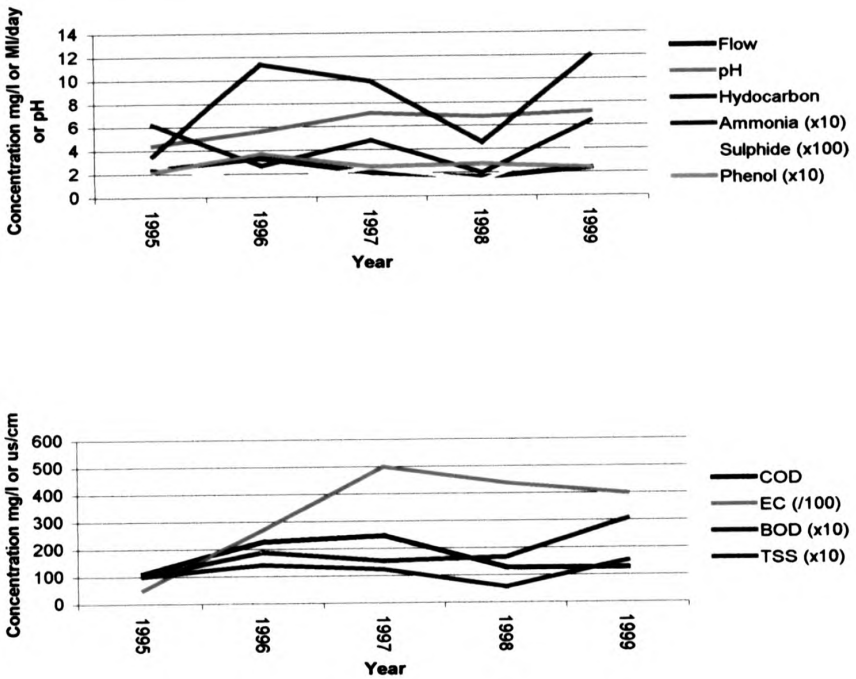


Figure 3.9. Change over time from 1995 to 1999 for the variables measured in the ballast water. All measured in mg/l except Flow (Ml/day), EC (us/cm) and pH.

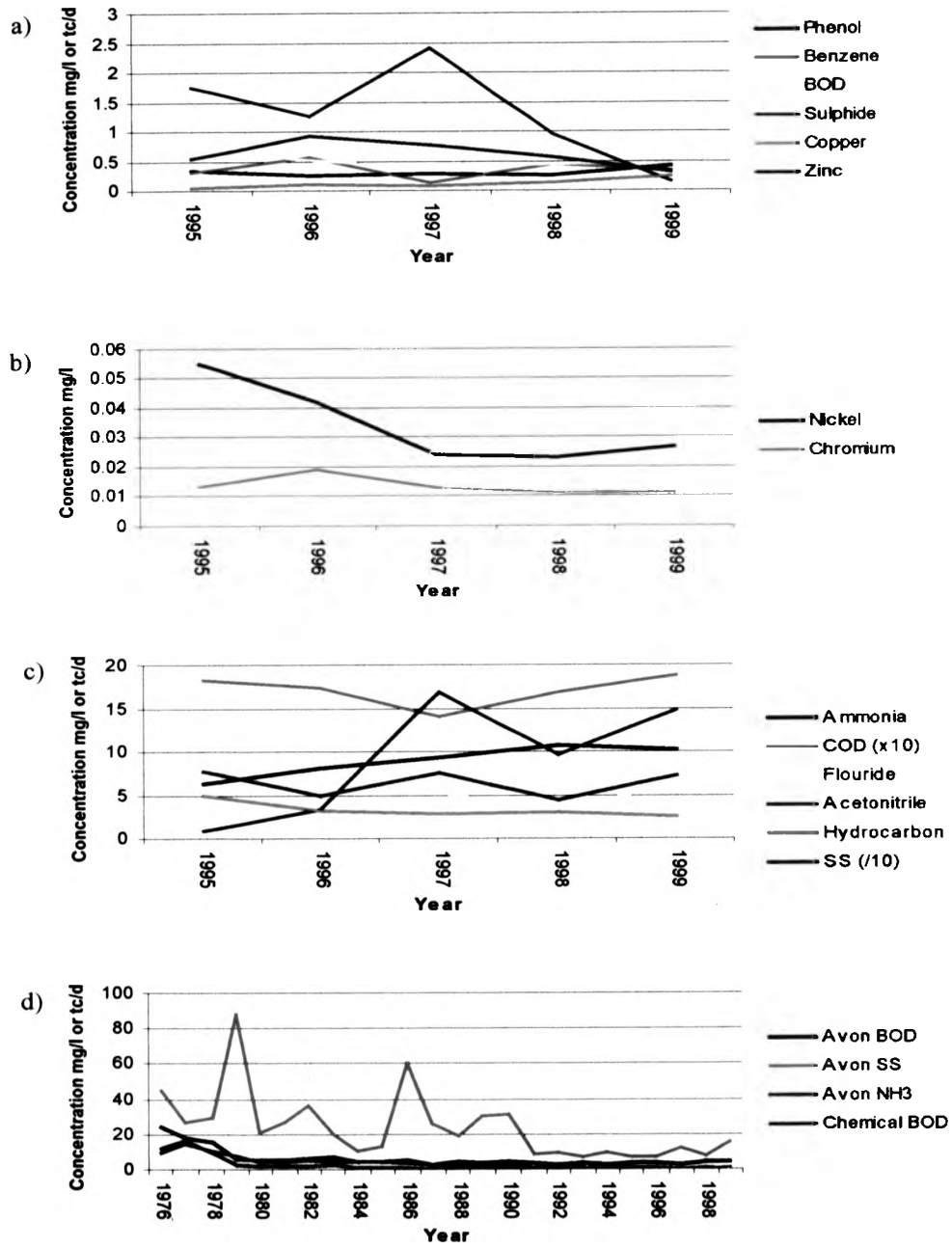


Figure 3.10. The change over time from 1995 to 1999 for the variables measured in the chemical effluent (a, b, c) and the change from 1976 to 1999 in the chamical/Avon variables (d). All measured in mg/l except BOD, COD and chemical BOD – tc/d.

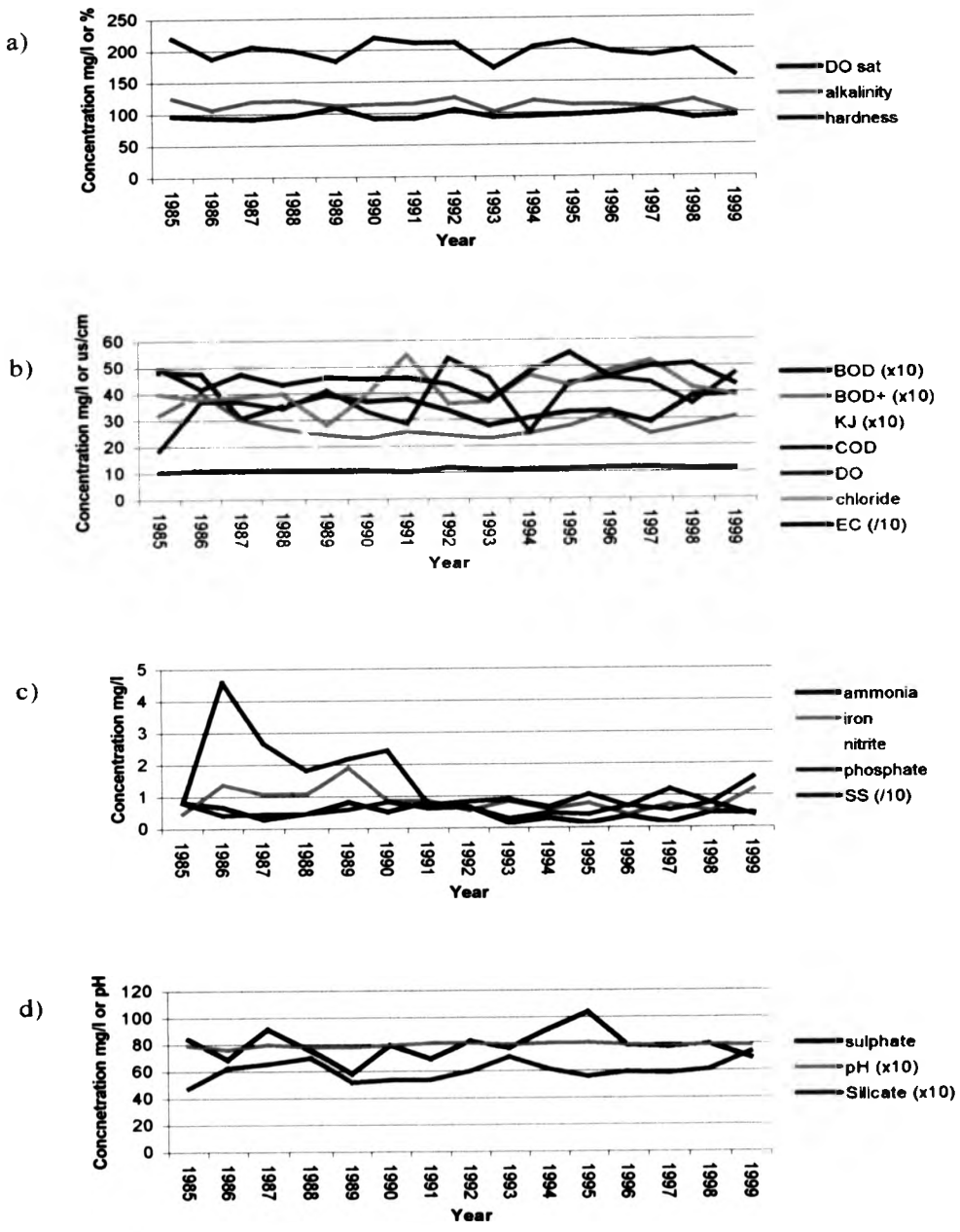


Figure 3.11. The change over time from 1985 to 1999 in the water quality variables measured in the Avon all measured in mg/l except EC - us/cm, DO sat - % and pH.

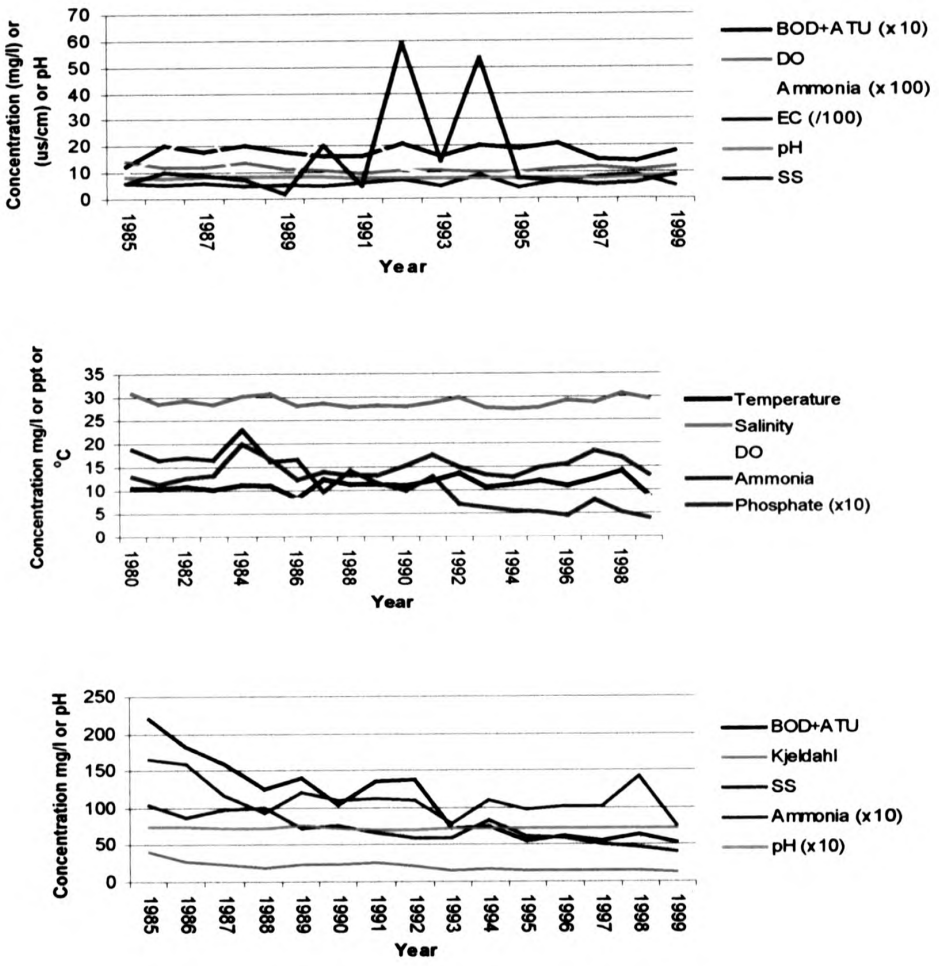


Figure 3.12. Change over time for the variables measured in from Grangeburn (Top), Kinneil channel (Middle) and the Sewage works (Bottom). All variables measured in mg/l except Temperature (°C), Salinity (ppt), EC (us/cm) and pH.

Table 3.3. Stepwise multiple regression and BIO-ENV results for the different pollution sources and climate data for each of the different time periods. The p and Rsq(adj) values for the stepwise regressions (Reg) and the correlation coefficient (p_s) from the BIO-ENV analyses are shown. Those with an Rsq(adj) or p_s above 70% are in bold.

	1976-99		**1980-99**		**1985-99**		**1993-99**		**1995-99**	
	Reg	BIO-ENV	Reg	BIO-ENV	Reg	BIO-ENV	Reg	BIO-ENV	Reg	BIO-ENV
	(p, Rsq(adj)%)	(ps)	(p, Rsq(adj)%)	(ps)	(p, Rsq(adj)%)	(ps)	(p, Rsq(adj)%)	(ps)	(p, Rsq(adj)%)	(ps)
Chemical/Avon	0.000, 72.8%	0.596	-	-	-	-	-	-	-	-
Temperature	None sig	0.048	None sig	0.086	0.046, 21.6%	0.085	None sig	0.062	None sig	0.297
NAO	None sig	0.02	None sig	-0.001	None sig	0.058	None sig	0.012	None sig	0.236
Kinneil channel	-	-	**0.000, 78.9%**	0.539	**0.000, 78.6%**	0.564	0.012, 69.6%	0.261	None sig	0.527
Avon	-	-	-	-	0.025, 27.9%	0.43	0.015, 67.3%	0.74	**0.003, 100%**	**0.915**
Grange burn	-	-	-	-	Not normal	0.155	None sig	0.494	None sig	0.188
Sewage works	-	-	-	-	0.000, 59.6%	0.667	None sig	0.623	None sig	-0.248
Refinery effluent	-	-	-	-	-	-	**0.002, 84.3%**	**0.832**	**0.010, 97.9%**	**0.879**
Chemicals effluent	-	-	-	-	-	-	-	-	Not normal	**0.83**
Ballast water	-	-	-	-	-	-	-	-	None sig	**0.83**

Table 3.4 The stepwise regression models that have an Rsq(adj) above 70%, including the p, F and Rsq(adj) values from the mean data for the different sources and time periods.

Source	Time period	Regression equation
Kinneil channel	1980 - 1999	Diversity = 2.58 - 1.25 log Ammonia, p=0.000, F=72.06, Rsq(adj)=78.9%
Kinneil channel	1985 - 1999	log Diversity = -0.232 - 0.318 log Ammonia + 0.0236 Salinity, p=0.000, F=26.65, Rsq(adj)=78.6
Refinery effluent	1993 - 1999	log Diversity = 1.36 - 0.345 log EC, p=0.002, F=33.16, Rsq(adj)=84.3%
Refinery effluent	1995 - 1999	Diversity = 0.977 Hydrocarbon + 0.0309 Temperature, p=0.010, F=95.97, Rsq(adj)=97.9%
River Avon	1995 – 1999	Diversity = -3.24 + 1.13 Ammonia + 0.597 pH - 0.0625 Iron, p=0.003, F=67371.52, Rsq(adj)=100%
Chemical/Avon	1976 - 1999	Diversity = 1.46 – 0.617 log ChemicalBOD, p=0.000, F=62.68, Rsq(adj)=72.8%

Table 3.5. The identified variables from the BIO-ENV analyses for the mean data that have high correlation coefficients.

Source	Time	Variables	P_s
Chemical/Avon	1976	Avon ammonia, Chemical BOD	0.596
Kinneil channel	1980	Ammonia, Phosphate	0.539
Sewage works	1985	BOD	0.667
Refinery effluent	1993	Flow, Zinc	0.832
Refinery effluent	1995	Flow, EC, Hydrocarbon	0.879
Ballast Water	1995	COD	0.830
Avon	1995	BOD+ATU, DO, Chloride, Nitrite, pH	0.915
Chemicals effluent	1995	Benzene, BOD, COD, Zinc	0.830

4. LONG TERM DATA – SPATIO-TEMPORAL ANALYSIS

4.1 INTRODUCTION

4.1.1 Spatial effects of refinery effluents

Areas that are impacted by refinery effluents or other pollutants often show spatial differences in community distribution, which are typically limited to a certain distance from the outfall (Wharfe, 1975; Petpirron & Dicks, 1982; Saha & Konar, 1984a). Close to the discharge point fauna is absent or at a low abundance and diversity. With increasing distance from the outfall, the diversity and abundance increases, eventually reaching levels that are typical of unpolluted areas (Wharfe, 1975; Petpiroon & Dicks, 1982; Saha & Konar, 1984a; Talsi, 1987; Dicks & Levell, 1989). The distance over which this effect is detected varies from site to site and is dependent on the effluent composition and the conditions of the recipient area (Lehtinen, 1986). If the refinery effluent at Kinneil has had an effect on the benthic community then a spatial difference should be expected to be seen.

4.1.2 Previous spatial analysis of the Kinneil long-term data

McLusky (1982a) investigated the spatial distribution of the benthic community at Kinneil. Four zones of pollution were observed around the effluent outfalls. 1) Gross pollution occurred within 250m of the outfall, where no fauna was found. 2) Between 250 and 500 m from the outfall (severe pollution) the community was characterised as having a low abundance, diversity and biomass, with *Manayunkia aestuarina* and oligochaetes as the dominant species. 3) Between 0.5 and 1.5 km the fauna had a high abundance and biomass but still a relatively low diversity. 4) At the greatest distance from the outfall (1.5 to 2.25 km) the abundance had decreased but the diversity was higher.

4.1.3 Requirement for further spatial-temporal analysis

It has already been shown that there has been a temporal change in the annual mean abundance for the whole Kinneil mudflat over the monitoring period (Chapter 3). The temporal changes observed could have been due to two reasons. Firstly, there could have been a change that has affected the entire area. Secondly, there could have been a change in only one or more localised areas, which has caused the observed changes in the overall mean abundance. The fact that it has been previously shown that the spatial distribution of the benthos at Kinneil is not uniform (McLusky, 1982a), suggests that certain areas of the mudflat may have the possibility to show a greater change with time than other areas. There is the possibility that it may have involved different species in different areas. It is also possible that that the area may have been affected by pollution sources other than the petro-chemical effluents. Although no obvious relationships were detected for the River Avon, Grange burn River and the Kinneil sewage works using the annual mean abundance data for the whole Kinneil area (Chapter 3), it is possible that these pollution sources may have had a localised effect.

4.1.4 Aims

The long-term data set has therefore been reanalysed by dividing it into 10 smaller groups (See 2.3.1) on which the analyses have been carried out, with the following aims: -

- To determine whether all ten groups have shown the same changes in community composition over time.
- To determine if the changes in the community composition of the ten groups are due to the same species.
- To determine if the changes in the community composition for each group have been caused by the same environmental factors.

4.2 RESULTS

The process of grouping the stations into smaller groups is shown first and then the analysis of the changes in the community over time for each group are shown.

4.2.1 Grouping of stations

The dendrogram from the cluster analysis of the year 1994 (Figure 4.1) groups the 90 stations into five groups (Figure 4.2 and Table 4.1). These groups were then further divided into smaller groups to produce 10 groups (Figure 4.3 and Table 4.2) (See 2.3.1 for details of the method).

4.2.2 Spatio-temporal analysis

The same analyses that were used for the temporal analysis of the mean abundance data for the whole mudflat (Chapter 3) have been used on the individual group abundance data. The results of the analysis for each of the 10 groups will be considered together, looking at community changes as well as the environmental changes.

4.2.2.1. Changes in the benthic community composition

4.2.2.1.1 Diversity

The change in the diversity for each group can be seen in Figures 4.4 to 4.6. These show that all ten groups have shown a gradual increase in diversity over the monitoring period although there are yearly fluctuations. By examining the change in diversity for all ten groups simultaneously (Figure 4.7) it can be seen that most of the groups have shown a generally similar increase in diversity, although some have shown a slightly different pattern. Group 1 stands out as having had a particularly low diversity throughout the whole monitoring period, although the diversity has still increased over time. Group 2 also seems to have a slightly lower diversity than most of the groups. On the other hand groups 5 and 8 began the monitoring period with relatively high diversities and they did not start to show an increase until after 1984. The regression analysis (Table 4.3) indicates that all groups have shown a significant increase in diversity over the study period. All the groups have shown

similar rates of increase except for groups 1 and 8, which have shown the least increase over time.

4.2.2.1.2 Evenness

The change in the evenness over the 24 years for each group can be seen in Figures 4.8 to 4.10. The graphs indicate that most of the ten groups have shown a gradual increase in evenness over time except for groups 5, 8, 9 and 10. The evenness for groups 5, 8 and 9 seems to have remained relatively constant over time, whilst the evenness for group 10 has been very variable especially during the early years from 1976 to 1988 after which it seems to have been less variable. The graph combining all ten groups (Figure 4.11) together shows that group 1 has the lowest evenness, whilst groups 5 and 8 had a relatively high evenness in the beginning. It can be seen that there was greater variation in the evenness between the ten groups at the beginning of the monitoring period than at the end. The regression analysis (Table 4.4) indicates that all the groups except groups 8 and 10 have shown a significant increase in the evenness over time, although the relationship tends to be fairly weak. The increase is only slight compared to that of the diversity.

4.2.2.1.3 Number of species per station

All the graphs showing the change in the number of species per station over time (Figures 4.12 to 4.14) indicate that there has been an increase over time for all groups but with yearly variations, however some groups have shown a greater increase than others. By assessing the groups together (Figure 4.15) it can be seen that most groups have shown a similar increase in the number of species per station. Group 1 had the lowest number of species per station throughout and groups 4 and 6 had relatively low number of species at certain times during the monitoring period. The regression analysis (Table 4.5) indicates that, like the diversity, all groups have shown a significant increase in the species richness over time. They again show varying rates of increase with group 1 the slowest followed by group 4.

4.2.2.1.4 Number of individuals

Over time there has been a fluctuation in the number of individuals $50cm^{-2}$ for each group (Figures 4.16 to 4.18). Some groups show peak years when a particularly high number of individuals occurred. From comparing all groups (Figure 4.19) it can be seen that peak years include 1980, 1982, 1986 and 1992. Groups 2, 4 and 7

showed peaks of 2139, 2269, 2033 individuals $50cm^{-2}$ respectively in 1980, but group 3 showed the largest ever peak of 3126 individuals $50cm^{-2}$. In 1982, group 2 had a peak of 1323 and group 7 of 859 individuals $50cm^{-2}$. Only Group 7 had a high number of individuals $50cm^{-2}$ from 1984 to 1988 with a peak of 2133 individuals $50cm^{-2}$ in 1986. Lastly in 1992 group 2 and group 10 showed peaks of 859 and 1213 individuals $50cm^{-2}$ respectively.

4.2.2.1.5 Individual species

The change in the individual species abundance of the common species for all groups can be seen in figures 4.20 to 4.23. The abundance of *Macoma balthica* remained fairly constant until 1994, after which its abundance increased in most groups. Group 1 had the lowest abundance of *M. balthica* out of the 10 groups, with generally less than 5 individuals $50cm^{-2}$.

Most groups had a low abundance of *Cerastoderma edule*, with only groups 5,.9 and 10 showing an abundance greater than 5 $50cm^{-2}$. The most notable peaks are of 44 individuals $50cm^{-2}$ in 1985 by group 10, of 18 individuals $50cm^{-2}$ in 1981 by group 9 and lastly of 14 individuals $50cm^{-2}$ in 1990 by group 5.

The abundance of *Hydrobia ulvae* shows peaks between 70 - 177 individuals $50cm^{-2}$ in the years 1980/81, 1985/86, 1990/92, 1994/95. Although not all groups showed the increased abundance at each of these times a majority did. Groups 1 and 2 had a low abundance throughout the monitoring period.

The abundance of *Nereis diversicolor* has shown much variation over the years. Groups 2, 6, 8 and 10 seem to have a higher abundance $50cm^{-2}$ than the other groups.

The change in the abundance of Oligochaetes also shows much variation. All of the groups showed a peak abundance around 1985/86 of between 200 to 400 individuals $50cm^{-2}$, except for group 1 and 2 which had peaks in 1982 of 304 and 222 individuals $50cm^{-2}$ respectively. Groups 1 and 4 had a relatively low abundance compared to most groups.

The abundance of *Nephtys hombergii* appears to have increased over the years for most groups since 1984. The majority of individuals of this species were found in groups 3, 4, 5, 6, 7 and 10. Peak abundances occurred in 1985 for groups 3, 4, 5, and 10 and in 1992 for groups 4,5 and 6.

The change in the abundance of the spionid *Pygospio elegans* shows two distinct peaks. One was in 1985 that included groups 3, 4, 7 and 10 and a smaller peak occurred in 1995 for groups 3, 5, 6, 8 and 9. The abundance of the spionid *Streblospio shrubsolii* shows a very different pattern. This species was not present in any of the groups until 1994. After this date however the abundance of *S. shrubsolii* increased for all groups. Some groups still had a relatively low abundance, specifically groups 1, 2 and 4.

Manayunkia aestuarina has shown an extremely high peak abundance of 1926 - 2859 individuals $50cm^{-2}$ in 1980 in four of the groups, namely 2, 3, 4 and 7. After 1980 groups 2 and 7 remained as having the highest abundance but at lower levels than those found in 1980. All groups showed a decline in abundance after 1994 and remained at very low levels for the rest of the monitoring period.

Groups 5 and 10 showed a large peak in *Eteone longa* abundance of 17 and 14 individuals $50cm^{-2}$ in 1980. Most groups showed a fluctuating abundance throughout, although group 8 has shown an overall increase in abundance over time having a very high abundance from 1994 to 1999.

The abundance of *Corophium volutator* has shown an increase for most groups after 1984 but especially for group 8, which showed the highest abundance over all groups until 1994. After 1994 the abundance for groups 2, 3, 5, 6 and 10 increased again.

4.2.2.1.6 MDS

The multivariate MDS plots showing the change in the community composition over time for each group can be seen in figures 4.24 to 4.27. Each group shows a slightly different pattern of community change. The MDS plot for group 1 shows that the majority of the years have a similar community composition, however the years 1976 to 1978 and 1995 to 1999 are slightly apart from the main group. This

suggests that there was a change in the community between 1978 and 1979 and again between 1994 and 1995. Group 2 shows a similar pattern except that this time 1981 is grouped with 1976 to 1978 and 1994 to 1999 are grouped together. This implies that there was a change between 1993 and 1994 and another between 1978 and 1979. After the change in 1979 the community undergoes large fluctuations until 1982 when it seems to stabilise. Group 3 shows the same pattern of change except that this time the large fluctuations did not stabilise until 1983. Group 4 shows a completely different pattern of change. It seems that there has been little change in the community composition with only a few years standing out as being different (1979 1980, 1985, 1986). This may suggest that there was a change in the community between 1978/79 and 1984/85, however the change did not last long and the community eventually went back to its previous state. Group 5 showed yearly fluctuations until 1993/94, after which a change in the community composition occurred. The MDS plots for both groups 6 and 8 show a pattern similar to that of group 2, whereby a change seems to have occurred between 1978 and 1979 and again between 1993 and 1994. Group 7 again is very similar except that the change occurred between 1979 and 1980 and between 1993 and 1994. Group 9 shows yearly fluctuation until 1987 after which there was a gradual change until 1993. Then there was a change in the community between 1993 and 1994. Group 10 showed another different pattern, with changes occurring between 1981 and 1982 and again between 1994 and 1995.

4.2.2.1.7 SIMPER

SIMPER analysis was used to assess exactly what individual species changes occurred between the years identified when a change occurred in the MDS plots. For group 1 (Table 4.6), there was an increase in the abundance of Oligochaetes, *M. aestuarina* and *H. ulvae* and a decrease in *N. hombergii* between the periods 1976-1978 and 1979-1994 and Oligochaetes can be considered as a good discriminating species. Between the periods 1979-1994 and 1995 –1999 there was an increase in the abundance of Oligochaetes, *H. ulvae*, *P. elegans* and *S. shrubsolii* and a decrease in *M. aestuarina*.

Group 2 (Table 4.7) shows a similar increase in the abundance of *M. aestuarina* and Oligochaetes between 1976-1978 and 1979-1993 but also a decrease in *H. ulvae*. Between the periods 1979-1993 and 1994-1999 a decrease in *M. aestuarina* was

seen and an increase in Oligochaetes, *M. balthica* and *H. ulvae*. *M. aestuarina* was a good discriminating species for both time periods.

Between the periods 1976-1978 and 1979-1999 for group 3 (Table 4.8) there was an increase in the abundance of *M. aestuarina*, Oligochaetes, *H. ulvae* and *P. elegans*. Between the periods 1979-1993 and 1994-1999 there was a decrease in *M. aestuarina*, Oligochaetes and *P. elegans* and an increase in *S. shrubsolii*, *C. volutator*, and *M. balthica*. *M. balthica* was considered to be a good discriminating species.

Group 4 (Table 4.9) showed changes during slightly different years. Between the periods 1976-1978 and 1979-1984 there was an increase in the abundance of *M. aestuarina* and a decrease in Oligochaetes, *H. ulvae* and *P. elegans*. Between the periods 1979-1984 and 1985-1999 a decrease *in M. aestuarina* and an increase in the abundance of Oligochaetes, *P. elegans*, *H. ulvae* and *M. balthica* was seen.

Group 5 (Table 4.10) shows only one change between the period 1979-1994 and 1995-1999 which was marked by an increase in the abundance of Oligochaetes, *S. shrubsolii*, *P. elegans*, *M. balthica* and *C. volutator* and a decrease in *M. aestuarina* and *H. ulvae*. The species *S. shrubsolii*, *H. ulvae* and *M. balthica* can be considered as good discriminating species here.

Group 6 (Table 4.11) shows a change between 1976-1978 and 1979-1993 that can be attributed to the increase in the abundance of *M. aestuarina*, Oligochaetes and *H. ulvae*. *M. aestuarina* and Oligochaetes can be considered good discriminating species between these to time periods. Between the periods 1979-1993 and 1994-1999 there was a decrease in *M. aestuarina*, Oligochaetes and *H. ulvae* and an increase in *S. shrubsolii* and *M. balthica*. Of these species *M aestuarina*, *S. shrubsolii* and *M. balthica* were good discriminating species.

The change between the periods 1976-1979 and 1980-1993 for group 7 (Table 4.12) indicates that there was an increase in the abundance of *M. aestuarina* and Oligochaetes and a slight decease in *H. ulvae*. Between the periods 1980-1993 and 1994-1999 the abundance of *M. aestuarina*, Oligochaetes and *H. ulvae* decrease and

S. shrubsolii increase. *M. aestuarina* was a good discriminating species for the differences between the two different changes.

Group 8 (Table 4.13) shows a change between the periods 1976-1978 and 1979-1993 that can be attributed to an increase in *M. aestuarina*, Oligochaetes and *C. volutator* abundance and a decrease in *H. ulvae*. Whereas the change between 1979-1993 and 1994-1999 can be attributed to a decrease in *M. aestuarina*, *H. ulvae* and *C. volutator* and an increase in the abundance of Oligochaetes, *S. shrubsolii* and *M. balthica*. For the first change Oligochaetes and for the second change *M. balthica* were good discriminating species.

The SIMPER results for the changes between the period 1976-1986 and 1987-1993 for group 9 (Table 4.14) indicates that there was an increase in the abundance of *M. aestuarina* and *M. balthica* and a decrease in Oligochaetes, *H. ulvae* and *P. elegans*. Of these *Oligochaetes* were a good discriminating species. Between the periods 1987-1993 and 1994-1999 there was an increase in *S. shrubsolii*, Oligochaetes and *M. balthica* and a decrease in *M. aestuarina*, *H. ulvae* and *P. elegans*, with *S. shrubsolii* and Oligochaetes as the discriminating species.

Group 10 (Table 4.15) shows changes between 1976-1981 and 1982-1994 that can be attributed to the increased abundance of *M. aestuarina*, Oligochaetes, *H. ulvae* and *P. elegans*. The difference between the periods 1982-1994 and 1995-1999 was due to a decrease in *M. aestuarina*, Oligochaetes and *H. ulvae* and an increase in *S. shrubsolii*, *M. balthica* and *P. elegans*. *M. aestuarina* and Oligochaetes were good discriminating species for the first change and *S. shrubsolii* and *M. balthica* for the second change.

4.2.2.2 Community change in relation to environmental factors

The changes in the water quality, climatic and effluent data have been discussed in chapter 3 (See 3.2.2). The changes in the species diversity and the community composition have been compared to the effluent, water quality and environmental data sets, using stepwise regressions and the BIO-ENV analysis (Tables 4.16 to 4.21).

4.2.2.2.1 Climate

The BIO-ENV results for the North Atlantic Oscillation (NAO) data did not produce any significant correlations and the Stepwise regressions produced only one significant regression model with a high Rsq(adj) for group 3 over the period 1995-1999. The air temperature data produced similar results with the multivariate method producing no highly correlated models and the stepwise regressions only producing this time two significant models with high Rsq(adj) values. The two models were for groups 3 and 9 for the period 1995-1999.

4.2.2.2.2 Refinery effluent

The refinery effluent data produced high correlation coefficients from the BIO-ENV analysis for groups 3, 5, 7, 8, and 10 over the period 1993-1999 and for all groups except 6, 7, and 10 over the period 1995-1999. The multiple regression analysis also produced significant models with high Rsq(adj) values for the period 1993-1999 for groups 4, 6, and 7 and for the period 1995-1999 for groups 3, 7, 8, and 9.

4.2.2.2.3 Ballast water

The ballast water data produced only two significant regression models during the period 1995-1999 for groups 1 and 3. The BIO-ENV analysis however found that groups 1, 3, 4, 5, 6, and 8 all had a significant correlation with the ballast water data.

4.2.2.2.4 Chemcial effluent

The chemical effluent was significantly correlated with all of the groups during the period 1995-1999, except group 8 using the multivariate method. The regression analysis found that the only groups 1, 4, 5 and 8 produced models with a high Rsq(adj). The chemical BOD (Chemical/Avon data) produced significant models that were fairly accurate at predicting the change in the diversity, for most groups, for the entire monitoring period (1976 to 1999).

4.2.2.2.5 River Avon

The data for the water quality of the river Avon was found to be significant with a large Rsq(adj) over the period 1985-1999 for groups 3 and 9. Also for the period 1993-1999 for groups 2, 3, 4, 5, 6, and 9 and from 1995-1999 for groups 3, 4, 5, 6, 7, and 9. The BIO-ENV analysis produced significant correlations for group 3 from 1993-1999 and for groups 2, 3, 5, 6, 7, 8, 9 and 10 from 1995-1999.

4.2.2.2.6 Grange burn River

The data from the smaller river Grange burn did not produce any significant regression and only one significant BIO-ENV result during the period 1995-1999 for group 7.

4.2.2.2.7 Kinneil channel

The data for the Kinneil channel did not correlate with the change in community composition for any time period or any group using the BIO-ENV analysis. The stepwise regressions produced only four significant models with high Rsq(Adj) values. These were during the period 1980-1999 for groups 3, 5 and 6 and during the period 1995-1999 for group 7.

4.2.2.2.8 Kinneil sewage works

The sewage works water quality data did not produce any significant BIO-ENV or regression results for the period 1985-1999. Group 7 produced a significant stepwise regression model for the period 1993-1999 and groups 3, 5, 6, 7, and 10 produced a significant correlation coefficient with the BIO-ENV analysis. For the period 1995-1999 the only significant correlation was for group 5 and the only significant regression models were for groups 7 and 10.

The significant stepwise regression models for each group can be seen in Tables 4.22 to 4.24. The variables that were identified by the significant BIO-ENV results can be seen in Tables 4.25 to 4.27. It can be seen that there is no distinct variable that stands out as being important for all groups.

4.3 DISCUSSION

4.3.1 Spatial pattern of community composition

The spatial distribution patterns observed in areas that are subjected to disturbance, including pollution, can often be explained by the intermediate disturbance hypothesis (Connell, 1978). This hypothesis predicts that where disturbance severity is high, i.e. close to an outfall, a low diversity is found. As the severity decreases i.e. with increasing distance, the diversity increases, whilst at very low disturbance levels the diversity would decrease again. This spatial change in diversity can be

explained by succession. Pearson & Rosenberg (1978) observed that in areas subjected to organic pollution a successional gradient with distance from the source was found. Close to the pollution source, at high disturbance levels, the community was kept at the start of succession, where diversity was low. With increasing distance and decreasing disturbance, the level of succession that is attained by the community increased. As with temporal succession the same main stages were seen, the peak of opportunists, the ecotone point and the transition zone (See 3.3.2). The state of a benthic community around a refinery outfall often shows a change in the community composition with distance comparable to that of the intermediate disturbance hypothesis and succession. Generally the area in close proximity to the outfall has a low diversity or is even devoid of fauna, whilst with increasing distance the diversity and abundance increases (Wharfe, 1975; Petpirron & Dicks, 1982; Saha & Konar, 1984a). Most areas that have been studied that are affected by refinery effluents show this pattern of spatial distribution of the community, although the extent and the size of the affected area is variable (Wharfe, 1975; Petpiroon & Dicks, 1982; Saha & Konar, 1984a; Talsi, 1987; Dicks & Levell, 1989). McLusky (1982a) found the same spatial distribution in the benthic community at Kinneil around the petrochemical outfalls, which was also observed in the present study. At the beginning of the monitoring period in 1976 groups 1 and 2, the areas closest to the refinery and chemical outfalls, had the lowest diversity, evenness and species richness. Whilst groups 5 and 8, those furthest from the outfalls, had the highest diversity, evenness and species richness. The abundance of the opportunistic species (Oligochaetes and *Manayunkia aestuarina*) was generally higher for the groups 3 and 7, which are at an intermediate distance from the two outfalls. The abundance of the larger non-opportunistic species, such as *Macoma balthica*, *Hydrobia ulvae* and *Nephtys hombergii*, was higher for the groups 5, 8 and 9, which are furthest from the outfall. It can be concluded that there was a spatial difference in the community composition at Kinneil similar to that observed at other sites affected by refinery effluents, and that the intermediate disturbance hypothesis and succession can explain this spatial distribution pattern.

4.3.2 Spatio-temporal changes in community composition

4.3.2.1 State of the benthic community

As a spatial distribution pattern has been detected in the community composition at Kinneil it is possible that these different areas will have shown different temporal changes. The temporal change in the annual mean abundance data for the whole mudflat indicated that there was an increase in the diversity, evenness and species richness that was indicative of an increase in the health of the community (See 3.3.1). This increase was observed in all ten groups, indicating that the whole intertidal area was impacted to some level and that over time it had reduced. Although all ten groups have shown an improvement in the state of the benthic community three groups still show signs of being impacted. Group 1 is the most highly impacted area, with the lowest diversity, whilst groups 2 and 4 are slightly less impacted. The other seven groups, in 1999, all had similar values of diversity, evenness and species richness, which may indicate that the community composition of the majority of the Kinneil mudflat has reached the same successional phase and possibly a steady state (Connell & Slatyer, 1977). It can therefore be concluded that the recovery of the benthic community that was detected as an increase in diversity and species richness by the analysis of the annual mean abundance for the whole mudflat did occur across the whole area. There were however spatial differences in the initial level of impact and the extent of the recovery.

4.3.2.2 Succession

The analysis of the annual mean abundance data for the whole area indicated that temporal succession had occurred. The MDS indicated that there were community composition changes in 1979 and 1994, which were marked by the appearance of *Manayunkia aestuarina* and *Streblospio shrubsolii*. A peak of opportunists was identified in 1980, which was followed by an increase in the abundance of the non-opportunistic species (See 3.3.2). The present spatio-temporal analysis also indicates that there was a change in the species composition in 1979 for all groups, except 9 and 10, which was caused by the increase in *M. aestuarina*. In 1994 all groups except group 4 showed a change in the community structure, which was caused by the appearance of *S. shrubsolii*. The temporal MDS analysis of the

individual smaller areas of the mudflat did highlight that in three groups additional community changes had occurred. Group 10 showed a change in 1982, Group 4 in 1985 and group 9 in 1987. The peak of opportunists in 1980 was also detected, although not in all groups indicating that it was restricted to a specific area of Kinneil intertidal area. The opportunist peak was only detected in groups 2, 3, 4 and 7, which span from the chemical outfall to the main channel. Smaller peaks of opportunistic species were also detected in 1982 in groups 2 and 7, 1986 in group 7 and 1992 in group 2. This shows that the peak of opportunists decreased in size and moved closer to the chemical outfall over time. An increase in the abundance of the non-opportunistic species was detected. All species showed an increase in abundance over the monitoring period in at least one group except *Hydrobia ulvae*, Oligochaetes and *Manayunkia aestuarina*. Although increases were seen for most species the groups in which they were seen varied and none of the species showed an increase in group 1, indicating a temporal difference in the community distribution.

It can therefore be concluded that the analysis of the annual mean abundance data for the whole area did detect the major changes that had occurred either to the entire area or to a large proportion of the area. The additional spatio-temporal analysis has however shown the different spatial distributions of the successional changes previously detected and has highlighted additional localised changes not previously detected.

4.3.3 Factors causing the changes in the benthic community

There are several different factors that could have caused the changes in the benthic community at Kinneil that have been detected. The same factors that were considered in chapter 3 are discussed in relation to the new spatial difference that have been found.

4.3.3.1 Climate

Although changes in the climate can effect the geographical distribution of species (De Jong, 1999), it is unlikely that it will cause any spatial differences in the benthic community at the small scale considered in this study. The air temperature and NAO was not found to be important in structuring the benthic community at Kinneil

as a whole (3.3.3.1). The analysis of the temporal changes in the abundance of smaller areas of the Kinneil intertidal areas also confirms this. None of the groups produced significant BIO-ENV results and only three groups in the period 1995 to 1999 produced significant multiple regression models. It is therefore concluded that the change in the air temperature and NAO index were not responsible for the temporal or spatial changes in the benthic community, although other climatic factors can not be totally ruled out.

4.3.3.2 Improved quality of the effluents

4.3.3.2.1 Refinery effluent

As has previously been discussed, refinery effluents have been shown to have spatial effects on the distribution of a community (See 4.3.1). The typical pattern of increasing diversity with distance from a refinery outfall (Wharfe, 1975; Petpiroon & Dicks, 1982; Saha & Konar, 1984a; Talsi, 1987; Dicks & Levell, 1989) is probably due to the decrease in toxicity and load with distance. Once the effluent is discharged it undergoes modifications through several different processes which reduce its toxicity. The effluent is firstly diluted within the receiving water, which will reduce its toxicity dramatically (McLusky & Colbourne, 1995). Any volatile chemicals will be lost through weathering and the remaining chemicals will undergo sedimentation and biodegradation (Cranthorne *et al.*, 1989). The concentration of the chemicals that enter the sediments are usually greater closest to the discharge point and decrease with distance. Le Dreau *et al.* (1997) found that in the Gulf of Fos, Southern France, there were three zones of hydrocarbon contamination of the sediment. A highly contaminated zone near the refinery outfall, followed by a less contaminated zone and then finally a slightly contaminated zone. This means that the toxicity will be highest in the area closest to the outfall. The reduction in the toxicity of the effluent will have an impact on this spatial pattern. In other areas where the toxicity of the refinery effluent has been reduced, the spatial pattern has remained but the areas affected became smaller (Dicks, 1976a). If the toxicity is removed then the whole area that was impacted will eventually recover (Dicks & Levell, 1989). The analysis of the mean abundance data for the whole mudflat indicated that the reduction in the toxicity of the refinery effluent in 1994, by the addition of the biological treatment plant, allowed *Streblospio shrubsolii* to colonise

and then further succession of the benthic community occurred (See 3.3.3.2.1). The temporal analysis of smaller areas of the mudflat has also found that all groups, except groups 9 and 10 showed a change in the community composition in 1994 that was due to *S. shrubsolii*. It was observed however that this species colonised the area around the refinery and chemical outfalls (groups 1 and 2) last and remained at relatively low abundance levels there until the end of the monitoring period. The diversity and species richness for group 1 were also the lowest of all groups throughout the monitoring period. The results of the multiple regression and BIO-ENV analyses for the period 1995 and 1993 to 1999 indicate that the refinery effluent was probably important in structuring the communities of all ten groups. It can therefore be concluded that the refinery effluent has been an important factor affecting the benthic community across the entire mudflat. It appears that the reduction in the toxicity of the effluent in 1994 caused an increase in diversity and allowed the introduction of the new species. There has been a spatial effect with the area around the outfall (group 1) showing the highest level of impact throughout the study.

4.3.3.2.2 Ballast water

As previously discussed (See 3.3.3.2.2) the effects of the ballast water can not be determined as it is discharged along with the refinery effluent and they will therefore act together. The lack of data before 1995 also restricts this analysis and means that its likely impact before this period can not be determined.
The multiple regression results for the period 1995 to 1999 only produced models with a high coefficient of determination for groups 1 and 3. The BIO-ENV analysis however produced significant correlations for all groups except 2, 7 and 9. It therefore seems that the ballast water may have played a role in structuring the community since 1995, particularly around the refinery outfall (Group 1).

4.3.3.2.3 Chemical effluent

As with the refinery effluent the chemical effluent will also have a spatial effect on the benthic community. The toxicity of the chemical effluent, which is known to be greater than the refinery effluent (Smith, 1987), is likely to cause a reduced diversity and abundance close to the outfall. As the chemical effluent is more toxic it might be expected that it would have a more pronounced effect on the benthos than the refinery effluent. The refinery effluent however is discharged in greater volume than

the chemical effluent and therefore its toxicity may be more readily apparent within the water column and sediments. The temporal analysis indicated that the reduction in the toxicity of the chemical effluent during the monitoring period may have been responsible for the increased diversity for the whole mudflat (See 3.3.3.3). The toxicity of the chemical effluent was reduced in 1985 when the phenol plant was closed and again in 1992 when the acetonitrile plant was closed. The MDS analysis indicated that group 4 showed a change in its community composition in 1985 which was due to a decrease in *Manayunkia aestuarina* and an increase in Oligochaetes, *Pygospio elegans, Hydrobia ulvae* and *Macoma balthica*. It could be hypothesised that this change in the community was due to the reduced toxicity of the chemical effluent at this time. The multiple regression and BIO-ENV analyses on the individual areas of the mudflat, using the chemical BOD for the 1976 to 1999 period, indicate that the overall decrease in BOD is negatively correlated with the increase in diversity over time. This was seen for most groups but the highest correlations or coefficients of determination were found for groups 6, 7 and 8. The more detailed chemical composition data for the chemical effluent, which was only available for the period 1995 to 1999, also produced significant regression models and BIO-ENV results for most groups especially 2, 4 and 7. These are the three groups which are closest to the track of the chemical effluent. The apparent decrease in the size of the area that the effluent is impacting is also substantiated by the temporal change in the spatial distribution of the peak of opportunists (See 4.3.2). The reduction of the size of the area and the movement of the peak of opportunists toward the chemical discharge are consistent with a reduction in the toxicity of this effluent. It can therefore be concluded that the toxicity of the chemical effluent has been important in determining the temporal changes that have occurred in the benthic community. It has affected the whole mudflat although it seems that the reduction in the toxicity of the effluent has also reduced the size of the area it is impacting.

4.3.3.3 Movement of the chemical outfall

The movement of the outfall from the river Avon site to the site closer to the refinery outfall in 1979 may have affected the spatial distribution of the benthic community. McLusky (1982a) showed that after the movement of the outfall in 1979 there was an increase in the diversity and species richness in the area around

the old outfall. Around the new outfall site a change in the species composition occurred, both *Macoma balthica* and *Nereis diversicolor* disappeared, whilst *Manayunkia aestuarina* appeared and *Hydrobia ulvae* and oligochaetes persisted. The present study has shown that most groups showed a change in the community composition around 1979 except for groups 9 and 10. This change in the benthic community can be attributed to the arrival of the new species *Manayunkia aestuarina*. It seems probable that this change in the community composition was caused by the disturbance of moving the chemical outfall. Therefore it seems that the movement of the outfall did cause a change in the community not only in the areas next to the new and old outfall sites but also across a large area of the Kinneil mudflat.

4.3.3.4 Other pollution sources

The other sources of pollution such as the rivers, estuary and sewage works may also cause spatial differences in the benthic community.

4.3.3.4.1 River Avon

The changes in the water quality of the river Avon did not seem to have a detectable effect on the mudflat as a whole (See 3.3.3.4.1). It is possible however that the water quality of the river may have had a localised effect. The multiple regression and BIO-ENV results indicate that the water quality of the Avon was correlated with the changes in the benthic community from 1985 for groups 3 and 9. These two groups include stations that are closest to the Avon River track that runs across Kinneil. This could also be the cause of the change in the community composition that was detected in 1987 for group 9, that was attributed to an increase in the abundance of *Manayunkia aestuarina* and *Macoma balthica* and a decrease in Oligochaetes, *Hydrobia ulvae* and *Pygospio elegans*. The majority of the groups did produce significant models from 1993 and 1995. It therefore seems that the River Avon may have had a localised effect on the benthic community since 1985 and the changes in its water quality may have caused the change in the community composition of group 9 in 1987. It also seems that the impact from the River Avon has become more important in recent years, which was also found by the temporal analysis of the whole area.

4.3.3.4.2 Grange burn

The Grange burn River was not shown to have a detectable temporal effect on the whole Kinneil mudflat benthic community (See 3.3.3.4.2). Again like the Avon River it may have had a localised effect. The multiple regression and BIO-ENV analysis did not however produce any evidence that this was the case since 1985. The MDS results indicated that group 10, which is close to the Grange burn, showed a change in the community in 1982 that was caused by the increase in abundance of *Manayunkia aestuarina*, Oligochaetes, *Hydrobia ulvae* and *Pygospio elegans*. It could be hypothesised that it was a change in the water quality of the Grange burn that caused this change, however, due to the lack of environmental data before 1985 this could not be tested. It can therefore only be concluded that the water quality of the Grange burn has had no significant spatial or temporal effects on the community composition of the Kinneil intertidal area since 1985.

4.3.3.4.3 Kinneil channel

As previously discussed the Kinneil channel water quality data represents pollution from many different sources including from the petrochemical effluents (See 3.3.3.4.3). The temporal analysis indicated that the general increase in the water quality of the Kinneil channel may have affected the invertebrate community at Kinneil. From considering the individual areas at Kinneil the multiple regression analysis produced significant models with high coefficients of determination for groups 3, 5 and 6 for the period 1980 to 1999. All models use the change in the concentration of ammonia. During the following time period (1985 to 1999) the models for these three groups are less accurate i.e. they have a lower coefficient of determination (Rsq(adj)). This suggests that the water quality of the Kinneil channel, particularly the concentration of ammonia may have had only a localised effect on the benthic community.

4.3.3.4.4 Kinneil sewage works

Organic effluents can cause spatial effects in benthic communities (Pearson & Rosenberg, 1978). The temporal analysis indicated that the Kinneil sewage works did not seem to have an important effect on structuring the benthic community across the whole of Kinneil (See 3.3.3.4.4). It is likely however that the organic effluent may have had a localised effect around the outfall, represented by group 8.

Although the water quality of the sewage effluent does produce a significant model for group 7 it does not seem to have affected group 8. This lack of temporal change is probably due to the lack of change in the quality of the organic effluent during the study period. Therefore although no temporal change was seen it is still possible that the sewage works was impacting the area. A spatial change in the benthic community around the outfall could not have been detected by the spatio-temporal analysis due to the large number of stations in group 8, of which only a few are close to the sewage outfall. It can not be ruled out that that the sewage works is having a localised impact on the benthic community, which may change if the effluent quality changed.

4.4 CONCLUSIONS

In response to the main objectives for this chapter it is possible to conclude that firstly not all the groups have shown the same changes in the community structure. Most notably group 1 has been the most impacted group throughout the monitoring period and still is today. Groups 2 and 4 are also still impacted but not to the same extent as group 1. As all groups have shown an improvement the whole of the mudflat must have been affected to some extent at the beginning. The majority of the mudflat now has the same community structure and it is hypothesised that these areas have completely recovered to a stable community.

The different groups have shown slight differences in when they have shown changes in the community composition. Most showed a change around 1979 and/or 1994 and a few have had changes in the 1980s. Different species have been responsible for the changes at the different times, but generally the same species have been responsible for the changes at the same time for the different groups. These changes have involved either an increase in the opportunistic species or the decrease in the opportunistic species and a subsequent increase in the less pollution tolerant species.

It seems from the environmental analysis that it is the same selection of pollution sources that have been acting over the entire mudflat. These are the chemicals and refinery effluent along with the ballast water. However the data for these pollution

sources is incomplete so that their role at the beginning of the monitoring period is hard to determine. The fact that the changes in the community happened when known events in the chemicals and refinery effluents occurred suggests that they have been the major source of the community change. The spatial distribution of the opportunistic species suggests that the distance from the effluents has been important in determining the level of impact.

All this again confirms the hypothesis that was proposed in chapter 3. The movement of the chemicals outfall in 1979 caused the area around the new oufall to become affected, which can be seen by the large peak of opportunists that was localised to the area where the chemical effluent was discharged. The chemical effluent was having a toxicity and enrichment effect at this time. The number of opportunists increased with distance from the outfall as the toxic effect suppressed their numbers close to the discharge point. Until 1994 the refinery effluent was also having a combined enrichment and toxicity effect. The addition of a biological treatment plant to the refinery effluent in 1994 reduced the toxicity of this effluent. This caused a reduction in the number of opportunistic species and the increase in less tolerant species across the whole areas except for the outer edge stations (Group 4).

It is also hypothesised that there was a change in the water quality of the Grange burn around 1982 which caused the enrichment of group 10. There was a reduction in the toxicity of the chemicals effluent in 1985 which caused the increase in less pollution tolerant species in group 4. Lastly that there was a change in the water quality of the River Avon in 1987 which caused an effect in group 9. The general increase in diversity, number of species and evenness during the 1980s was due to the cleaning up of the chemicals effluent and the River Avon at this time, which together reduced the enrichment effect.

The spatio-temporal analysis is in general agreement with results from the temporal analysis of the whole area. It has however also highlighted some localised changes that have occurred during the monitoring period that were not detected in the mean analysis. This data could not detect if any change had occurred due to the movement of the chemicals outfall in 1999, as there was insufficient post movement data. To

assess the effects of this disturbance to the community further monitoring was needed, the results of which are considered in chapters 5, 6 and 7.

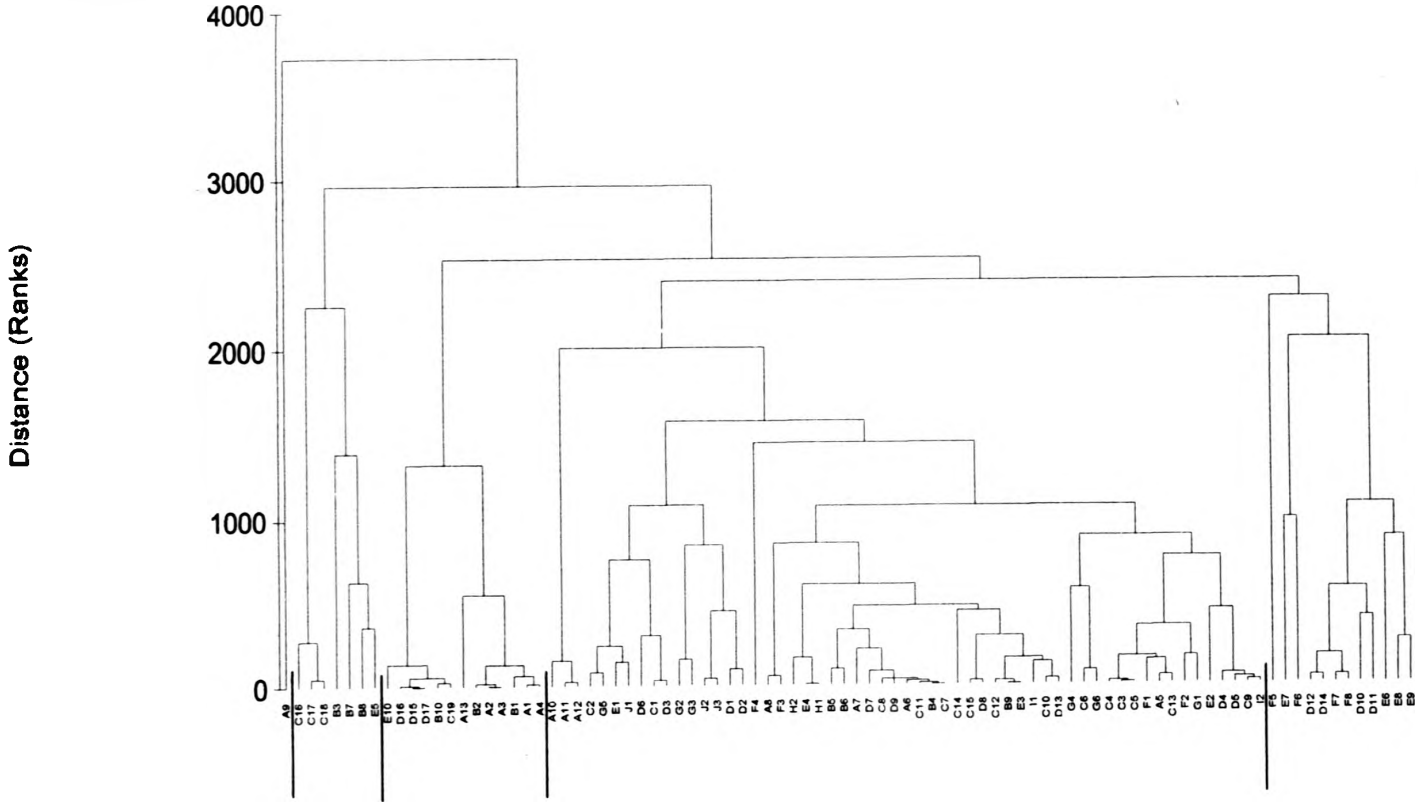


Figure 4.1. Dendrogram of the results of the cluster analysis on the community data of all 90 stations for 1994.

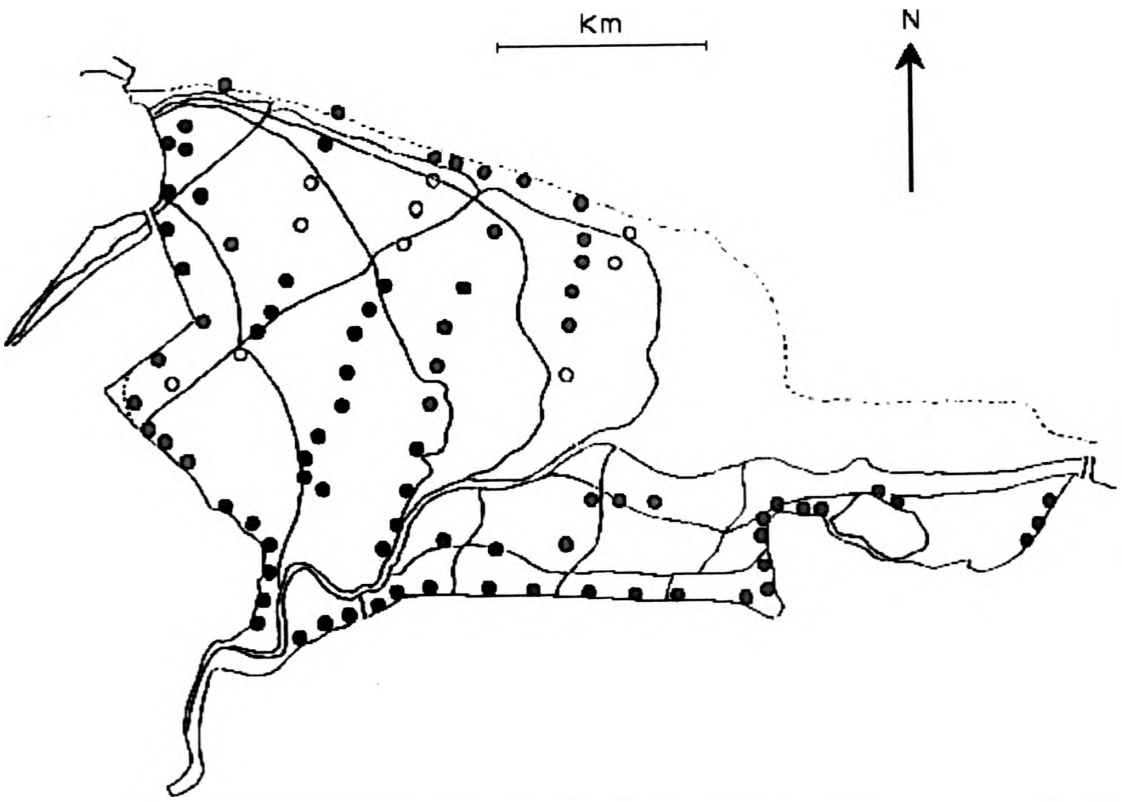


Figure 4.2. Map showing the group of the stations into five groups from the cluster analysis. Group 1 – Red, Group 2 – Yellow, Group 3 – Green, Group 4 – Blue, Group 5 - Pink

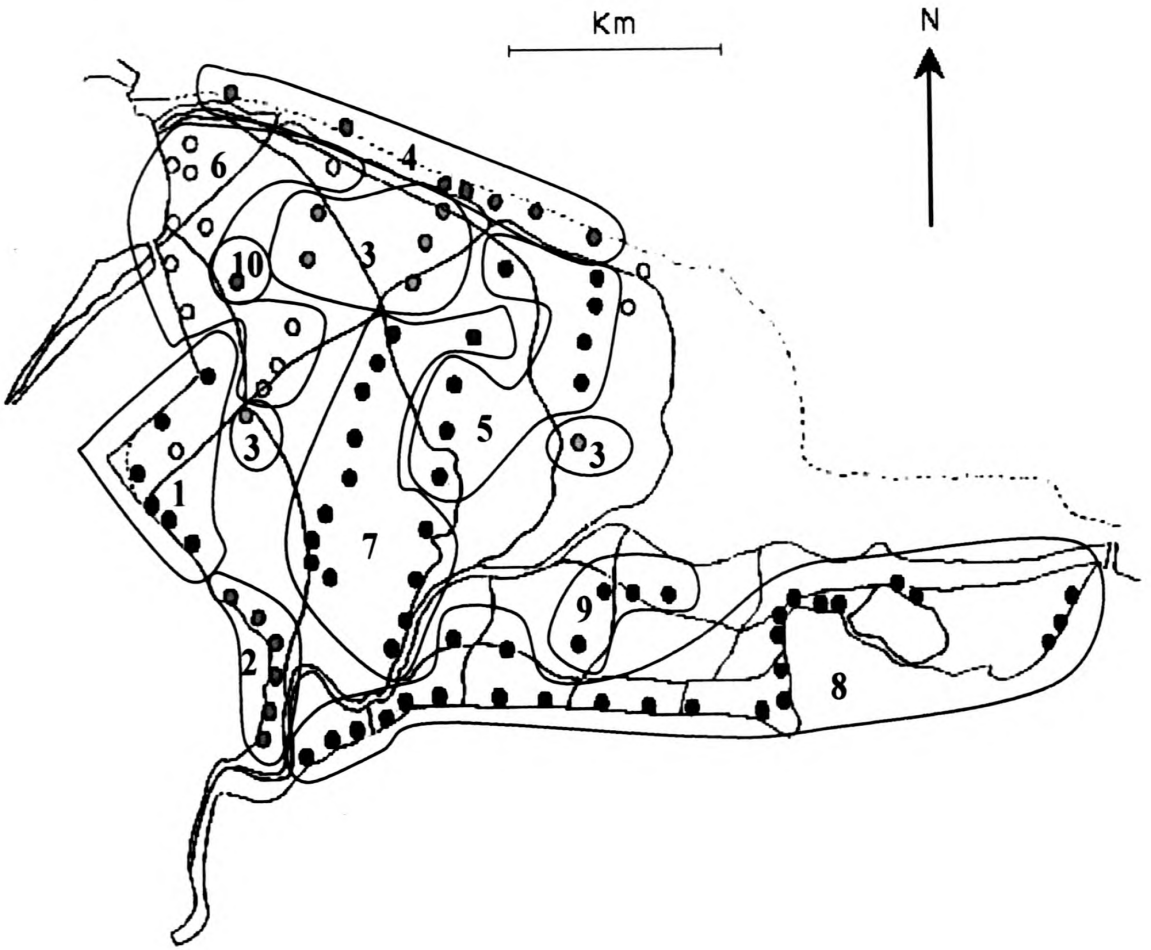


Figure 4.3. Map showing the grouping of stations into the ten groups. Group 1 – Black, Group 2 –Pink, Group 3 – Orange, Group 4 – Light blue, Group 5 – Purple, Group 6 – Yellow, Group 7 – Light green, Group 8 – Dark blue, Group 9 – Dark green, Group 10 – Red, Not sampled - White.

Table 4.1. The grouping of the stations into the five groups.

Group	Stations
1	A1-4, A13, B1, B2, B10, C19, D15-17, E10
2	B3, B7, B8, C16-18, E5
3	A5-8, A10-12, B4-6, B9, C1-15, D1-9, D13, E1-4, F1-4, G1-6, H1-2, I1-2, J1-3
4	D10-12, D14, E6-9, F5-8
5	A9

Table 4.2. The grouping of the stations into the 10 groups.

Group	Stations
1	A1-4, B1, B2
2	C1-6
3	B3, B7, B8, C16-18, E5
4	A13, B10, C19, D15-17, E10
5	D10-12, D14, E6-9
6	A5-8, A10-12, B4-6, B9
7	C7-15, D6-9, D13
8	D1-5, E1-4, F1-4, G1-6, H1, H2, I1, I2, J1-3
9	F5-8
10	A9

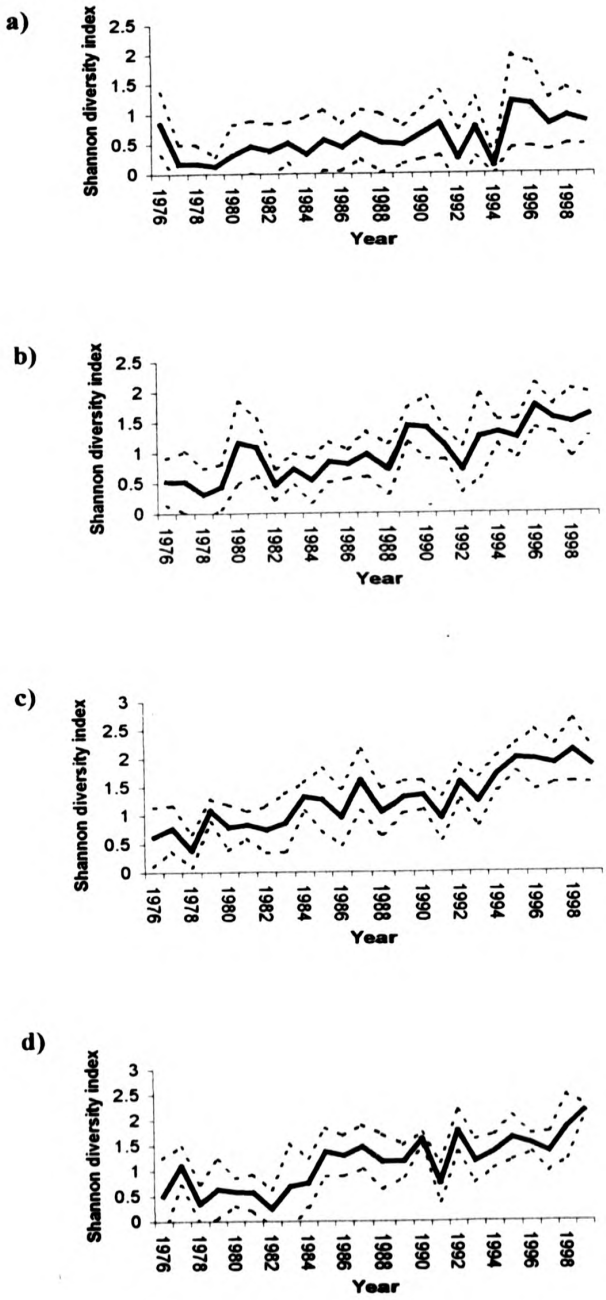


Figure 4.4. Change in the mean diversity (—) over time with 95% confidence limits (---) for a) Group 1, b) Group 2, c) Group 3, d) Group 4.

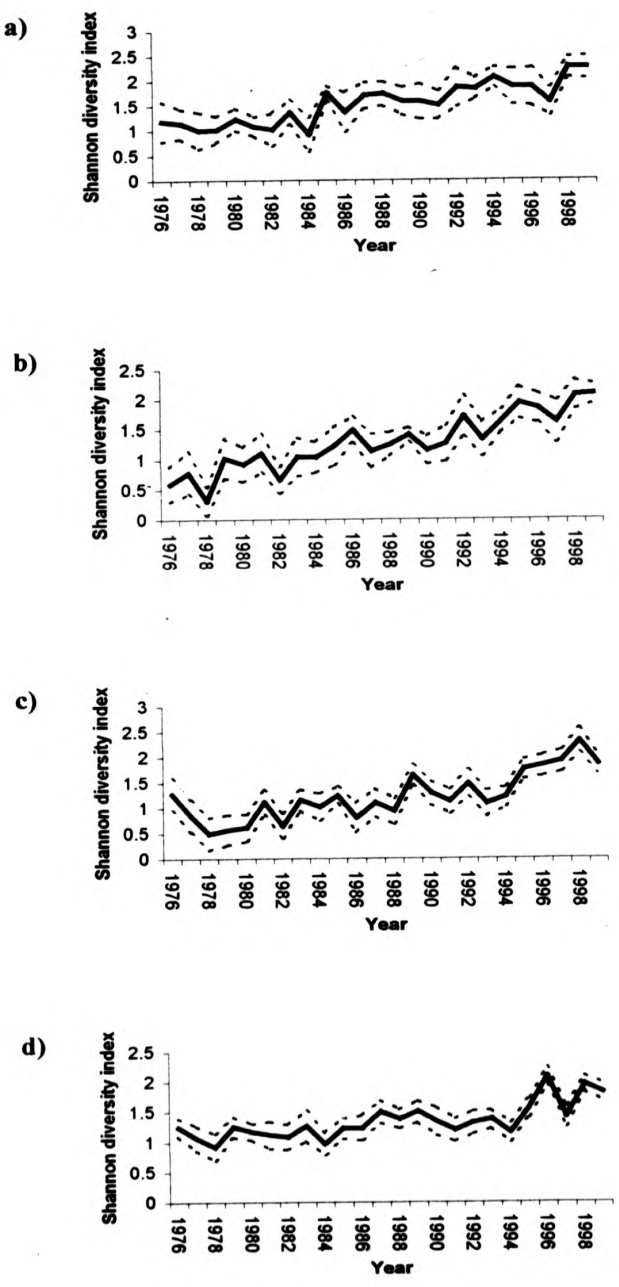


Figure 4.5. Change in the mean diversity (—) over time with 95% confidence limits (---) for a) Group 5, b) Group 6, c) Group 7, d) Group 8.

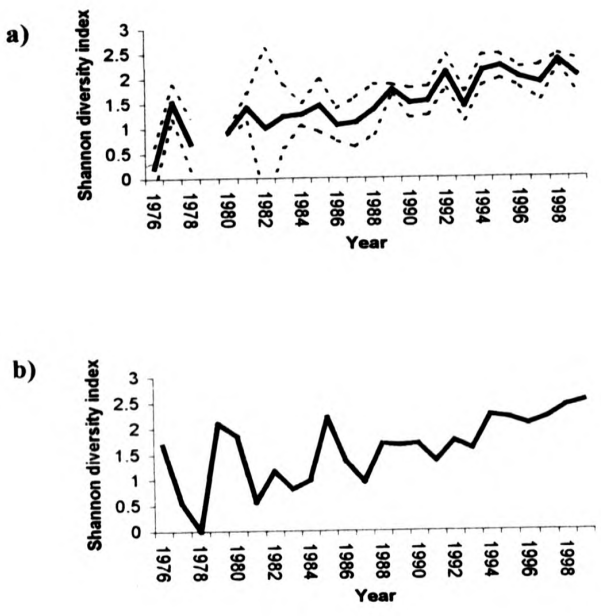


Figure 4.6. Change in the mean diversity (—) over time with 95% confidence limits (---) for a) Group 9, b) Group 10.

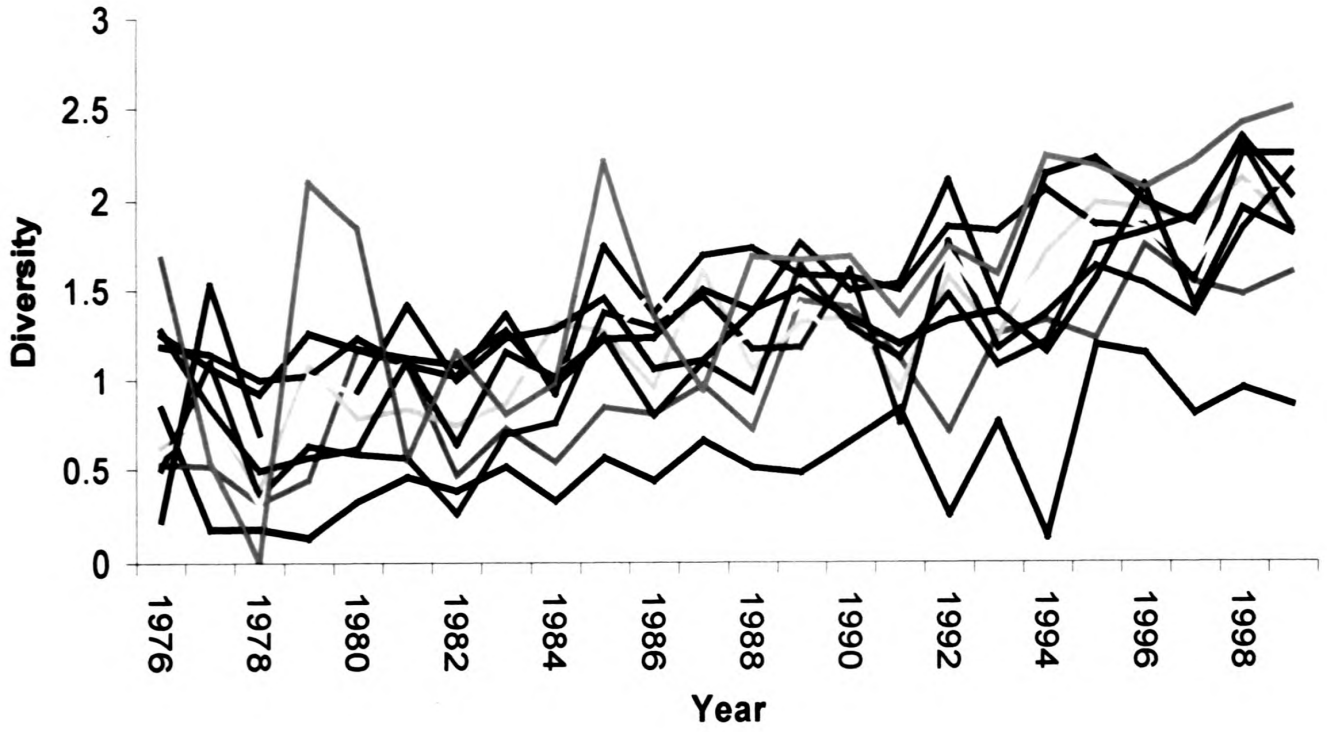


Figure 4.7. Change in the mean diversity over time for all ten groups. Group 1 – black, Group 2 – Pink, Group 3 – Orange, Group 4 – Light blue, Group 5 – Purple, Group 6 – Yellow, Group 7 – Light green, Group 8 – Dark blue, Group 9 – Dark green, Group 10 – Red.

Table 4.3. Regression results for the change in diversity over time.

Group	Regression results
1	Diversity = -52.2 + 0.0265 Year, p=0.002, F=12.53, Rsq=36.3%
2	Diversity = -95.4 + 0.0485 Year, p=0.000, F=42.07, Rsq=65.7%
3	Diversity = -120 + 0.0610 Year, p=0.000, F=77.63, Rsq=77.9%
4	Diversity = -116 + 0.0587 Year, p=0.000, F=41.82, Rsq=62.5%
5	Diversity = -95.6 + 0.0489 Year, p=0.000, F=65.63, Rsq=74.9%
6	Diversity = -117 + 0.0595 Year, p=0.000, F=101.95, Rsq=82.3%
7	Diversity = -104 + 0.0530 Year, p=0.000, F=37.49, Rsq=63%
8	Diversity = -57.6 + 0.0296 Year, p=0.000, F=24.73, Rsq=52.9%
9	Diversity = -124 + 0.0630 Year, p=0.000, F=48.85, Rsq=69.9%
10	Diversity = -121 + 0.0617 Year, p=0.000, F=16.90, Rsq=43.4%

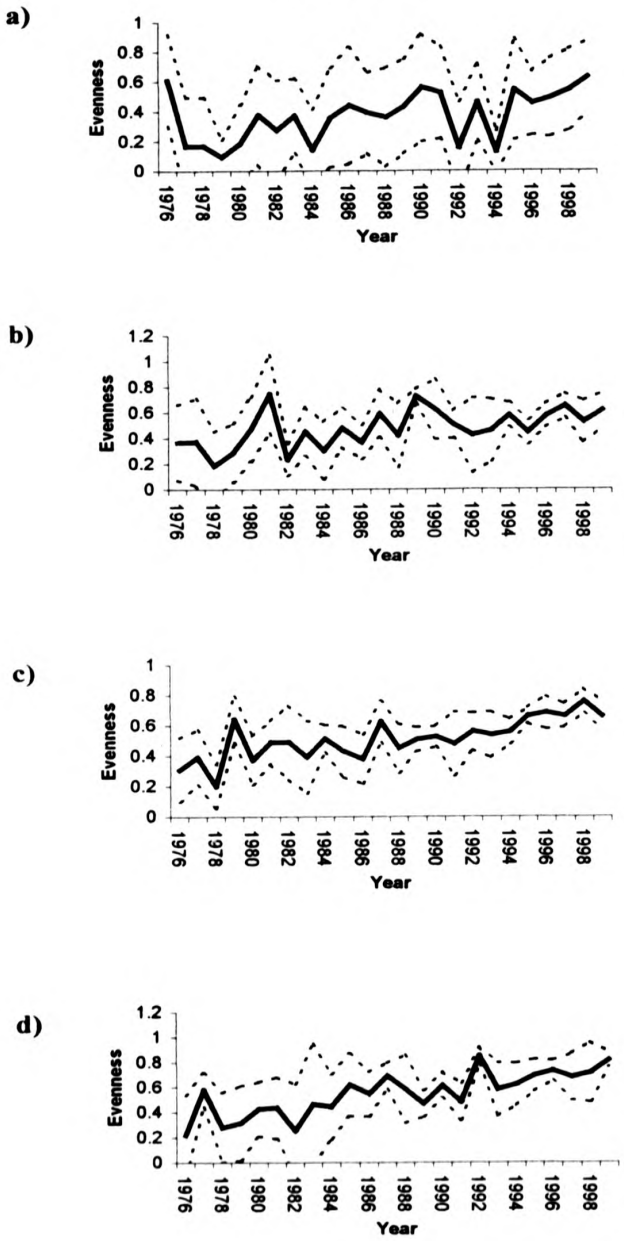


Figure 4.8. Change in the mean evenness (—) over time with 95% confidence limits (---) for a) Group 1, b) Group 2, c) Group 3, d) Group 4.

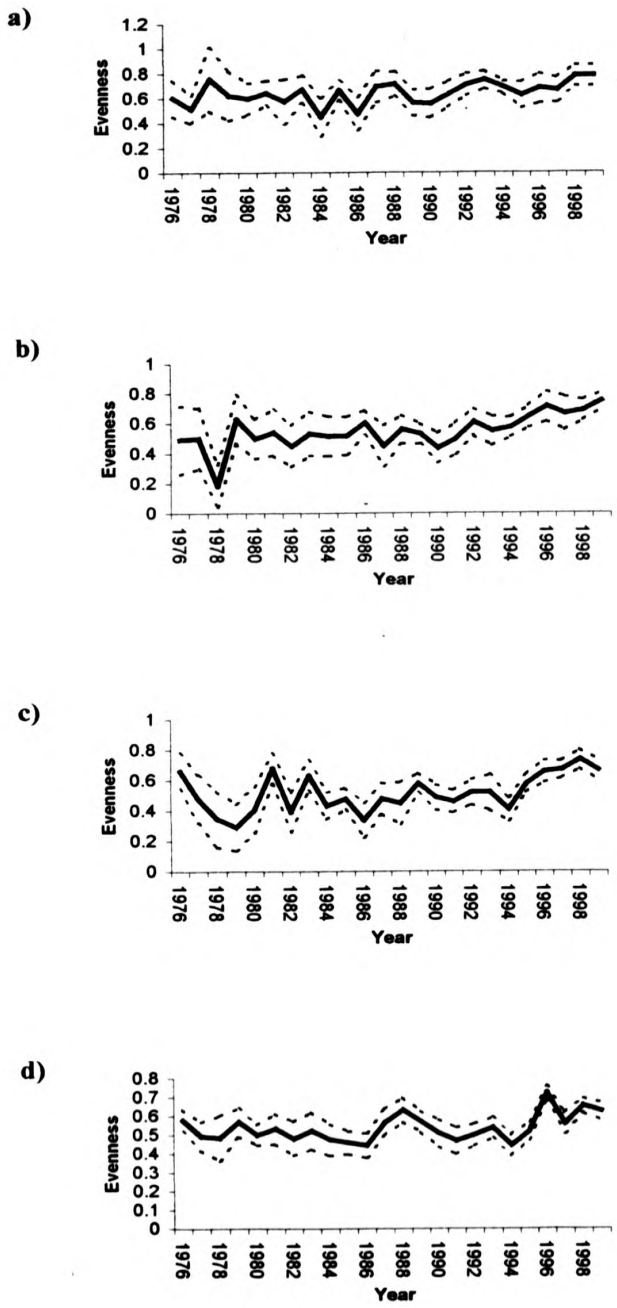
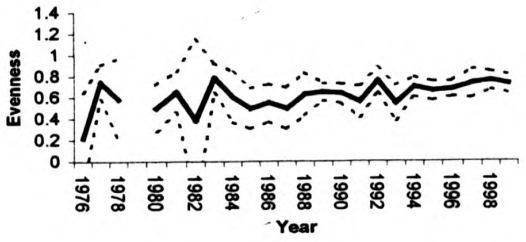


Figure 4.9. Change in the mean evenness (—) over time with 95% confidence limits (---) for a) Group 5, b) Group 6, c) Group 7, d) Group 8.

a)



b)

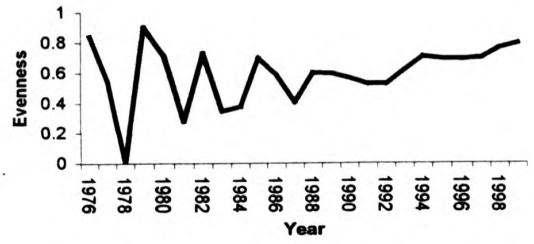


Figure 4.10. Change in the mean evenness (—) over time with 95% confidence limits (---) for a) Group 9, b) Group 10.

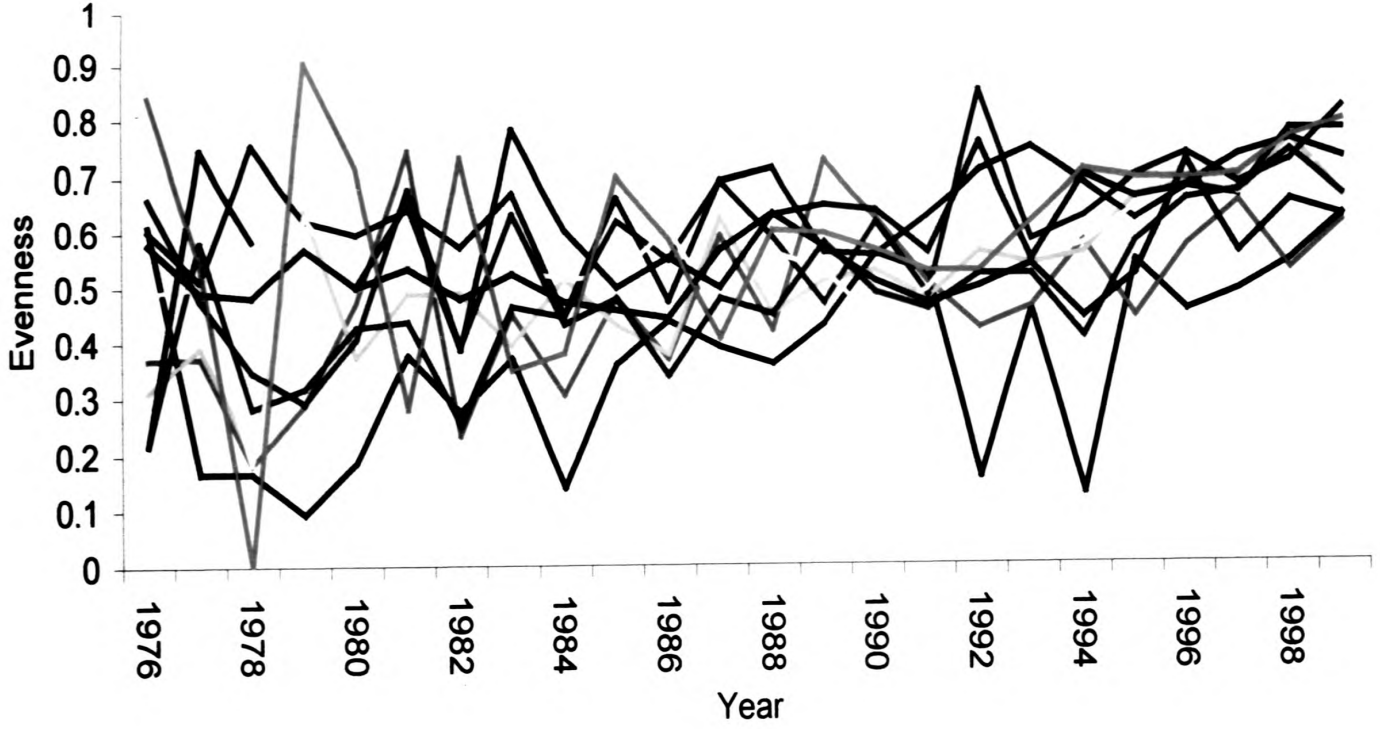


Figure 4.11. Change in the mean evenness over time for all groups. Group 1 – Black, Group 2 – Pink, Group 3 – Orange, Group 4 - Light blue, Group 5 – Purple, Group 6 – Yellow, Group 7 – Light green, Group 8 – Dark blue, Group 9 – Dark green, Group 10 – Red.

Table 4.4. Regression results for the change in evenness over time.

Group	Regression results
1	Evenness = -22.4 + 0.0114 Year, p=0.016, F=6.75, Rsq=20.0%
2	Evenness = -21.3 + 0.0110 Year, p=0.008, F=8.57, Rsq=28.0%
3	Evenness = -28.0 + 0.0143 Year, p=0.000, F=30.28, Rsq=57.9%
4	Evenness = -38.2 + 0.0195 Year, p=0.000, F=39.25, Rsq=64.1%
5	Evenness = -10.4 + 0.00558 Year, p=0.030, F=5.39, Rsq=19.7%
6	Evenness = -20.2 + 0.0104 Year, p=0.001, F=15.55, Rsq=41.4%
7	Evenness = -15.2 + 0.00790 Year, p=0.027, F=5.60, Rsq=20.3%
8	N/S
9	Evenness = -18.9 + 0.00979 Year, p=0.012, F=7.62, Rsq=26.6%
10	N/S

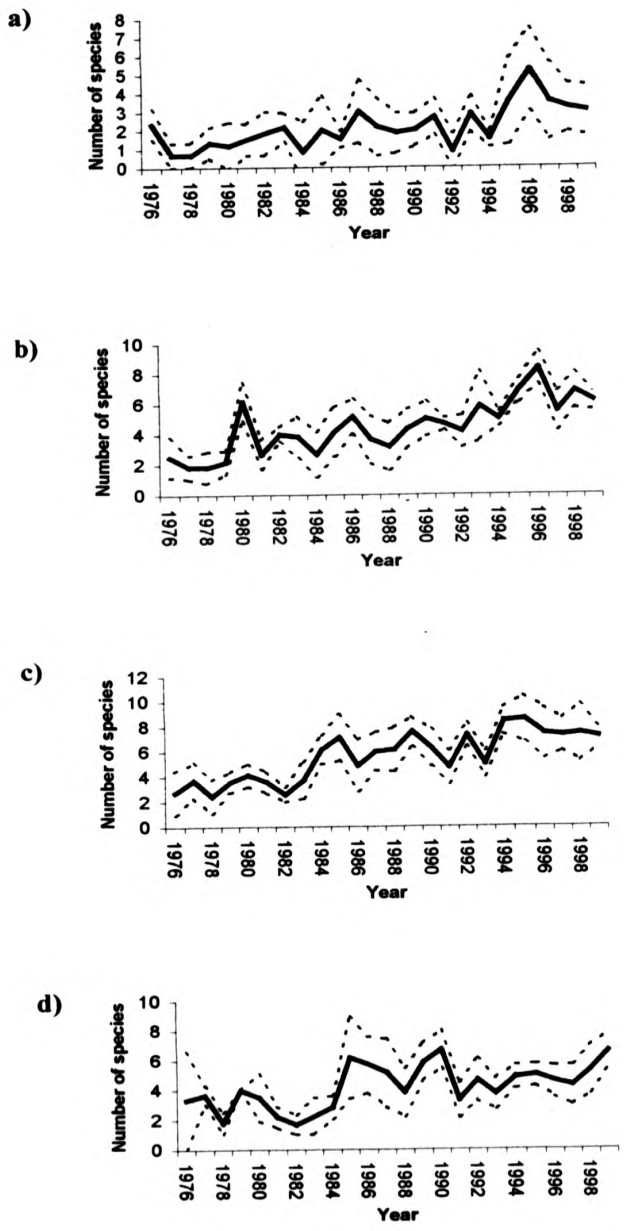


Figure 4.12. Change in the mean number of species per station (—) over time with 95% confidence limits (---) for a) Group 1, b) Group 2, c) Group3, d) Group 4.

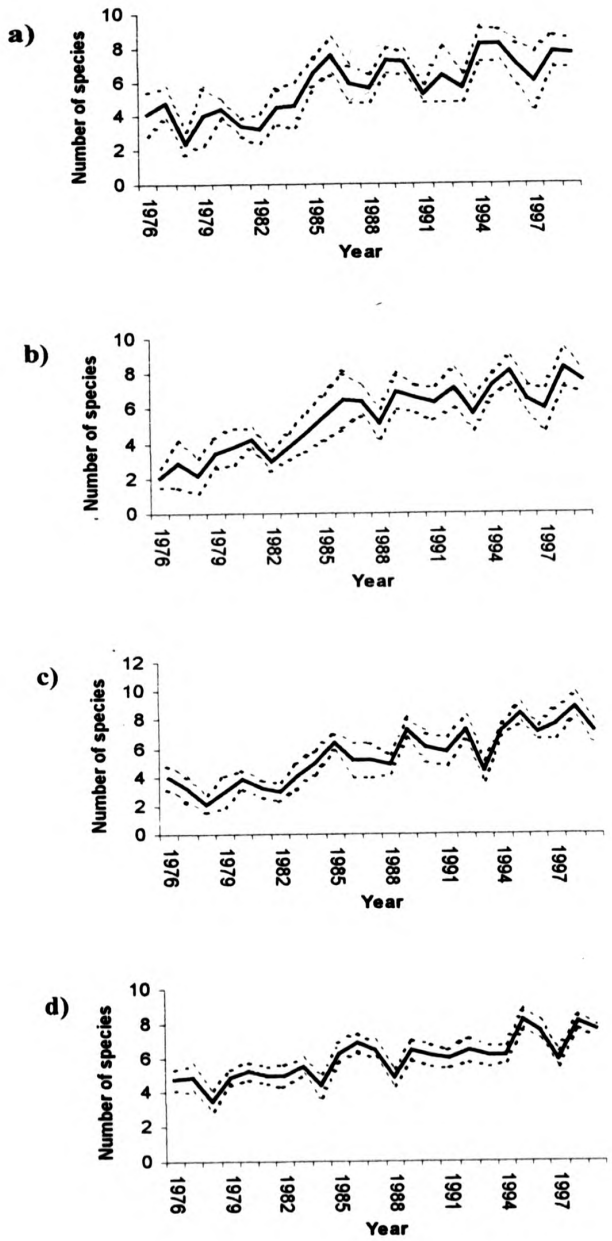


Figure 4.13. Change in the mean number of species per station (—) over time with 95% confidence limits (---) for a) Group 5, b) Group 6, c) Group7, d) Group 8.

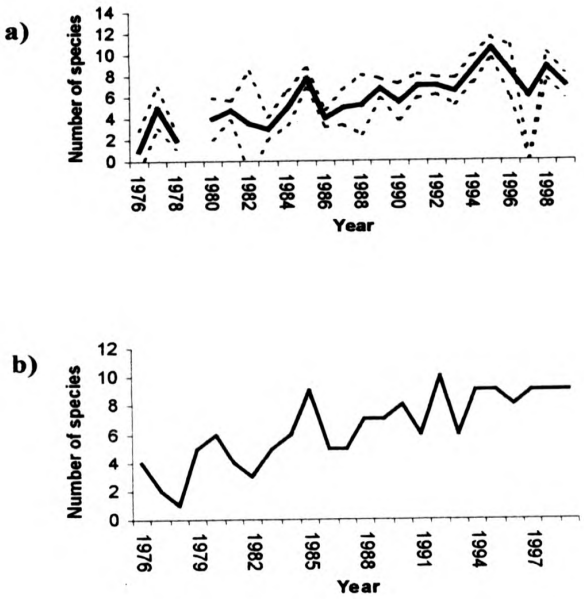


Figure 4.14. Change in the mean number of species per station (—) over time with 95% confidence limits (---) for a) Group 9, b) Group 10.

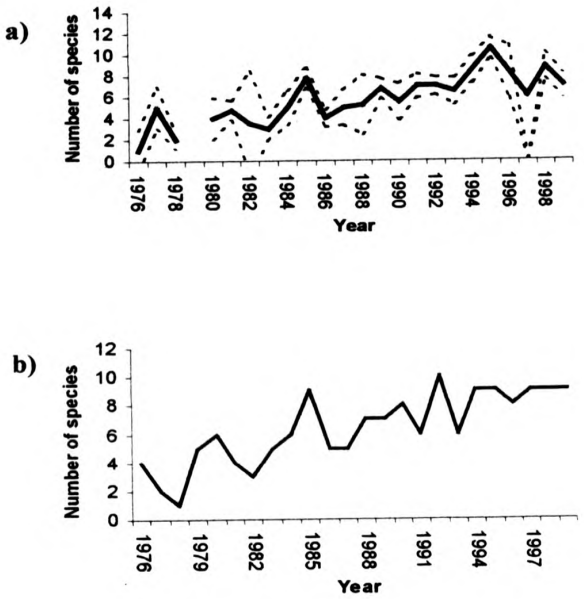


Figure 4.14. Change in the mean number of species per station (—) over time with 95% confidence limits (---) for a) Group 9, b) Group 10.

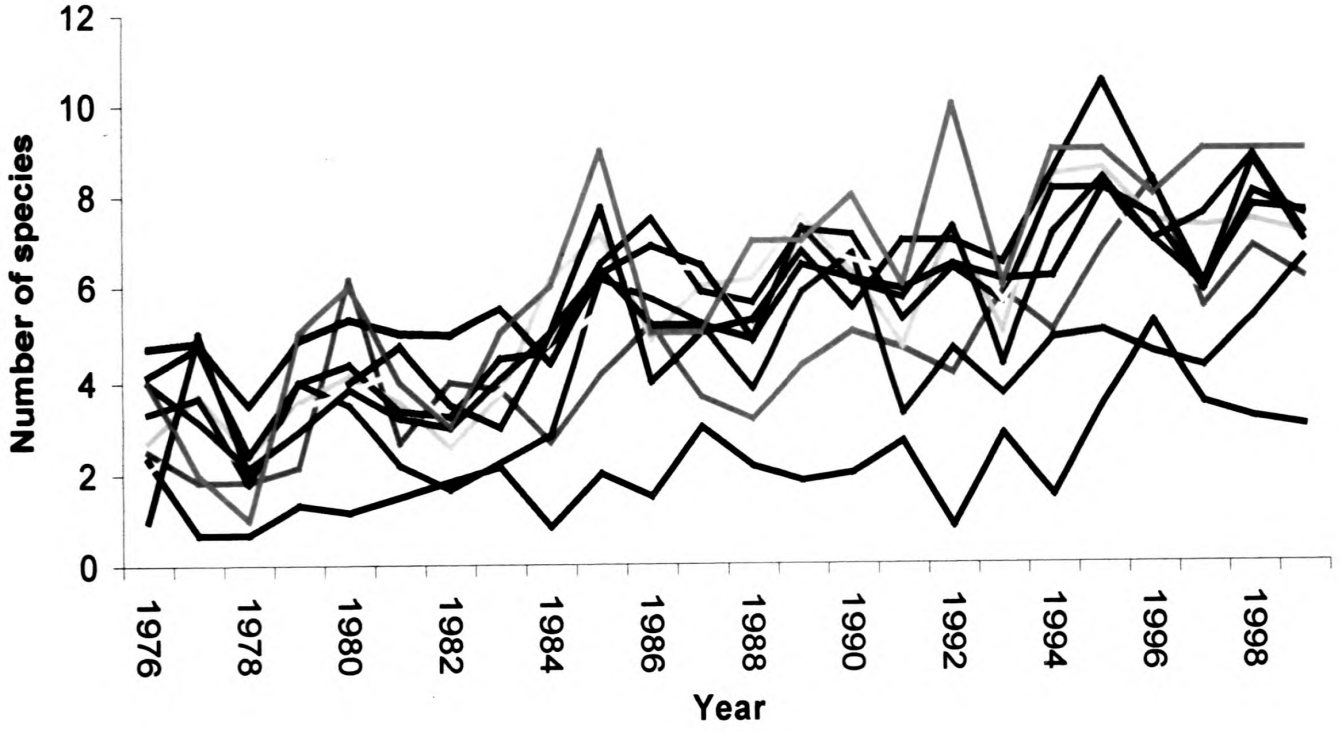


Figure 4.15. Change in the number of species per station over time for all groups. Group 1 – Black, Group 2 – Pink, Group 3 – Orange, Group 4 - Light blue, Group 5 – Purple, Group 6 – Yellow, Group 7 – Light green, Group 8 – Dark blue, Group 9 – Dark green, Group 10 – Red.

Table 4.5. Regression results for the change in species richness over time.

Group	Regression results
1	No Species = -200 + 0.102 Year, p=0.000, F=17.30, Rsq=44.0%
2	Log No Species = -41.0 + 0.0209 Year, p=0.000, F=40.66, Rsq=64.9%
3	No Species = -444 + 0.226 Year, p=0.000, F=47.99, Rsq=68.6%
4	No Species = -231 + 0.118 Year, p=0.003, F=10.71, Rsq=32.7%
5	No Species = -365 + 0.187 Year, p=0.000, F=38.28, Rsq=63.5%
6	No Species = -460 + 0.234 Year, p=0.000, F=91.05, Rsq=80.5%
7	No Species = -459 + 0.233 Year, p=0.000, F=68.52, Rsq=75.7%
8	No Species = -2.62 + 0.135 Year, p=0.000, F=40.62, Rsq=64.9%
9	No Species = -514 + 0.261 Year, p=0.000, F=38.0, Rsq=64.4%
10	No Species = -561 + 0.285 Year, p=0.000, F=44.95, Rsq=67.1%

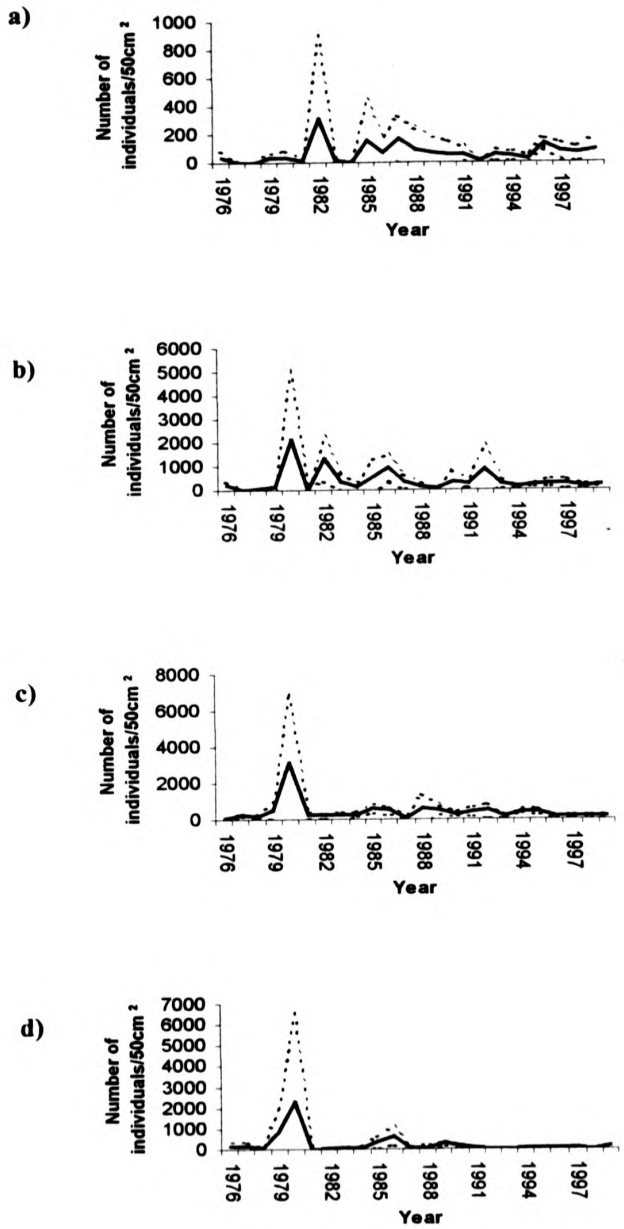


Figure 4.16. Change in the mean number of individuals / 50cm^2 (—) over time with 95% confidence limits (---) for a) Group 1, b) Group 2, c) Group 3, d) Group 4.

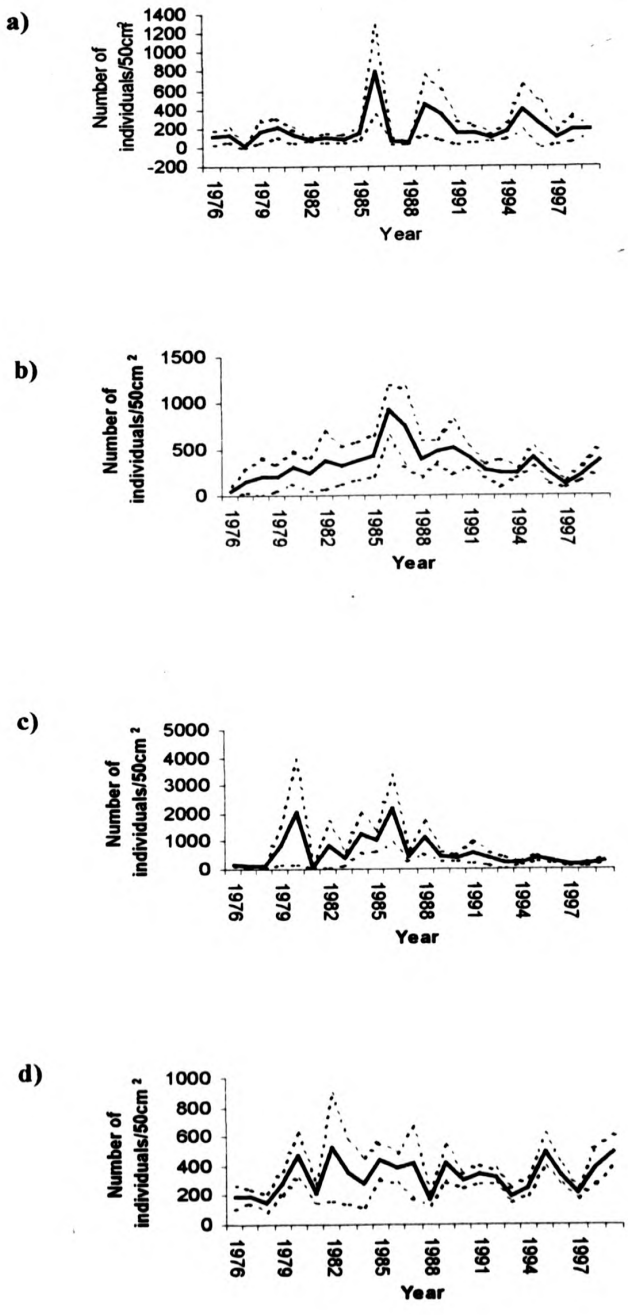
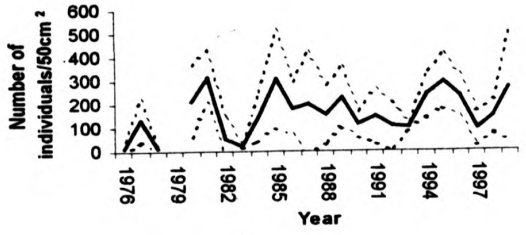


Figure 4.17. Change in the mean number of individuals / 50cm^2 (—) over time with 95% confidence limits (---) for a) Group 5, b) Group 6, c) Group 7, d) Group 8.

a)



b)

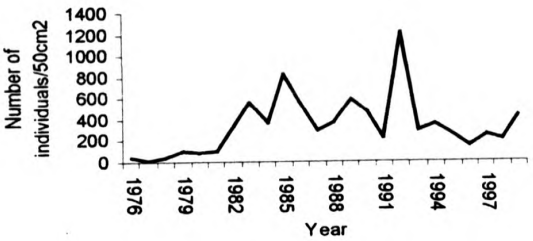


Figure 4.18. Change in the mean number of individuals / 50cm^2 (—) over time with 95% confidence limits (---) for a) Group 9, b) Group 10.

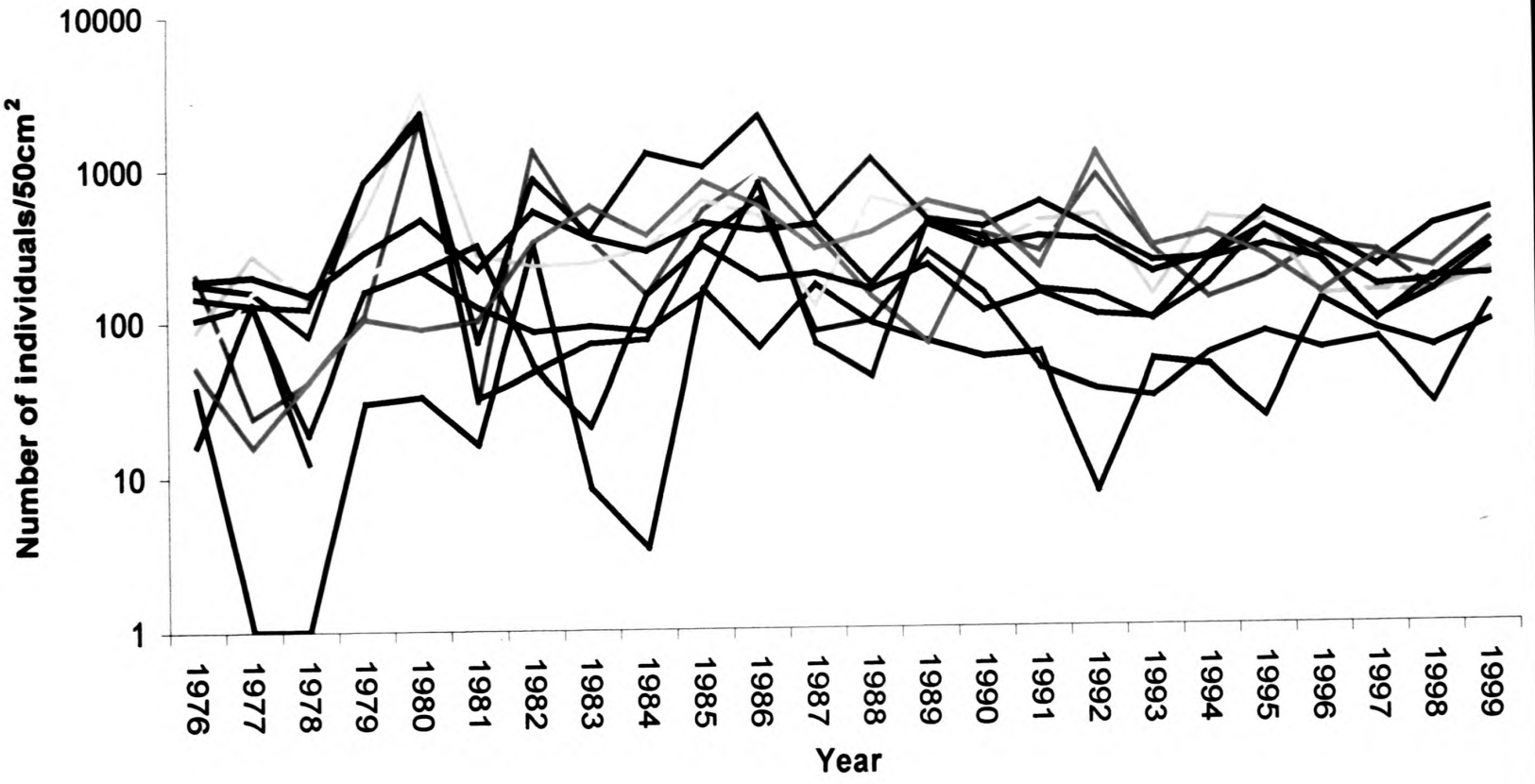


Figure 4.19. Change in the number of individuals/50cm^2 over time for all groups. Group 1 – Black, Group 2 – Pink, Group 3 – Orange, Group 4 - Light blue, Group 5 – Purple, Group 6 – Yellow, Group 7 – Light green, Group 8 – Dark blue, Group 9 – Dark green, Group 10 – Red.

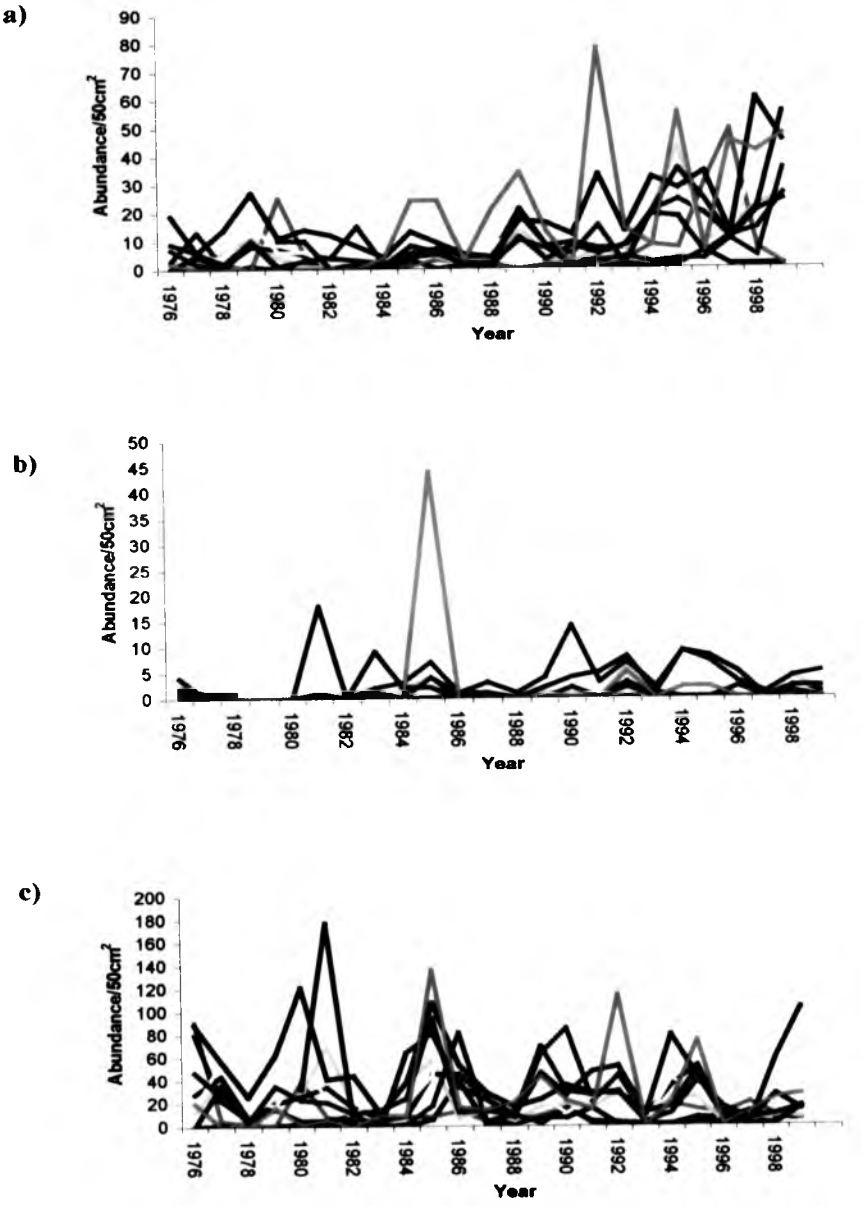


Figure 4.20 Change over time of the mean abundance of a) *Macoma balthica*, b) *Cerastoderma edule*, c) *Hydrobia ulvae* for all groups. Group 1 – Black, Group 2 – Pink, Group 3 – Orange, Group 4 – Light blue, Group 5 – Purple, Group 6 – Yellow, Group 7 – Light green, Group 8 – Dark blue, Group 9 – Dark green, Group 10 – Red.

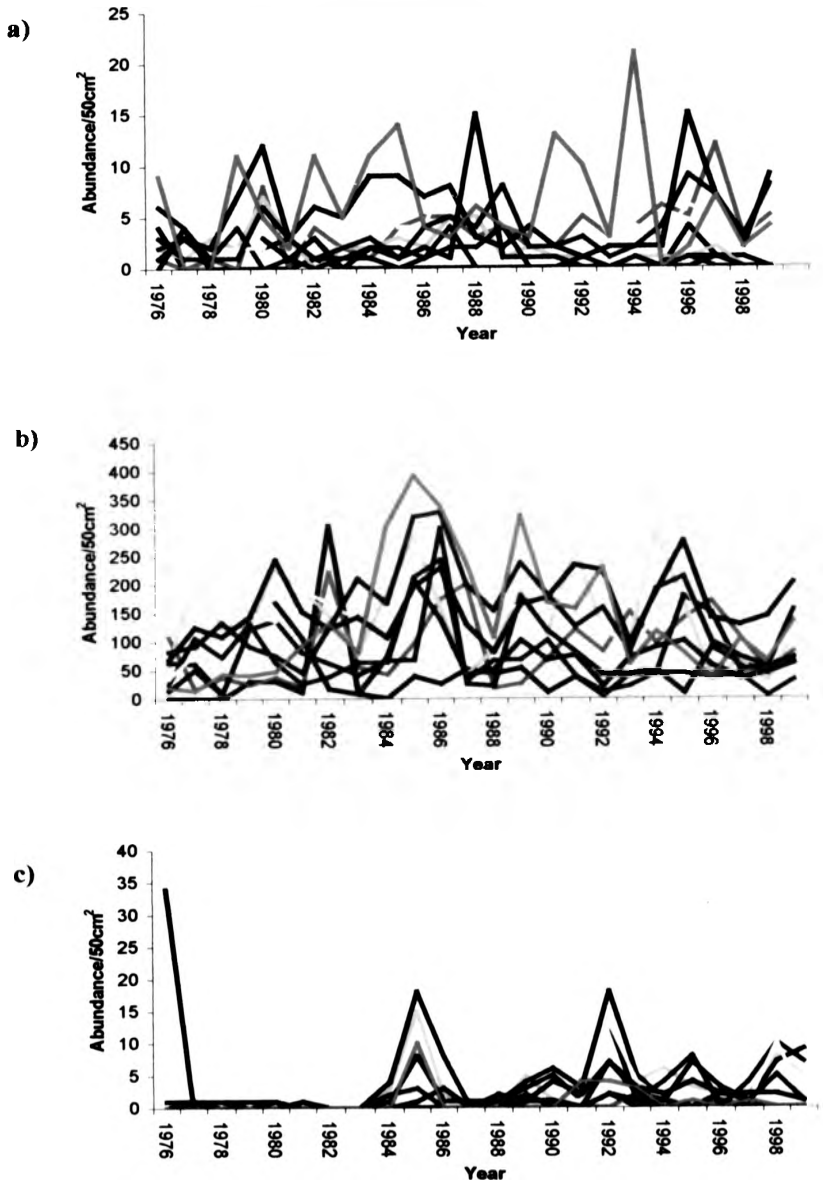


Figure 4.21. Change over time of the mean abundance of a) *Nereis diversicolor*, b) *Oligochaetes*, c) *Nephtys hombergii* for all groups. Group 1 – Black, Group 2 – Pink, Group 3 – Orange, Group 4 – Light blue, Group 5 – Purple, Group 6 – Yellow, Group 7 – Light green, Group 8 – Dark blue, Group 9 – Dark green, Group 10 – Red.

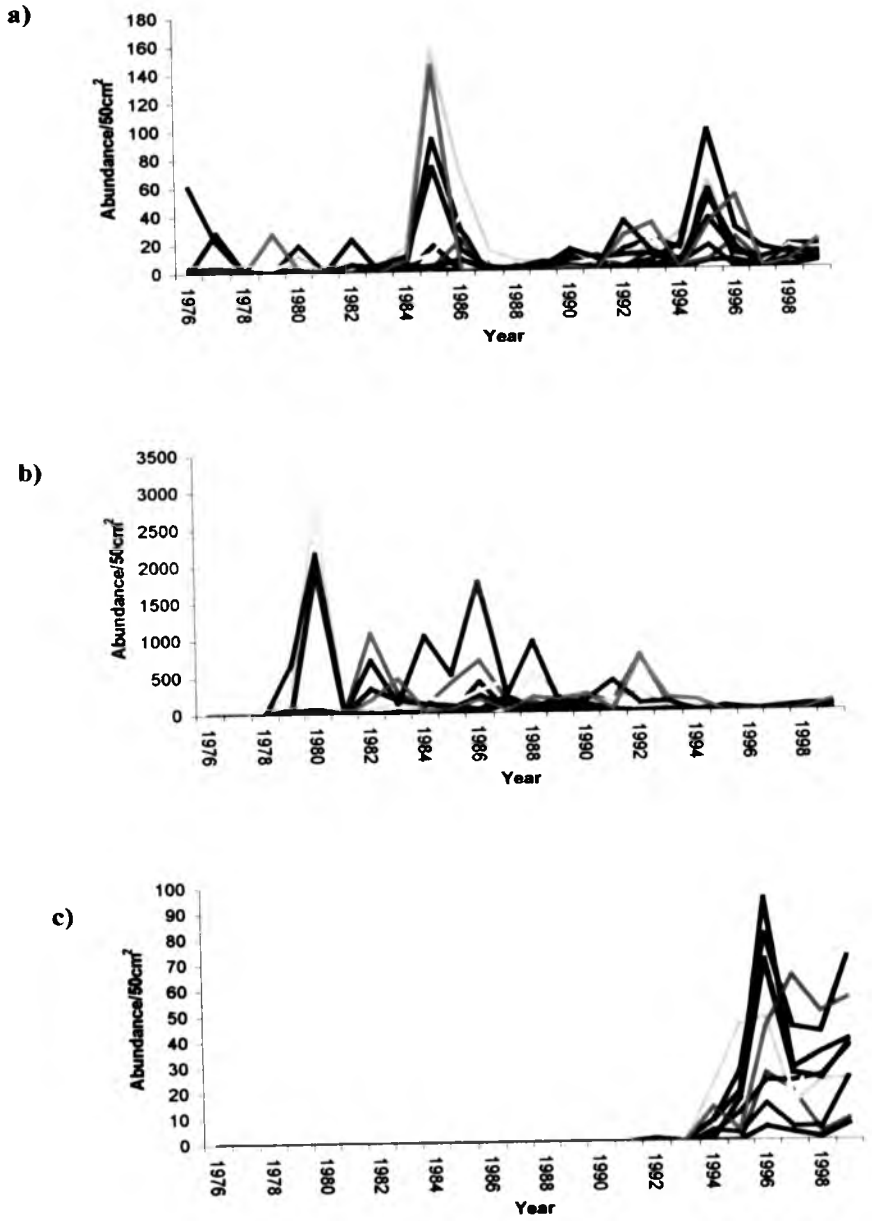


Figure 4.22. Change over time of the mean abundance of a) *Pygospio elegans*, b) *Manayunkia aestuarina*, c) *Streblospio shrubsolii* for all groups. Group 1 – Black, Group 2 – Pink, Group 3 – Orange, Group 4 – Light blue, Group 5 - Purple, Group 6 – Yellow, Group 7 – Light green, Group 8 – Dark blue, Group 9 – Dark green, Group 10 – Red.

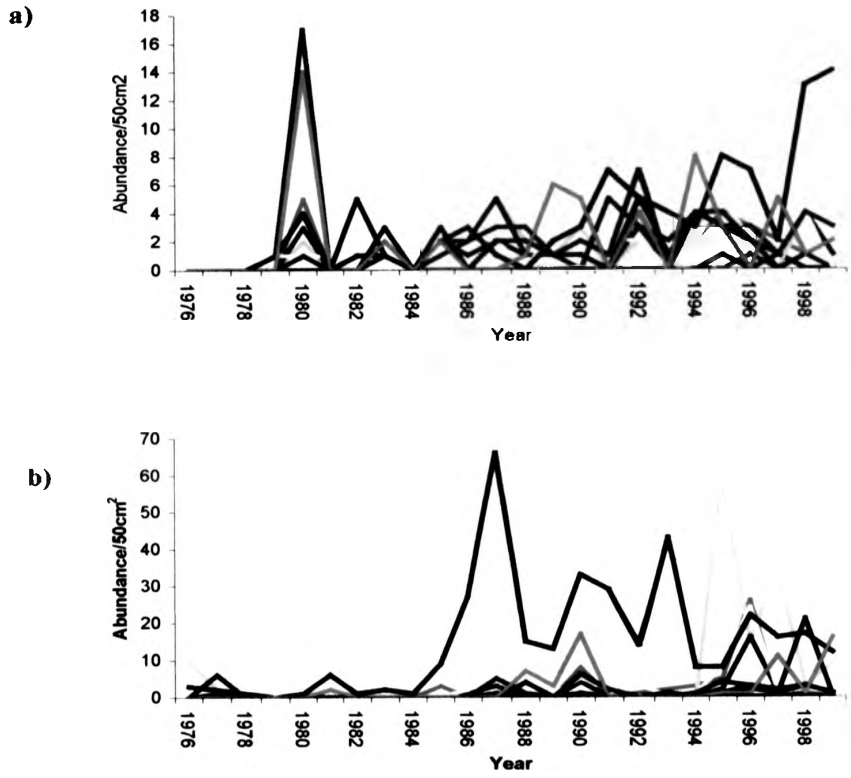


Figure 4.23. Change over time of the mean abundance of a) *Eteone longa*, b) *Corophium volutator* for all groups. Group 1 – Black, Group 2 – Pink, Group 3 – Orange, Group 4 – Light blue, Group 5 - Purple, Group 6 –Yellow, Group 7 – Light green, Group 8 – Dark blue, Group 9 – Dark green, Group 10 – Red.

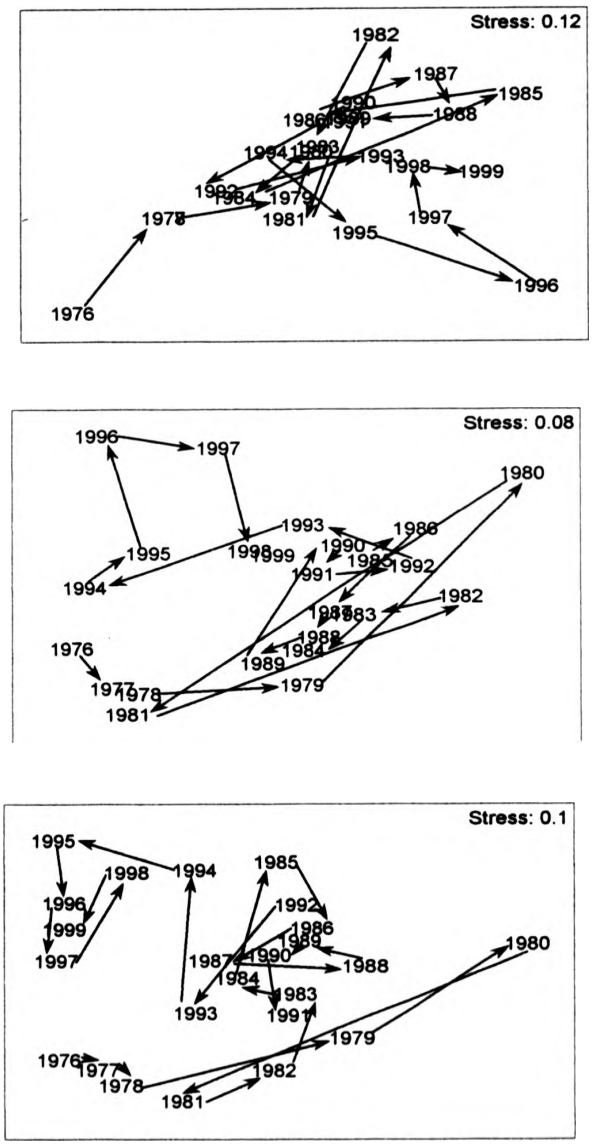


Figure 4.24. MDS plots showing the change in the community from 1976 to 1999 for Group 1 (Top), Group 2 (Middle) and Group 3 (Bottom).

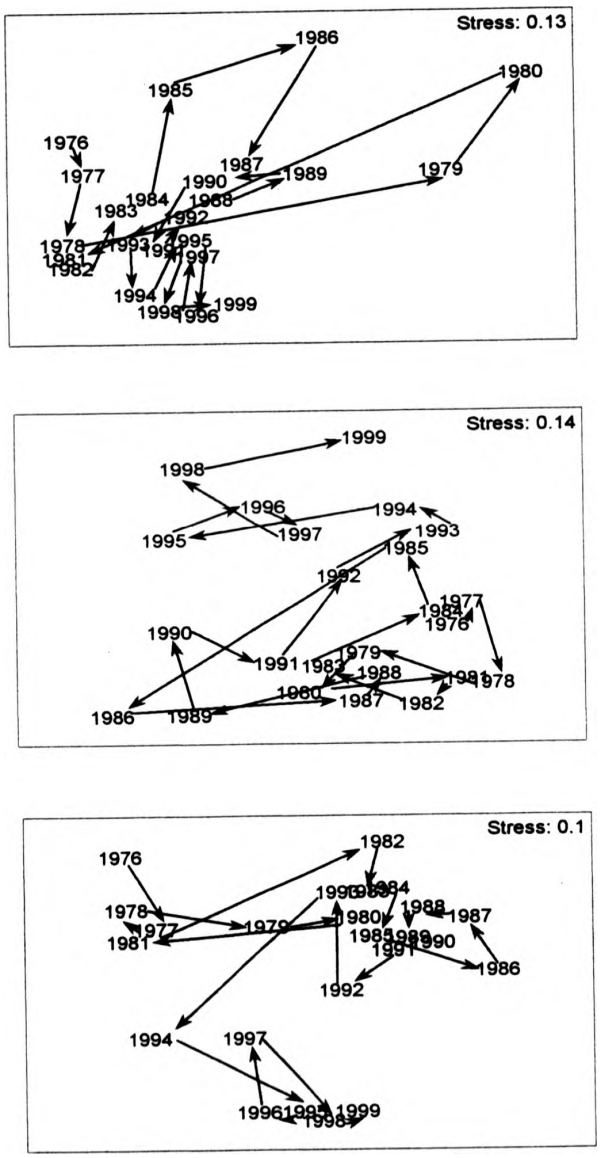


Figure 4.25. MDS plots showing the change in the community from 1976 to 1999 for Group 4 (Top), Group 5 (Middle) and Group 6 (Bottom).

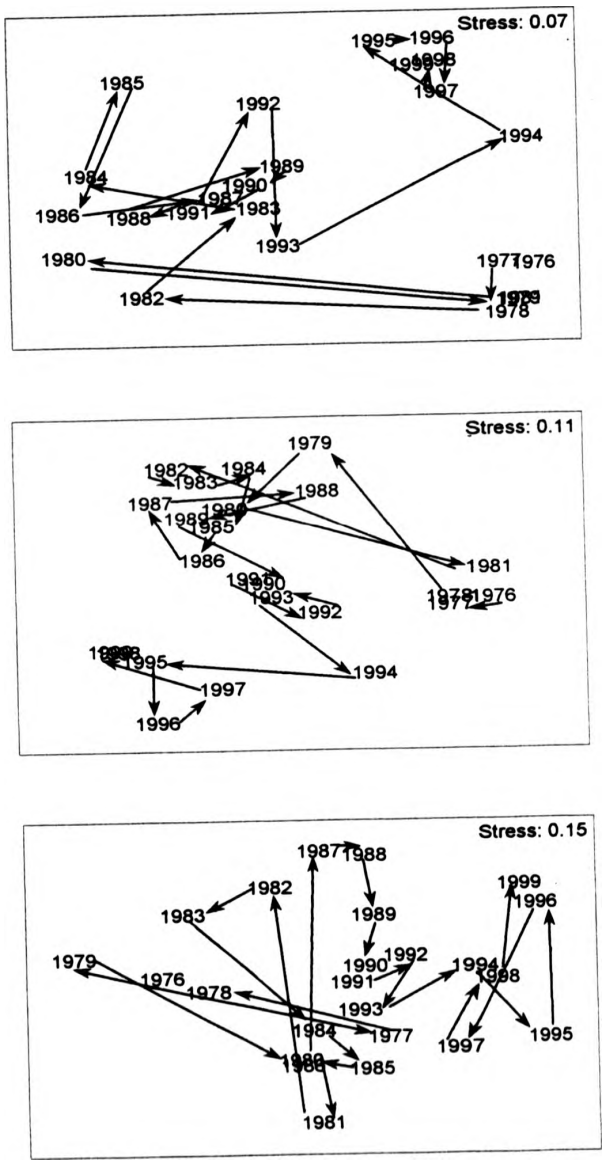


Figure 4.26. MDS plots showing the change in the community from 1976 to 1999 for Group 7 (Top), Group 8 (Middle) and Group 9 (Bottom).

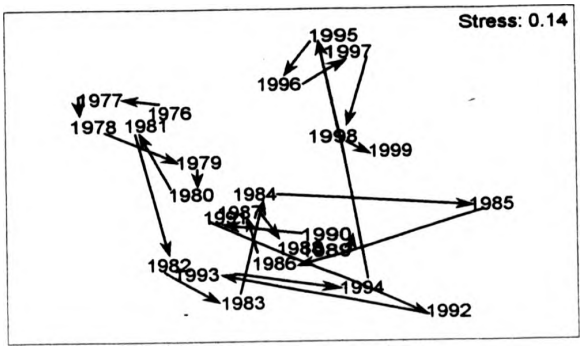


Figure 4.27. MDS plots showing the change in the community from 1976 to 1999 for Group 10.

Table 4.6. SIMPER results for Group 1 (* = good discriminating species).

Average dissimilarity = 96.24					
Species	**1976-1978 Average abundance**	**1979-1994 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Oligochaete	0	46.13	1.84*	51.74	51.74
Manayunkia	0	20.81	0.85	20.7	72.43
Nephtys	11.33	0.19	0.65	15.33	87.77
Hydrobia	0.67	5.56	0.57	8.21	95.97
Average dissimilarity = 56.78					
Species	**1979-1994 Average abundance**	**1995-1999 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Oligochaete	46.13	52.2	1.37	51.58	51.58
Manayunkia	20.81	5.4	0.82	20.9	72.48
Hydrobia	5.56	7.2	0.91	10.8	83.28
Pygospio	1.38	3.4	0.98	5.83	89.11
Streblospio	0	3	1.22	3.54	92.65

Table 4.7. SIMPER results for Group 2 (* = good discriminating species).

Average dissimilarity = 76.77					
Species	**1976-1978 Average abundance**	**1979-1993 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	0	416.93	2.06*	68.58	68.58
Oligochaete	53.67	89.13	1.17	19.05	87.63
Hydrobia	32.67	8.27	0.74	8.97	96.6
Average dissimilarity = 61.81					
Species	**1979-1993 Average abundance**	**1994-1999 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	416.93	36.17	1.53*	61.18	61.18
Oligochaete	89.13	106.5	1.11	22.62	83.79
Macoma	3.47	17	0.84	4.64	88.43
Hydrobia	8.27	13	1.14	2.81	91.24

Table 4.8. SIMPER results for Group 3 (* = good discriminating species).

Average dissimilarity = 55.75					
Species	**1976-1978 Average abundance**	**1979-1993 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	0	345.47	1.33	56.94	56.94
Oligochaete	132.67	153.8	1.27	26.36	83.3
Hydrobia	19	23.67	0.97	6.18	89.48
Pygospio	5.33	21.13	0.61	5.64	95.12
Average dissimilarity = 61.91					
Species	**1979-1993 Average abundance**	**1994-1999 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	345.47	19.83	1.15	43.04	43.04
Oligochaete	153.8	116.17	1.37	25.5	68.54
Streblospio	0	29.33	1.5*	8.12	76.66
Pygospio	21.13	20.5	0.79	5.82	82.48
Corophium	0.13	19.83	0.98	5.24	87.72
Macoma	4.13	22.33	1.49*	4.91	92.63

Table 4.9. SIMPER results for Group 4 (* = good discriminating species).

Average dissimilarity = 61.72					
Species	**1976-1978 Average abundance**	**1979-1984 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	0	475.17	0.69	43.55	43.55
Oligochaete	69	64.17	1.01	22.86	66.4
Hydrobia	36.33	6.17	0.83	16.82	83.23
Pygospio	27.67	4.83	0.87	13.95	97.18
Average dissimilarity = 63.12					
Species	**1979-1984 Average abundance**	**1985-1999 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	475.17	33.2	0.88	51.06	51.06
Oligochaete	64.17	66	1.1	25.45	76.52
Pygospio	4.83	11.67	0.64	5	81.52
Hydrobia	6.17	10.87	0.93	4.76	86.27
Macoma	2	6.27	0.61	4.1	90.38

Table 4.10. SIMPER results for Group 5 (* = good discriminating species).

Average dissimilarity = 53.17					
Species	**1979-1994 Average abundance**	**1995-1999 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Oligochaete	82.37	100	1.37	30.6	30.6
Manayunkia	40.79	18.4	0.84	15.65	46.25
Streblospio	0.16	23.6	1.77*	14.33	60.58
Hydrobia	29.89	15.6	1.43*	12.64	73.21
Pygospio	4.37	18	1.1	7.55	80.77
Macoma	7.89	18.88	1.47*	6.64	87.41
Corophium	0.32	8	0.82	4.46	91.86

Table 4.11. SIMPER results for Group 6 (* = good discriminating species).

Average dissimilarity = 56.47					
Species	**1976-1978 Average abundance**	**1979-1993 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	0	172.87	1.83*	51.28	51.28
Oligochaete	97.67	178.13	1.47*	32.33	83.62
Hydrobia	32	33.8	1.14	8.02	91.64
Average dissimilarity = 52.03					
Species	**1979-1993 Average abundance**	**1994-1999 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	172.87	19.83	1.68*	42.16	42.16
Oligochaete	178.13	113.83	1.39	22.47	64.63
Streblospio	0	42	1.52*	12.21	76.84
Hydrobia	33.8	24.83	1.14	7.6	84.44
Macoma	9.07	29.33	1.58*	6.15	90.59

Table 4.12. SIMPER results for Group 7 (* = good discriminating species).

Average dissimilarity = 65.79					
Species	**1976-1979 Average abundance**	**1980-1993 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	0	587.86	1.8*	72.01	72.01
Oligochaete	100	172	1.32	17.92	89.94
Hydrobia	28.25	27.29	1.1	5.07	95.01
Average dissimilarity = 65.08					
Species	**1980-1993 Average abundance**	**1994-1999 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	587.86	13	1.63*	63.77	63.77
Oligochaete	172	130.33	1.1	15.75	79.52
Streblospio	0	45.33	1.21	9.02	88.54
Hydrobia	27.29	16.33	0.93	3.53	92.07

Table 4.13. SIMPER results for Group 8 (* = good discriminating species).

Average dissimilarity = 43.05					
Species	**1976-1978 Average abundance**	**1979-1993 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	0	93.67	1.13	37.84	37.84
Oligochaete	101	162.87	1.49*	29.89	67.73
Hydrobia	57.33	43.33	1.35	15.95	83.68
Corophium	2	17.33	0.92	7.51	91.18
Average dissimilarity = 37.30					
Species	**1979-1993 Average abundance**	**1994-1999 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	93.67	38.83	1.04	28.69	28.69
Oligochaete	162.87	179	1.35	23.03	51.72
Hydrobia	43.33	40	1.3	13.79	65.51
Streblospio	0	29	1.25	11.32	76.84
Macoma	13.2	35.17	1.53*	8.8	85.64
Corophium	17.33	13.83	1.17	5.75	91.38

Table 4.14. SIMPER results for Group 9 (* = good discriminating species).

Average dissimilarity = 63.74					
Species	**1976-1986 Average abundance**	**1987-1993 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Oligochaete	72.18	60.86	1.53*	38.32	38.32
Hydrobia	39.09	30.71	1.25	23.02	61.34
Manayunkia	0.73	39.71	0.8	22.09	83.43
Pygospio	5.27	2	0.71	4.18	87.61
Macoma	3.64	5.29	1.09	3.33	90.94
Average dissimilarity = 52.34					
Species	**1987-1993 Average abundance**	**1994-1999 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	39.71	20.5	0.99	21.45	21.45
Streblospio	0.14	37	1.41*	20.34	41.79
Oligochaete	60.86	64	1.43*	15.5	57.29
Hydrobia	30.71	28.83	1.26	13.83	71.13
Pygospio	2	1.18	1.03	13.38	84.5
Macoma	5.29	21.5	1.18	8.62	93.12

Table 4.15. SIMPER results for Group 10 (* = good discriminating species).

Average dissimilarity = 76.96					
Species	**1976-1981 Average abundance**	**1982-1994 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	4.5	205.23	1.54*	43.29	43.29
Oligochaete	43.17	203.08	1.58*	39.5	82.79
Hydrobia	7.17	32.23	1.04	5.25	88.04
Pygospio	5.17	16.15	0.62	3.66	91.7
Average dissimilarity = 63.20					
Species	**1982-1994 Average abundance**	**1995-1999 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia	205.23	27.8	1.36	37.18	37.18
Oligochaete	203.08	82.6	1.3	28.05	65.22
Streblospio	1	43.4	1.75*	9.97	75.2
Macoma	17.23	38.8	1.62*	7.06	82.26
Hydrobia	32.23	27.6	1.08	6.62	88.88
Pygospio	16.15	21.6	0.99	6.24	95.11

Table 4.16. Stepwise regression and BIO-ENV group results for the different time periods for the NAO data. The p and Rsq(adj) values for the stepwise regressions and the correlation coefficient (p_s) from the BIO-ENV analyses are shown. Those with a high Rsq(adj) or p_s are highlighted in bold.

	NAO									
Group	1976-1999		1980-1999		1985-1999		1993-1999		1995-1999	
	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s
1	None sig	0.1	None sig	0.153	0.011, 35.7%	0.35	0.048, 49.2%	0.24	None sig	0.0358
2	None sig	0.078	None sig	0.086	None sig	0.114	None sig	0.182	None sig	0.442
3	None sig	0.054	None sig	0.095	None sig	0.085	None sig	0.019	**0.009, 90.3%**	0.442
4	None sig	0.179	None sig	0.134	None sig	0.245	None sig	0.306	None sig	0.685
5	None sig	0.011	None sig	0.002	None sig	-0.008	None sig	-0.064	None sig	0.608
6	None sig	0.005	None sig	0.06	None sig	0.076	None sig	0.06	None sig	0.333
7	None sig	0.034	None sig	0.032	None sig	0.057	None sig	0.043	None sig	0.139
8	None sig	0.058	None sig	0.073	None sig	0.128	None sig	0.23	None sig	0.115
9	None sig	0.056	None sig	-0.03	None sig	0.001	None sig	0.029	None sig	0.109
10	None sig	0.21	None sig	0.201	None sig	0.231	None sig	0.225	None sig	0.006

Table 4.17. Stepwise regression and BIO-ENV group results for the different time periods for the temperature data. The p and Rsq(adj) values for the stepwise regressions and the correlation coefficient (p_s) from the BIO-ENV analyses are shown. Those with a high Rsq(adj) or p_s are highlighted in bold.

	Temperature									
Group	1976-1999		1980-1999		1985-1999		1993-1999		1995-1999	
	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s
1	0.008, 28.3%	0.03	None sig	-0.034	None sig	-0.101	None sig	0.053	None sig	-0.139
2	0.016, 20.3%	-0.009	0.045, 37.9%	0	None sig	-0.023	None sig	-0.117	None sig	-0.564
3	0.032, 15.6%	-0.016	None sig	-0.009	None sig	-0.082	None sig	0.186	**0.007, 91.6%**	0.467
4	None sig	0.113	None sig	0.103	None sig	0.19	None sig	-0.242	None sig	-0.127
5	None sig	0.354	None sig	0.339	None sig	0.318	None sig	0.368	None sig	0.333
6	None sig	0.12	None sig	0.118	None sig	0.134	None sig	-0.07	None sig	-0.164
7	0.001, 40.2%	0.001	0.005, 33.2%	0.009	0.012, 35.0%	0.008	0.023, 61.2%	-0.049	Not normal	0.37
8	0.030, 16.0%	0.026	None sig	0.007	None sig	0.032	None sig	-0.249	None sig	0.236
9	None sig	-0.021	0.021, 22.1%	-0.006	None sig	-0.019	None sig	-0.36	**0.025, 80.3%**	-0.164
10	None sig	-0.056	None sig	-0.102	None sig	-0.157	None sig	-0.018	None sig	-0.479

Table 4.18. Stepwise regression and BIO-ENV group results for the different time periods for the Avon River and refinery effluent data. The p and Rsq(adj) values for the stepwise regressions and the correlation coefficient (p_s) from the BIO-ENV analyses are shown. Those with a high Rsq(adj) or p_s are highlighted in bold.

	Avon						Refinery effluent			
Group	1985-1999		1993-1999		1995-1999		1993-1999		1995-1999	
	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s
1	None sig	0.291	**0.003, 100%**	0.394	Not normal	0.552	None sig	0.216	None sig	**0.721**
2	0.001, 57.5%	0.317	**0.027, 75.6%**	0.616	Not normal	**0.709**	None sig	0.443	None sig	**0.855**
3	**0.000, 74.2%**	0.427	**0.001, 99.9%**	**0.703**	**0.001, 99.8%**	**0.867**	Not normal	**0.816**	**0.000, 100%**	**0.782**
4	0.025, 27.9%	0.672	**0.000, 97.6%**	0.652	**0.001, 100%**	0.564	**0.001, 98.5%**	0.217	None sig	**0.939**
5	0.009, 46.9%	0.387	**0.005, 89.2%**	0.614	**0.000, 100%**	**0.988**	None sig	**0.847**	None sig	**0.891**
6	0.013, 33.9%	0.383	**0.000, 100%**	0.656	**0.004, 99.1%**	**0.794**	**0.009, 72.8%**	0.686	None sig	0.491
7	0.007, 48.7%	0.448	0.046, 50%	0.616	**0.002, 99.7%**	**0.976**	**0.008, 74.2%**	**0.704**	**0.012, 100%**	0.685
8	0.050, 20.8%	0.233	0.017, 65.8%	0.406	None sig	**0.867**	Not normal	**0.881**	**0.001, 98.4%**	**0.903**
9	**0.001, 72.8%**	0.411	**0.001, 95.2%**	0.688	**0.000, 100%**	**0.964**	Not normal	0.486	**0.047, 70.5%**	**0.745**
10	0.014, 42.7%	0.641	0.042, 57.4%	0.543	None sig	**0.939**	0.047, 49.5%	**0.734**	None sig	0.527

Table 4.19. Stepwise regression and BIO-ENV group results for the different time periods for the Grangeburn and ballast water data. The p and Rsq(adj) values for the stepwise regressions and the correlation coefficient (p_s) from the BIO-ENV analyses are shown. Those with a high Rsq(adj) or p_s are highlighted in bold.

	Grangeburn						Ballast water	
Group	1985-1999		1993-1999		1995-1999		1995-1999	
	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s
1	0.002, 57.1%	0.275	Not normal	0.642	None sig	0.43	**0.014, 86.5%**	**0.952**
2	None sig	0.196	Not normal	0.397	None sig	0.261	Not normal	0.685
3	None sig	0.111	None sig	0.584	None sig	**0.709**	**0.038, 74.5%**	**0.782**
4	None sig	0.185	None sig	0.021	None sig	-0.018	None sig	**0.879**
5	None sig	0.154	None sig	0.322	None sig	**0.879**	None sig	**0.964**
6	0.006, 50.7%	0.307	None sig	0.558	None sig	0.479	None sig	**0.709**
7	0.003, 62.4%	0.245	None sig	0.535	None sig	**0.794**	None sig	0.697
8	None sig	0.246	None sig	0.695	None sig	0.527	None sig	**0.879**
9	0.001, 63.1%	0.213	None sig	0.313	None sig	0.612	None sig	0.697
10	0.012, 35.2%	0.22	None sig	0.636	None sig	0.673	None sig	**0.733**

Table 4.20. Stepwise regression and BIO-ENV group results for the different time periods for the Kinneil channel and chemical effluent data. The p and Rsq(adj) values for the stepwise regressions and the correlation coefficient (p_s) from the BIO-ENV analyses are shown. Those with a high Rsq(adj) or p_s are highlighted in bold.

	Kinneil Channel								Chemical Effluent	
Group	1980-1999		1985-1999		1993-1999		1995-1999		1995-1999	
	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s
1	.0007, 30.4%	0.327	None sig	0.239	None sig	0.119	None sig	-0.042	**0.000, 99.4%**	**0.842**
2	0.000, 49.4%	0.249	0.007, 40.0%	0.153	None sig	0.151	None sig	0.042	None sig	**0.976**
3	**0.000, 78.3%**	0.347	0.000, 64.1%	0.425	None sig	0.069	None sig	0.697	None sig	**0.721**
4	0.000, 59.2%	0.313	0.008, 38.2%	0.508	None sig	0.078	None sig	-0.091	**0.002, 99.6%**	**0.988**
5	**0.000, 74.9%**	0.337	0.001, 58.3%	0.358	0.045, 50.4%	0.364	None sig	0.479	**0.030, 78.2%**	**0.903**
6	**0.000, 73.3%**	0.418	0.000, 66.9%	0.474	None sig	-0.023	None sig	0.648	None sig	**0.806**
7	0.000, 65.2%	0.511	0.001, 62.6%	0.624	0.026, 59.5%	0.09	**0.018, 96.4%**	0.479	Not normal	**0.952**
8	0.000, 58.6%	0.414	0.017, 31.5%	0.352	0.026, 59.5%	0.313	None sig	0.503	**0.004, 99.1%**	0.636
9	0.000, 62.1%	0.414	0.001, 53.8%	0.481	None sig	0.552	None sig	0.685	None sig	**0.842**
10	0.001, 57.5%	0.212	0.010, 36.4%	0.23	None sig	0.004	None sig	0.406	**0.003, 99.4%**	**0.842**

Table 4.21. Stepwise regression and BIO-ENV group results for the different time periods for the Sewage works and Chemical/Avon. The p and Rsq(adj) values for the stepwise regressions and the correlation coefficient (p_s) from the BIO-ENV analyses are shown. Those with a high Rsq(adj) or p_s are highlighted in bold.

	Sewage works						Chemical/Avon	
Group	1985-1999		1993-1999		1995-1999		1976-1999	
	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s	Regression p,Rsq(adj)	BIO-ENV p_s
1	None sig	0.231	None sig	0.182	None sig	0.018	0.001, 35.6%	0.355
2	0.000, 67.5%	0.266	None sig	0.318	None sig	0.43	0.000, 60.4%	0.335
3	0.001, 57.4%	0.594	0.004, 43.8%	**0.723**	Not normal	0.152	0.000, 68.7%	0.345
4	None sig	0.624	None sig	0.139	None sig	0.43	0.000, 48.1%	0.432
5	0.022, 29.4%	0.388	None sig	**0.788**	None sig	**0.915**	0.000, 66.2%	0.16
6	0.001, 58.8%	0.511	None sig	**0.747**	None sig	0.503	**0.000, 76.4%**	0.467
7	0.001, 53.5%	0.641	**0.002, 98.0%**	**0.757**	**0.002, 99.6%**	0.236	0.000, 56.6%	0.454
8	0.032, 25.5%	0.513	None sig	0.674	None sig	-0.176	0.000, 43.7%	0.518
9	0.004, 43.9%	0.519	0.027, 59.0%	0.347	None sig	0.345	0.000, 62.7%	0.415
10	0.002, 60.3%	0.46	None sig	**0.739**	**0.030, 78.4%**	0.321	0.001, 40.3%	0.324

Table 4.22. The significant stepwise regression models including the p, F and Rsq(adj) values for groups 1 to 3.

	Scource	Time period	Regression equation
Group 1	Avon	1993-1999	Diversity = -9.12 + 0.0727COD + 0.0641Alkalinity - 0.0137Chloride + 0.378Iron + 0.0841BOD+ATU, p=0.003, F=48578.46, Rsq(adj)=100.0%
	Chemcial effluent	1995-1999	logDiversity = 0.0886 - 0.0107Acetonitrile, p=0.000, F=674.58, Rsq(adj)=99.4%
	Ballast water	1995-1999	Diversity = 1.84 - 0.137pH, p=0.014, F=26.36, Rsq(adj)=86.5%
Group 2	Avon	1993-1999	Diversity = 10.6 + 0.350BOD - 1.27pH, p=0.027, F=10.28, Rsq(adj)=75.6%
Group 3	Avon	1985-1999	logDiversity = -1.59 + 0.132DO + 0.00157EC -0.00209Hardness, p=0.000, F = 14.43, Rsq(adj) = 74.2%
		1993-1999	Diversity = -0.821+ 0.00510EC + 2.08logBOD+ATU - 0.0108Sulphide + 0.162Kjeldahl, p=0.001, F=1136.36, Rsq(adj)=99.9%
		1995-1999	Diversity = 1.69 + 0.0110Alkalinity - 0.00957DOsat, p=0.001, F=1148.81, Rsq(adj)=99.8%
	Temperature	1995-1999	Diversity = 0.082 + 0.143MaxTemperature, p=0.007, F=44.36, Rsq(adj)=91.6%
	NAO	1995-1999	Diversity = 2.19 + 0.124MinNAO, p=0.009, F=38.17, Rsq(adj)=90.3%
	Refinery effluent	1995-1999	Diversity = 1.71 + 0.00197Nickel + 0.00308TSS, p=0.000, F=4697.23, Rsq(adj)=100%
	Ballast water	1995-1999	Diversity = 2.24 - 0.0234TSS, p=0.038, F=12.71, Rsq(adj)=74.5%

Table 4.23. The significant stepwise regression models including the p, F and Rsq(adj) values for groups 4 to 6.

	Scource	Time period	Regression equation
Group 4	Refinery effluent	1993-1999	Diversity = -1.55 + 34.6(1/Flow) + 0.155Chromium + 0.0118TSS, p=0.001, F=134.35, Rsq(adj)=98.5%
	Avon	1993-1999	Diversity = -0.800 + 0.646BOD + 0.0267SS, p=0.000, F=120.75, Rsq(adj)=97.6%
		1995-1999	Diversity = -0.498 + 0.687BOD + 0.275Iron - 0.242Kjeldahl, P=0.001, F=1918e+06, Rsq(adj)=100%
	Chemcial effluent	1995-1999	Diversity = 2.38 - 0.233Sulphide - 0.600Zinc, p=0.002, F=460.22, Rsq(adj)=99.6%
Group 5	Kinneil channel	1980-1999	Diversity = 2.28 - 0.0582Ammonia, p=0.000, F=57.70, Rsq(adj)=74.9%
	Avon	1993-1999	Diversity = 4.73 + 1.08Ammonia - 0.0319DOsat, p=0.005, F=25.89, Rsq(adj)=89.2%
		1995-1999	Diversity = -0.710 + 0.695BOD + 0.00146Hardness, p=0.000, F=8466.88, Rsq(adj)=100%
	Chemcial effluent	1995-1999	Diversity = 2.38 - 0.319Sulphide, p=0.030, F=15.35, Rsq(adj)=78.2%
Group 6	chemcial/Avon	1976-1999	Diversity = 1.56 - 0.819logChemicalBOD, p=0.000, F=75.44, Rsq(adj)=76.4%
	Kinneil channel	1980-1999	Diversity = 0.780 + 5.36(1/Ammonia), p=0.000 F=53.01 Rsq(adj)=73.3%
	Refinery effluent	1993-1999	Diversity = 3.13 - 0.0912Flow, p=0.009, F=17.06, Rsq(adj)=72.8%
	Avon	1993-1999	Diversity = -0.655 + 4.88logBOD + 0.00109EC - 0.361Kjeldahl - 0.0755Iron + 0.00753Ammonia, p=0.000, F=8.99e+07, Rsq(adj)=100.0%
		1995-1999	logDiversity = 2.04 - 0.149DO - 0.000804Sulphide, P=0.004, F=222.42, Rsq(adj)=99.1%

Table 4.24 The significant stepwise regression models including the p, F and Rsq(adj) values for groups 7 to 10.

	Scource	Time period	Regression equation
Group 7	Sewage works	1993-1999	Diversity = -36.6 + 5.20pH - 0.0340SS + 0.178Kjeldahl, p=0.002, F=98.21, Rsq(adj)=98.0%
		1995-1999	Diversity = 1.70 + 0.0847Ammonia - 0.0125SS, p=0.002, F=452.53, Rsq(adj)=99.6%
	Refinery effluent	1993-1999	Diversity = 0.659 + 2009(1/AvEC), p=0.008, F=18.25, Rsq(adj)=74.2%
		1995-1999	Diversity = 0.161 + 0.0488Copper - 0.00375COD + 0.250pH, p=0.012, F=3685.54, Rsq(adj)=100%
	Kinneil channel	1995-1999	Diversity = -0.234 + 0.15Salinity - 0.270DO, p=0.018, F=54.01, Rsq(adj)=96.4%
	Avon	1995-1999	Diversity = 4.60 - 0.0483COD - 0.00712Sulphide, P=0.002, F=5866.20, Rsq(adj)=99.7%
Group 8	Refinery effluent	1995-1999	Diversity = 1.14 + 0.0251TSS, p=0.036, F=13.11, Rsq(adj)=75.2%
	Chemcial effluent	1995-1999	Diversity = 0.745 + 1.72Benzene + 0.0479Ammonia, p=0.004, F=224.71, Rsq(adj)=99.1%
Group 9	Avon	1985-1999	Diversity = 0.778 - 0.0151SS - 0.173Kjeldahl + 0.00342EC, p=0.001, F=13.46, Rsq(adj)=72.8%
		1993-1999	Diversity = -0.388 + 0.00437EC + 1.18Ammonia, p=0.001, F=60.44, Rsq(adj)=95.2%
		1995-1999	Diversity = 11.7 - 0.0804DOsat - 0.533BOD, p=0.000, F=23110.30, Rsq(adj)=100%
	Refinery effluent	1995-1999	Diversity = 1.74 + 0.0396Nickel, p=0.047, F=10.58, Rsq(adj)=70.5%
	Temperature	1995-1999	Diversity = -1.32 + 0.259MaxTemperature, p=0.025, F=17.34, Rsq(adj)=80.3%
Group 10	Sewage works	1995-1999	Diversity = 3.27 - 0.0191SS, p=0.030, F=15.29, Rsq(adj)=78.1%
	Chemcial effluent	1995-1999	Diversity = 2.14 - 0.547Zinc + 0.0540Ammonia, p=0.003, F=334.02, Rsq(adj)=99.4%

Table 4.25. The chosen variables from the BIO-ENV analyses that have high correlation coefficients for groups 1 to 4

	Source	Time period	Vaiables	Ps
Group 1	Refinery effluent	1995-1999	Ammonia	0.721
	Chemicals effluent	1995-1999	Chromium, Nickel, Zinc	0.842
	Ballast water	1995-1999	EC, Phenol, Ammonia(log+1)	0.952
Group 2	Avon	1995-1999	BOD+ATU, Iron, pH	0.709
	Refinery effluent	1995-1999	Flow, Temperature, EC, Ammonia, Lead, Chromium	0.855
	Chemicals effluent	1995-1999	Benzene, BOD, Acetonitrile, Chromium, Zinc	0.976
Group 3	Sewage works	1993-1999	BOD+ATU, AvpH	0.723
	Avon	1993-1999	BOD, DO, EC, Nitrite, pH, COD(log50-x)	0.703
		1995-1999	COD, DO, Kjeldahl, pH, Sulphide	0.867
	Refinery effluent	1993-1999	pH, Zinc	0.816
		1995-1999	Temperature, TSS	0.782
	Grangeburn	1995-1999	DO, pH(log8.5-x)	0.709
	Chemcials effluent	1995-1999	Ammonia, Hydrocarbon, SS	0.721
	Ballast water	1995-1999	EC, TSS, COD	0.782
Group 4	Refinery effluent	1995-1999	Ammonia, Lead	0.939
	Chemicals effluent	1995-1999	SS, Chromium, Zinc	0.988
	Ballast water	1995-1999	Flow, Sulphide, Phenol	0.879

Table 4.26. The chosen variables from the BIO-ENV analyses that have high correlation coefficients for groups 5 to 7.

	Source	Time period	Vaiables	Ps
Group 5	Sewage works	1993-1999	pH	0.788
		1995-1999	pH	0.915
	Refinery effluent	1993-1999	Flow, pH, Chromium	0.847
		1995-1999	Flow, Chromium (lnx+1), Copper (ln)	0.891
	Avon	1995-1999	EC, Alkalinty, Hardness, pH	0.988
	Grangeburn	1995-1999	DO, SS	0.879
	Chemicals effluent	1995-1999	Phenol, Hydrocarbon	0.903
	Ballast water	1995-1999	pH, BOD, Sulphide, Hydrocarbon	0.964
Group 6	Sewage works	1993-1999	pH, SS	0.747
	Avon	1995-1999	BOD, DOsat, Chloride, Iron	0.794
	Chemicals effluent	1995-1999	Phenol, Acetonitrile, Chromium, Zinc	0.806
	Ballast water	1995-1999	Flow, BOD, COD, Sulphide	0.709
Group 7	Sewage works	1993-1999	pH	0.757
	Refinery effluent	1993-1999	pH, Copper, Zinc	0.704
	Avon	1995-1999	BOD+ATU, Nitrite, Phosphate, pH, Sulphide	0.976
	Grangeburn	1995-1999	BOD+ATU, EC, Ammonia, pH(log8.5-x)	0.794
	Chemicals effluent	1995-1999	BOD, Flouride, Acetonitrile, SS	0.952

Table 4.27. The chosen variables from the BIO-ENV analyses that have high correlation coefficients for groups 8 to 10.

	Source	Time period	Vaiables	Ps
Group 8	Refinery effluent	1993-1999	Flow	0.881
		1995-1999	Flow	0.903
	Avon	1995-1999	DOsat, Chloride, Nitrite, pH	0.867
	Ballast water	1995-1999	COD	0.879
Group 9	Avon	1995-1999	BOD+ATU, DO, EC Sulphide	0.964
	Refinery effluent	1995-1999	Flow, Temperature	0.745
	Chemicals effluent	1995-1999	Benzene, Sulphide, Hydrocarbon	0.842
Group 10	Sewage works	1993-1999	BOD+ATU, pH, SS	0.739
	Refinery effluent	1993-1999	pH, TSS, Zinc, AvCOD(log)	0.734
	Avon	1995-1999	BOD+ATU, Ammonia, Iron, pH	0.939
	Chemicals effluent	1995-1999	Benzene, Hydrocarbon, Copper	0.842
	Ballast water	1995-1999	pH, BOD, Phenol	0.733

5. FIELD SURVEY – SPATIAL VARIATION

5.1 INTRODUCTION

5.1.1 Processes affecting the spatial distribution of benthic communities

Benthic communities have been characterised as having patchy species distributions (Thrush, 1991). These patches occur on a range scales from millimetres to kilometres (Hall *et al.*, 1994). The patches are often created by disturbances, which can be caused by many different factors, including natural physical disturbances such as wave action, hypoxia and temperature stress as well as man-made stresses (Dayton, 1984). Disturbance may, clear an area of the sediment allowing colonisation and succession (Wilson, 1991). A mudflat can therefore contain a variety of different patches that are at different successional phases, which relate to the time and severity of the disturbance. Disturbance is not the only factor controlling the spatial distribution of a species, larval supply, sediment characteristics, predation and competition are also important (Thrush, 1991). Many benthic invertebrate rely on passive transport of their larvae whilst in the water column. This means that on a large scale the water currents determine the settlement area (Thrush, 1991). On a smaller scale however, the larvae are known to actively select a suitable substratum on which to settle (Wolff, 1983) and therefore the sediment characteristics could potentially play an important role. The level of competition between and within species can influence the small scale spacing of individuals (Thrush, 1991) and the diversity of species within an area (Dayton, 1984). Predation also influences the spatial distribution either through the disruption of the sediment or through direct mortality of the prey (Thrush, 1991). The importance of these different factors in determining species distributions varies from site to site. Within soft-substratum communities diversity is thought to be more often related to niche variation than to the effects of competition and predation (Dayton, 1984).

5.1.2 Spatial distributions at Kinneil

The spatio-temporal analysis of the long-term data set indicated that there were spatial differences in the community composition (Chapter 4). The upper shore areas around the refinery and chemical outfall had a different benthic community composition from most of Kinneil and were considered to be impacted. It is likely that the upper and lower shore areas that were examined in the two-year field survey will therefore have different community compositions. It is also possible that small-scale effects will mean that there will be community compositional difference within the two areas. The two-year survey data will allow these small-scale spatial distributional patterns to be assessed in greater detail. In the two year field survey not only was the abundance of each species measured, including that of the oligochaetes to species level, the biomass of each species was also measured. This may provide further information as to the current state of the two areas. The survey assessed the benthic community every three months and therefore any seasonal effects on the spatial distribution may also be detected. It is important to understand what factors are causing the spatial distributional patterns in the two areas as it may help to explain any temporal effects of the movement of the chemical outfall.

5.1.4 Aims

This chapter will consider the spatial differences in abundance and biomass. The analysis of the two year survey data has been carried out with the following aims:-

- To determine if there is a difference in the abundance or biomass between the upper and lower shore areas.
- To determine if there is a difference in the abundance and biomass within the upper shore and the lower shore areas.
- To determine if there has been any change in the spatial distribution over the survey period.
- To determine what factors may have caused the spatial distributional patterns.

5.2 RESULTS

The community structure and composition of the two areas named upper shore (Stations A1-A4, B1, B2, C1-6) and lower shore (stations B5-7, C12-18, D11-14) (Figure 5.1 and Table 2.3 for GPS fixes), are described. The possible cause of any spatial difference will be considered.

5.2.1 Spatial differences in the hydrocarbon content of the sediment

The hydrocarbon results from M-Scan, using saponifaction extraction method and Uvf for total hydrocarbon expression, for the selected 14 station can be seen in Table 5.1. The total aliphatic hydrocarbon (TAH) concentrations were highest in the inner corner and at stations C2 and B7. It may be expected that the concentration of TAH would decrease with distance from the source but although station A1 has fairly high concentrations the highest were found at Station A4. The unresolved complex mixture (UCM) shows a similar pattern and the presence of a UCM indicates that there is an input of petroleum hydrocarbons in this area. The concentration of the n-C_{26-33} series of alkanes is attributed to higher plant leaf waxes typically from terrestrial runoff, the relative importance of this input can be gauged by the ratio (n-C_{26-33}/UCM) x 100. From this it can be seen that the leaf waxes were more important in the lower shore stations and especially at station B5. The concentration of n-alkanes at station A2a was distorted by a large contribution of natural n-C_{17}, also indicating an input from plants. The alkane series n-C_{14-23} is representative of a fresh petrogenic input to the sediments. High concentrations were seen at stations A1, A2a, A3 and A4 although the highest concentration was at A3 and not directly outside the outfall at A1. The ratio of UCM/n-C_{14-23} shows the relative importance of long-term chronic relative to fresh petrogenic input. The low values that were found at stations A1, A2a and A3 indicate that that either little biodegradation is occurring or that there is a continuing input of fresh petrogenic hydrocarbons to this area. The large values at stations B1, B7, B5, A4 and C18 indicate that the petroleum hydrocarbons found in the sediments at these stations may have been present for some time. The ratio of Ph/n-C_{18} indicates the level of microbial degradation, as it causes the level of the isopreniods (Phytane and pristane) to increase. The high values found at stations A2a, A4, and B1 all indicate high levels of biodegradation, although the lowest levels are indicative of a medium

rate of degradation. The concentration of total oil was measured and the values show the same patterns as the TAH which is what would be expected as the TAH usually makes up around 30-50% of crude oil. The distribution of aromatic compounds in the sediments was assessed and it was found that the samples were dominated by petrogenic aromatic hydrocarbons, probably 3 and 4 ring polycyclic aromatic hydrocarbons (PAH). Smaller contributions were made by lighter and heavier aromatics.

5.2.2 Spatial differences in abundance

5.2.2.1 Differences in the community composition

5.2.2.1.1 Diversity

All the sampling periods showed very similar spatial patterns. All lower shore stations and upper shore stations C1-6 all had a similar diversity of 2 (Figures 5.2 to 5.4). The upper shore stations in the inner corner (A1-4, B1, 2) however often had a lower diversity, with stations A1, A2a and A2b having the lowest diversity. There were some slight differences between the sampling periods in that the inner corner shore stations showed an increased diversity in May 1999 and May 2000. C1 had a diversity of 0, which was indicative of the presence of only one species at this site in May 1999.

5.2.2.1.2 Evenness

All stations except A1, A2a and A2b had a similar evenness (Figures 5.5 to 5.7) between 0.5 and 1. The three other stations usually had a lower evenness especially during November 1998, 1999 and February 1999. C1 had a notably low evenness of 0 in May 1999.

5.2.2.1.3 Species richness

The stations had similar numbers of species per station (Figures 5.8 to 5.10), with the shore stations in the inner corner having the lowest value of usually less than 5 species per station. The other stations generally had between 5 and 10 species per station. In February 2000 and May 2000 D11 had the largest number of 14 species $50cm^{-2}$. This figure is considerably larger than those found for the long-term data

due to the identification of the Oligochaetes to species level for the two year survey data.

5.2.2.1.4 Number of individuals

The number of individuals $50cm^{-2}$ is fairly constant for the different stations in each sampling period (Figures 5.11 to 5.15). The stations usually had between 1 and 600 individuals $50cm^{-2}$. The only notable exceptions were for station A1 in November 1998 which had a large number of individuals (1276 $50cm^{-2}$) and in November 1999 when it had 799 individuals 50 cm^{-2}. November 1998 had the largest variation in the number of individuals $50cm^{-2}$ out of all the sampling periods. From assessing the species contributions it can be seen that the large abundance at station A1 was due to *Lumbricillus spp.* This species was mainly found in the upper shore area and particularly at the inner corner stations (A1, A2a and A2b). The lower shore stations were dominated by varying contributions of *Streblospio shrubsolii*, *Tubificoides swirenocoides*, *Tubificoides benedii* and *Manayunkia aestuarina*.

5.2.2.1.5 MDS

The MDS plots (Figures 5.16 to 5.18) for the different sampling periods show that the lower shore stations are grouped more closely than the upper shore stations. In most cases there seems to be a difference in the community between the upper and lower shore stations. Stations A1, A2a and A2b are always the further stations away from the lower shore stations indicating that they have the least similarity of the community. The upper shore stations C1-6 had a similar community to the highest lower shore stations (B5-7).

5.2.2.1.6 k-dominance curves

The k-dominance curves for the difference between upper and lower shore stations (Figures 5.19 to 5.21) show that in November 1998 the upper shore stations had a slightly increased dominance, suggesting an impact. The increase had disappeared by February 1999 and the two areas remained very similar until May 2000. In May and July 2000 the upper and lower shore stations showed very different dominance curves. In both cases the upper shore stations are above the lower shore stations. In November 2000 they both go back to being the same again.

The k-dominance plot showing the difference between the stations within the upper shore area (Figures 5.22 to 5.24) show that the inner corner stations (A1, A2a, A2B and A3) were often dominated by one or two species. Whereas the least dominated stations are usually along C1-6. The exact order of dominance does change between sampling times. For the lower shore stations (Figures 5.25 to 5.27) there does not seem to be any consist order of stations in the different sampling times. The stations that can be considered highly dominated varied throughout the two-year period.

5.2.2.1.7 ANOSIM and dispersion

The ANOSIM results (Table 5.2) confirm that there was a significant difference in each sampling period between the community of the lower and upper shore areas. The communities were however more similar although still different in July 1999 and May 2000. The measure of dispersion (Table 5.3) also confirms that in all sampling periods except May 2000 the lower shore stations showed less variation than the upper shore stations. The greatest variation difference between the upper and lower shore stations was in July and November 1999.

5.2.2.1.8 SIMPER

The SIMPER analysis results (Tables 5.4 to 5.12) show that there was a large difference in the community composition between upper shore and lower shore sites as they all have an average dissimilarity above 83.5. The species that were responsible for the differences between the upper and lower shore stations are mainly the oligochaete species, the spionids and *Manayunkia aestuarina*. It can be seen that certain species were more predominant either in the upper or lower shore stations, very few species have high abundance in both areas. Typically *Lumbricillus* species, *M. aestuarina*, *Tubificidae* species, *Paranais litoralis* and *Heterochaeta costata* had a high abundance in the upper shore stations. On the other hand *Tubificoides swirenocoides*, *Streblospio shrubsolii* and *Macoma balthica* had a higher abundance in the lower shore stations. Both *Pygospio elegans* and *T. benedii* are fairly abundant in both areas but tend to have higher abundance in lower shore stations.

5.2.2.2 Community differences in relation to environmental factors

5.2.2.2.1 Distance from pollution sources, shore height and sediment characteristics

The BIO-ENV results (Table 5.13) indicate that for all sampling times except May 2000 the distance from the refinery outfall was important in determining the community distribution. The distance from the new lower shore chemicals outfall was also important in February 1999, May 1999, November 1999 and July 2000. In November 1999 and July 2000 the percentage clay may also have been important. For the sampling time May 2000 height alone had the highest correlation. All the variables selected have high Spearmans correlation coefficient (p_s) above 70% except Jul 1999, which had a value of 68%. It should be noted that the distance from the refinery outfall and the height are highly correlated (-0.950, p=0.000) and can therefore be considered interchangeable.

The variables chosen by the BIO-ENV analysis were superimposed onto the MDS plots for each sampling time (Figures 5.28 to 5.32). The same patterns can be seen for each sampling time. Stations A1, A2a, A2b, B1 and B2 are closest to the refinery outfall and they are generally grouped together away from the other stations on one side. In the middle are the intermediate stations and on the other side are the stations furthest away, the lower shore stations. The distance from the new lower shore outfall (site 3) does not show such a clearly defined pattern. In general the upper shore stations are further away and the lower shore stations closer. The percentage clay also does not show a completely clear picture but generally the lower shore stations have a relatively intermediate percentage and are grouped together on one side. The stations C1-6, B1 and B2 have a low percentage of clay and are grouped together in the middle of the plot and the stations A1- 4 have a high percentage and are grouped on the other side. Lastly the height shows that the stations at a lower tidal height differ from the upper shore stations but within each area the values are very similar.

The Stepwise regression analysis (Table 5.14) shows that the change in the number of species can only be explained by the spatial variables in November 1998, May 2000 and July 2000. All three models included the distance from the refinery outfall often with the distance from Grange burn and/or a measure of the sediment. The fact

that very significant models explain the variation in diversity, evenness and the number of individuals suggests that the relationship may not be linear or that other factors than those examined are important.

5.2.2.2.2 Sediment hydrocarbon content

The results of the spatial regression and BIO-ENV analyses on the 14 stations, which included the hydrocarbon results and the other spatial variables that were previously used (distances from pollution sources, height and sediment characteristics), are seen in Table 5.15. These indicate that the levels of TAH, n-C_{14-23}, UCM/n-C_{14-23} ratio or the crude oil may help to explain the spatial distribution in diversity and species richness along with other factors such as distance from the refinery and the chemical outfall (site 3). The concentration of the different hydrocarbon fractions did not seem important in determining the evenness or the number of individuals.

5.2.2.2.3 Salinity

The salinity showed a slightly different pattern for the upper shore stations for February and July 1999 (Figure 5.33). There was a decreased salinity at B2 and in February 1999 at C6 as well. In general the salinity was higher in February 1999 than in July 1999. The difference in the salinity for February 1999 did not correlate with the differences in diversity, evenness, species richness and number of individuals. The salinity data for July 1999 did however significantly correlate with the number of species and the number of individuals (No. Species = - 4.81 + 0.495 Salinity, P=0.13, F=8.79, Rsq(adj)=39.4%; No. Individuals = -337 + 22.6 Salinity, P=0.021, F=7.23, Rsq(adj)=34.2%). The BIO-ENV analysis did not produce highly significant results for either February or July 1999. The data for July 1999 did however produce a more significant result than the February 1999 data (Feb 99 – p_s = 0.063; Jul 99 – p_s= 0.258).

5.2.3 Spatial differences in biomass

5.2.3.1 Biomass regression equations

The regression equations for the biomass/size relationship for *Macoma balthica*, *Hydrobia ulvae*, *Cerastoderma edule*, *Eteone longa*, *Nephtys hombergii* and *Nereis*

diversicolor (Figures 5.34 to 5.36) all showed an increase in biomass with increasing size. These equations were used to determine the biomass for each station, along with the estimated mean biomass per individual for the other species (Table 5.16).

5.2.3.2 Differences in the community composition

5.2.3.2.1 Total biomass

It can be seen that generally the biomass was greater for the lower shore stations than the upper shore stations although it was very variable both within and between each sampling period (Figures 5.37 to 5.39). The stations that tended to have the highest biomass were C14, D11, D12 and D13. The species responsible for the increased biomass at these stations were *Mytilus edulis* and *Cerastoderma edule*. July 2000 had the highest biomass levels of all sampling periods. Station D11 had the largest biomass which was attributed to *Mytilus edulis*, whilst stations C16 and D12 had an exceptionally high biomass of *Nephtys hombergii*.

5.2.3.2.2 MDS

The MDS plots (Figures 5.40 to 5.42) indicate that there was a difference in the biomass between the upper and lower shore stations. For most of the sampling times the variability between the upper shore stations looked to be greater than that of the lower shore stations. It can also be noted that most of the plots indicate that the stations A1, A2a or A2b are often on one side whilst the stations D11, D12 or D13 are on the other, suggesting that they had different community compositions.

5.2.3.2.3 k-dominance curves

The k-dominance plots (Figures 5.43 to 5.45) also showed a difference in the biomass between the upper and lower shore stations. The lower shore area tends to have a more dominant biomass, especially during February 1999, May 1999, November 1999 and February 2000, indicating that one or two species were influencing the biomass distributions.

The k-dominance plots for the differences within the upper shore and the lower shore areas (Figures 5.46 to 5.51) indicate that like those for the abundance data

there was variability between the sampling periods. In general though for the upper shore area the inner corner stations (A1, A2a, A2b and A3) tended to have a high dominance, although in both July periods B2 also seemed to become highly dominated. For the lower shore the stations D11 and D12 tended to show the highest dominance. This indicates that the biomass at these stations was governed by one or two species. The fact that the upper shore results for the biomass and abundance data are very similar indicates that the higher biomass was due to a larger abundance of individuals at these stations. On the other hand the lower shore results for the biomass and abundance data are very different suggesting that the high biomass at these stations was due to a few large individuals.

5.2.3.2.4 ANOSIM and dispersion

The ANOSIM results (Table 5.16) confirms that for all sampling times a difference in the biomass could be detected between the upper and lower shore areas. This difference was least obvious during the sampling period February 2000. The measure of dispersion (Table 5.17) indicates that there is a higher variability between the upper shore stations in November 1998, 1999 and 2000, May 1999 and July 1999 and 2000 and a higher variability within the lower shore area during all other sampling periods.

5.2.3.2.5 SIMPER

The SIMPER analysis compared the species composition of the upper and lower shore areas during each sampling time. The results of the analysis (Tables 5.18 to 5.26) indicate that the species *Macoma balthica, Cerastoderma edule, Streblospio shrubsolii, Mytilus edulis* and *Nephtys hombergii* all had a greater biomass in the lower shore area. On the other hand *Manayunkia aestuarina* and *Nereis diversicolor* generally had a greater biomass at the upper shore stations. The Oligochaetes showed a fluctuating pattern with higher biomass at the upper shore stations during November and February and higher at the lower shore stations during May and July.

5.2.3.2.6 Comparison of biomass to previous studies

Yule (1996) previously measured the benthic invertebrate biomass of stations at Kinneil in August 1996. The comparison of this data with the present data collected in July 1999 and 2000 (Figure 5.52) indicates that there has been little change in the

biomass at the upper shore stations. These stations and particularly the inner corner stations still have a very low biomass compared to stations elsewhere. The lower shore stations however do show variations between the different sampling times. For most of the lower shore stations the biomass was higher in 1996, except for stations D11 and D12 when the biomass was greatest in 2000. The species responsible for the high biomass levels for the two sampling times were different. In 1996 *Hydrobia ulvae*, *Macoma balthica* and *Cerastoderma edule* were the main contributors to the high biomass at these two stations, whilst in 2000 it was *Mytilus edulis*, *Nephtys hombergii and C. edule*.

5.2.3.3 Community difference in relation to environmental factors

5.2.3.3.1 Distance from pollution sources, shore height and sediment characteristics

The spatial distribution of the biomass was tested against the distance from the different pollution sources, station height and the sediment properties to see if any of these could potentially explain the differences. The BIO-ENV results (Table 5.27) suggested that the distance from the refinery and/or the height may have been important factors in determining the species distributions, although none of the results are highly significant. For May 1999 and November 1999 the percentage organic matter content of the sediment and in May 2000 the distance from the new lower shore chemicals outfall, may also have been important. The variables selected show the least correlation during both February sampling periods and the highest during May 2000.

The Stepwise regression results (Table 5.28) produced only one highly significant result with a large Rsq(adj) value in May 2000. The model for May 2000 included the height, mean particle size and distance from the sewage works and Grange burn. As with the diversity evenness and abundance data it seems as if the relationship for the biomass may not be linear or can not be fully explained by the factors tested in this study.

5.2.3.3.2 Sediment hydrocarbon content

The hydrocarbon results have been used along with the other variables such as distances, height and sediment characteristics, in stepwise regression and BIO-ENV

analyses to see if they were affecting the species distributions of biomass (Table 5.29). Neither the stepwise regression nor the BIO-ENV analysis found any of the hydrocarbon measures to be important in explaining the spatial distribution of the biomass.

5.2.3.3.3 Salinity

The salinity at the upper shore stations in February 1999 and July 1999 were collected and examined to see if this might possibly have an effect. The BIO-ENV analysis indicated that it was probably not important in governing the spatial distribution in either February 1999 ($p_s = 0.046$) or July 1999 ($p_s = 0.300$). It was however more significant during July than February. A regression analysis also had similar results with February 1999 not producing a significant result, whilst July 1999 did produce a significant result (log biomass = -7.06 + 0.216 salinity, p=0.003, F=15.05, Rsq(adj)=53.9%). It therefore may have been more important during certain months along with other factors.

5.3 DISCUSSION

5.3.1 Spatial difference in the benthic community

5.3.1.1 Between upper and lower shore areas

The upper shore and lower shore areas could potentially have different benthic communities caused by differences in the environmental conditions. The upper shore area receives effluents from the petrochemical complex that have been previously shown to affect the distributions of certain species (McLusky, 1982a; Chapter 4). The two areas also have slightly different physical conditions. The upper shore area is exposed to a higher level of stress from desiccation due to the increased time the area is uncovered by the tide. The lower shore area has been shown to have a slightly different sediment composition, with it having a higher percentage of silt and a lower percentage of sand than the upper shore area. The spatio-temporal long-term data analysis (Chapter 4) indicated that the upper shore area around the petrochemical outfall had a different community to the lower shore areas. Yule (1996) also found that there was a spatial difference in the biomass, being reduced in the area around the petrochemical effluent. A difference in the

diversity, evenness, species richness, abundance and biomass was detected between the upper and lower shore areas during each sampling period in the present study. The upper shore area generally had a lower abundance, diversity, evenness, species richness and biomass than the lower shore areas. Opportunistic species were found at both areas, but this is not unexpected, as these species are particularly capable of living in stressful environments such as estuaries (Pearson & Rosenberg, 1978). The abundance data indicated that the upper shore area had a higher abundance of *Lumbricillus spp, Manayunkia aestuarina, Paranais litoralis* and *Heterochaeta costata*, whereas the lower shore area had a higher abundance of *Tubificoides swirenocoides, Streblospio shrubsolii* and *Macoma balthica*. The biomass indicated that the upper shore area had a higher biomass of *M. aestuarina* and *Nereis diversicolor*, whereas the lower shore area had a higher biomass of *M. balthica, Cerastoderma edule, S. shrubsolii, Mytilus edulis* and *Nephtys hombergii*. The higher abundance and biomass of the opportunistic species and the lower diversity, species richness and biomass all indicate that the upper shore area is under greater stress that the lower shore area (Dauer & Ranasingle, 1992).

5.3.1.2 Within the upper and lower shore areas

It is also possible that there are differences in the community composition between the stations within each area. Effects of the effluents, predation, competition and sediment characteristics can occur on very small scales (Hall *et al.*, 1994). The analysis of the survey data did detect variation in the abundance and biomass of the benthic community within each of the areas. The upper shore area showed greater variation than the lower shore area. Both the abundance and biomass data indicated that the stations A1, A2a and A2b were the most impacted as they had the lowest diversity, abundance and biomass values. This agrees with the findings of McLusky & McCrory (1989), Yule (1996), and Chapter 4 that the inner corner area had a reduced abundance and biomass compared to the rest of the mudflat. The biomass data indicated that the stations C14, D11, D12, and D13 had the highest biomass, which was attributed to one species, *Mytilus edulis*. It can therefore be concluded that the upper shore area is the most impacted of the two areas, however not all stations within the area are impacted and the impact is restricted to the inner corner stations.

5.3.1.3 Changes in the spatial community distribution over time

The spatial distribution of the benthic community at Kinneil was assessed for each sampling period over the two years. It is possible that the spatial distributional patterns may have changed from one period to the next. At Kinneil there are many possible factors that could cause a change in the spatial distribution of the benthic community including seasonal factors such as recruitment (David *et al.*, 1997), changes in the pollution levels i.e. from the refinery effluent (Dicks, 1976a; Leppakoski & Lindstrom, 1978), a disturbance (Hall *et al.*, 1994) or the movement of the chemical outfall. Holland (1987) observed that spring was the critical period for the development of distributional patterns. The difference between and within the two areas remained relatively constant throughout the two years. The only differences that were detected between the different sampling periods were a particularly high abundance of *Lumbricillus spp* at station A1 in November 1998 and 1999, the variable dispersion of the biomass within the upper and lower shore areas and the variable dominance of the two areas. The comparison of the biomass with previous studies also indicated that there had been a change over time in the lower shore area but not in the upper shore area. The reasons and implications of these temporal changes in the spatial community distributions will be considered in Chapter 6.

5.3.2 Reasons for spatial distribution of the benthic community

Although it has been shown that there were spatial differences in the benthic community composition within and between the two areas, the question remains what factor(s) are responsible for these distributional patterns? This study has considered a number of different factors, including the distance from the different pollution sources, shore height, sediment composition, hydrocarbon concentrations and salinity. It should be noted however that there are other possible factors such as predation, competition and larval supply (Thrush, 1991) that were not quantified in this study.

5.3.2.1 Shore height

Moore *et al.* (1987) found that shore height was an important factor in explaining the differences in the community composition of meiofauna at Kinneil. Gonzalez-Oreja & Saiz-Salinas (1999) found that in the Bilbao estuary, intertidal height was the most important factor controlling the species distributions. The multiple regression and BIO-ENV both indicated that the differences in the heights of the stations on the shore did explain the spatial distribution of the benthic community. The stations at the greatest tidal height, the most impacted in the inner corner stations, will be subjected to greater stress from prolonged exposure to the air. Therefore only those species that can tolerate highly stressful conditions, such as opportunistic species, will be able to survive in this area. It therefore seems that the shore height is an important factor governing the spatial community distribution. It should be noted however that the shore height is highly correlated with the distance from the refinery outfall (See 5.3.2.3), which therefore means that the effects from these two factors can not be separated.

5.3.2.2 Distance from pollution sources

As has been previously shown, the effects from a pollution source, such as a refinery effluent, often cause a spatial distribution pattern in the benthic community which is related to the distance from the source (Wharfe, 1975; Petpiroon & Dicks, 1982; McLusky, 1982a; Saha & Konar, 1984a; Talsi, 1987; Dicks & Levell, 1989; McLusky & McCrory, 1989; Estacio et al., 1997; Cardell *et al.*, 1999; Mucha & Costa, 1999). The potential pollution sources at Kinneil are the two effluents from the petrochemical complex, the sewage effluent, the two rivers (Avon and Grangeburn) and the main estuary channel (Kinneil channel). The distance from these sources as well as the distance from the refinery track and the end of the refinery track were assessed to see if they were important in structuring the benthic community.

The results indicated that the distance from the refinery outfall was an important factor that explained the species distributions during many of the sampling periods. The bubble plots indicated that the abundance and biomass increased with

increasing distance from the outfall, which is the typical pattern seen around refinery outfalls (Wharfe, 1975; Petpiroon & Dicks, 1982; Saha & Konar, 1984a; Talsi, 1987; Dicks & Levell, 1989). Again it should be noted that the distance from the refinery outfall is highly correlated with the shore height and therefore the individual effects of these two factors can not be determined.

The distance from the new chemical outfall was also implicated during several of the sampling periods suggesting that it may be important at certain times i.e. under certain climate or hydrological conditions or when the effluent has a certain chemical composition. The toxicity of many chemicals is known to increase or decrease with temperature, pH, hardness and the presence of other compounds (Cote, 1976; Burks, 1982) and the chemical composition of petrochemical effluents are known to vary on a day to day basis. The analysis suggested that the chemical effluent may be having a positive effect on the community, by increasing the diversity, abundance and biomass in the areas close to the outfall. It is possible that the organic compounds within the effluent, such as ammonia, are causing a slight enrichment effect (Pearson & Rosenberg, 1978; Mucha & Costa, 1999).

The distance from the other pollution sources did not seem to be important in determining the benthic community structure. The multiple regression analysis does implicate the distance from the sewage works and the distance from the Grangeburn River but it is unlikely that either of these will have effected the two areas, as they are not very close. These findings therefore agree with those found in Chapter 4 that the pollution sources other than the petrochemical effluents have had little effect on the spatial distributions of the Kinneil benthos.

5.3.2.3 Sediment characteristics

There is some controversy over the role the sediment characteristics play in determining species distributional patterns. Many studies have found that the type of sediment correlated with infaunal invertebrate distributions (Gray, 1974), however, Snelgrove & Butman (1994) show that there is in fact little evidence to indicate that the sediment characteristics are the primary determinant of infaunal species distributions. The present study has shown that there were differences in the particle

size and organic matter content of the sediment at the different stations. Pearson & Rosenberg (1987) theorised that food availability is the most fundamental variable underlying the structure of marine benthic communities. It therefore seems possible that the organic matter content of the sediment may be important in determining the community composition. The sediment particle size may also affect species distributions, as larvae are known to actively select a suitable substratum on which to settle (Gray, 1974). The difference in the sediment particles size or the organic matter content were implicated by the multiple regression and BIO-ENV analysis to be important in determining the species abundance and to some extent the species biomass distributions. It should be noted, however, that these factors were only implicated along with other variables and therefore on their own did not explain the spatial community distributions.

5.3.2.4 Sediment hydrocarbon concentration

The sediment is an important sink for hydrocarbons and other compounds and they can remain in the sediments for many years (Knap & Williams, 1982). Hydrocarbons are known to be toxic to many marine organisms (Cote, 1976; Hall *et al.*, 1978; Reece & Burks, 1985; Das & Konar, 1988) and can affect species distributions (Houston *et al.*, 1983). The petrochemical effluents, the sewage effluent and the rivers all potentially contain hydrocarbons that may be deposited within the sediment. Several studies have looked at the spatial distribution of hydrocarbons around refinery outfalls, usually finding the concentration to be highest next to the outfall and decreasing with increasing distance (Knap *et al.*, 1982; Houston *et al.*, 1983; Armannsson *et al.*, 1985; Talsi, 1987; Le Dreau *et al.*, 1997). Previous studies have also measured the hydrocarbon concentrations in the Forth estuary, with generally the greatest concentrations detected around Grangemouth (Ajayi & Poxton, 1987; Elliott & Griffiths, 1987; Cranthorne *et al.*, 1989) and specifically at Kinneil close to the refinery outfall (Mohd Long, 1987). The hydrocarbon content of the sediment at Kinneil in this study was only measured for samples taken in May 2000 (See 2.1.2.3). The analysis of sediment samples (M-Scan, 2001) indicated that there was an accumulation of fresh petrogenic hydrocarbons within the sediment around the refinery outfall (stations A1, A2a, A3 and A4). Several stations had a large concentration of hydrocarbons that could be

attributed to plant leaf waxes, specifically stations A1, A4, B5 and B7. Stations A4, B5 and B7 are all close to the Grangeburn River. Rivers are a source of terrestrial run off and therefore often contain hydrocarbons (Latimer & Quinn, 1998). There are however two possible reasons why A1 has a high load from plant waxes. Firstly, the refinery effluent does contain some terrestrial runoff and secondly, around stations A1 and A2a marsh plants are starting to colonise the mudflat. The presence of marsh plants may also explain why station A2a has a high concentration of natural n-C_{17}. The values found for the TAH around the refinery outfall are lower than those found by Mohd Long (1987). Along the shore Mohd Long (1987) measured concentrations from 2451 to 5981 µg/g, compared to the 390 to 2500 µg/g from this study. This difference can largely be attributed to the different methods used in measuring the aliphatic fraction. The levels of hydrocarbons found at Kinneil in the present study are high compared to other areas of the Forth estuary (Elliot & Griffiths, 1987) and are four times higher than those found at the offshore Kinneil diffuser site in 1998 (M-Scan, 2001). The multiple regression and BIO-ENV analysis indicate that the concentration of TAH, the petrogenic hydrocarbons (n-C14-23) or the crude oil, may play a role in determining the diversity and number of species, although they do not seem to control the number of individuals, evenness or biomass. It therefore seems that the hydrocarbons may be having a toxic effect, which is excluding those species that can not survive in these conditions, from the areas around the refinery outfall. It can be concluded that the hydrocarbons in the refinery effluent, although at very low levels, are still able to affect the species distributions through accumulation within the sediment.

5.3.2.5 Salinity

Salinity is an important factor governing the distribution of species within estuaries (McLusky, 1989; Wolf, 1983). Many species have a specific salinity range within which they can survive (Hunter, 1981). Holland (1987) noted that salinity was a major factor in determining spatial distributions. It is possible that the discharge of the largely freshwater refinery and chemical effluents may indeed have a localised effect on the distribution of some species. Changes in salinity may also affect the toxicity of the effluents, for example the toxicity of some heavy metals is dependent on salinity (Cote, 1976). The regression and BIO-ENV analysis indicate that salinity

may play a role along with other variables in determining the spatial distribution, although its effects may be limited to certain months.

5.4 CONCLUSIONS

In response to the main objectives of this chapter it can be concluded that there was a difference in the abundance and biomass of the community between the upper and lower shore areas, which was consistent throughout the two-year study period. The upper shore area had a higher abundance and biomass of the more opportunistic species, whereas the lower shore area tended to have a higher abundance and biomass of the non-opportunistic species. Within the lower shore area there was little variation between the stations in abundance, however there was variation in biomass, with stations C14, D11, D12, and D13 being the least impacted with the highest biomass. Within the upper shore area there were detectable differences in the abundance and biomass, which indicate that stations A1, A2a, A2b and A3 were impacted, having a decreased diversity, evenness, species richness and biomass. The most probable cause for the distributional differences was found to be the distance from the refinery outfall and/or shore height. It was hypothesised that it was likely that the combined effects of the refinery effluent and the shore height work together to produce the community distribution that was seen. The shore height determines which species are able to live in that environment. At the highest tidal height only those species that are able to withstand long periods of exposure to air can survive. The refinery effluent may further reduce the species to those that can survive the concentrations of chemicals within the effluent, such as the hydrocarbons. The accumulated hydrocarbon content of the sediments was implicated for the species richness and diversity.

This spatial analysis was in general agreement with the spatio-temporal analysis but has highlighted the specific stations that are highly impacted in the inner corner. It has also confirmed the hypothesis that the desiccation effects of shore height are important in determining the spatial species distributions, along with the effects of the refinery effluent.

Table 5.1. Hydrocarbon results for the selected stations at Kinneil in May 2000, using saponification extraction method and Uvf for total hydrocarbon expression. Concentrations in µg/g dry sediment. TAH – Total aliphatic hydrocarbons. UCM = Unresolved complex mixture (See text for explanation).

Station	TAH	UCM	n-C_{14-23}	UCM/n-C_{14-23}	n-C_{26-33}	n-C_{26-33}%UCM	Ph/n-C_{18}	Total Oil
A1	2100	1600	74	21	24	1.5	1.1	4800
A2A	1300	1000	35	29	16	1.5	2.3	3700
A3	2500	1900	86	22	9.5	0.51	1.4	5500
A4	2200	1900	20	92	21	1.1	2.1	8500
B1	1500	1200	12	100	14	1.2	2.9	3500
B5	880	730	7	105	23	3.1	1.5	2900
B7	1800	1600	11	147	28	1.8	1.7	6200
C2	1600	1300	5.3	246	11	0.86	1.9	3200
C6	390	330	5.7	57	5.5	1.7	1.5	1100
C12	390	330	4.7	70	10	2.9	1.4	1200
C15	250	210	4	54	5.6	2.6	1	800
C18	270	230	2.3	102	6.9	3	1	1100
D12	300	250	4	64	9	3.6	1.2	1000
D14	400	350	3.8	93	9	2.6	1	1400

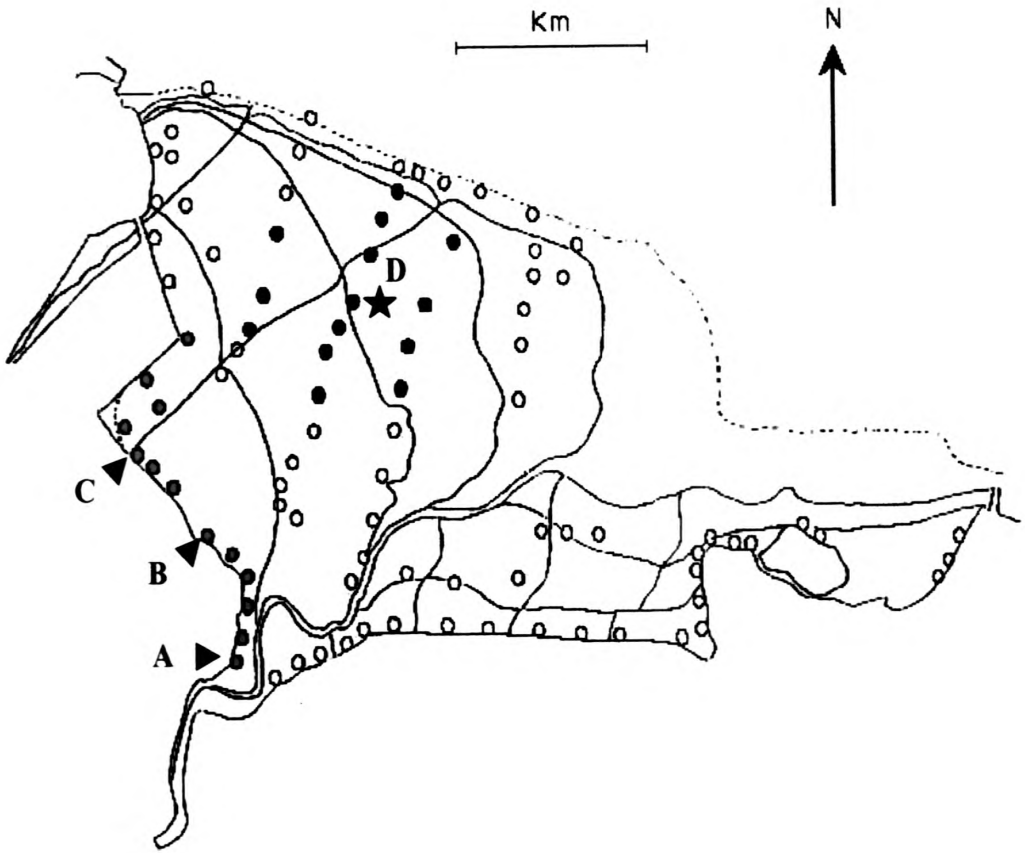


Figure 5.1. Location of sampling stations, Upper shore stations - Red, Lower shore stations - Blue, and of effluent outfalls, A – Chemical outfall until 1979, B – Chemical outfall 1979-1999, C – Refinery + Ballast water outfall, D – Lower shore chemical outfall from 1999.

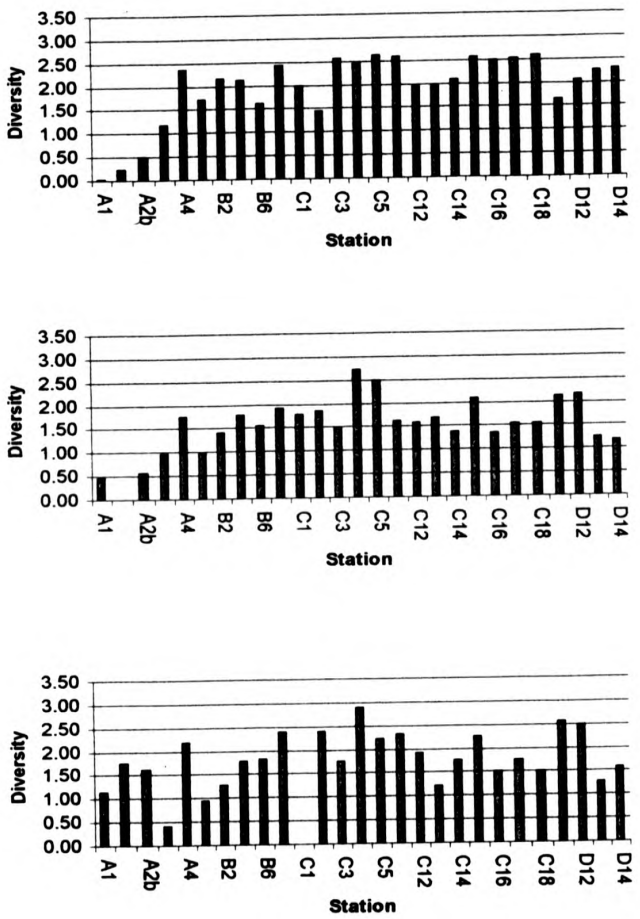


Figure 5.2. The average diversity for each station for November 1998 (top), February 1999 (middle) and May 1999 (bottom).

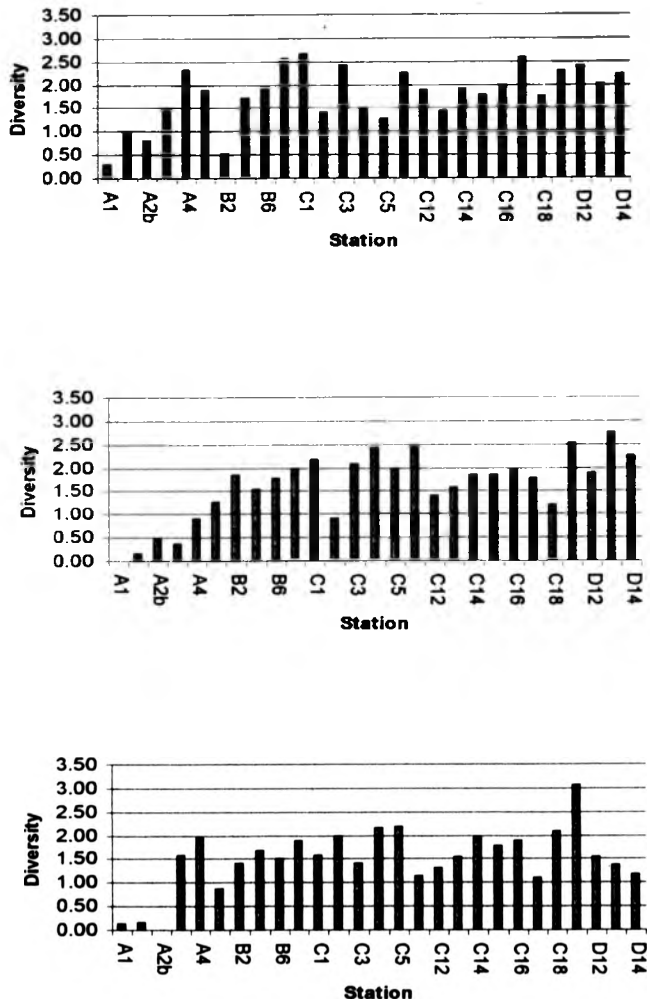


Figure 5.3. The average diversity for each station for July 1999 (top), November 1999 (middle) and February 2000 (bottom).

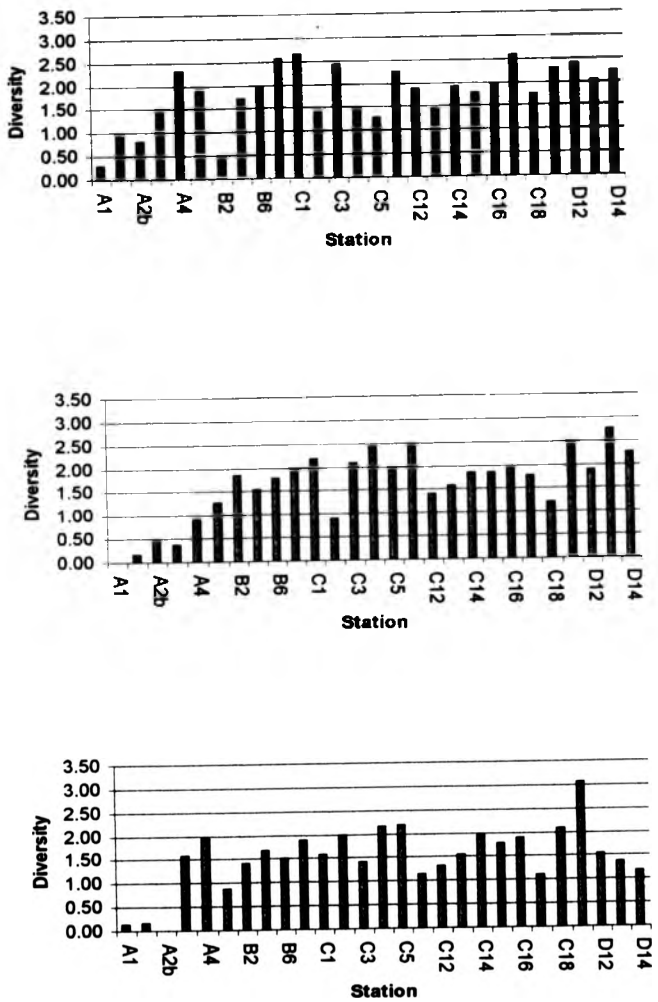


Figure 5.3. The average diversity for each station for July 1999 (top), November 1999 (middle) and February 2000 (bottom).

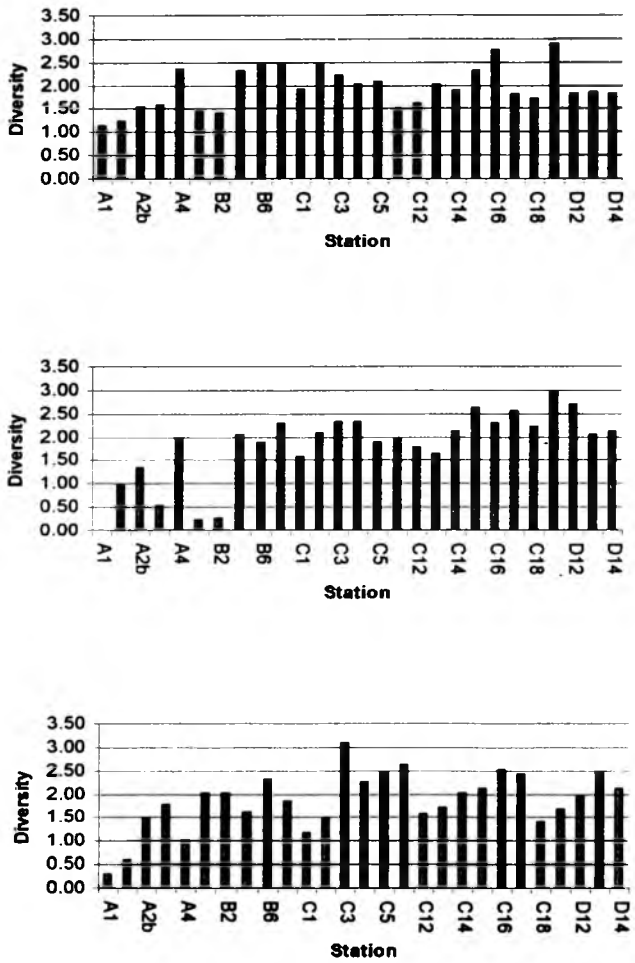


Figure 5.4. The average diversity for each station for May 2000 (top), July 2000 (middle) and November 2000 (bottom).

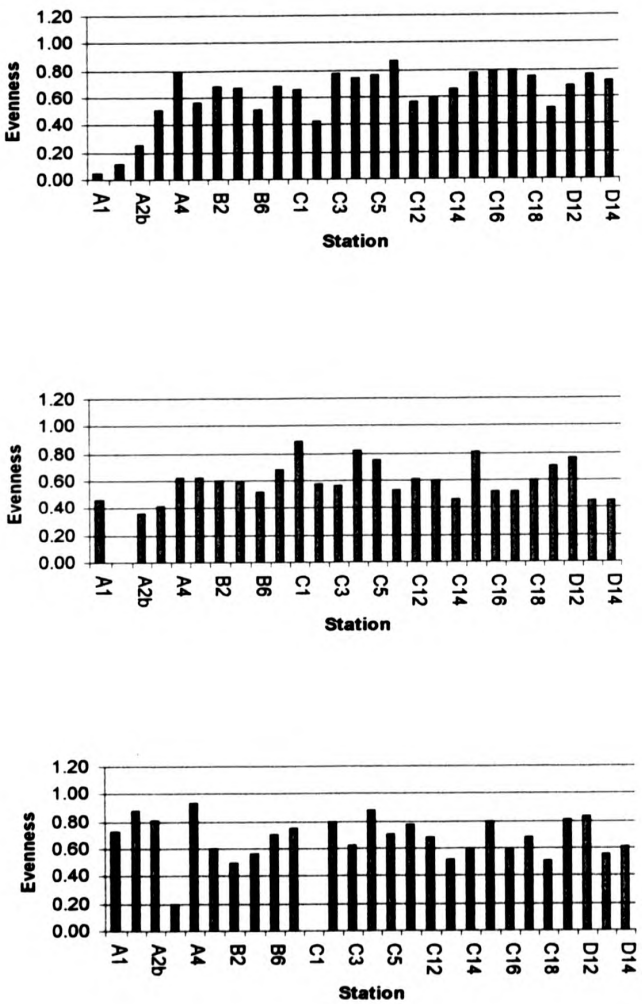


Figure 5.5. The average evenness for each station for November 1998 (top), February 1999 (middle) and May 1999 (bottom).

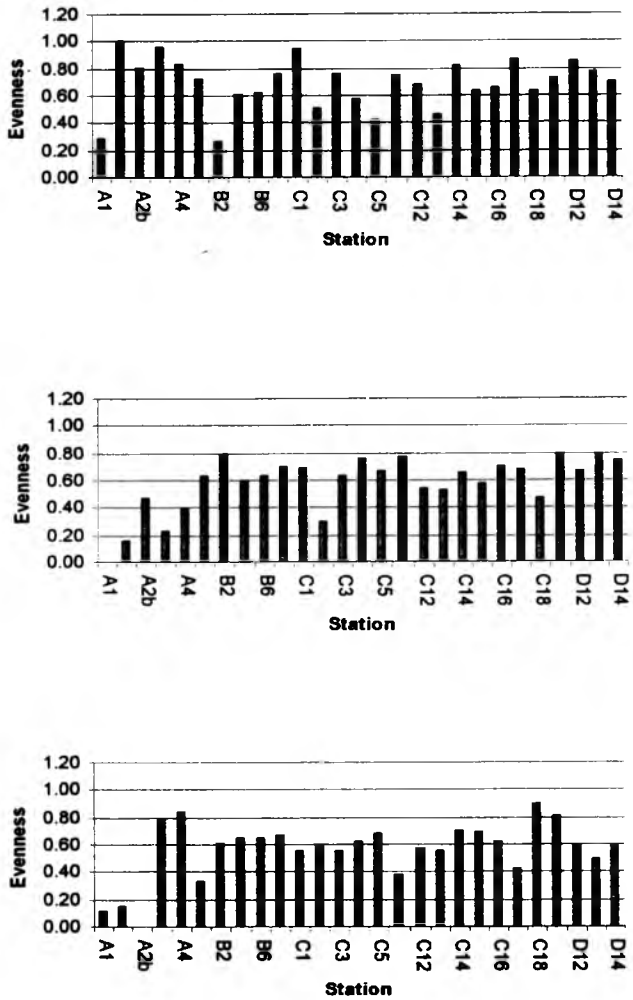


Figure 5.6. The average evenness for each station for July 1999 (top), November 1999 (middle) and February 2000 (bottom).

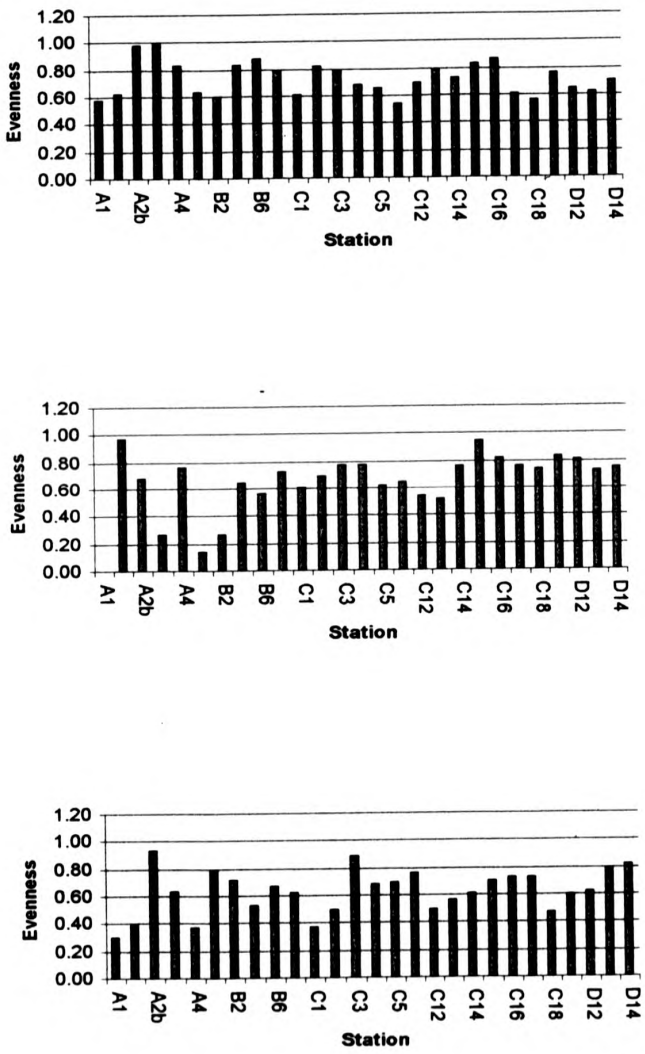


Figure 5.7. The average evenness for each station for May 2000 (top), July 2000 (middle) and November 2000 (bottom).

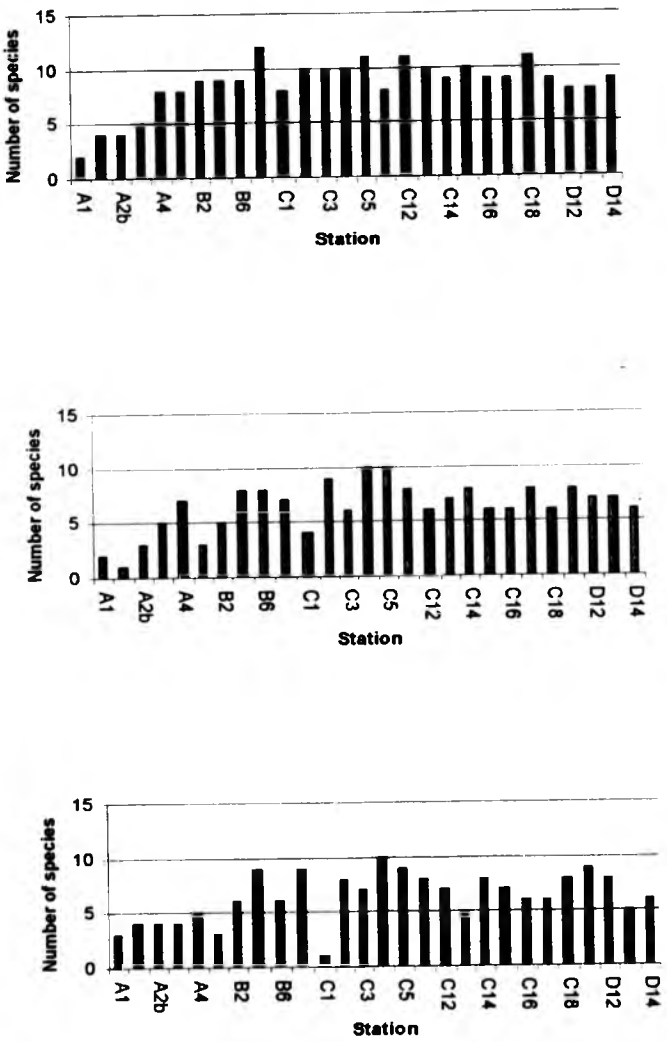


Figure 5.8. The average number of species per station for each station for November 1998 (top), February 1999 (middle) and May 1999 (bottom).

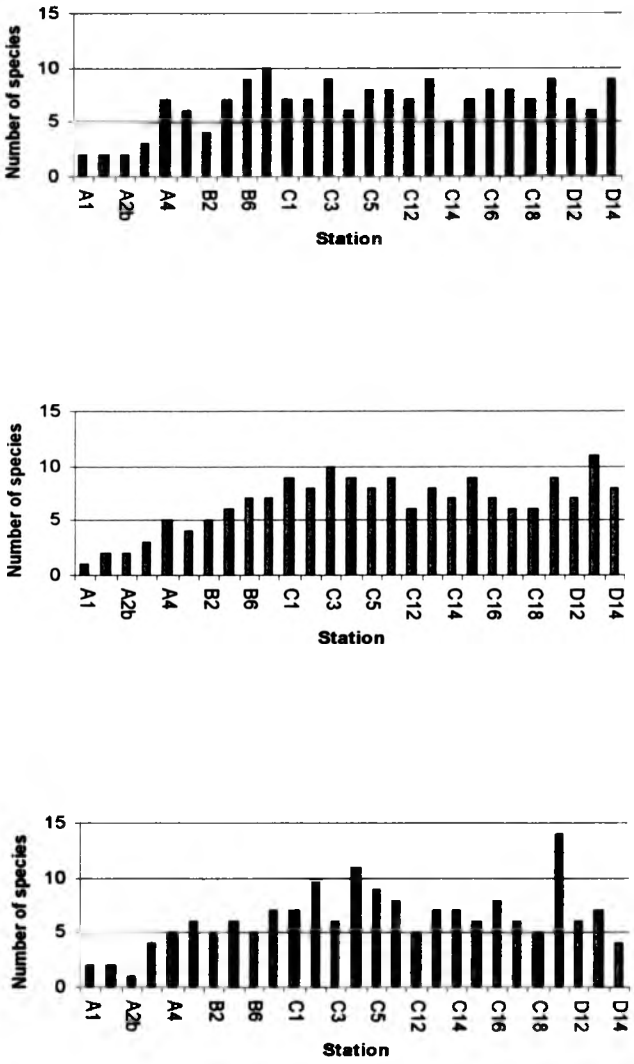


Figure 5.9. The average number of species per station for each station for July 1999 (top), November 1999 (middle) and February 2000 (bottom).

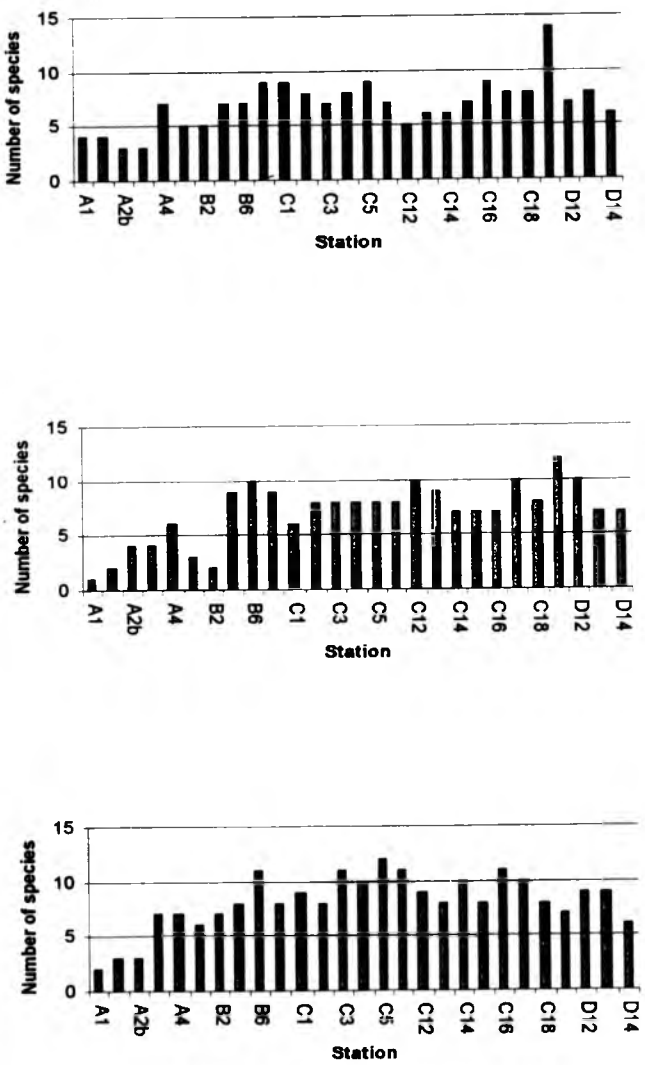


Figure 5.10. The average number of species per for each station for May 2000 (top), July 2000 (middle) and November 2000 (bottom).

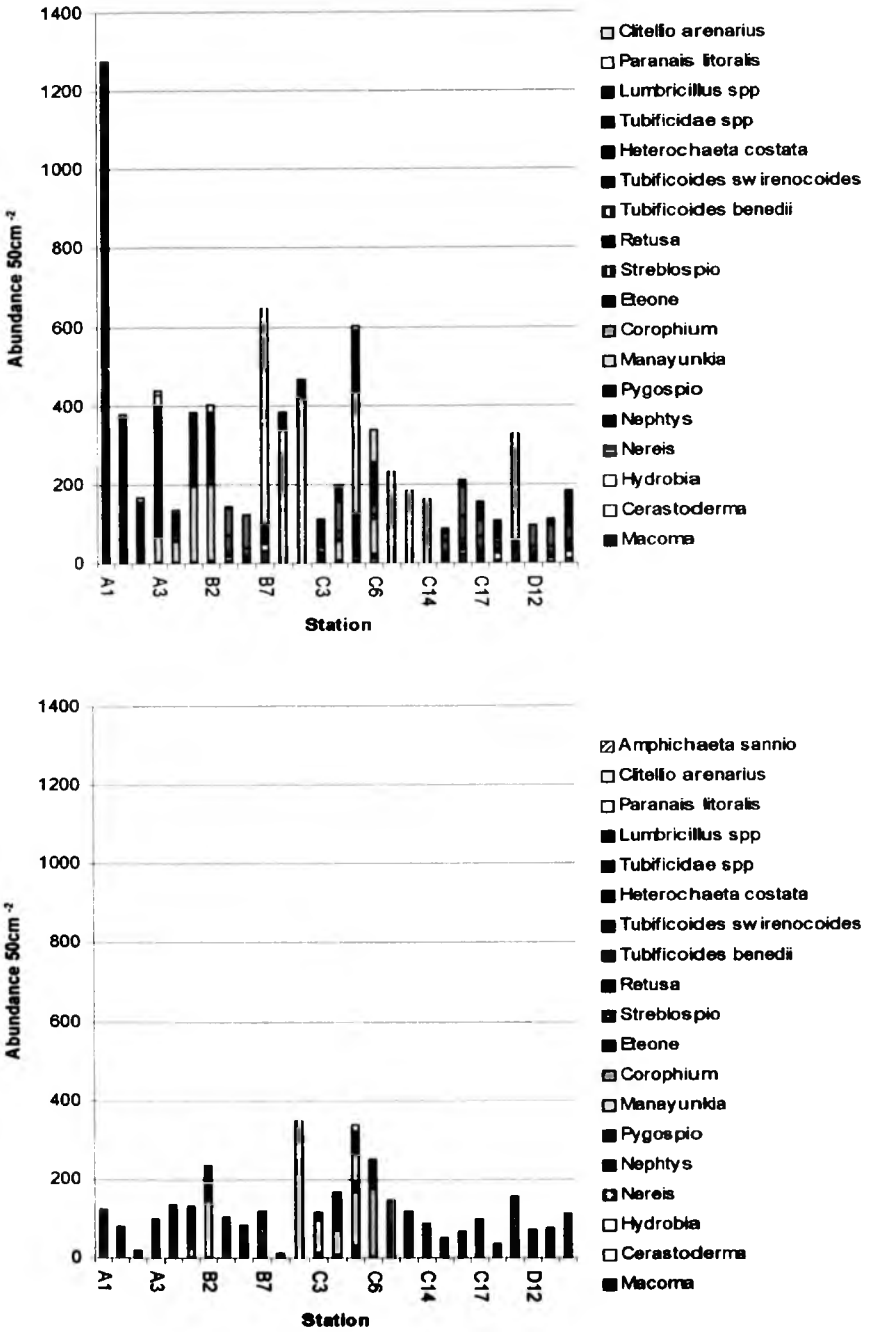


Figure 5.11.The abundance at each station and the contribution of each species for November 1998 (Top) and February 1999 (Bottom).

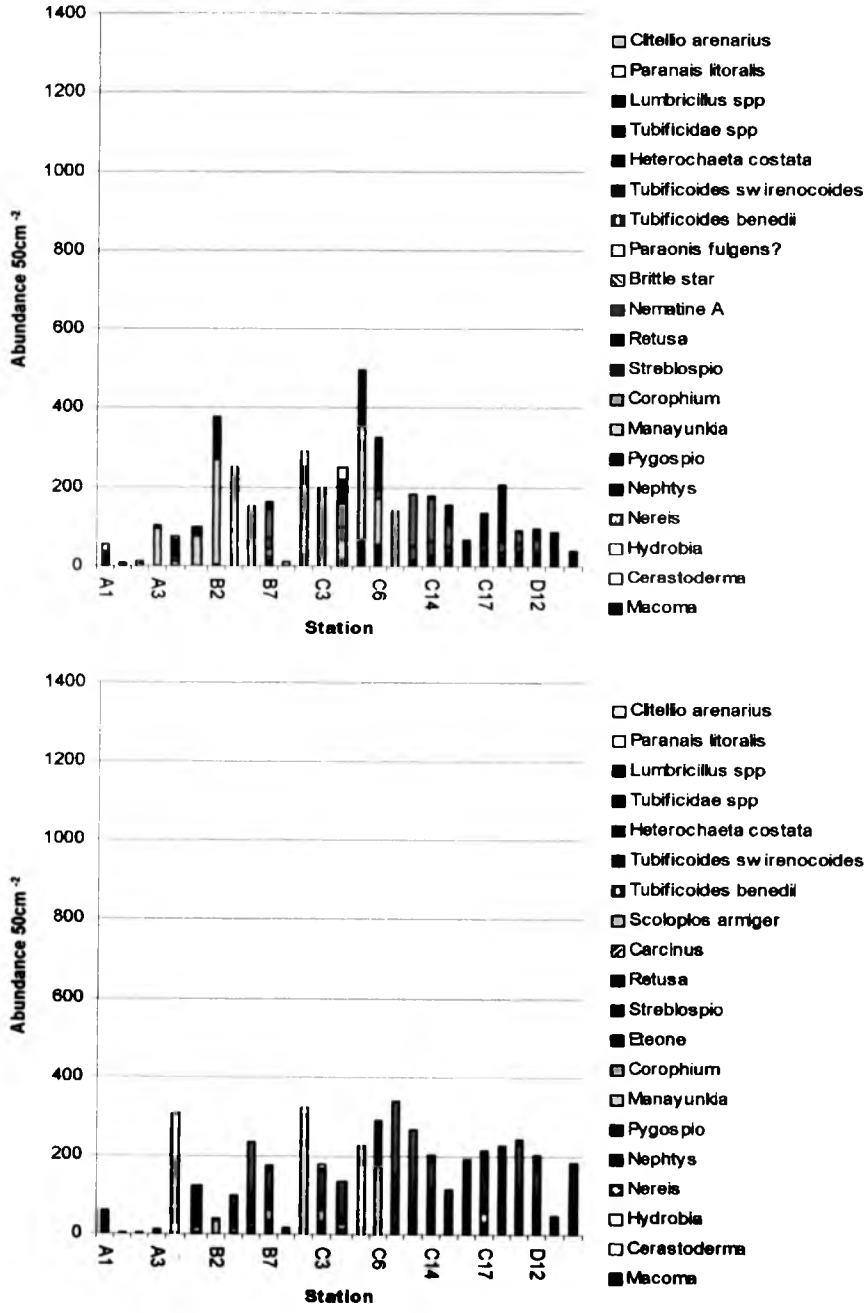


Figure 5.12. The abundance at each station and the contribution of each species for May 1999 (Top) and July 1999 (Bottom).

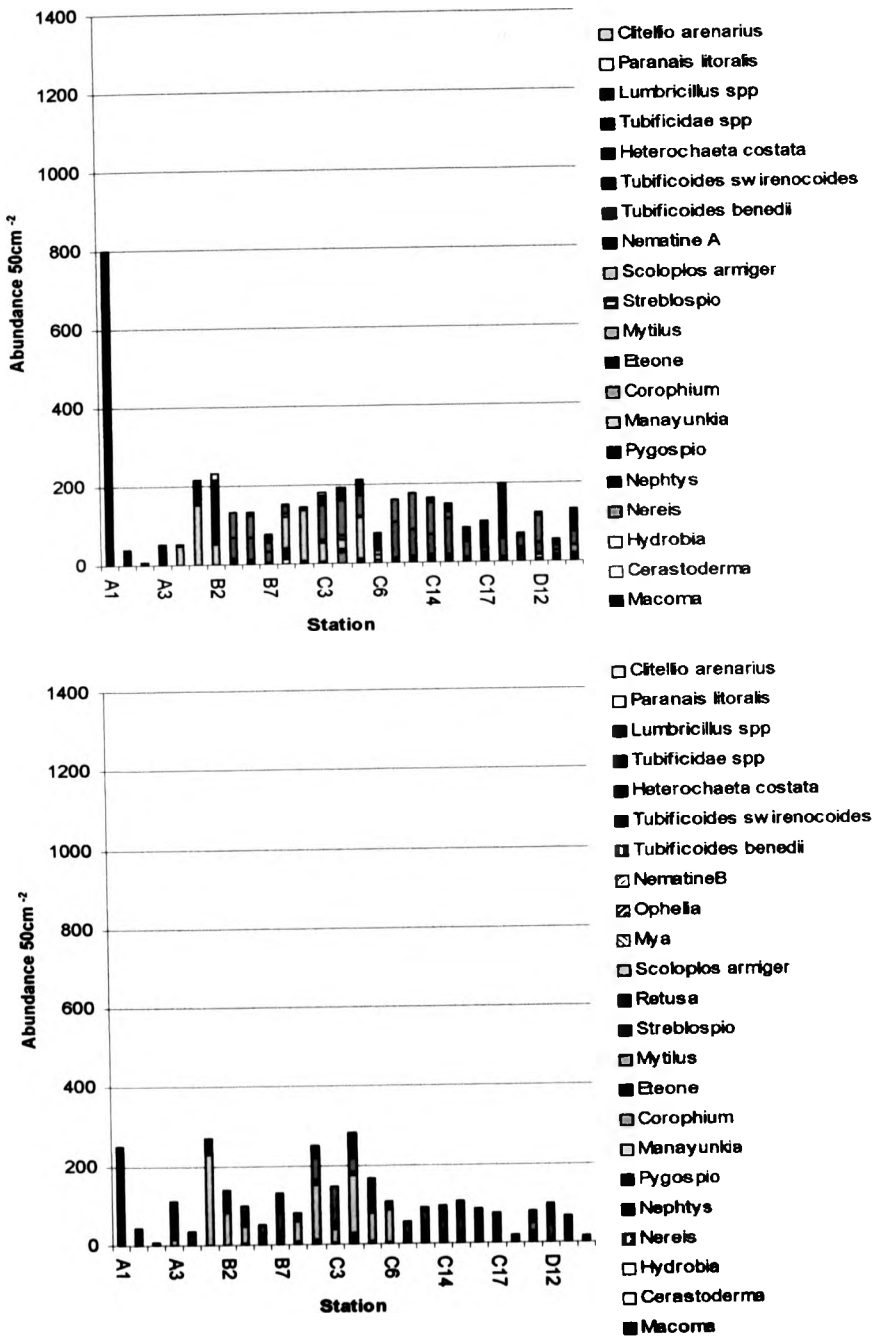


Figure 5.13. The abundance at each station and the contribution of each species for November 1999 (Top) and February 2000 (Bottom).

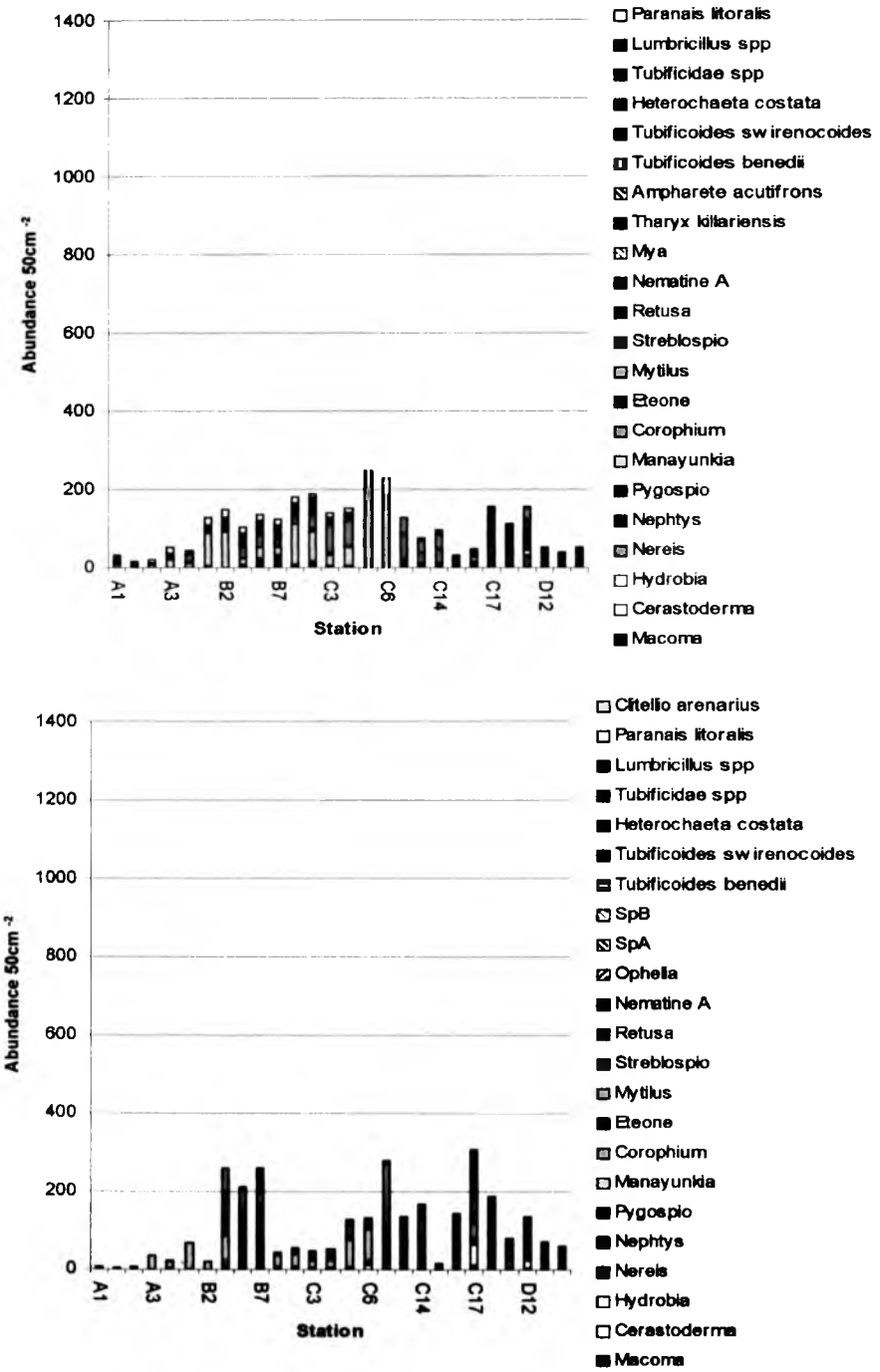


Figure 5.14. The abundance at each station and the contribution of each species for May 2000 (top) and July 2000 (Bottom).

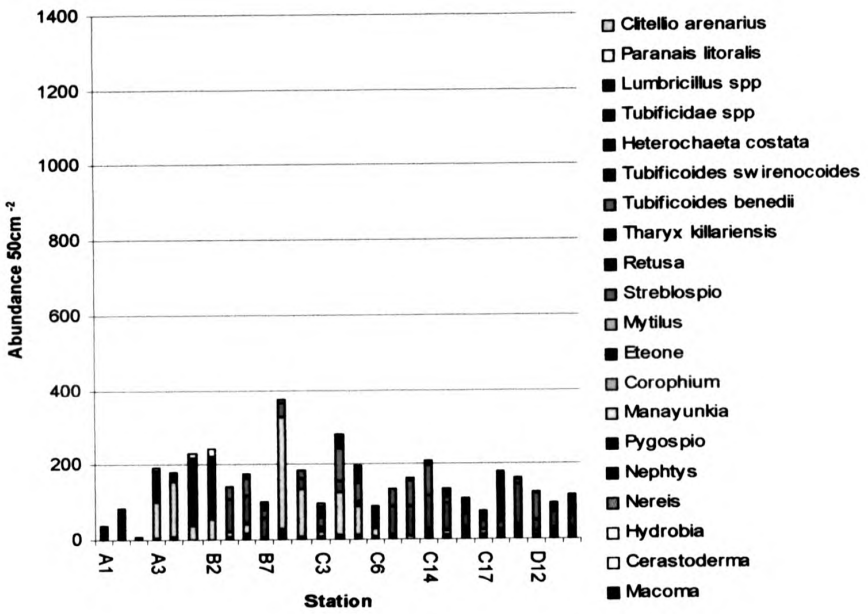


Figure 5.15. The abundance at each station and the contribution of each species for November 2000.

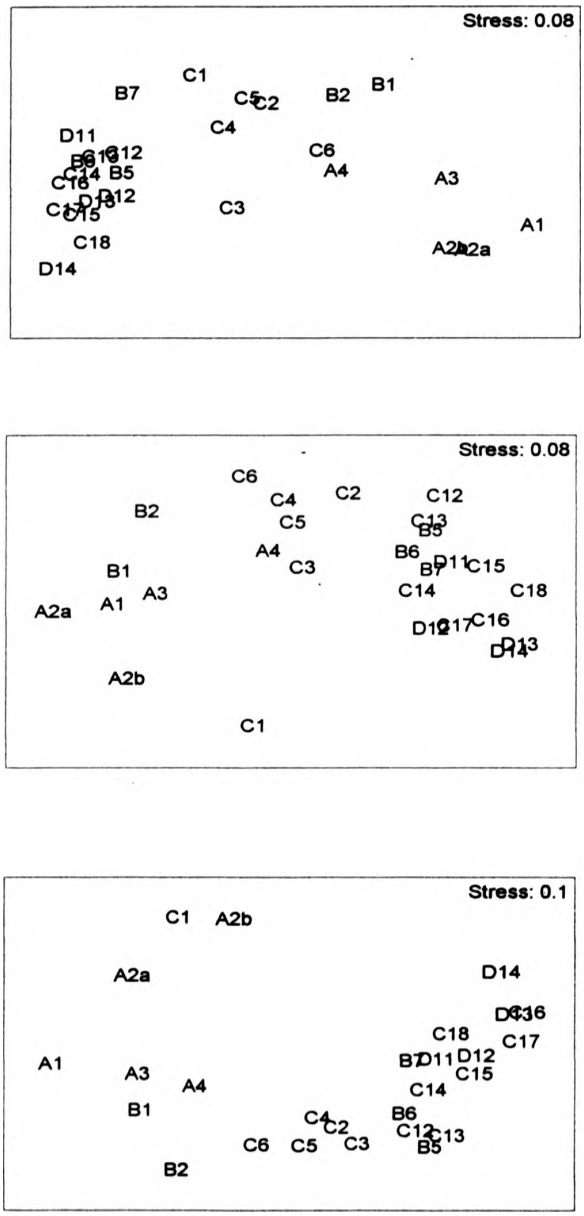


Figure 5.16. MDS plots for November 1998 (top), February 1999 (middle) and May 1999 (bottom).

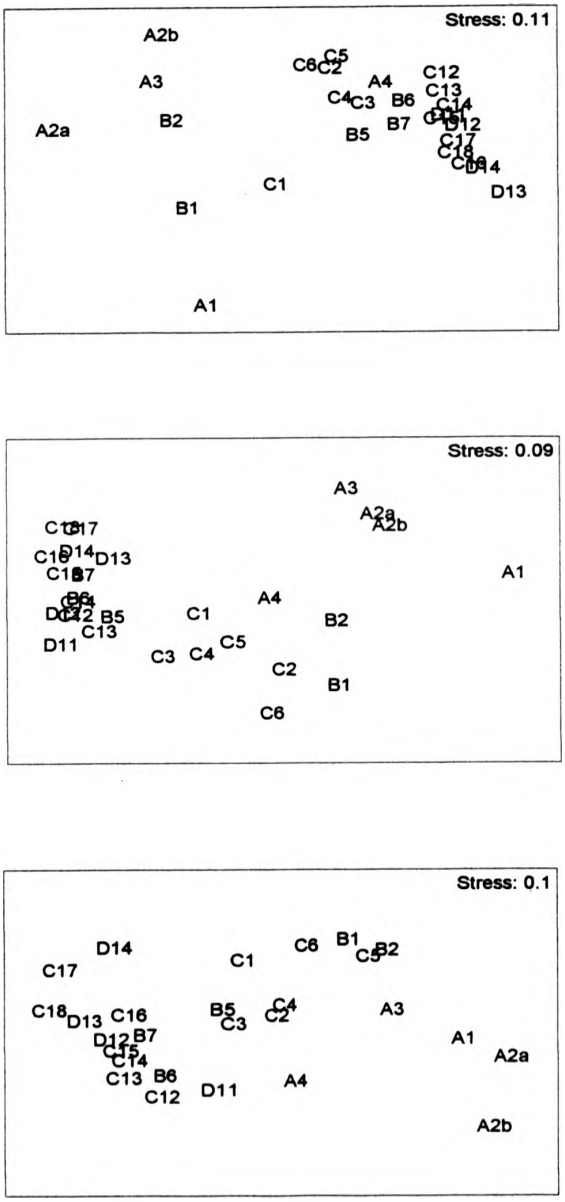


Figure 5.17. MDS plots for July 1999 (top), November 1999 (middle) and February 2000 (bottom).

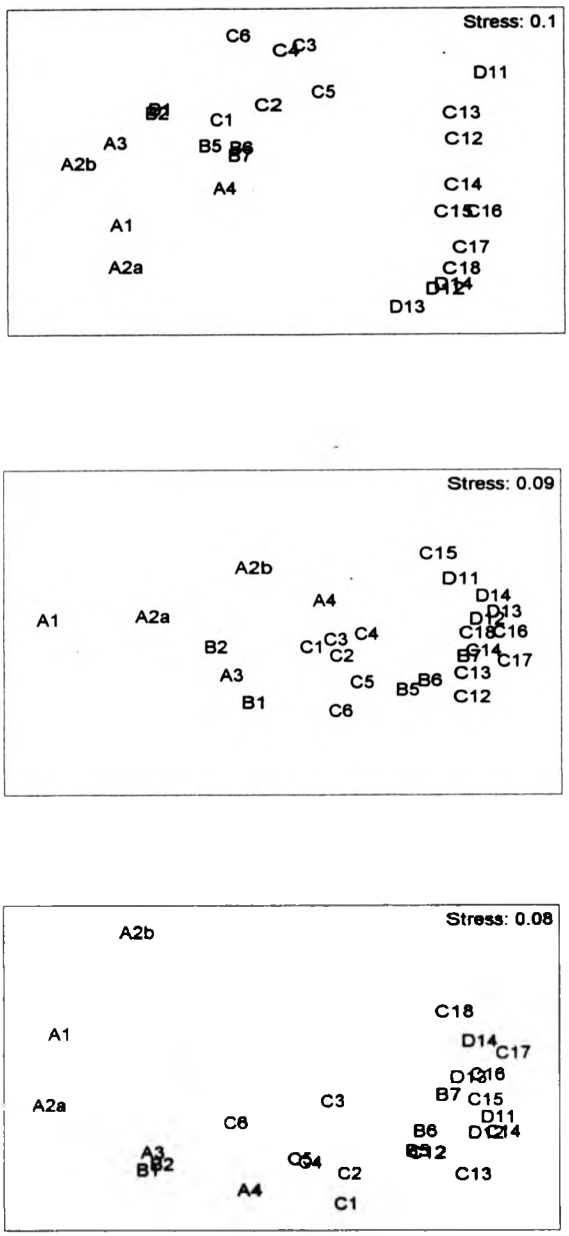


Figure 5.18. MDS plots for May 2000 (top), July 2000 (middle) and November 2000 (bottom).

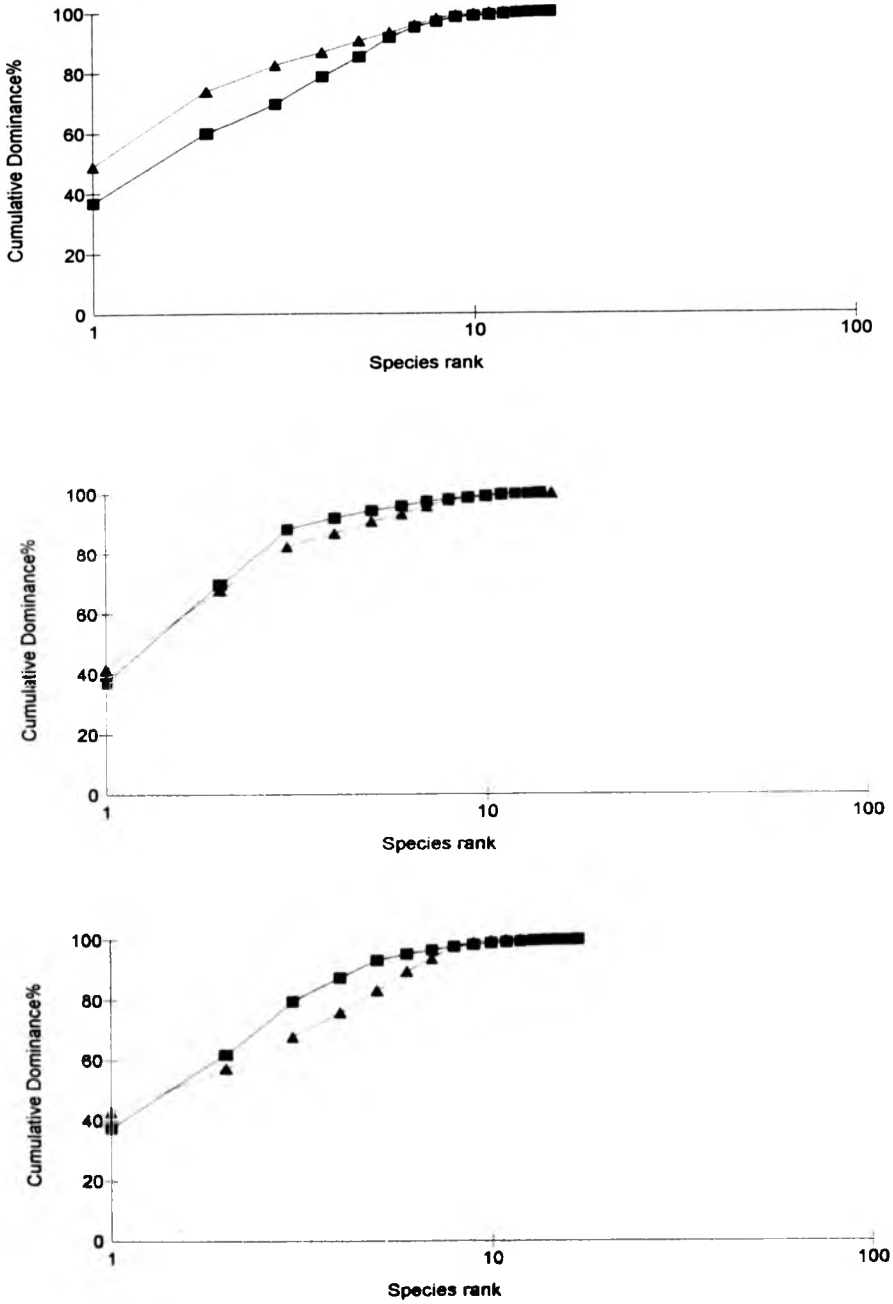


Figure 5.19. k- dominance curves showing the difference between upper shore (Triangle) and lower shore (Square) sites for November 1998 (top), February 1999 (middle) and May 1999 (bottom).

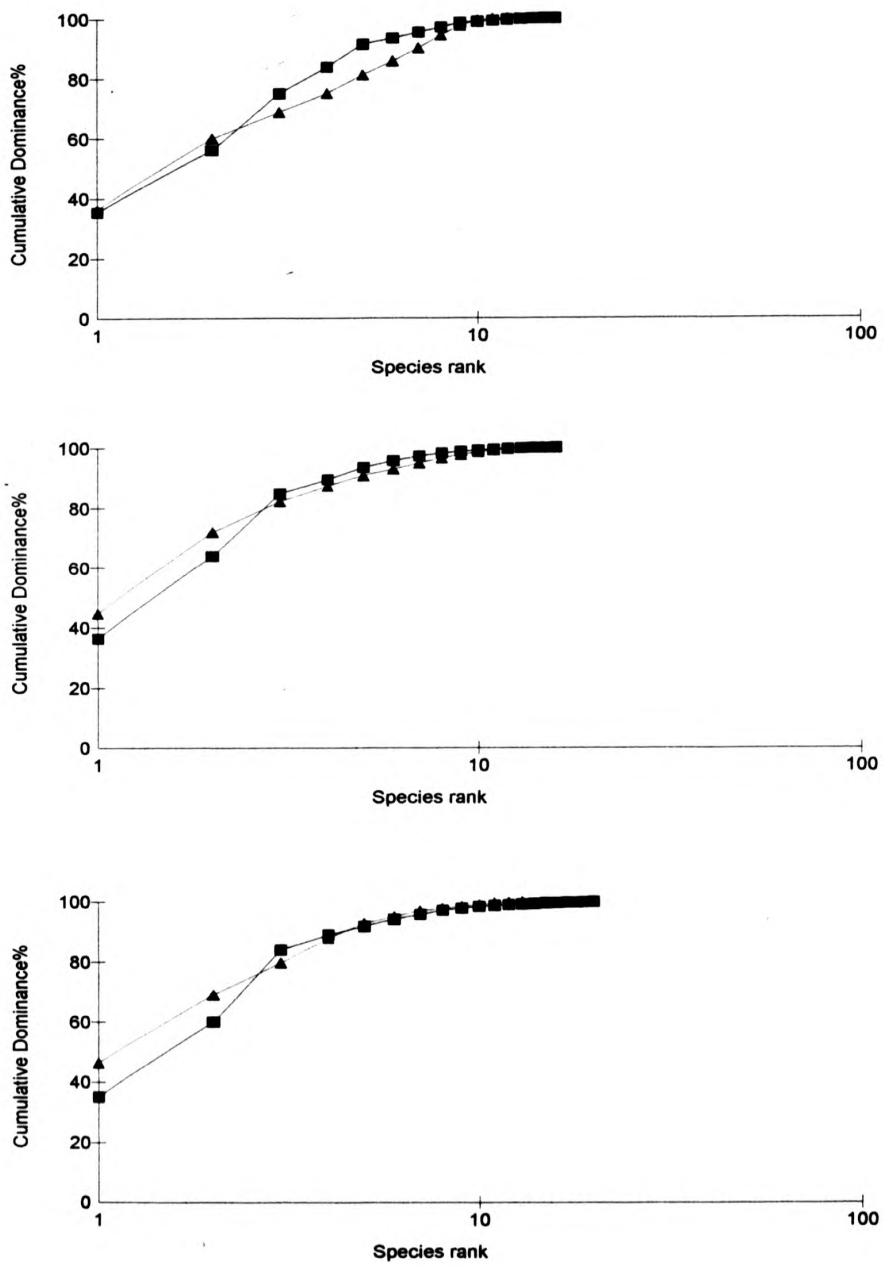


Figure 5.20. k- dominance curves showing the difference between upper shore (Triangle) and lower shore (Square) sites for July 1999 (top), November 1999 (middle) and February 2000 (bottom).

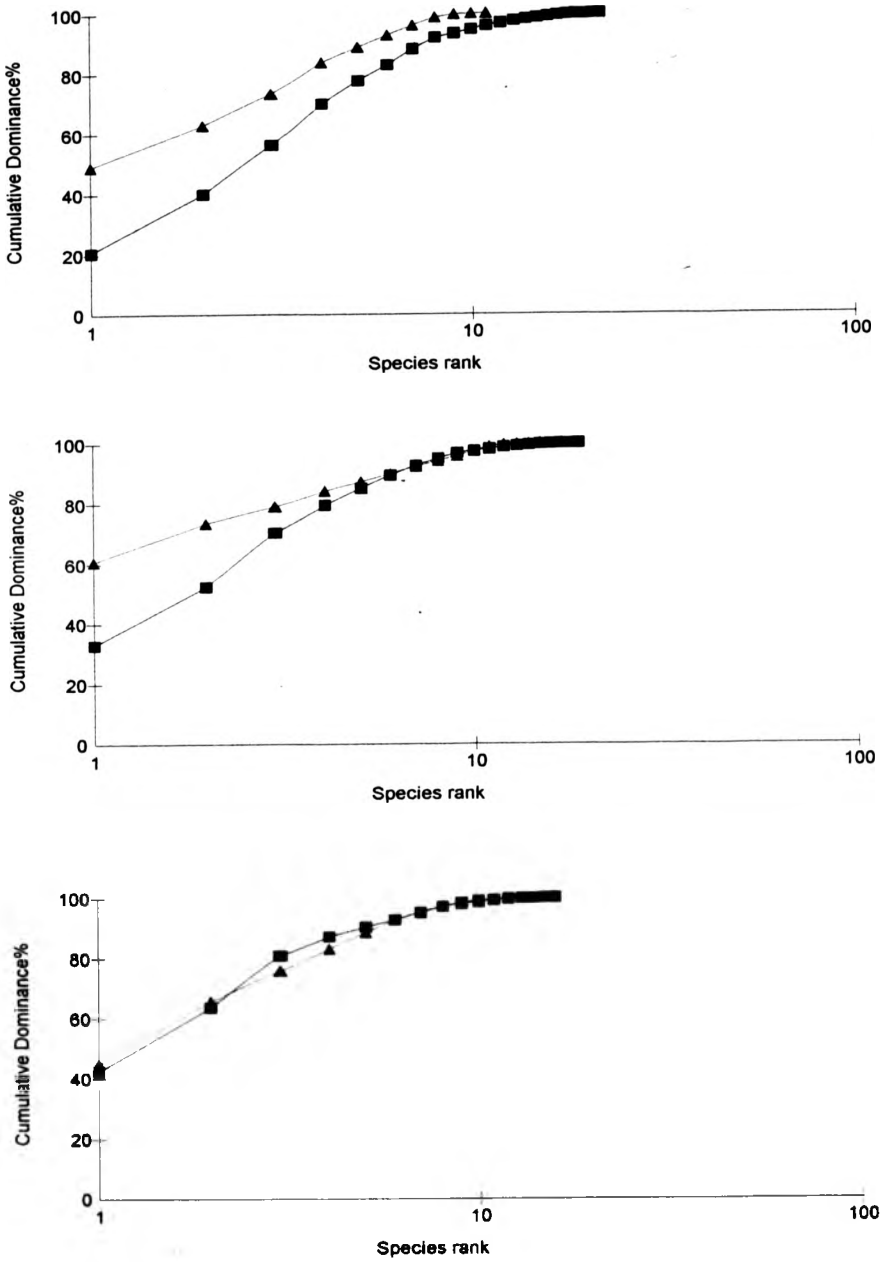


Figure 5.21. k- dominance curves showing the difference between upper shore (Triangle) and lower shore (Square) sites for May 2000 (top), July 2000 (middle) and November 2000 (bottom).

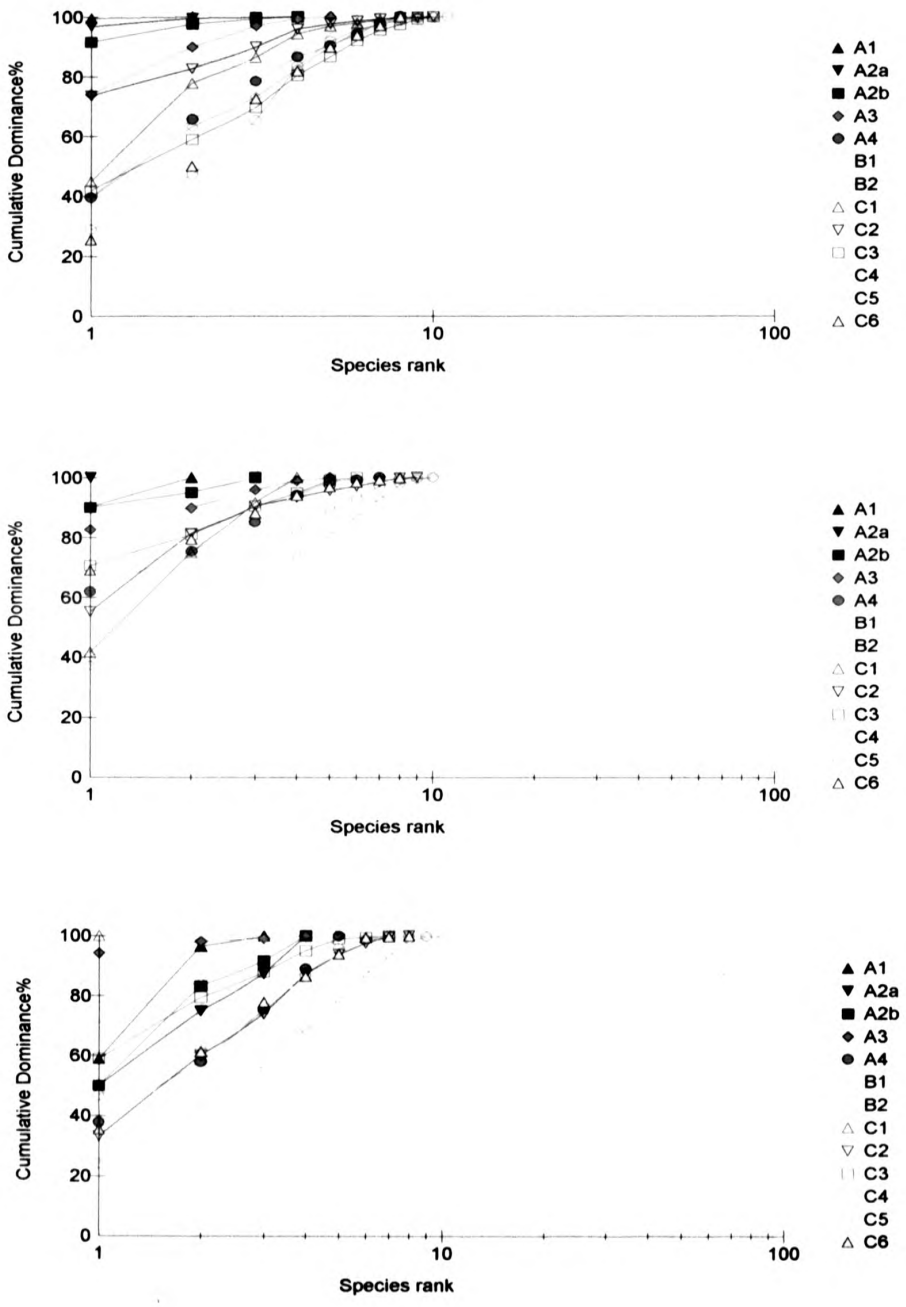


Figure 5.22. k- dominance curves for each of the upper shore stations for November 1998 (top), February 1999 (middle) and May 1999 (bottom).

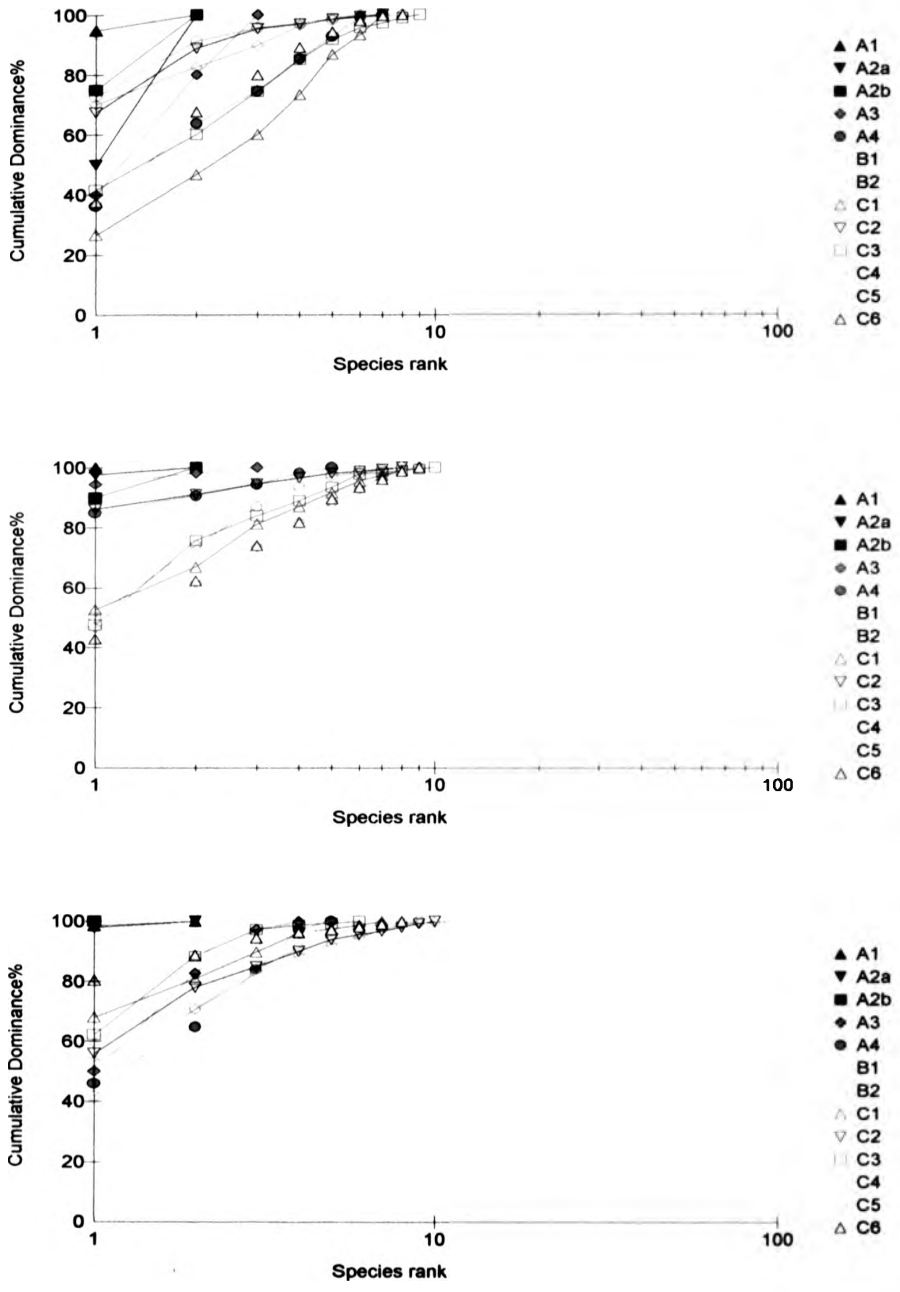


Figure 5.23. k- dominance curves for each of the upper shore stations for July 1999 (top), November 1999 (middle) and February 2000 (bottom).

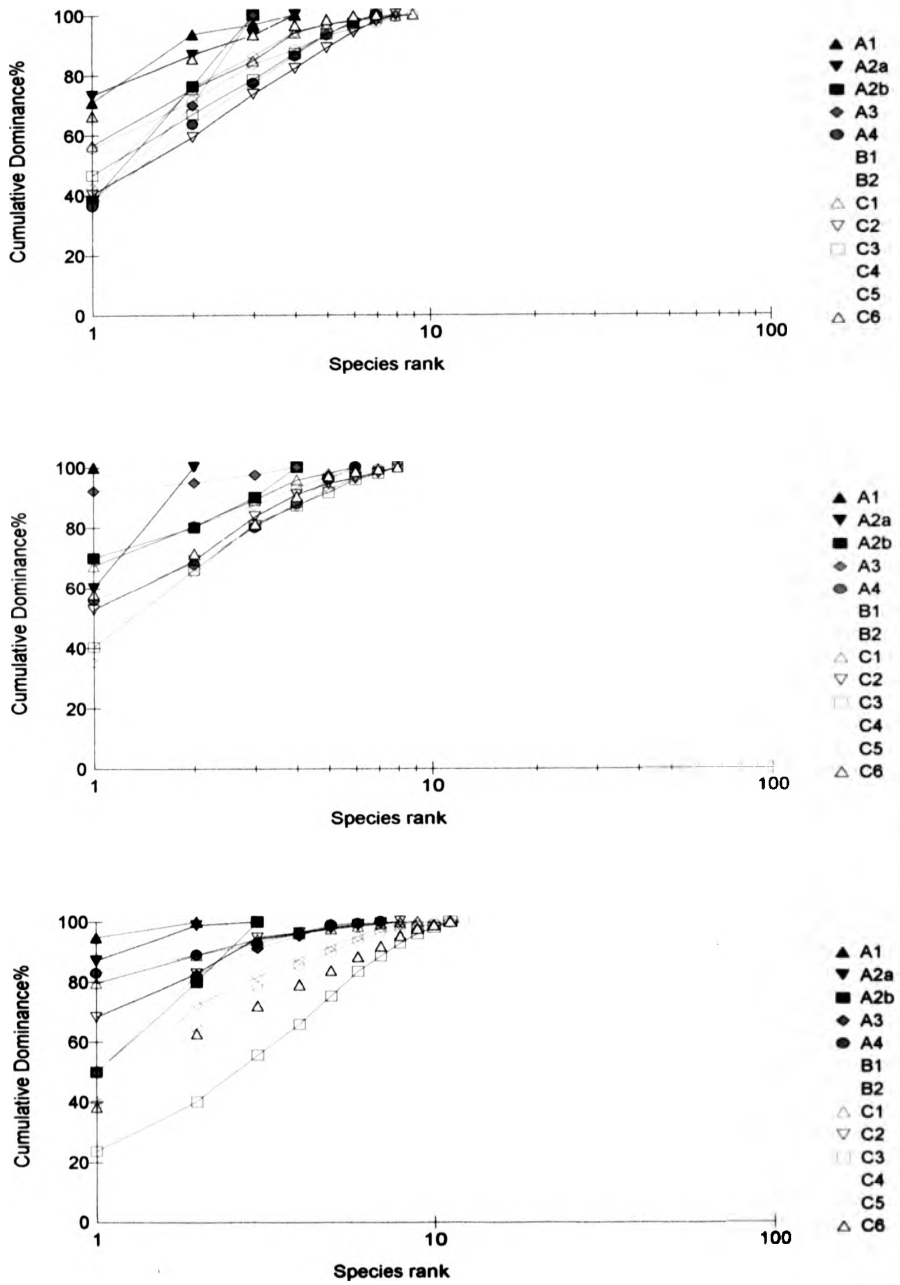


Figure 5.24. k- dominance curves for each of the upper shore stations for May 2000 (top), July 2000 (middle) and November 2000 (bottom).

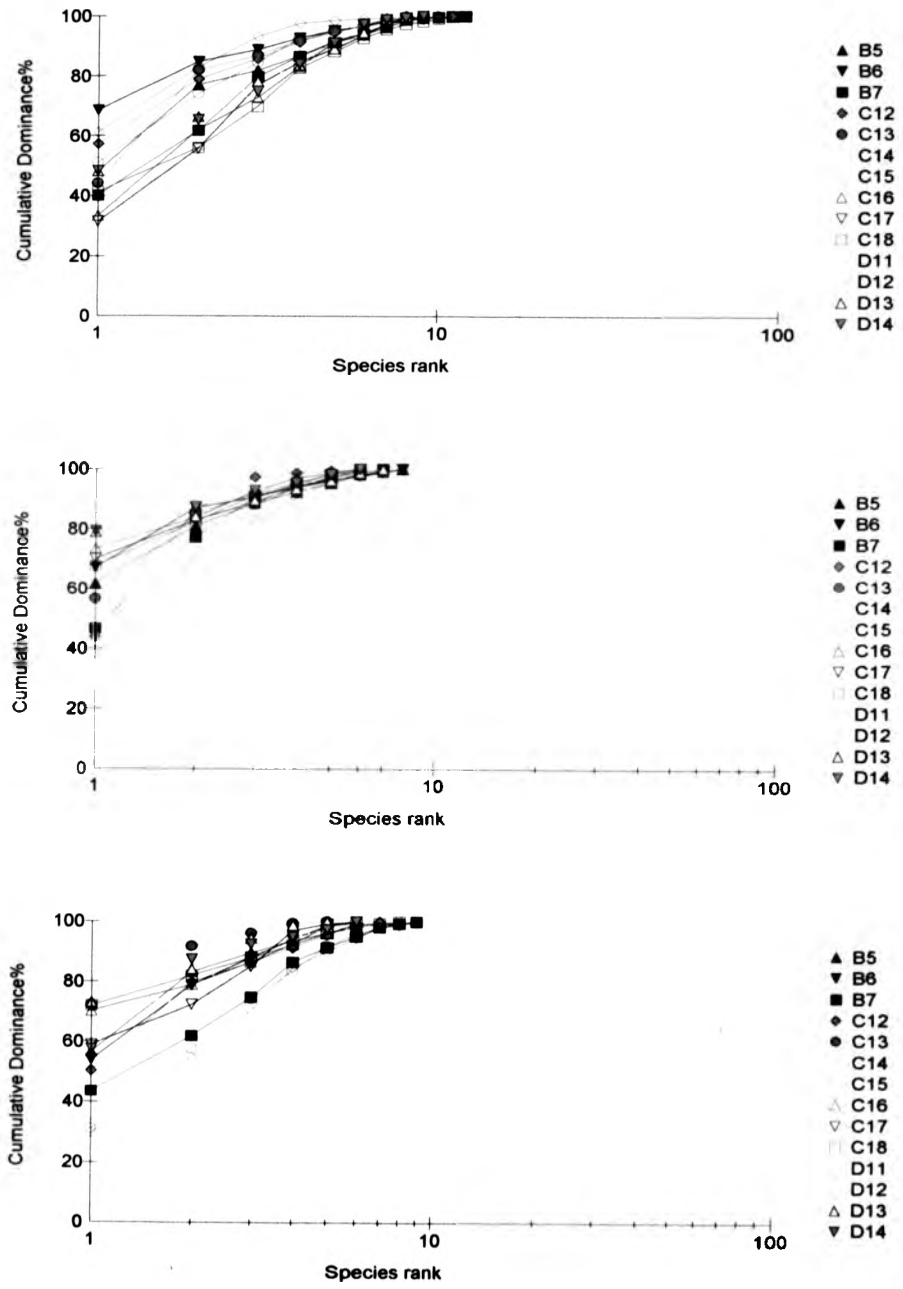


Figure 5.25. k- dominance curves for each of the lower shore stations for November 1998 (top), February 1999 (middle) and May 1999 (bottom).

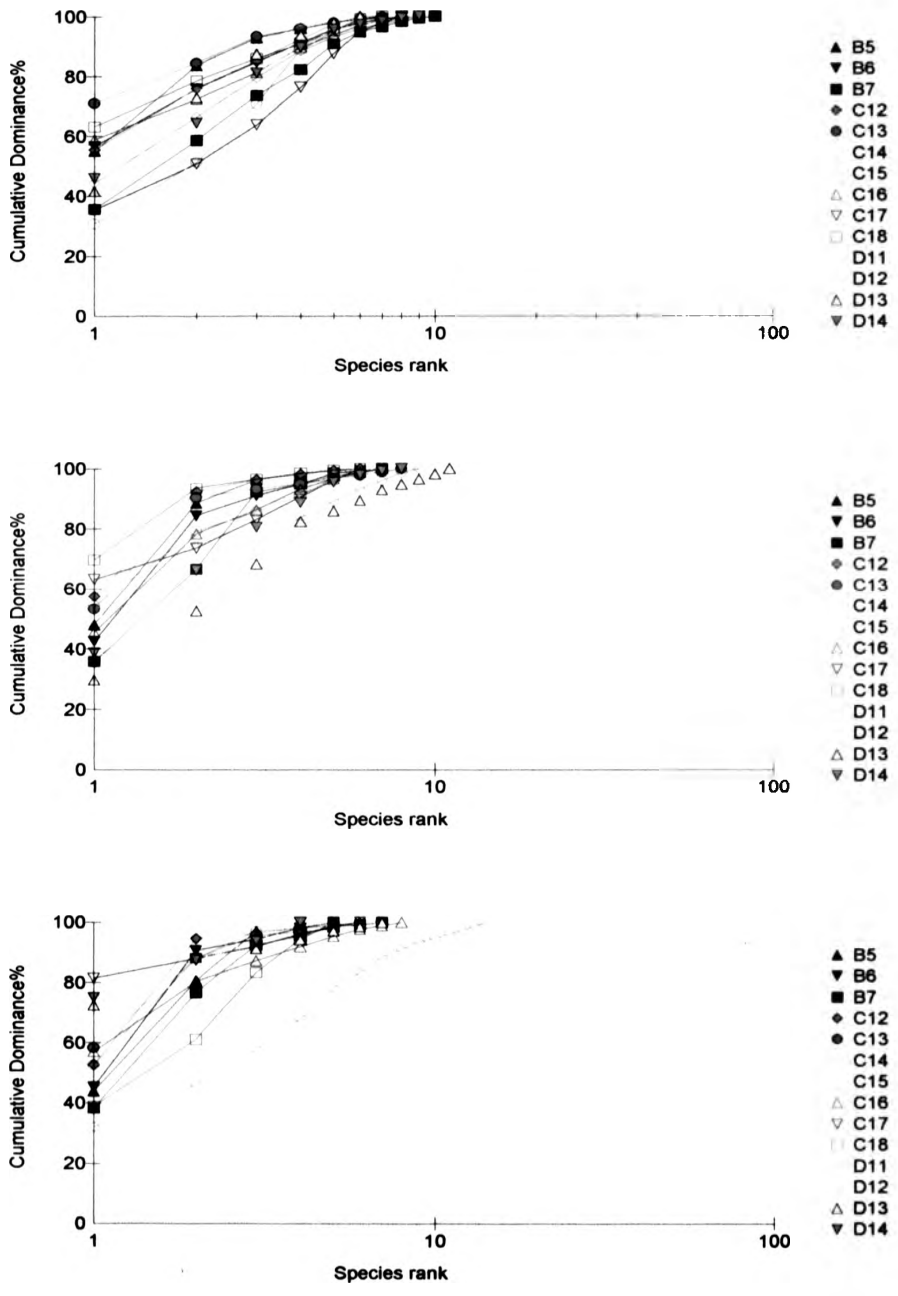


Figure 5.26. k- dominance curves for each of the lower shore stations for July 1999 (top), November 1999 (middle) and February 2000 (bottom).

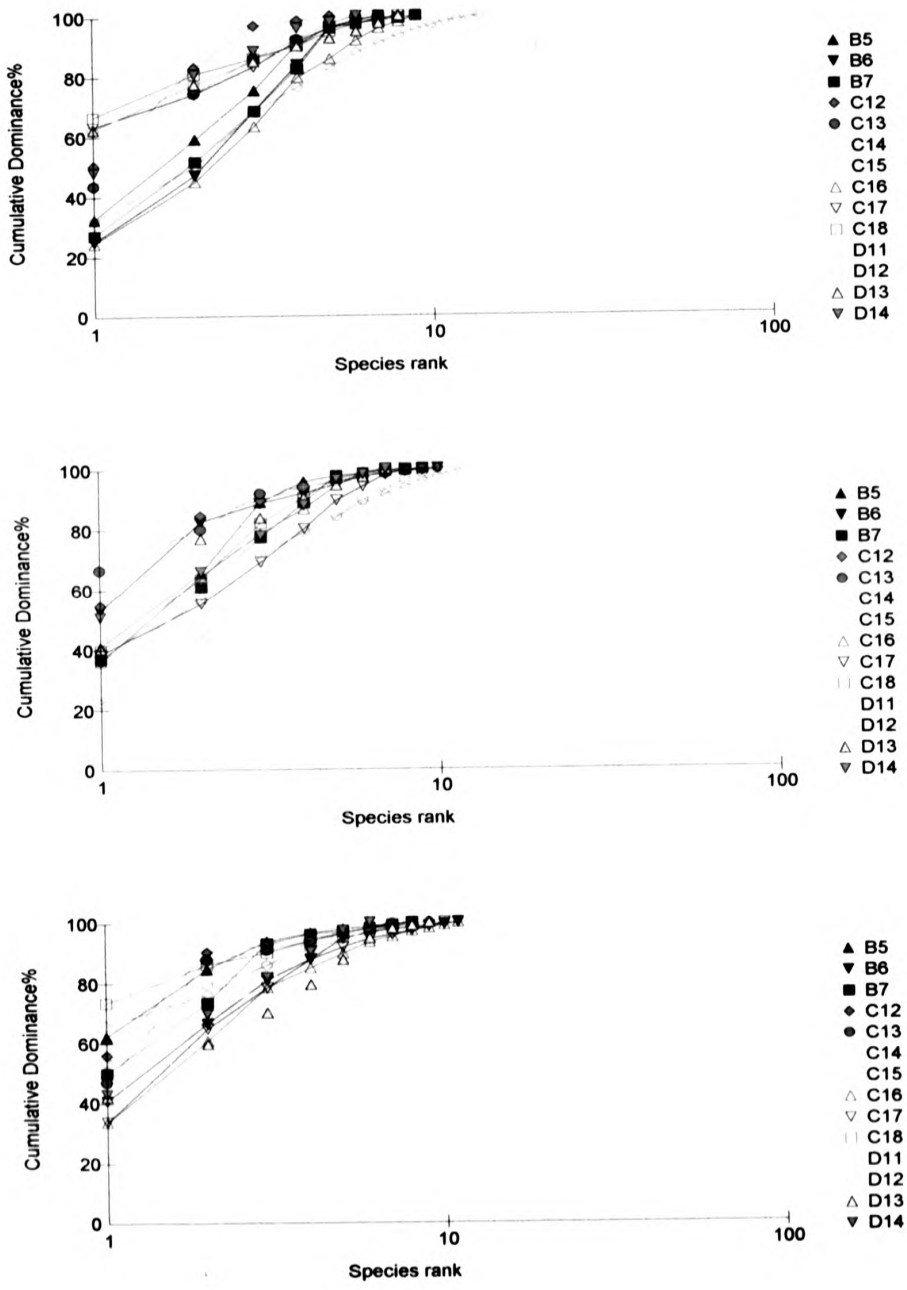


Figure 5.27. k- dominance curves for each of the lower shore stations for May 2000 (top), July 2000 (middle) and November 2000 (bottom).

Table 5.2. ANOSIM results testing the hypothesis that there is no difference between upper shore and lower shore stations for each sampling time.

Sampling time	Global R	Significance
Nov-98	0.766	0.10%
Feb-99	0.782	0.10%
May-99	0.704	0.10%
Jul-99	0.543	0.10%
Nov-99	0.756	0.10%
Feb-00	0.706	0.10%
May-00	0.602	0.10%
Jul-00	0.732	0.10%
Nov-00	0.738	0.10%

Table 5.3. Measure of dispersion for upper shore and lower shore stations and the Index of Multivariate Dispersion (IMD) between the two areas, for each sampling time.

Sampling time	Shore	Offshore	IMD
Nov-98	1.368	0.685	0.687
Feb-99	1.302	0.741	0.564
May-99	1.353	0.698	0.659
Jul-99	1.412	0.647	0.77
Nov-99	1.401	0.656	0.749
Feb-00	1.237	0.797	0.443
May-00	0.925	1.064	-0.14
Jul-00	1.231	0.802	0.432
Nov-00	1.317	0.729	0.591

Table 5.4. SIMPER results showing the species differences between upper shore and lower shore sites in November 1998 (* = good discriminating species).

Average dissimilarity = 83.5					
Species	**Upper shore Average abundance**	**Lower shore Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Lumbricillus spp	198.77	0.71	0.99	31.9	31.9
Manayunkia aestuarina	100.46	19.07	1.18	21.79	53.69
Tubificoides benedii	35.08	73.21	1.31	14.78	68.47
Streblospio shrubsolii	8.54	45.36	1.55*	8.06	76.53
Tubificoides swirenocoides	0	18	0.64	4.49	81.02
Tubificidae spp	17.31	0.21	0.68	3.33	84.34
Pygospio elegans	15.15	12.93	0.85	3.25	87.6
Heterochaeta costata	11.31	0	0.8	2.82	90.42

Table 5.5. SIMPER results showing the species differences between upper shore and lower shore sites in February 1999.

Average dissimilarity = 89.2					
Species	**Upper shore Average abundance**	**Lower shore Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia aestuarina	65.69	0.71	1.07	22.71	22.71
Lumbricillus spp	41.31	0	0.98	21.81	44.51
Tubificoides benedii	23.23	35.21	1.13	18.08	62.6
Tubificoides swirenocoides	0	30.79	0.97	16.87	79.46
Streblospio shrubsolii	6.08	17.5	1	8.28	87.74
Tubificidae spp	6.85	0.5	0.91	2.64	90.38

Table 5.6. SIMPER results showing the species differences between upper shore and lower shore sites in May 1999.

Average dissimilarity = 88.15					
Species	**Upper shore Average abundance**	**Lower shore Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia aestuarina	76.08	1	1.11	21.93	21.93
Tubificoides benedii	25.46	52.14	1.06	20.95	42.88
Tubificoides swirenocoides	0.08	33.93	0.8	16.73	59.61
Streblospio shrubsolii	7.46	24.43	1.22	9.75	69.36
Lumbricillus spp	18.69	0	0.89	6.38	75.74
Pygospio elegans	11.38	11	1.32	6.31	82.04
Tubificidae spp	14.08	1.79	0.9	3.78	85.82
Heterochaeta costata	12.85	0.43	0.58	3.61	89.43
Macoma balthica	0.62	7.86	1.18	3.55	92.98

Table 5.7. SIMPER results showing the species differences between upper shore and lower shore sites in July 1999 (* = good discriminating species).

Average dissimilarity = 84.26					
Species	**Upper shore Average abundance**	**Lower shore Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Tubificoides benedii	31.15	69.57	1.17	24.1	24.1
Streblospio shrubsolii	6.08	40.71	1.29	16.1	40.2
Tubificoides swirenocoides	0	37.21	0.79	15.81	56.02
Manayunkia aestuarina	48	2.71	0.82	13.95	69.97
Macoma balthica	0.62	17.21	1.47*	6.75	76.72
Pygospio elegans	5.62	15.07	1.23	5.59	82.31
Lumbricillus spp	8.31	0	0.42	3.91	86.22
Paranais litoralis	11.69	0	0.43	3.08	89.29
Tubificidae spp	8.08	0.36	0.4	2.31	91.6

Table 5.8. SIMPER results showing the species differences between upper shore and lower shore sites in November 1999 (* = good discriminating species).

Average dissimilarity = 90.14					
Species	**Upper shore Average abundance**	**Lower shore Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia aestuarina	49.62	0.5	1.02	19.02	19.02
Streblospio shrubsolii	3.15	46.14	1.5*	18.88	37.9
Lumbricillus spp	81.38	0	0.63	16.75	54.65
Tubificoides benedii	18.46	35	1.09	15.88	70.53
Tubificoides swirenocoides	0.69	26.36	0.75	11.41	81.94
Heterochaeta costata	9.38	0	0.58	3.67	85.61
Macoma balthica	0.31	5.86	0.89	2.67	88.27
Pygospio elegans	3.92	5.14	0.99	2.49	90.77

Table 5.9. SIMPER results showing the species differences between upper shore and lower shore sites in February 2000.

Average dissimilarity = 89.99					
Species	**Upper shore Average abundance**	**Lower shore Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia aestuarina	67.46	3.79	1.22	28.74	28.74
Lumbricillus spp	32.92	0.07	0.66	16.13	44.87
Tubificoides swirenocoides	0	26.93	0.99	15.74	60.61
Tubificoides benedii	15.54	19.21	1.09	13.13	73.74
Streblospio shrubsolii	3.31	18.43	1	9.97	83.71
Heterochaeta costata	12	0	0.66	6.03	89.74
Tubificidae spp	7	0.21	0.66	2.97	92.71

Table 5.10. SIMPLER results showing the species differences between upper shore and lower shore sites in May 2000 (* = good discriminating species).

Average dissimilarity = 83.6					
Species	**Upper shore Average abundance**	**Lower shore Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia aestuarina	59.62	4.93	1.45*	27.39	27.39
Tubificoides swirenocoides	0	19.36	0.67	12.61	40
Tubificoides benedii	16.62	12.86	1.04	12.23	52.23
Streblospio shrubsolii	3.92	18.29	0.95	10.22	62.45
Lumbricillus spp	12.77	7.29	1.16	9.89	72.34
Pygospio elegans	3.15	15.43	1.14	8.35	80.69
Paranais litoralis	12.62	3.64	1.24	6.78	87.47
Tubificidae spp	6.15	0.86	0.74	2.97	90.44

Table 5.11. SIMPER results showing the species differences between upper shore and lower shore sites in July 2000 (* = good discriminating species).

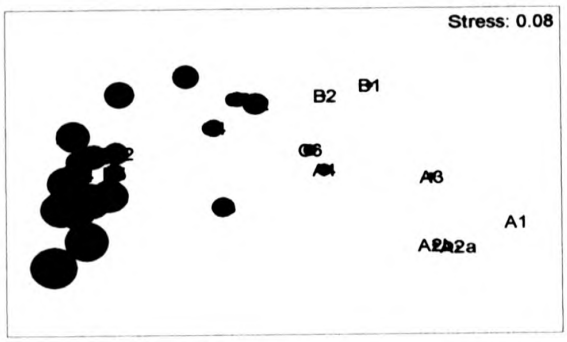
Average dissimilarity = 89.79					
Species	**Upper shore Average abundance**	**Lower shore Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Streblospio shrubsolii	2.54	54.64	1.67*	26.06	26.06
Tubificoides swirenocoides	0	32.14	1.13	17.52	43.58
Manayunkia aestuarina	29.92	5.29	1.15	17.18	60.76
Tubificoides benedii	6.23	30	0.94	12.7	73.46
Macoma balthica	0.77	15.29	1.63*	7.72	81.18
Pygospio elegans	2.77	9.21	1.16	4.62	85.8
Hydrobia ulvae	1.15	7.14	0.69	3.59	89.38
Nephtys hombergii	0.08	3.86	0.88	2.62	92

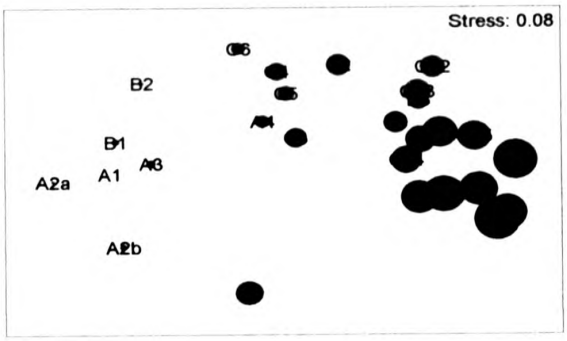
Table 5.12. SIMPER results showing the species differences between upper shore and lower shore sites in November 2000 (* = good discriminating species).

Average dissimilarity = 85.63					
Species	**Upper shore Average abundance**	**Lower shore Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Manayunkia aestuarina	75.85	3.14	1.11	23.59	23.59
Streblospio shrubsolii	9.23	58.29	1.46*	21.66	45.25
Lumbricillus spp	25.23	0	0.9	13.98	59.23
Tubificoides benedii	17	29.36	1.08	11.83	71.06
Tubificoides swirenocoides	0.69	23.5	0.73	9.91	80.97
Heterochaeta costata	12.31	0	0.84	4.72	85.69
Macoma balthica	1	8.64	1.07	3.45	89.14
Tubificidae spp	7.15	0.5	0.88	2.65	91.79

Table 5.13. BIO-ENV results for the abundance data showing the subset of variables selected for each sampling time.

Sampling time	p_s	Variables
Nov-98	0.736	Refinery distance
Feb-99	0.800	Refinery distance, Chemcials 3 distance
May-99	0.726	Refinery distance, Chemcials 3 distance
Jul-99	0.682	Refinery distance
Nov-99	0.733	Refinery distance, Chemcials 3 distance, % Clay
Feb-00	0.766	Refinery distance
May-00	0.744	Height
Jul-00	0.771	Refinery distance, Chemcials 3 distance, % Clay
Nov-00	0.804	Refinery distance





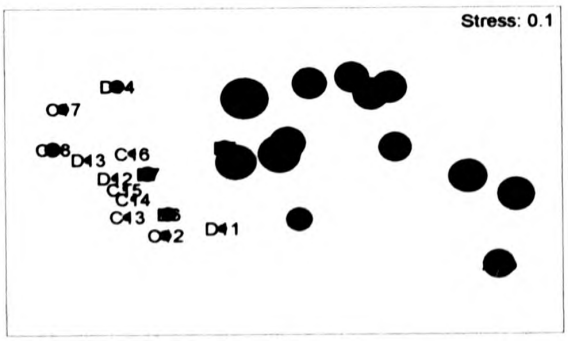


Figure 5.28. Bubble MDS plots. The distance from the refinery outfall for November 1998 (Top), and February 1999 (Middle) and the distance from the chemicals outfall 3 for February 1999 (Bottom).

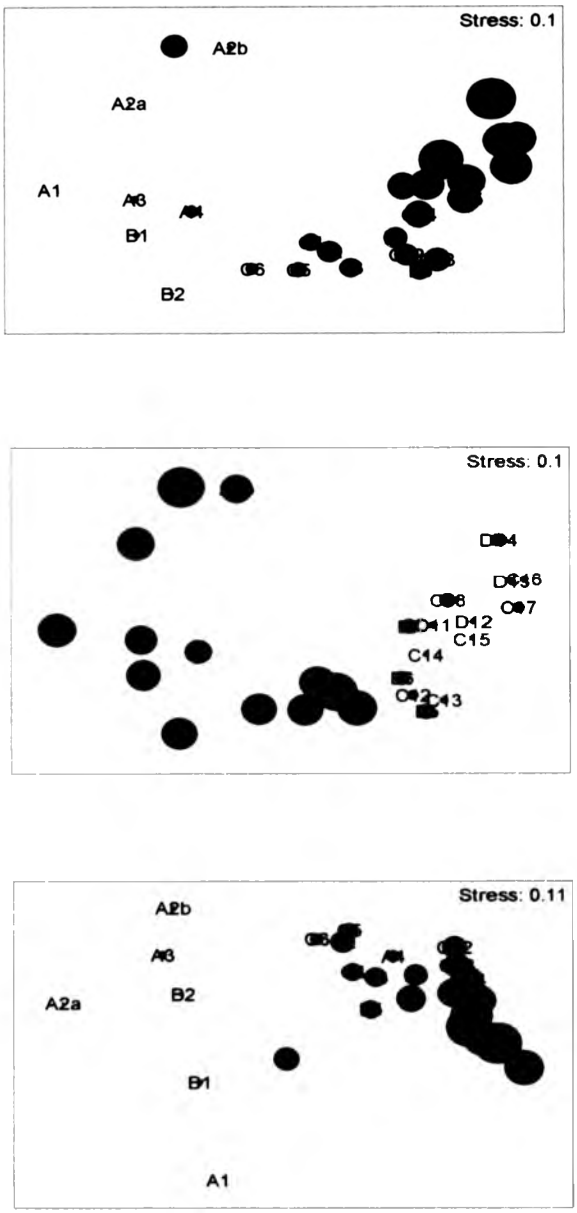


Figure 5.29. Bubble MDS plots. The distance from the refinery outfall for May 1999 (Top), and July 1999 (Bottom) and the distance from the chemicals outfall 3 for May1999 (Middle).

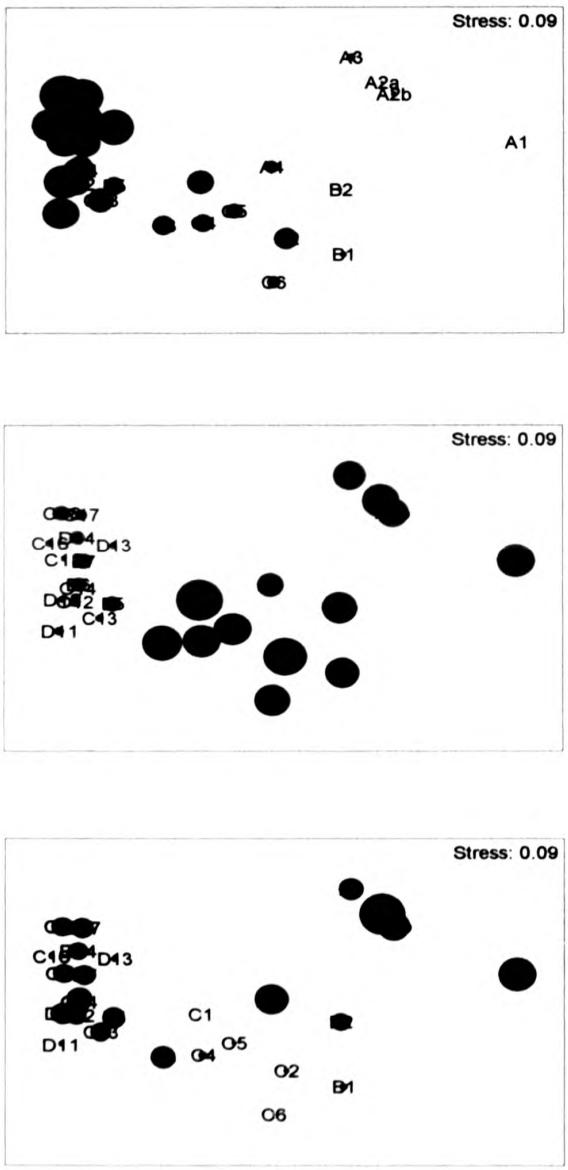


Figure 5.30. Bubble MDS plots. The distance from the refinery outfall (Top), the distance from the chemicals outfall 3 (Middle) and the % Clay (Bottom) for November 1999.

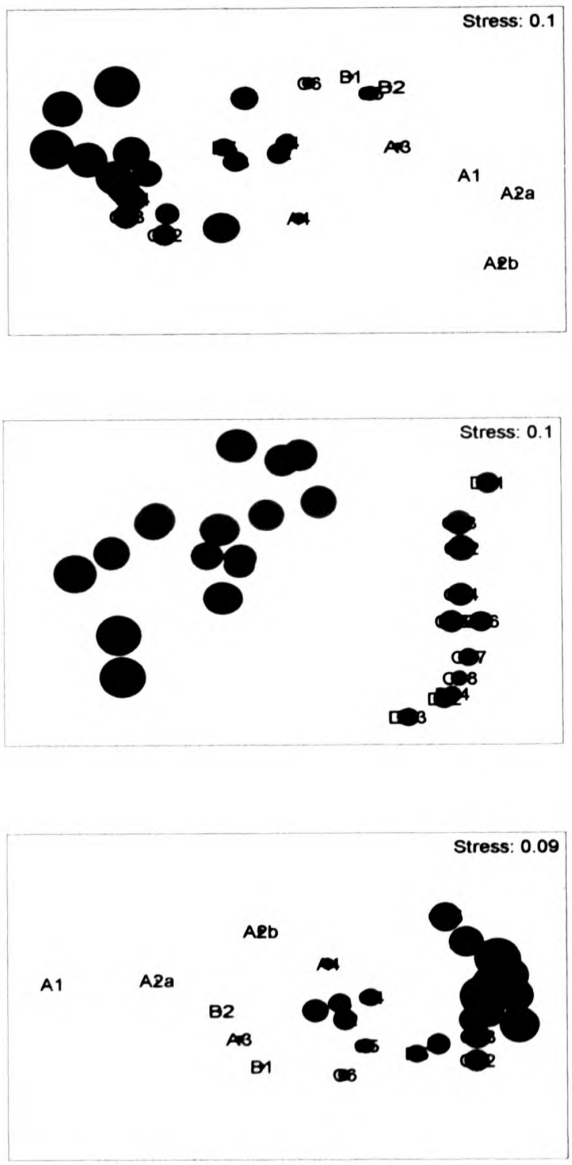


Figure 5.31. Bubble MDS plots. The distance from the refinery outfall for February 2000 (Top), and July 2000 (Bottom) and the height for May 2000 (Middle).

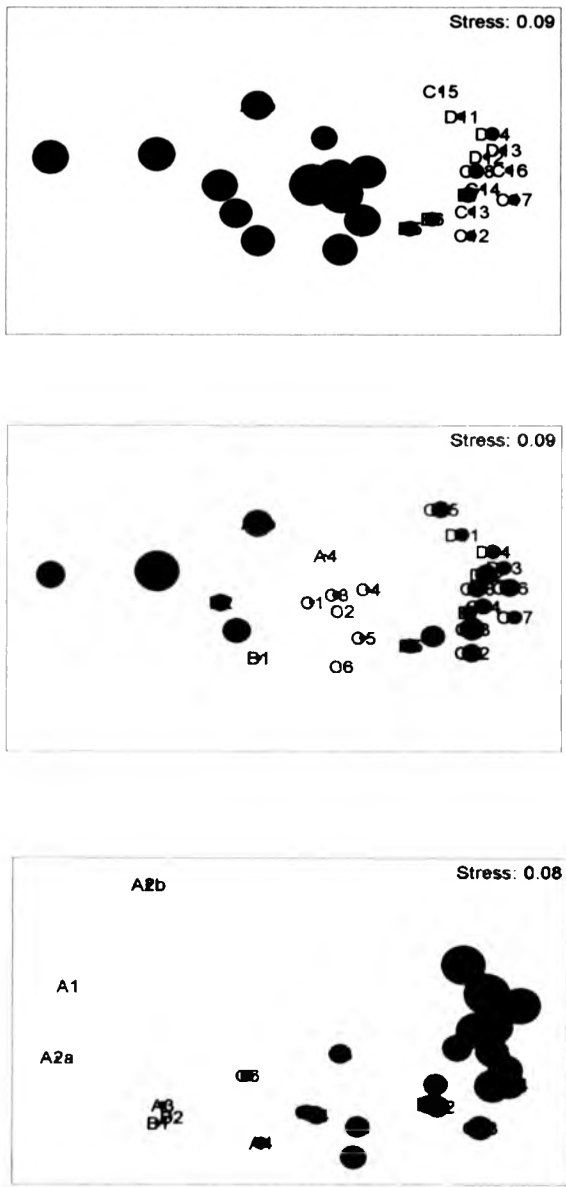


Figure 5.32. Bubble MDS plots. The distance from the Chemicals outfall 3 (Top) and the % Clay (Middle) for July 2000, and the Distance from the refinery outfall for November 2000 (Bottom).

Table 5.14. Stepwise regression results using distance, height and sediment characteristics, showing the p value and Rsq(adj) for each sampling time. Those With an Rsq(adj) above 70% are highlighted in bold. The equations for these three models are: Nov 98 No. species = 20.7 + 0.00538 refinery distance – 0.646 %Clay – 8.15 Grangeburn distance – 4.29 Avon distance, p=0.000, F=25.05, Rsq(adj)=78.7%. May 00 No. Species = 0.67 + 0.453 Mean particle size + 0.00273 refinery distance – 0.605 %Clay + 0.237 % organic matter, p=0.000, F=19.84, Rsq(adj)=74.3%. Jul 00 log No. Species = 1.85 + 0.000729 refinery distance – 0.673 sewage works distance – 0.704 grangeburn distance, p=0.000, F=50.11, Rsq(adj)=85%

Sampling time	Diversity p, Rsq(adj)	Evenness p, Rsq(adj)	No. Species p, Rsq(adj)	No. Individuals p, Rsq(adj)
Nov-98	0.000, 60.9%	0.000, 54.9%	**0.000, 78.7%**	0.002, 29.6%
Feb-99	0.000, 47.2%	0.001, 42.7%	0.001, 37.8%	0.000, 40.0%
May-99	None selected	None selected	0.044, 11.8%	0.005, 24.3%
Jul-99	0.000, 55.3%	None selected	0.000, 57.1%	0.010, 20.6%
Nov-99	0.000, 59.2%	0.000, 48.4%	0.000, 61.9%	None selected
Feb-00	0.001, 39.0%	0.000, 66.7%	0.000, 67.2%	0.002, 29.7%
May-00	0.008, 21.6%	0.029, 14.3%	**0.000, 74.3%**	0.000, 58.7%
Jul-00	0.000, 60.2%	Not Normal	**0.000, 85.0%**	0.003, 33.1%
Nov-00	0.001, 38.5%	None selected	0.000, 57.9%	0.010, 26.5%

Table 5.15. The regression and BIO-ENV results for the spatial analysis including the hydrocarbon results.

Diversity	1.62 + 0.00572 UCMC14-23 – 0.000606 Distance from chemical outfall 3 + 0.000082 Crude, p = 0.001, F = 12.92, Rsq(adj) = 73.3%
Evenness	None selected
No. individuals	178 – 27.1 %Clay, p = 0.003, F = 13.63, Rsq(adj) = 49.3%
No. species (log)	0.989 – 0.212 log C14-23, p = 0.002, F = 16.33, Rsq(Adj) = 54.1%
BIO-ENV	ρ_s = 0.766, Refinery distance, Chemical outfall 3 distance, Total Aliphatic Hydrocarbons

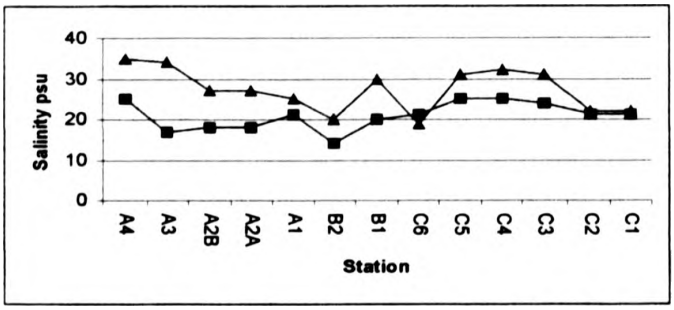


Figure 5.33. Salinity measured at the upper shore stations in February 1999 (Triangle) and July 1999 (Square).

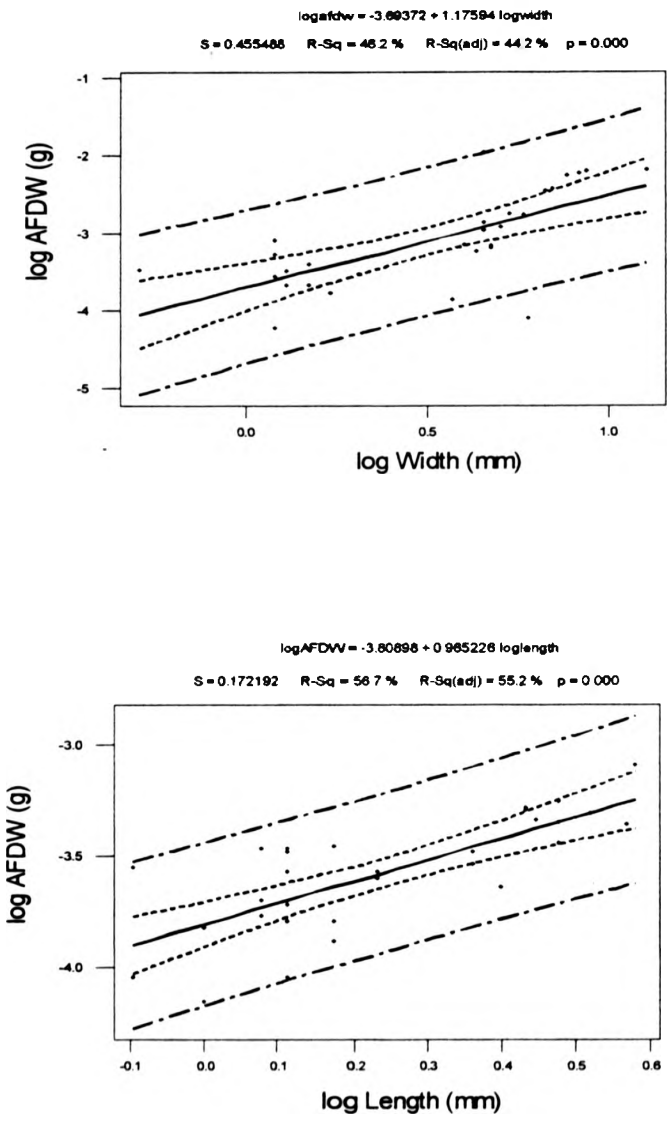


Figure 5.34. Regression plots for *Macoma balthica* (Top) and *Hydrobia ulvae* (Bottom), with 95% confidence limits (---) and predicted limits (– • –).

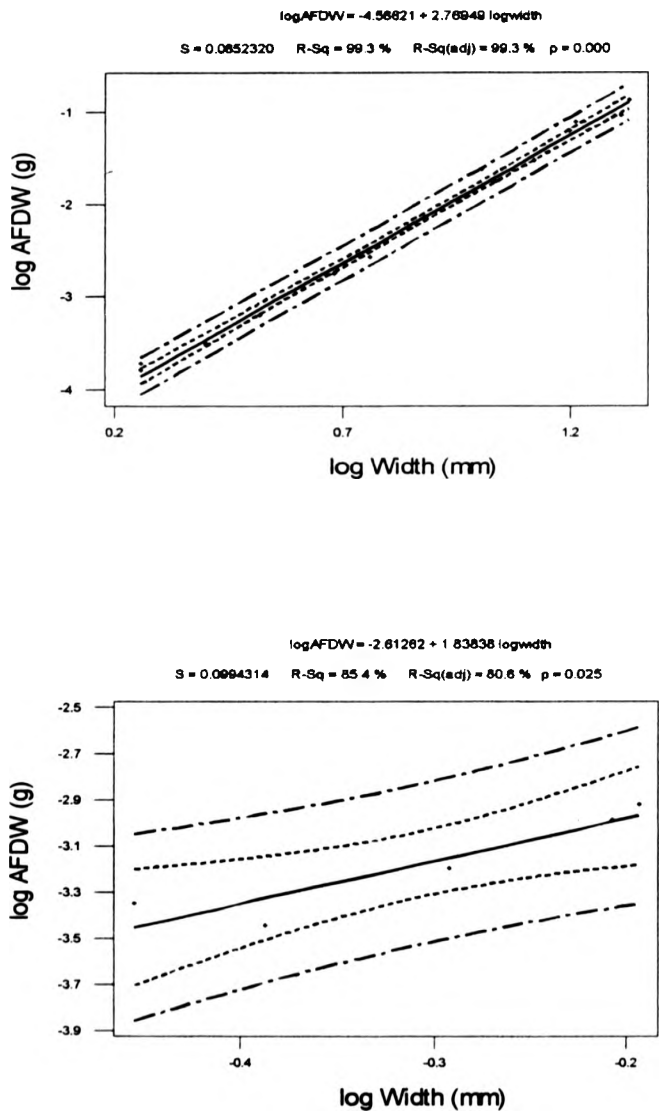


Figure 5.35. Regression plots for *Cerastoderma edule* (Top) and *Eteone longa* (Bottom), with 95% confidence limits (---) and predicted limits (– • –).

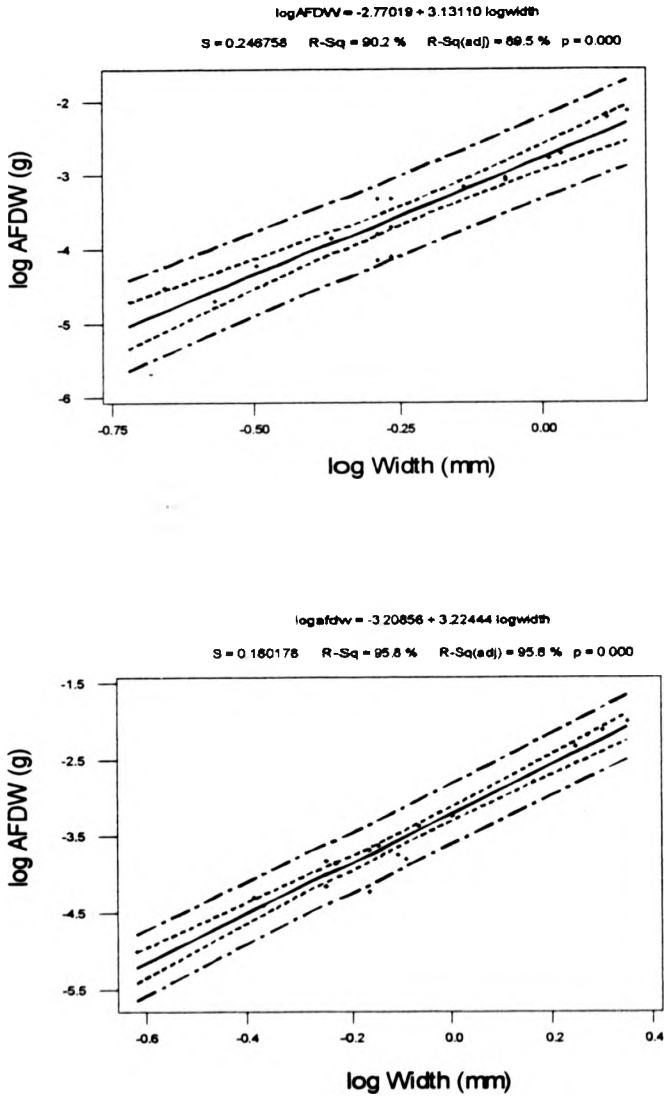


Figure 5.36. Regression plots for *Nephtys hombergii* (Top) and *Nereis diversicolor* (Bottom), with 95% confidence limits (---) and predicted limits (– • –).

Table 5.16. The mean individual ash free dry weights (AFDW) for those species whose sizes were not measured.

Species	Mean individual AFDW (g)
Corophium volutator	0.000405
Manayunkia aestuarina	0.00001567
Mytilus edulis	0.110092
Oligochaetes	0.000025
Pygospio elegans	0.000032
Streblospio shrubsolii	0.00003633

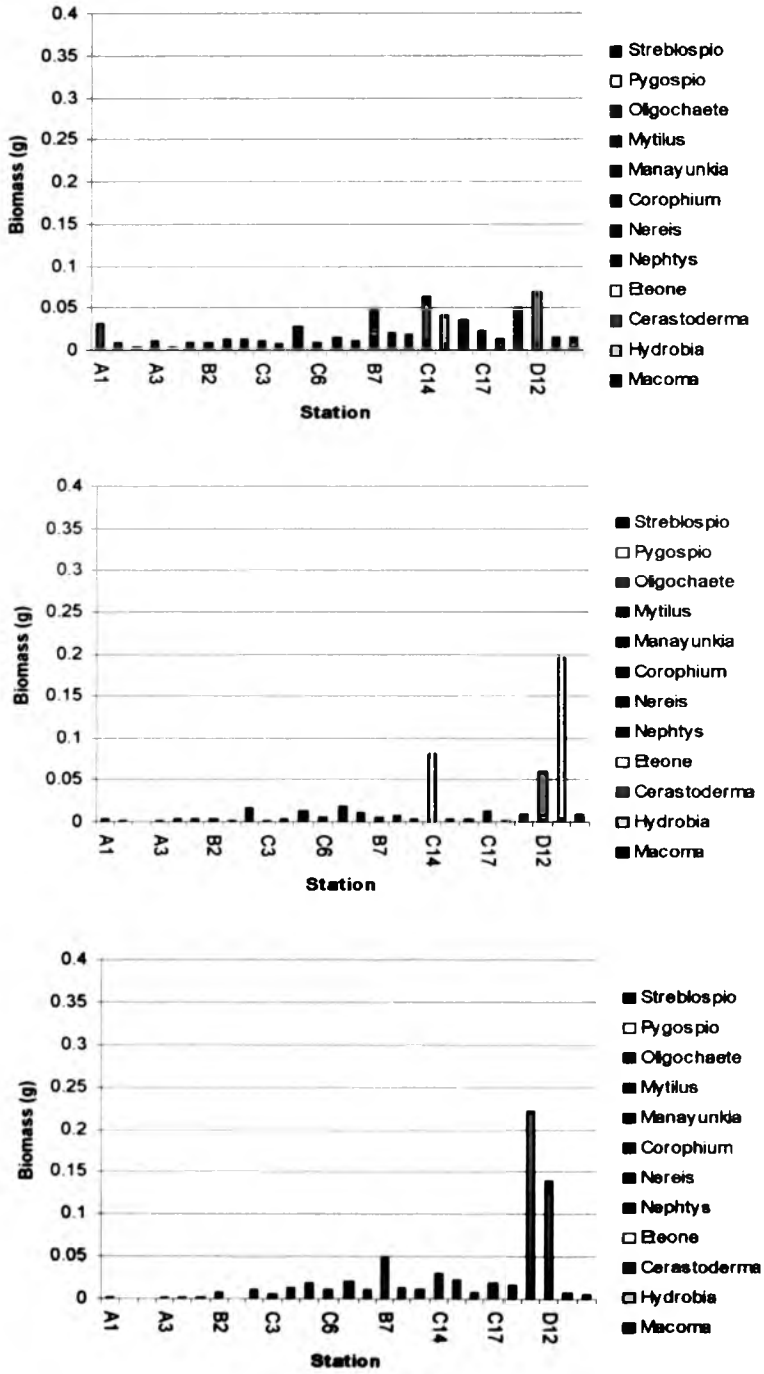


Figure 5.37. The total biomass at each stations and the contribution of each species for November 1998 (Top), February 1999 (Middle) and May 1999 (Bottom).

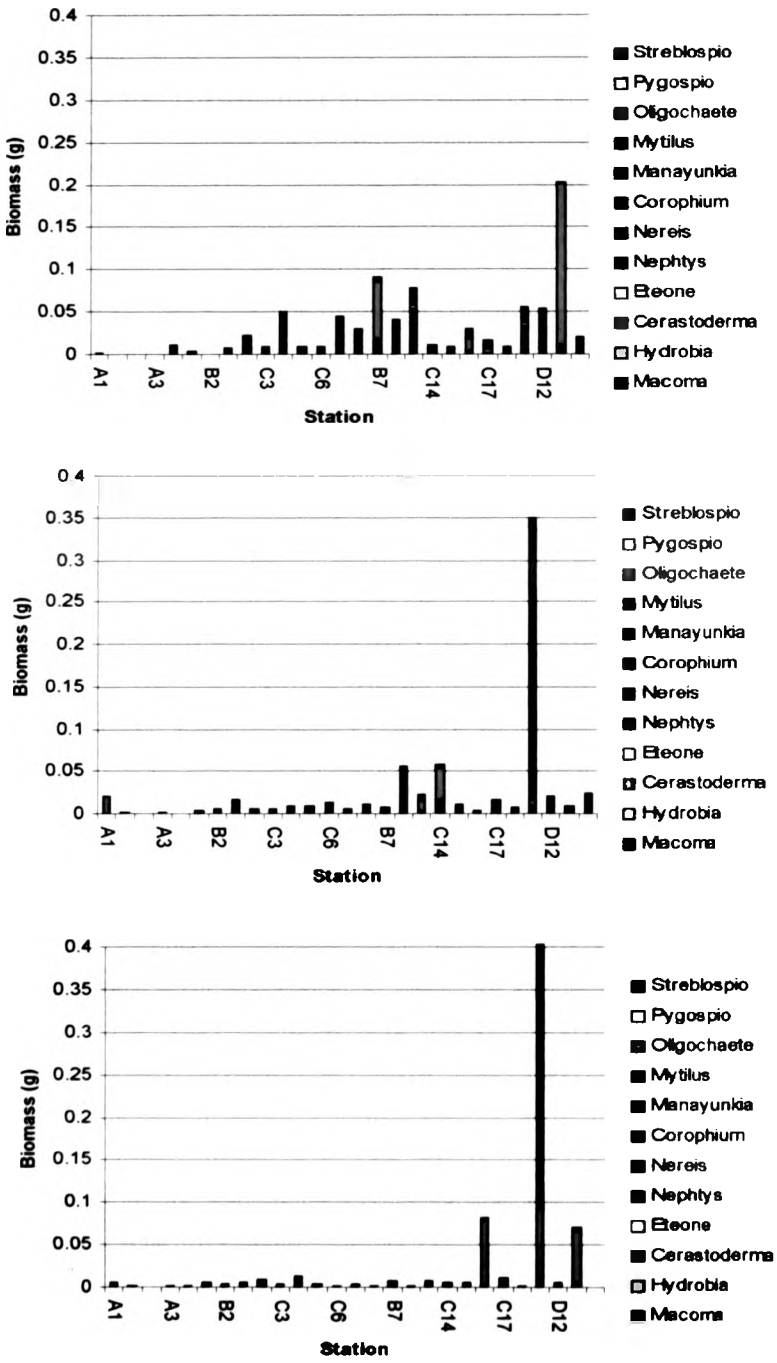


Figure 5.38. The total biomass at each station and the contribution of each species for July 1999 (Top), November 1999 (Middle) and February 2000 (Bottom).

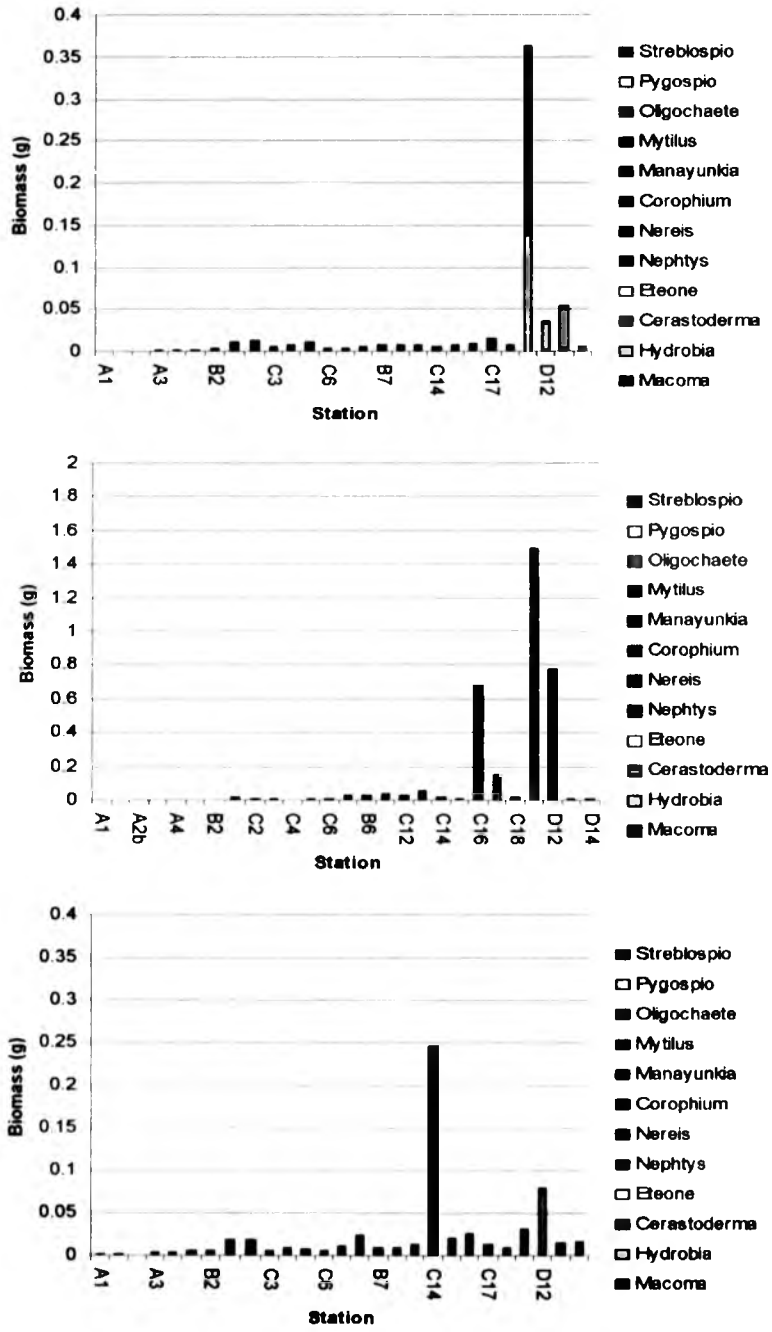


Figure 5.39. The total biomass at each station and the contribution of each species for May 2000 (Top), July 2000 (Middle, note different scale) and November 2000 (Bottom).

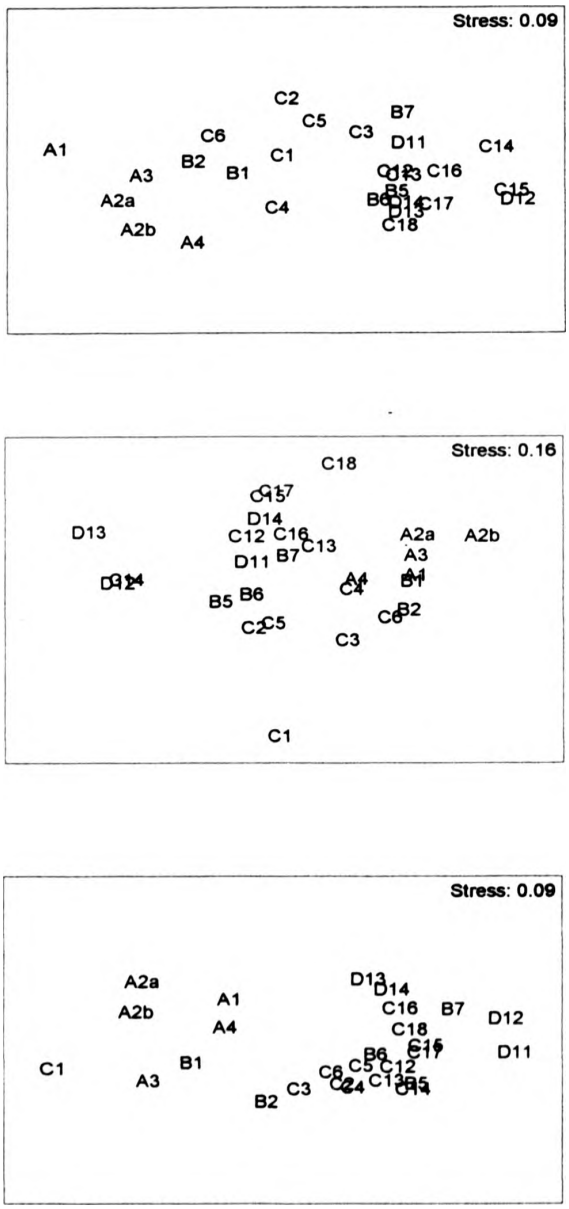


Figure 5.40. MDS plots showing the spatial distribution based on the biomass for November 1998 (Top), February 1999 (Middle) and May 1999 (Bottom).

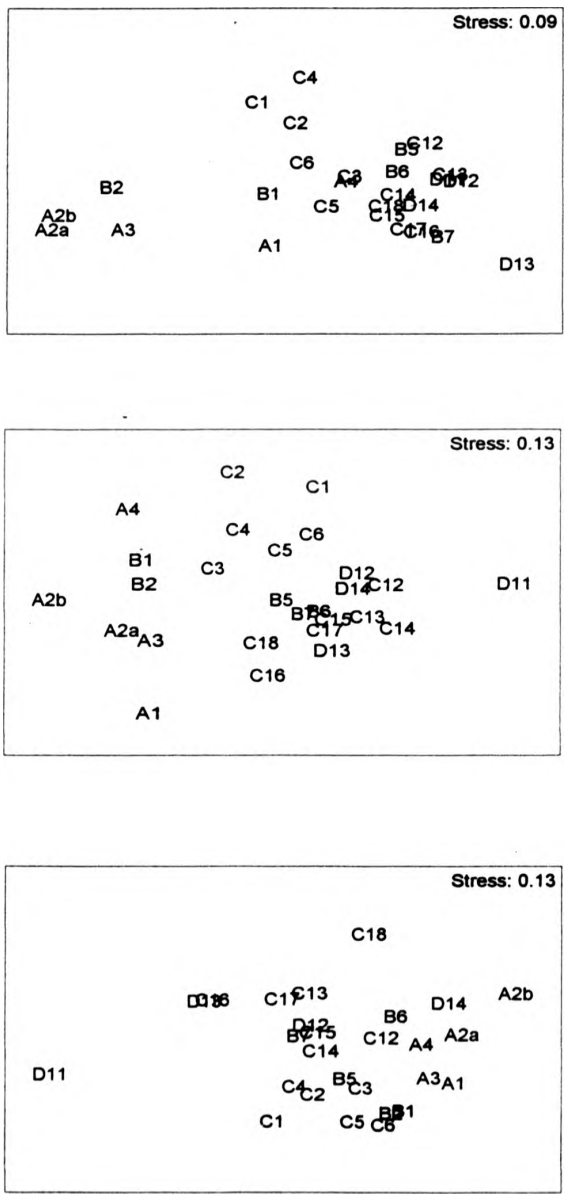


Figure 5.41. MDS plots showing the spatial distribution based on the biomass for July 1999 (Top), November 1999 (Middle) and February 2000 (Bottom).

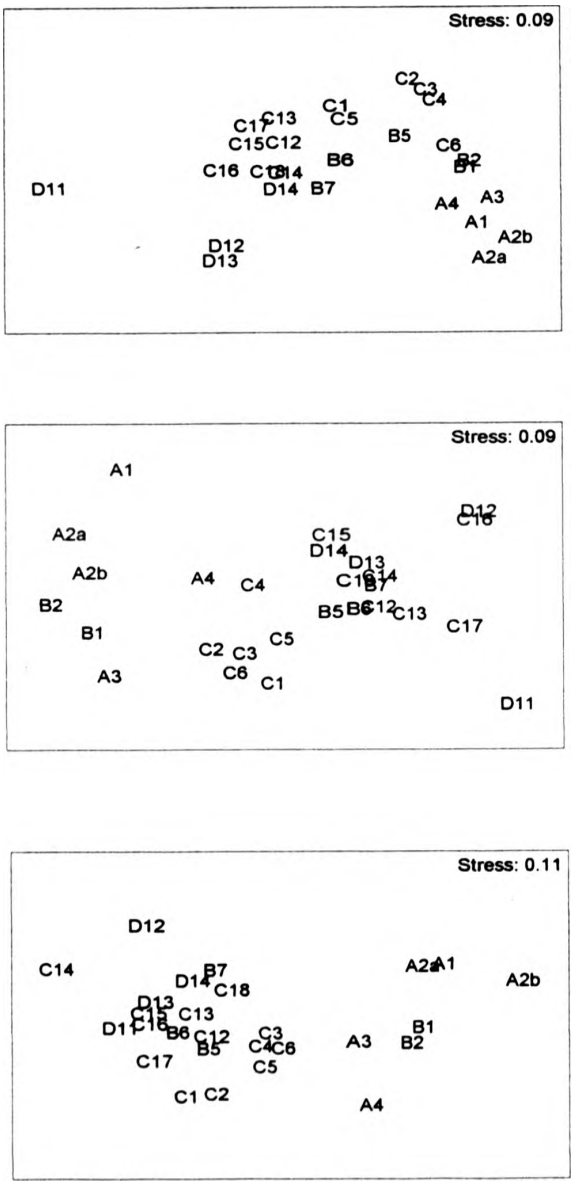


Figure 5.42. MDS plots showing the spatial distribution based on the biomass for May 2000 (Top), July 2000 (Middle) and November 2000 (Bottom).

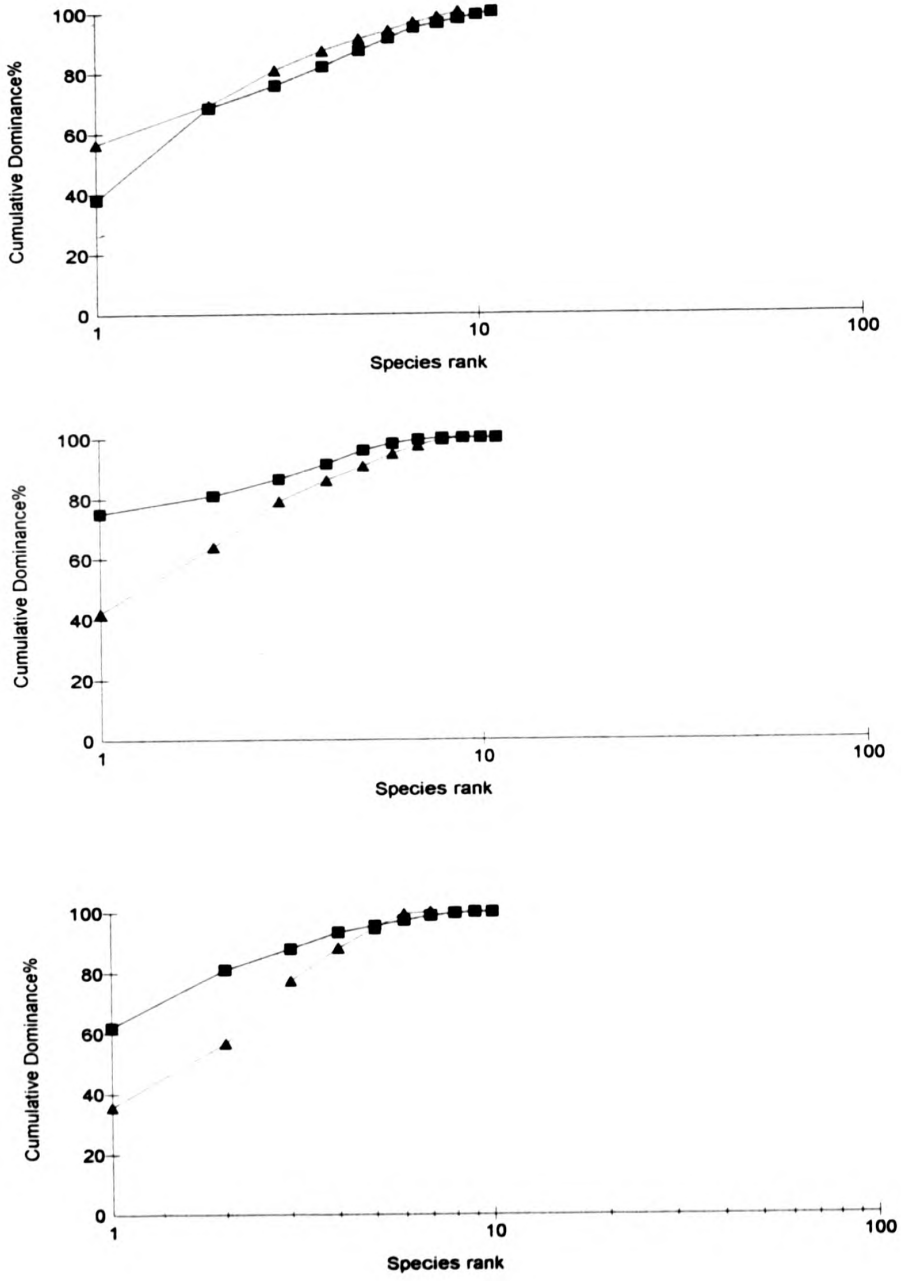


Figure 5.43. k-dominance plots for the biomass showing the difference between upper shore (Triangle) and lower shore (Square) areas for November 1998 (Top), February 1999 (Middle) and May 1999 (Bottom).

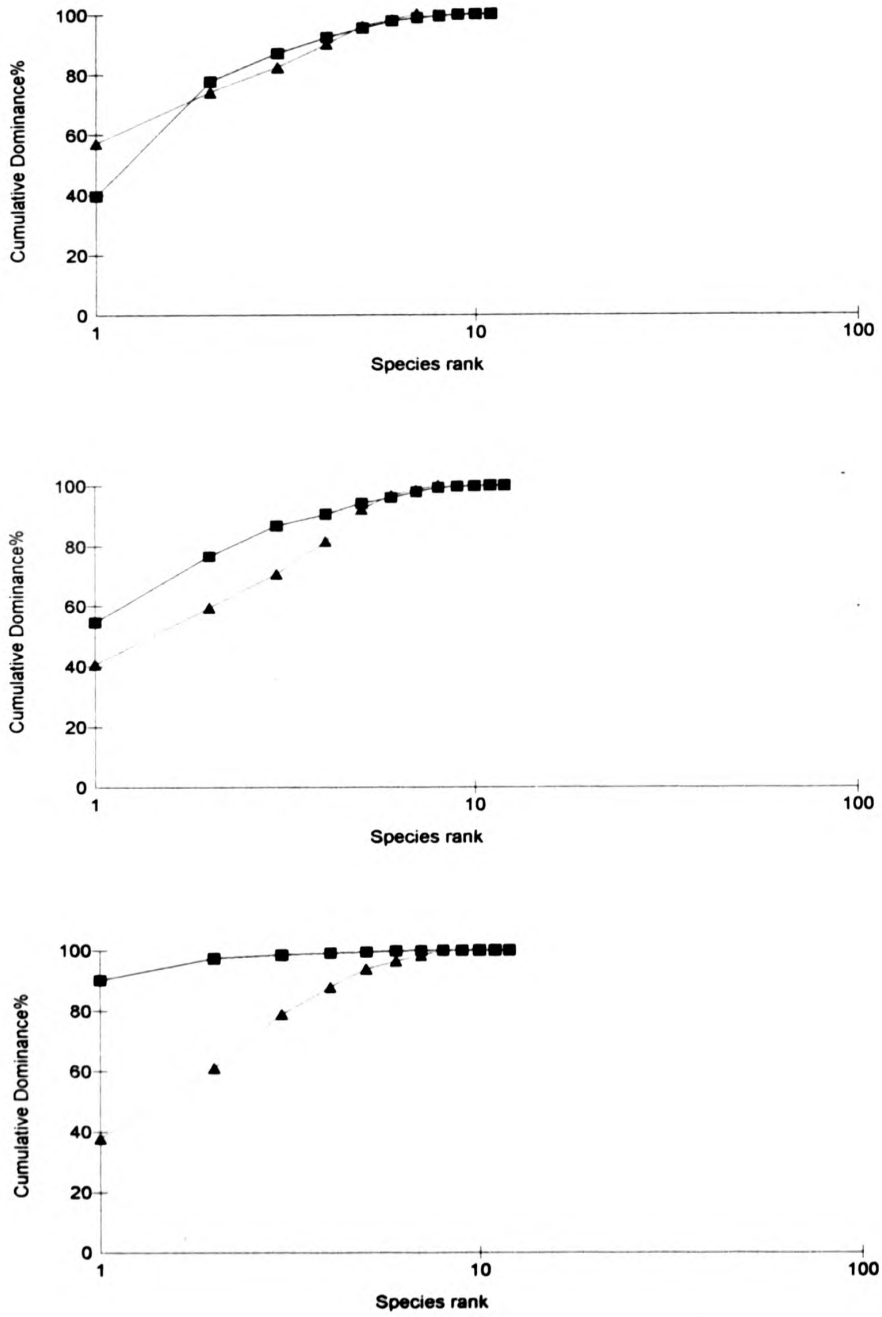


Figure 5.44. k-dominance plots for the biomass showing the difference between upper shore (Triangle) and lower shore (Square) areas for July 1999 (Top), November 1999 (Middle) and February 2000 (Bottom).

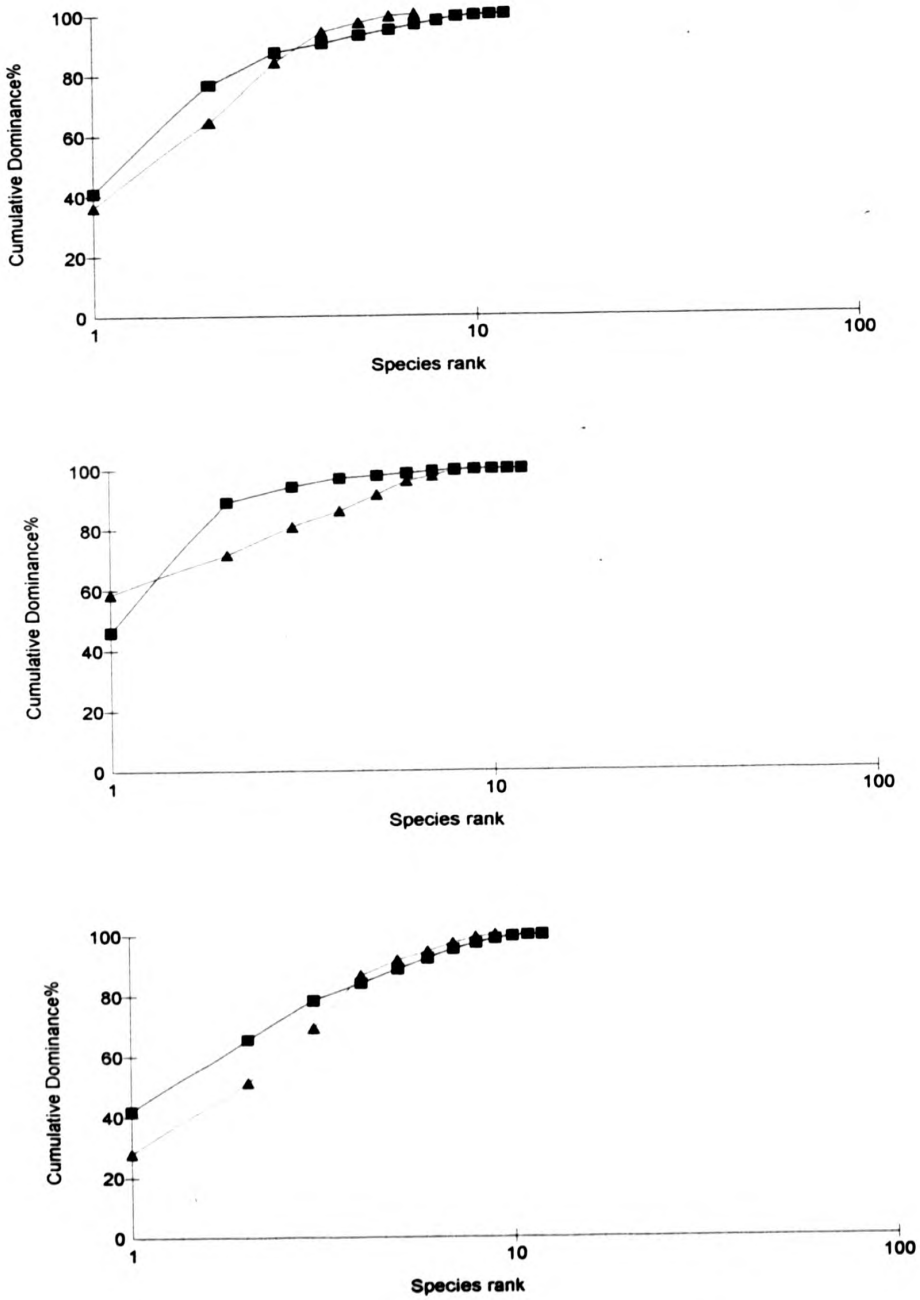


Figure 5.45. k-dominance plots for the biomass showing the difference between upper shore (Triangle) and lower shore (Square) areas for May 2000 (Top), July 2000 (Middle) and November 2000 (Bottom).

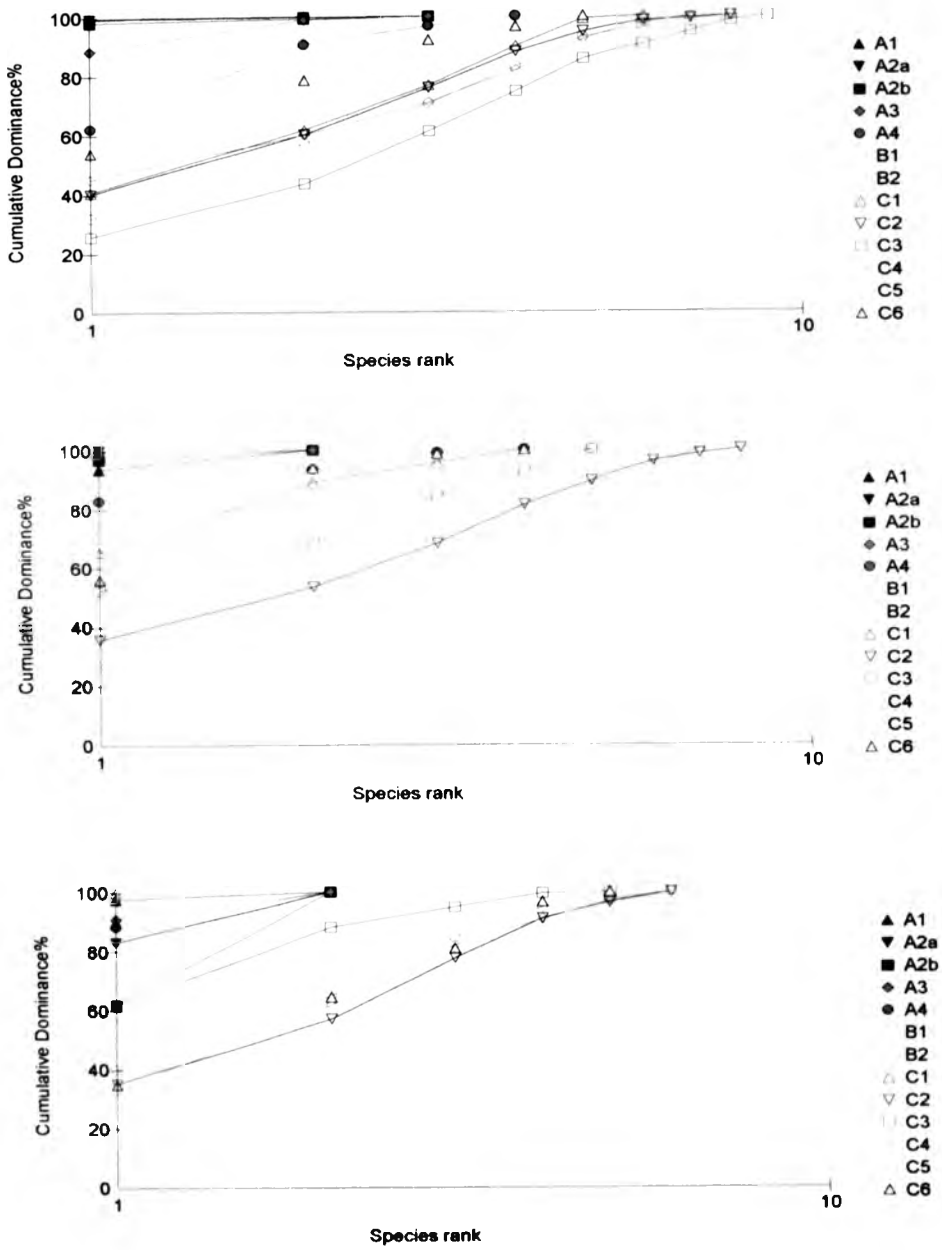


Figure 5.46. k-dominance curves for each of the upper shore stations for November 1998 (top), February 1999 (middle) and May 1999 (bottom).

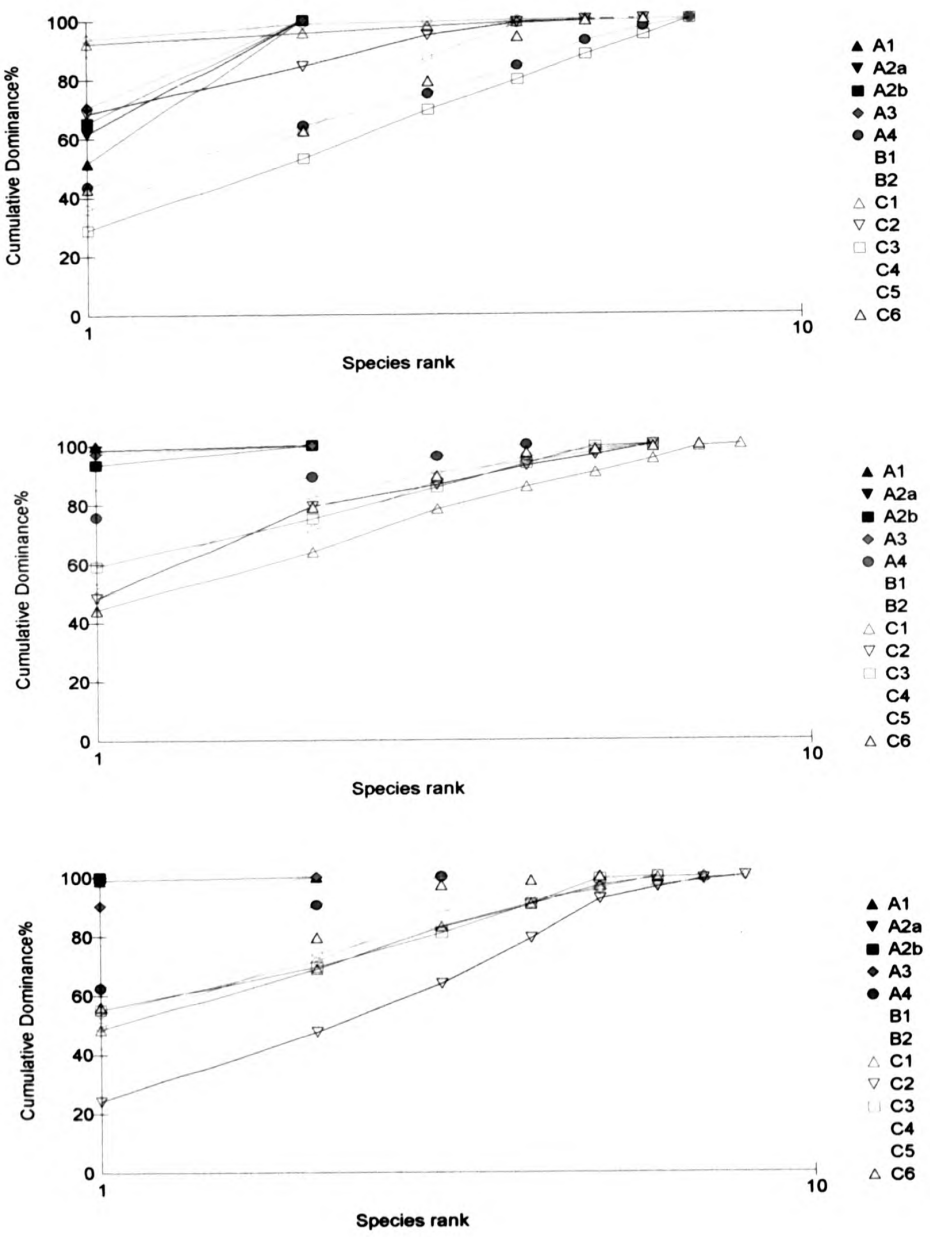


Figure 5.47. k-dominance curves for each of the upper shore stations for July 1999 (top), November 1999 (middle) and February 2000 (bottom).

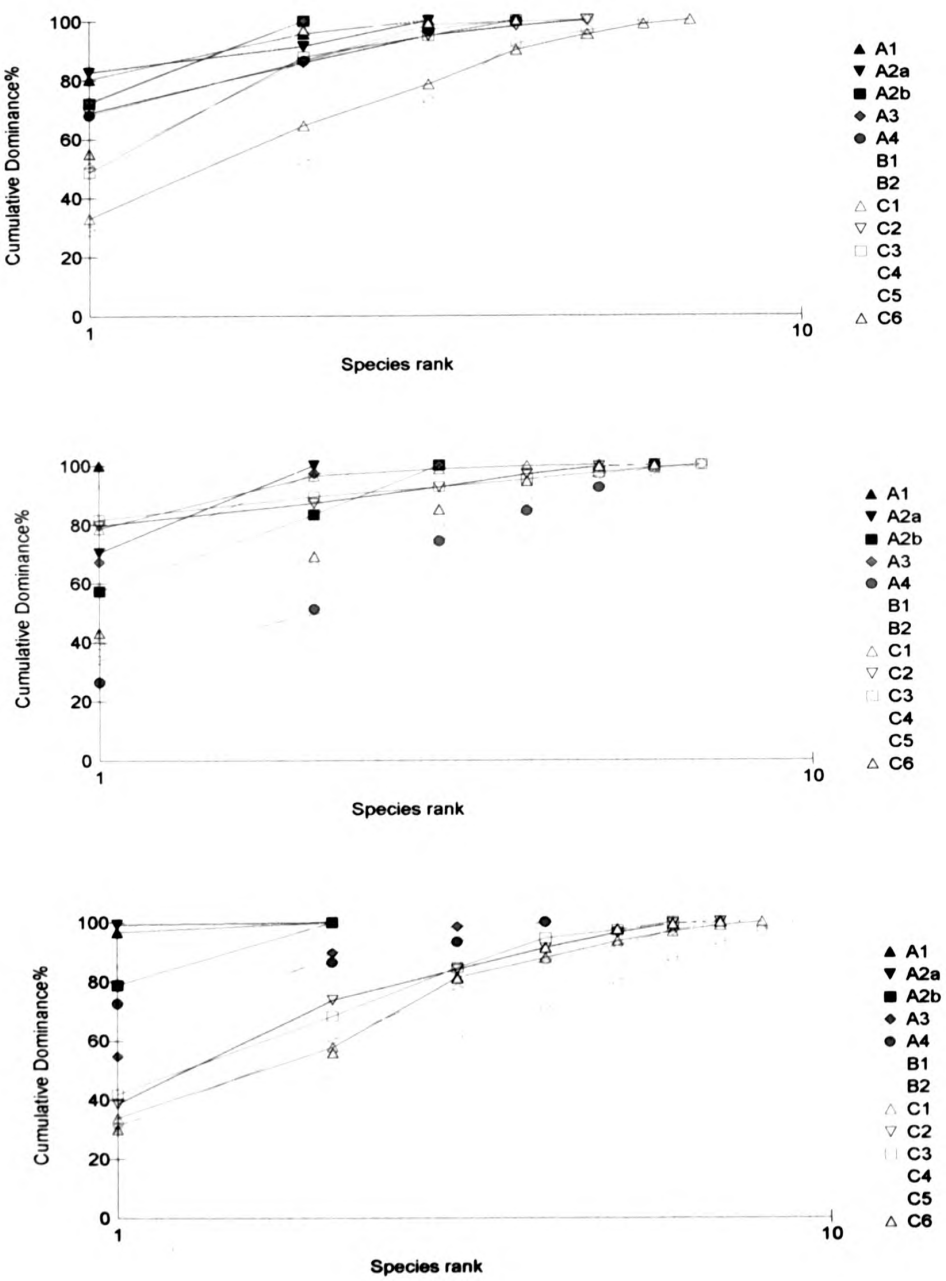


Figure 5.48. k-dominance curves for each of the upper shore stations for May 2000 (top), July 2000 (middle) and November 2000 (bottom).

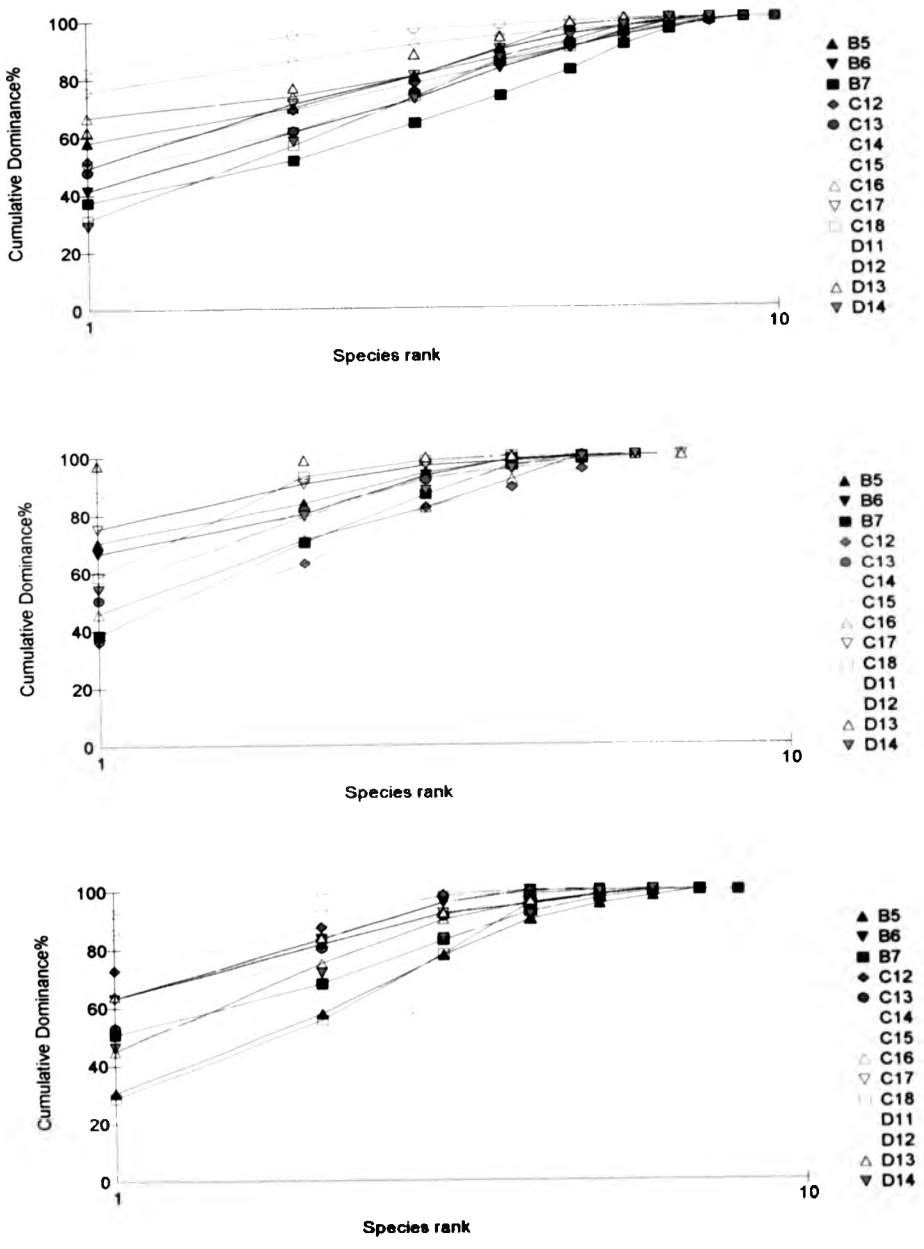


Figure 5.49. k-dominance curves for each of the lower shore stations for November 1998 (top), February 1999 (middle) and May 1999 (bottom).

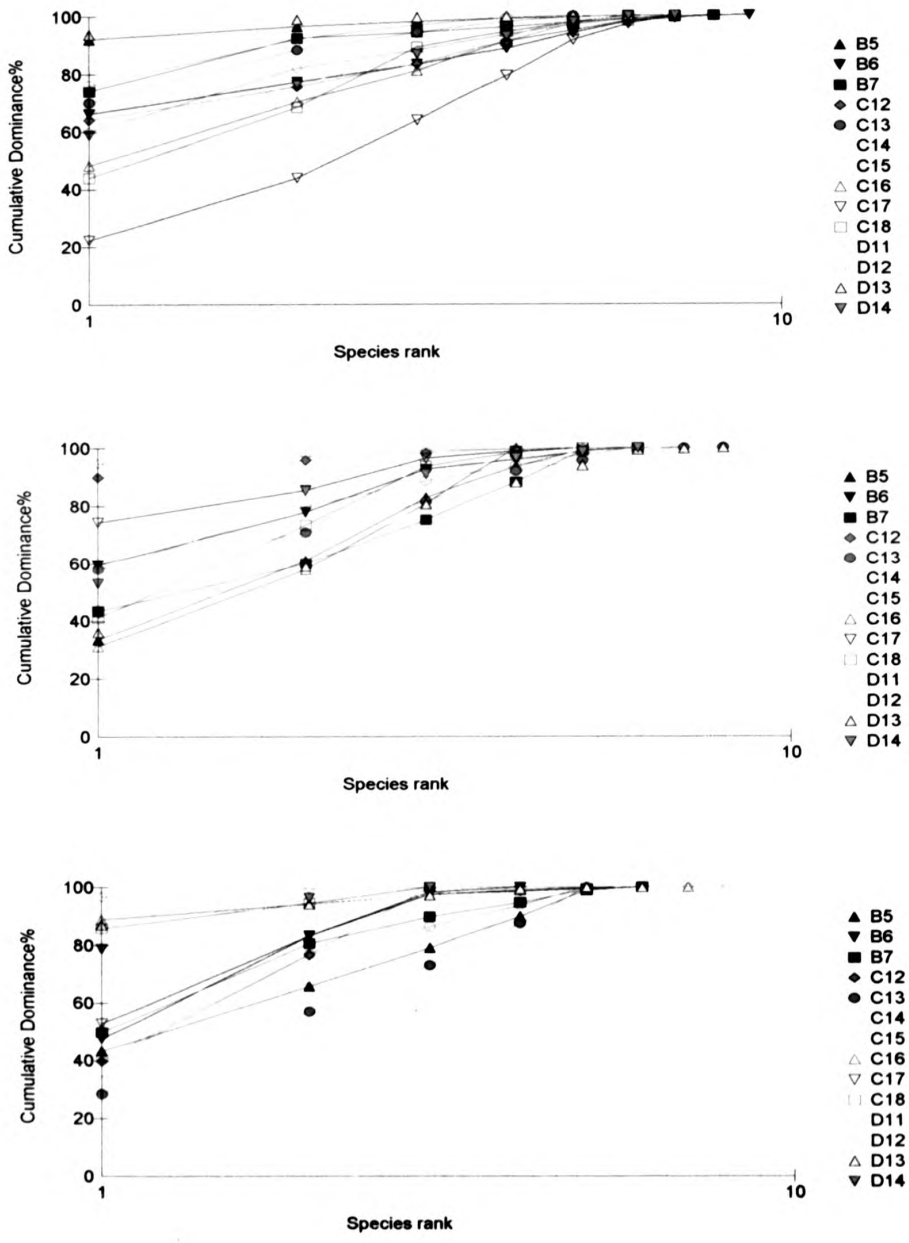


Figure 5.50. k-dominance curves for each of the lower shore stations for July 1999 (top), November 1999 (middle) and February 2000 (bottom).

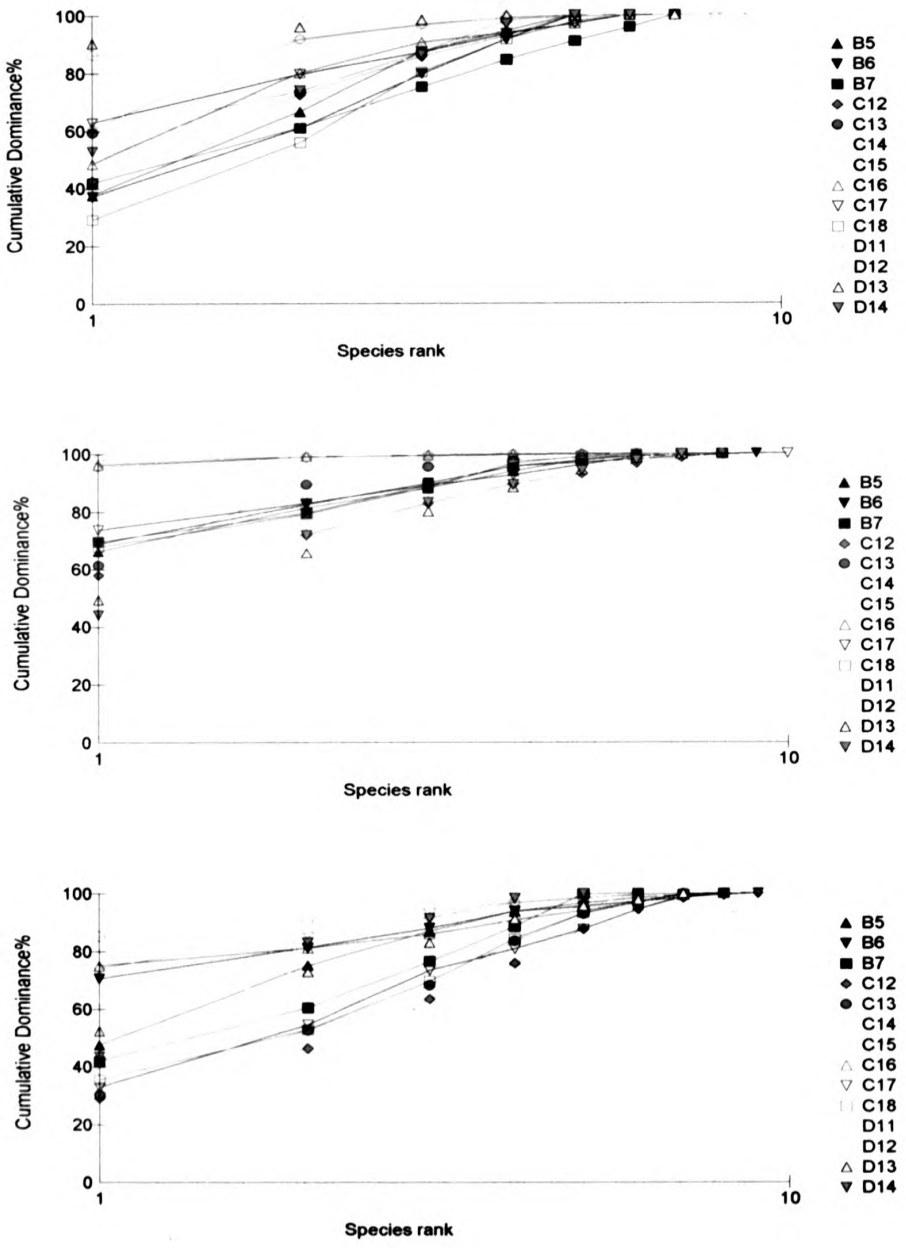


Figure 4.51. k-dominance curves for each of the lower shore stations in May 2000 (top), July 2000 (middle) and November 2000 (bottom).

Table 5.16. ANOSIM results testing the hypothesis that there is no difference between the upper shore and lower shore stations for each sampling time based on the biomass.

Sampling time	Global R	Significance
Nov-98	0.769	0.10%
Feb-99	0.433	0.10%
May-99	0.551	0.10%
Jul-99	0.556	0.10%
Nov-99	0.574	0.10%
Feb-00	0.302	0.10%
May-00	0.609	0.10%
Jul-00	0.669	0.10%
Nov-00	0.551	0.10%

Table 5.17. The measure of dispersion for upper shore and lower shore stations and the Index of multivariate dispersion (IMD) between the two areas based on the biomass, for each sampling time.

Sampling time	Shore	Offshore	IMD
Nov-98	1.245	0.79	0.458
Feb-99	0.953	1.04	-0.088
May-99	1.223	0.809	0.416
Jul-99	1.299	0.743	0.559
Nov-99	1.221	0.811	0.412
Feb-00	0.905	1.081	-0.177
May-00	0.954	1.039	-0.085
Jul-00	1.151	0.871	0.281
Nov-00	1.225	0.807	0.42

Table 5.18. SIMPER results for November 1998 based on the biomass comparing the upper shore and lower shore areas (* = good discriminating species).

Average dissimilarity = 78.58					
Species	**Upper shore Mean Biomass ($x10^4$)**	**Lower shore Mean Biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Macoma balthica	0.78	11.82	1.68*	34.42	34.42
Cerastoderma edule	0	9.43	0.53	17.26	51.67
Oligochaete	6.92	2.31	0.83	14.94	66.61
Hydrobia ulvae	0.21	1.92	1.03	6.65	73.26
Manayunkia aestuarina	1.57	0.3	0.96	5.72	78.98
Nereis diversicolor	1.42	0.45	0.73	4.61	83.59
Streblospio shrubsolii	0.31	1.65	1.49*	4.44	88.03
Corophium volutator	0.34	1.24	0.93	4.35	92.38

Table 5.19. SIMPER results for February 1999 based on the biomass comparing the upper shore and lower shore areas.

Average dissimilarity = 75.80					
Species	**Upper shore Mean Biomass ($x10^4$)**	**Lower shore Mean Biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Cerastoderma edule	0	28.95	0.61	31.6	31.6
Nephtys hombergii	0.06	1.71	0.68	16.6	48.19
Macoma balthica	0.95	1.7	0.86	13.04	61.23
Oligochaete	1.97	1.6	0.8	10.86	72.09
Manayunkia aestuarina	0.84	0	0.64	7.17	79.26
Nereis diversicolor	1.42	0.02	0.38	7.05	86.31
Streblospio shrubsolii	0.3	0.63	0.8	6.97	93.29

Table 5.20. SIMPER results for May 1999 based on the biomass comparing the upper shore and lower shore areas.

Average dissimilarity = 81.49					
Species	**Upper shore Mean Biomass ($x10^4$)**	**Lower shore Mean Biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Macoma balthica	1.19	7.81	1.31	33.45	33.45
Cerastoderma edule	0	25.4	0.62	24.18	57.63
Nephtys hombergii	0	2.81	0.84	12.04	69.66
Oligochaete	2.02	2.22	1.15	9.94	79.61
Manayunkia aestuarina	1.17	0.02	0.77	5.63	85.24
Nereis diversicolor	0.61	0.72	0.61	5.1	90.34

Table 5.21. SIMPER results for July 1999 based on the biomass comparing the upper shore and lower shore areas.

Average dissimilarity = 86.77					
Species	**Upper shore Mean Biomass ($x10^4$)**	**Lower shore Mean Biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Macoma balthica	0.55	18.94	1.31	39.49	39.49
Cerastoderma edule	0	19.66	0.53	17.49	56.98
Nephtys hombergii	0	4.62	0.89	12.75	69.74
Nereis diversicolor	5.51	0.33	0.52	10.21	79.95
Oligochaete	1.63	2.68	0.8	6.95	86.9
Streblospio shrubsolii	0.22	1.48	0.82	4.78	91.68

Table 5.22. SIMPER results for November 1999 based on the biomass comparing the upper shore and lower shore areas.

Average dissimilarity = 83.89					
Species	**Upper shore Mean Biomass ($x10^4$)**	**Lower shore Mean Biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Macoma balthica	0.8	9.48	1.15	32.03	32.03
Oligochaete	2.97	1.54	0.68	11.87	43.9
Cerastoderma edule	0	4.4	0.47	10.27	54.17
Streblospio shrubsolii	0.11	1.68	1.1	9.51	63.68
Mytilus edulis	0	23.59	0.28	7.9	71.58
Nephtys hombergii	0	0.81	0.76	6.48	78.06
Nereis diversicolor	1.37	0.05	0.59	6.27	84.33
Corphium volutator	0.81	0.78	0.58	5.55	89.88
Manayunkia aestuarina	0.78	0.01	0.74	4.49	94.37

Table 5.23. SIMPER results for February 2000 based on the biomass comparing the upper shore and lower shore areas.

Average dissimilarity = 77.72					
Species	**Upper shore Mean Biomass ($x10^4$)**	**Lower shore Mean Biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Macoma balthica	0.81	2.47	0.96	17.57	17.57
Oligochaete	1.72	1.16	0.83	16.35	33.91
Cerastoderma edule	0	15.68	0.42	15.56	49.47
Manayunkia aestuarina	1.06	0.06	0.77	11.76	61.23
Streblospio shrubsolii	0.12	0.67	0.86	9.59	70.82
Mytilus edulis	0	196.59	0.28	8.87	79.69
Nephtys hombergii	0	0.89	0.54	8.22	87.91
Hydrobia ulvae	0.27	0.26	0.66	4.97	92.88

Table 5.24. SIMPER results for May 2000 based on the biomass comparing the upper shore and lower shore areas.

Average dissimilarity = 80.94					
Species	**Upper shore Mean Biomass ($x10^4$)**	**Lower shore Mean Biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Macoma balthica	0.47	4.11	1.18	25.03	25.03
Cerastoderma edule	0	13.75	0.48	16.89	41.92
Nereis diversicolor	1.71	0.12	0.72	11.11	53.02
Nephtys hombergii	0	1	0.81	9.19	62.21
Oligochaete	1.33	1.11	1.11	7.25	69.46
Streblospio shrubsolii	0.14	0.66	0.8	7.13	76.59
Manayunkia aestuarina	0.93	0.08	0.98	7.05	83.64
Mytilus edulis	0	15.73	0.28	5.28	88.93
Corophium volutator	0	0.52	0.38	4.96	93.88

Table 5.25. SIMPER results for July 2000 based on the biomass comparing the upper shore and lower shore areas.

Average dissimilarity = 93.89					
Species	**Upper shore Mean Biomass ($x10^4$)**	**Lower shore Mean Biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Macoma balthica	0.66	12.21	1.29	34.83	34.83
Nephtys hombergii	0	102.89	0.66	22.62	57.45
Mytilus edulis	0	110.09	0.4	12.68	70.13
Nereis diversicolor	2.98	0.07	0.55	7.23	77.36
Cerastoderm edule	0	6.29	0.36	5.68	83.04
Streblospio shrubsolii	0.09	1.99	1.16	5.64	88.68
Oligochaete	0.27	1.58	1.04	4.13	92.81

Table 5.26. SIMPER results for November 2000 based on the biomass comparing the upper shore and lower shore areas.

Average dissimilarity = 78.79					
Species	**Upper shore Mean Biomass ($x10^4$)**	**Lower shore Mean Biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Macoma balthica	1.62	8.81	1.22	33.29	33.29
Nephtys hombergii	0	1.71	0.79	10.2	43.49
Streblospio shrubsolii	0.34	2.12	1.22	9.9	53.39
Mytilus edulis	0	15.73	0.28	7.91	61.29
Oligochaete	1.91	1.33	0.95	7.66	68.96
Cerastoderm edule	0	4.8	0.28	7.14	76.1
Nereis diversicolor	1.23	0.3	0.73	5.71	81.81
Manayunkia aestuarina	1.19	0.05	0.92	5.27	87.08
Corphium volutator	0.06	1.1	0.59	4.7	91.77

Table 5.27. The spatial BIO-ENV results based on the total biomass for the different sampling times.

Sampling time	p_s	Variables
Nov-98	0.531	Refinery distance
Feb-99	0.352	Height
May-99	0.47	Refinery distance, % organic matter
Jul-99	0.44	Refinery distance
Nov-99	0.456	Refinery distance, % organic matter
Feb-00	0.401	Height
May-00	0.611	Refinery distance, Chemicals 3 distance
Jul-00	0.509	Refinery distance
Nov-00	0.559	Refinery distance

Sampling time	Biomass p, Rsq(adj)
Nov-98	0.023, 15.7%
Feb-99	0.019, 16.9%
May-99	0.000, 48.0%
Jul-99	0.000, 55.6%
Nov-99	0.002, 34.7%
Feb-00	0.002, 36.4%
May-00	**0.000, 82.4%**
Jul-00	0.000, 56.8%
Nov-00	0.000, 58.8%

Table 5.28. Spatial stepwise regression results for each sampling time showing the p and Rsq(adj) values based on the total biomass. High Rsq(adj) values are highlighted in bold. The significant equation for May 2000 – Log Biomass = 1.69 – 0.714 Height + 0.00852 Mean particle size – 0.832 Sewage distance – 0.560 Grangeburn distance, p=0.000, F=31.45, Rsq(adj) = 82.4%.

Table 5.29. Regression and BIO-ENV results for the spatial biomass data, including the hydrocarbon results.

Regression	**log biomass** = -2.22 + 0.000887 **refinery distance** – 0.701 sewage distance, p = 0.000, F = 29.78, Rsq(adj) = 81.6%
BIO-ENV	p_s = 0.832, Refinery distance, Chemical outfall 3 distance

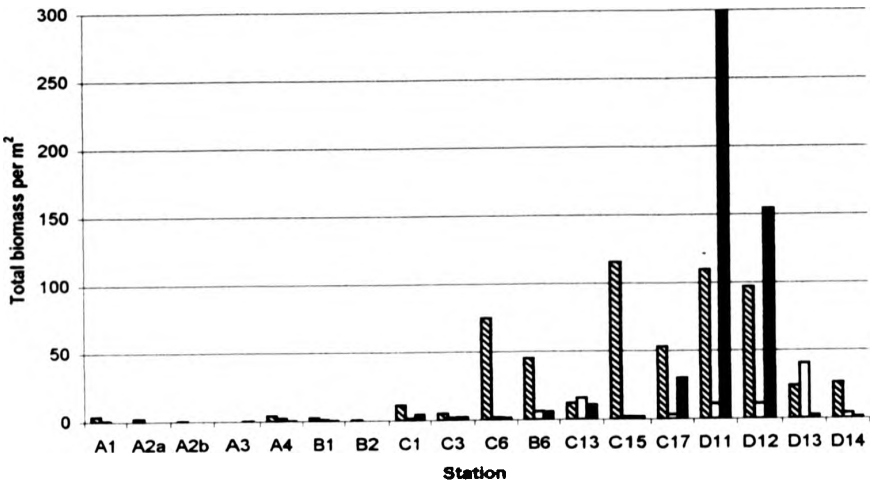


Figure 5.52. Comparison between the total biomass found at Eighteen stations in August 1996 (stripe) (Yule, 1996), July 1999 (white) and July 2000 (black).

6. FIELD SURVEY –TEMPORAL VARIATION

6.1 INTRODUCTION

6.1.1 Movement of the chemical outfall

The two-year field survey was undertaken to assess the impact on the benthic community of the movement of the chemical outfall in January 1999 to a lower shore site. This move was made to try to reduce any impact that the effluent was having on the environment. The intention being to dilute the effluent to reduce its toxicity by discharging it directly into the water column instead of onto the shore. The new outfall is still within the tidal range and is therefore uncovered at low tide. The effluent discharge is however controlled so that it is only released from the outfall whilst it is covered by water, generally for about 2 hours per high tide.

There are many different potential effects that may occur from moving the chemical outfall down shore. It would be expected that if the chemical effluent was having an impact on the benthic community then the removal of the effluent from the upper shore area would allow this area to recover. On the other hand it would be expected that the area around the new outfall should not show any signs of becoming impacted as a direct result of the toxicity of the effluent, due to the increased dilution effect. The movement of the outfall may also have indirect effects that could potentially cause a change in the benthic community composition. The discharge of the effluent into the water column will have an effect on the localised water currents, which in turn could effect the deposition of sediments and/or the distribution of settling larvae (Thrush, 1991). The species composition of an area is often related to the type of sediment (Gray, 1974). Adults and larvae have been shown to select suitable sites on the type of substratum and many are restricted to certain types of sediment. Suspension feeders are usually limited to fairly stable substrates as highly mobile sediment clog their filtering systems. Whitlatch (1981) also found that the species diversity especially of deposit feeders was correlated with the organic matter content of the sediment. The organic matter content is

usually correlated with the particle size of the sediment with finer sediments having higher organic matter content (McLusky, 1989).

6.1.2 The effect of the first movement of the chemical outfall

McLusky (1982a) observed the effects of the movement of the chemical outfall from its first position in the Avon to its second site in 1979. McLusky (1982a) observed that the area around the old outfall site (site 1) showed a rapid and dramatic recovery. Whilst the area around the new chemical outfall (site 2) showed a decrease in the biomass and a change in the community composition. It was noted that *Macoma balthica* and *Nereis diversicolor* disappeared, *Manayunkia aestuarina* appeared and *Hydrobia ulvae* and Oligochaetes persisted in the areas around the new outfall. It was proposed that it was the chemical nature of the effluent that caused these effects, and that the removal of the chemicals allowed the recolonisation of the closed site whilst their increased concentration caused the increased impact at the new site.

6.1.3 Aims

The abundance and biomass data collected by the two-year survey, before and after the installation of the lower shore chemical outfall in January 1999 has been analysed with the following aims:-

- To determine if there was a change in the community composition around the old shore chemical outfall.
- To determine if there was a change in the community competition around the new lower shore chemical outfall.
- To determine the cause of any changes in the upper or lower shore areas.

6.2 RESULTS

6.2.1 Sediment, climate, effluent and water quality changes

6.2.1.1 Sediment properties

The sediment properties (Figures 6.1 and 6.2) did not show any significant increase or decrease over time, although a difference between the upper and lower shore areas was detected. Both areas had a similar mean particle size, organic matter content and percentage clay fraction. The upper shore however generally had a higher percentage of sand and a lower percentage of silt than the lower shore area.

6.2.1.2 Climate

The apparent difference between the upper and lower shore areas is due to the slight differences in the exact times when the samples were collected. There was no significant increase or decrease of the climatic factors over time (Figure 6.3). The two areas show the same seasonal pattern of change in temperature and rainfall. In general the temperature was higher between July and November and the minimum and maximum temperatures show the same pattern. The rainfall was fairly high in November 1998 after which it decreased. There was an increase again in July 1999 after which it again decreased and remained at a low level throughout 2000. The North Atlantic Oscillation (NAO) for the sampling dates has shown slightly different patterns between the upper and lower shore areas again due to the slightly different sampling times. The NAO is negative only in November 1998 for the upper shore area and in November 1998 and May 1999 for the lower shore area.

6.2.1.3 Petrochemical effluents

The data on the change in the variables measured in the petrochemical effluents (Figures 6.4 to 6.7) indicate that most do not show a clear pattern, only fluctuations, except for ammonia, fluoride and the electrical conductivity (EC) in the chemical effluent which have all shown a decrease over the two-year study period. For the refinery effluent both chromium and lead showed a large decrease between November 1998 and February 1999.

6.2.1.4 Other pollution sources

The graphs for the change over time for the variables that were measured in the Avon and Kinneil channel (Figures 6.8 to 6.11) show no clear patterns just fluctuations for both the upper and lower shore areas. The slight differences between the two areas are again due to the slightly different times they were sampled. Most of the variables from the sewage works data (Figure 6.12 and 6.13) for both the upper and lower shore areas also show no pattern except zinc which increased in concentration between July and November 2000.

6.2.2 Temporal changes in abundance

6.2.2.2 Changes in the community composition

6.2.2.2.1 Diversity, evenness, species richness and number of individuals

When comparing the mean of the upper shore stations for each sampling period (Figure 6.14) it can be seen that there has been little change in the diversity, evenness or number of species per station. There may be a small seasonal cycle with a slight increase in diversity and evenness in May/July. The mean number of individuals has a peak of 400 individuals $50cm^{-2}$ in November 1998. For all other sampling periods the number of individuals remained much lower between 50 to 200 50 cm^{-2}.

The mean lower shore results (Figure 6.15) indicate that there has been little change in the diversity, evenness, number of species or number of individuals over the two year sampling period, except for a seasonal cycle with increases during the summer.

6.2.2.2.2 Individual species abundance

The changes in the abundance over time of the common species for the upper shore area (Figure 6.16 to 6.18) indicates that most species have shown fluctuations in abundance over the survey period. Many species showed a decrease in abundance between November 1998 and February 1999. The most abundant species in November 1998 were *Lumbricillus spp* and *Manayunkia aestuarina*. *Hydrobia*

ulvae showed a peak abundance in July 1999 and *Nereis diversicolor* in November 1999. Some of the oligochaete species such as *Heterochaeta costata*, *Lumbricillus spp* and *Paranais litoralis* showed a slight seasonal cycle of abundance. Other species like *Pygospio elegans* ($p = 0.039$, $F = 6.45$, Rsq = 48.0%), *Tubificoides benedii* ($p = 0.007$, $F = 13.83$, Rsq = 66.4%) and *Tubificidae spp* ($p = 0.029$, $F = 7.54$, Rsq = 51.8%) showed a significant decrease in abundance over time.

The lower shore area shows a different pattern for the individual species abundance changes (Figure 6.19 to 6.22). None of the species showed a clear decrease in abundance except *T. benedii* ($p = 0.038$, $F = 6.47$, Rsq = 48%), although most species did show seasonal fluctuations or peak months but to varying extents, but most have the highest abundance during July. Again many showed a decrease between November 1998 and February 1999 but this is often part of a continuing seasonal trend.

6.2.2.2.3 Cluster analysis, MDS and cyclicity

The cluster analysis dendrogram and MDS plot showing the relationship between the different sampling times for the upper shore stations (Figure 6.23) indicates that July 2000 had a very different community composition from the other sampling periods. November 1998 was also different but the rest of the sampling times had very similar community compositions. By looking at the MDS plot there does seem to be a pattern with most samples for the same months from the two years being fairly close or in the same area of the plot, suggesting that there may be a seasonal cycle.

The lower shore cluster analysis (Figure 6.24) shows that May 2000 had a very different community composition from the other sampling periods. February 1999 and February 2000 were very similar, whilst the rest of the sampling periods were grouped together. From looking at the MDS plot there does not seem to be any obvious season trend due to the large difference between May 1999 and 2000.

The cyclicity of the sampling times was tested to determine whether there was a seasonal pattern. Both the lower shore and upper shore sites where not found to

have any significant cyclic pattern (Lower shore - Rho = -0.031, 55.4%; Upper shore – Rho = -0.055, 72.7%).

6.2.2.2.4 SIMPER

SIMPER was used to look at the species difference between the sampling times indicated by the cluster analysis. The species that were found to be responsible for the differences between the sampling periods for the upper shore stations (Table 6.1) were again the Oligochaetes, *P. elegans* and *M. aestuarina*. The difference between November 1998 and all the other sampling times was that there was a higher abundance of *Lumbricillus sp.*, *M. aestuarina*, *T. benedii*, *Tubificidae sp.* and *P. elegans*. The difference between July 2000 and the most of the other sampling times was that it had a very low abundance of all the Oligochaetes and *M. aestuarina*.

The species that were found to be responsible for the differences for the lower shore stations (Table 6.2 and 6.3) were again Oligochaetes, Spionids, *M. aestuarina* and this time *M. balthica* and *Hydrobia ulvae*. The difference between May 2000 and all other sampling times was the high abundance of *P. elegans*, *Lumbricillus sp.* and *P. litoralis* and a low abundance of *T. benedii* and *T. swirenocoides*. The difference between the February sampling periods and all the other periods except November 1998 was there was a decreased abundance of *T. benedii*, *M. balthica*, *P. elegans*, *M. aestuarina* and *H. ulvae*.

6.2.2.3 Community change in relation to environmental factors

The temporal BIO-ENV (Table 6.4) analysed which variables from the different pollution sources as well as the sediment properties and climate may be causing the changes in the community over time. The results for the lower shore area indicate that none of the sources had a highly significant correlation. The highest was the climate data with the minimum temperature and the Kinneil water quality, with temperature, pH and ammonia combined, both having a correlation of 60%. The upper shore area produced two highly significant results. Firstly with the Avon water quality data, where combined BOD, Nitrate and Mercury had a correlation of

89%. Secondly with the Chemical effluent data, where combined pH, EC, Copper, Nickel and Phenol had a correlation of 86%..

The temporal stepwise regressions (Table 6.5) show that for the upper shore area the number of species produced highly significant models with the sediment and Avon variables. The chemicals effluent data produced significant models for evenness and the number of individuals although the number of individuals can also be modelled from the sewage work data and the Avon data. The lower shore area showed slightly different results. The Avon data produced significant models for diversity, evenness, species richness and the number of individuals. The Sewage data can predict the number of individuals and the Kinneil data the number of species per station. The equations for these models (Table 6.6 and 6.7) indicate that there was no obvious single variable that appears in the different models, although the dissolved oxygen (DO) and heavy metals are often identified.

6.2.3 Temporal changes in biomass

6.2.3.1 Changes in the community composition

6.2.3.1.1 Total biomass

The upper shore and lower shore areas have very different patterns of change over time of the biomass (Figure 6.25). The upper shore area had its highest biomass in November 1998 after which the biomass decreased. There was a further increase in July 1999 after which again there was a decrease, and then it remained fairly constant. The lower shore area biomass has remained fairly constant but has shown two peaks in February 2000 and July 2000. It should also be noted that the biomass for the lower shore stations is generally higher than that of the upper shore stations.

6.2.3.1.2 Individual species abundance

The change in the biomass of the individual species (Figures 6.26 to 6.30) indicates that for the lower shore area the species *Macoma balthica, Hydrobia ulvae, Pygospio elegans* and *Streblospio shrubsolii* all showed seasonal fluctuations with peaks in July or July/November. The *Oligochaetes* also seem to show a slight

seasonal cycle with peaks in July but the biomass also seemed to have decreased from the year 1999 to 2000. The biomass of *Cerastoderma edule* seems to remain fairly constant throughout the year except during November when its biomass tends to be lower. The biomass of *Eteone longa* has also shown changes in that it was relatively high in November 1998 after which it dropped. It did however show a steady increase from May 2000 till the end of sampling. Some species showed peak periods when biomass was exceptionally high. *Nephtys hombergii* showed a peak in July 2000, *Nereis diversicolor* in February 1999 and *Mytilus edulis* in February 2000 and July 2000. The upper shore area has fewer species but again *H. ulvae* and also *N. diversicolor* show a seasonal cycle with peaks in July, whilst *Corophium volutator* showed one large peak in November 1999. The species *M. aestuarina*, *P. elegans* and *S. shrubsolii* all showed a similar pattern of change over time. They all showed a slight decrease over time with small increases in biomass during the spring. *M. aestuarina* and *S. shrubsolii* however also showed an increase again between July and November 2000. The Oligochaetes showed a similar pattern with a general overall decrease but with a seasonal cycle that peaked during November.

6.2.3.1.3 ABC plots and the W statistic

The ABC plots for the shore area (Figures 6.31 to 6.33) show how the abundance and biomass has changed over the two years with respect to each other. The upper shore area ABC plots all have the abundance curve above the biomass curve. The two curves however do become close and cross over for July 1999 and July 2000. The lower shore ABC plots (Figures 6.34 to 6.36) again have the abundance curve above the biomass curve except in February, May and July 2000 when the biomass is above the abundance. By plotting the W statistic over time for both areas (Figure 6.37) it can be seen that the two areas show different patterns. The upper shore area seems to show a seasonal cycle with the W statistic (See 2.3.3.4) at its greatest in July. The lower shore area does not show a seasonal cycle, it does however show an overall increase from February 1999 till July 2000. Between November 1999 and February 2000 the W statistic goes from negative to positive. Although there has been an increase till July 2000 in November 2000 the W statistic drops and becomes negative again.

6.2.3.1.4 Cluster analysis and MDS

The cluster analysis and MDS plots of the change in biomass over the two year sampling period (Figures 6.38 and 6.39) indicate that there are differences in the biomass between the different sampling times. The results for the upper shore area show that all the sampling periods are similar except for November 1998, July 1999 and July 2000. These three times have a very different community biomass distribution than all the other times. The results for the lower shore area indicate that May 2000, November 1999 and 2000 have a similar community biomass composition. February 1999, November 1998, July1999 and May 1999 are also very similar, whereas February 2000 and July 2000 are very different from all other sampling times.

6.2.3.1.5 SIMPER

The SIMPER analysis has been used to determine what species differences are responsible for the difference in the sampling times that were found by the Cluster and MDS analysis. The upper shore analysis (Table 6.8 and 6.9) shows that November 1998 had a higher biomass of the opportunistic species, *Oligochaetes, M. aestuarina* and *P. elegans*. July 1999 had a higher biomass of *N. diversicolor* and *H. ulvae*. July 2000 also had a high biomass of *N. diversicolor* but less than that of July 1999 and it also has a lower biomass of *Oligochaetes* and *M aestuarina*.

The lower shore analysis (Table 6.10 and 6.11) shows that November 1998 to July 1999 had no *M. edulis* but a high biomass of *M. balthica* and *C. edule*. November 1999, 2000 and May 2000 also had a low biomass of *M. edulis* and also of *N. hombergii*. July 2000 however had a high biomass of *M. edulis* and of *N. hombergii* and *M. balthica*. February 2000 however can be distinguished from all other times by their extremely high biomass of *M. edulis*.

6.2.3.2 Environmental effects

The changes in the water quality from different sources, the effluents and the climatic conditions have been compared to the change in the biomass. The BIO-

ENV analysis (Table 6.12) found that for the lower shore area, the sewage and the chemical effluent both produced high correlations. In both of these the variable, suspended solids, was chosen along with the EC for the chemical effluent and two heavy metals (Nickel and Chromium) for the sewage data. The upper shore analysis found that the Avon had the highest correlation with the variables DO, BOD, phosphate, nickel and mercury. The chemical effluent also produced a fairly high correlation with the variables pH, EC, copper and phenol.

The stepwise regression analysis (Table 6.13) showed different results. The lower shore area did not produce any significant results with high Rsq(adj) values. The highest was for the water quality at Kinneil, which included the variables pH and turbidity. The upper shore area produced two highly significant models with the Temperature/NAO and the chemical effluent. The temperature/NAO model implicates minimum temperature and rainfall whilst the chemical effluent model selected the heavy metal copper.

6.3 DISCUSSION

6.3.1 The impact of moving the chemical outfall

The first movement of the chemical outfall in 1979 caused the areas around the new and the old outfall sites to show a change in their community composition. McLusky (1982a) noted that the area around the new outfall showed a decrease in the number of species, diversity and biomass, whilst the area around the old outfall showed an increase. It was noted however that the area around the new outfall was already impacted to some extent before the discharge of the chemical effluent to that part of the shore began. From this is would therefore be expected that the second movement of the outfall site in January 1999 would also cause changes in the community composition around the new and old outfall sites. This time however the new outfall has been placed further down shore in an attempt to reduce its impact, and it only opens when covered by the tide. It was therefore hypothesised that no impact should occur to the benthic community around this new site. The survey of the lower shore area does confirm this as no reduction in diversity, species richness

or biomass after January 1999 was detected. It was also hypothesised that the area around the old outfall would show a recovery, as an increase in diversity, species richness and /or biomass. The survey of the upper shore area however did not show any indication of these changes. The multivariate analysis did indicate that November 1998 was different from all the subsequent sampling periods. This difference was identified to be related to a larger abundance and therefore biomass of the species *Lumbricillus sp.*, *Manayunkia aestuarina*, *Tubificoides benedii*, *T. swirenocoides* and *Pygospio elegans*. It was also noted in Chapter 5 that in November 1998 station A1 had an abnormally high abundance of *Lumbricillus sp.*. The decrease in the abundance of these opportunistic species in February 1999 could potentially have been caused by the closure of the chemical outfall. A decrease in the number of opportunistic species can represent a lessening or removal of an impact (Pearson & Rosenberg, 1978). There is however another explanation for this increase in the opportunistic species in November 1998. It may have been that this was just a good year for these species in that either recruitment was high or predation was low allowing high population abundances for these species. It would also be expected that if the increased abundance in November 1998 was due to natural seasonal events then the abundance would be lower in July 1998. If however it was due to the movement of the outfall then the abundance before November should have been consistently higher. From the data collected for the long-term analysis the mean number of individuals for the shore stations in July 1998 was calculated at 95 individuals $50cm^{-2}$. This figure is within the range found after the movement of the chemical outfall, therefore suggesting that the peak of individuals in November 1998 was not caused by the movement of the outfall. The fact that each November period shows an increase in abundance although not to the same extent as in November 1998 and that it is not only the opportunistic species, although they show the largest increase, all supports this second hypothesis. It can therefore be concluded that neither area has shown any detectable change in the benthic community composition that can be attributed to the movement of the chemical outfall in January 1999.

6.3.2 Seasonal changes in the benthic community

Any population and therefore community will naturally undergo variations in abundance and biomass within and between years. A major source of variation are seasonal recruitment cycles (Holland, 1985). Many benthic invertebrates have specific recruitment periods within a year, the success of this recruitment and the extent of the subsequent mortality regulates their population sizes. Lillebo *et al.* (1996) noted that the density of *Hydrobia ulvae* peaked during settlement, whilst Udalov *et al.* (1996) found that the density of *H. ulvae* decreased after settlement due to the high mortality rates. Different species of benthic invertebrates are known to have different recruitment periods that can vary from site to site. *Nephtys hombergii* is known to undergo recruitment during the winter months (Davey & Geroge 1986), whilst other species such as *Manayunkia aestuarina* (Bagheri & McLusky, 1982) and *Macoma balthica* (Wolff, 1980) have a spring recruitment. The spawning and recruitment periods are often determined by physical conditions such as temperature (Orton, 1920). The recruitment of the spionid *Pygospio elegans* has been shown to be determined by temperature and salinity (Bolan & Fernandes, *Pers comms.*). It is therefore not surprising that both the upper and lower shore areas show seasonal fluctuations in abundance and biomass. The two areas have different cycles and the upper shore areas seasonal cycle is less defined that that of the lower shore area. This may be due to the wider variety of the recruitment periods of the species in the upper shore area, or more likely it is due to fact that recruitment is not successful at the impacted stations due to contaminant levels (Dicks & Levell, 1989). The seasonal cycle of the diversity, species richness and evenness for the upper shore area showed an increase in May or November. It has already been shown (Chapter 5 and 6.1.3) that the recruitment period for *Lumbricillus sp.* is in November. This is a dominant species in the upper shore area and will therefore greatly influence the seasonal patterns. The lower shore areas seasonal cycle on the other hand peaks during July in both years and there is a decline in the abundance of most species during February. Many species in both areas show peak times when their abundance are relatively high, these are likely to be exceptionally good recruitment and survival periods for these species. It can therefore be concluded that most of the changes that have been detected throughout the two year survey can be

explained by the natural seasonal recruitment and subsequent mortality cycle, the intensity of which can vary from year to year, and from place to place.

6.3.3 Influence of other factors on the temporal community change

6.3.3.1 Pollution sources

As discussed in chapters 3 and 4 there are several pollution sources at Kinneil as well as the petrochemical effluents that may influence the benthic community composition. The temporal change of the water quality of the main Kinneil channel, the sewage works, the River Avon and the refinery and chemical effluents has been assessed and related to the change in the benthic community. Most of the water quality variables that were measured for these pollution sources showed no clear trends, only fluctuations. The BIO-ENV and regression analysis did produce significant models for the pollution sources for the temporal change in diversity, evenness, species richness, abundance or biomass of the upper and lower shore areas except for the refinery effluent. This is likely to be a coincidence in that the fluctuations in these water quality variables coincide with the seasonal fluctuations of the benthic community. It is therefore unlikely that the changes in the effluent or the other pollution sources have caused the temporal changes in the community composition.

6.3.3.2 Sediment

It was found that the sediment might play a small role along with other factors in determining the spatial distribution of the benthic community at Kinneil (Chapter 5). It is therefore possible that a change in the sediment properties could cause a change in the benthic community composition. There was however no detectable temporal change in the sediment in either the upper shore or the lower shore areas. It therefore seems that the new lower shore outfall did not cause any great change to the hydrodynamics and therefore the settlement of the sediment in the area around the outfall. The regression analysis did however still produce significant models for the temporal change in diversity and species richness, but this again is likely to be coincidental as the seasonal fluctuations can be explained by the natural recruitment

patterns. The sediment has not therefore played a role in determining the temporal changes of the benthic community during the survey period.

6.3.3.3 Climate

Temperature regulates the reproduction of many benthic invertebrates (Orton, 1920) and therefore has the potential to alter the community composition if it changes. Neither the air temperature nor the NAO show any trend other than seasonal fluctuations, which again coincides with the seasonal fluctuations of the biomass of the upper shore area. It is possible that the air temperature and rainfall will effect the growth and survivorship of the organisms in the shore area as they are exposed to the air for greater periods. The regression model however suggests that the biomass increases as the temperature and rainfall decreases which is the opposite of what might be expected to occur. It is therefore concluded that this relationship is coincidental and that climate has had little effect over the two-year period in determining the community composition of either the upper shore or lower shore areas.

6.3.3.4 Sampling method

The method that was used to sample the macrobenthos was designed to accurately measure the majority of the benthic invertebrates at Kinneil, which are generally fairly small. There are however two species which are notably larger than most for which the size of the core used may not be large enough to accurately estimate their population size. Clarke & Warwick (1994) note that large species are liable to a higher sampling error. These species are namely *Mytilus edulis* and *Cerastoderma edule*, which are only found in the lower shore area. The fact that *M. edulis* was only found at the edge of the lower shore sampling area also meant that its sampling was less accurate. The lower shore sampling was undertaken by a grab from a boat, which was subject to tidal drift. This meant that the lower shore sampling of individual stations was less exact and therefore the small mussel bed was not always sampled. For the abundance data this did not seem to be a problem as these two species generally had a low abundance and therefore did not have a great influence on the benthic community. For the biomass data however these species proved to

have a great influence. These two species were found to be the main species along with *Nephtys hombergii* that determined the temporal and spatial (Chapter 5) differences in the biomass of the lower shore area. It can therefore be concluded that the sampling method used in this study caused large variations in the biomass data as it was not adequate for sampling the larger species that dominate the biomass of the community.

6.4 CONCLUSIONS

The upper and lower shore areas have both shown changes in their community composition over the two-year survey period. The change that was detected in both areas was a seasonal cycle that can be attributed to the natural recruitment and mortality cycle. The two areas did show slightly different seasonal cycles. The cycle for the upper shore area was less pronounced, which may be related to the increased level of impact to this area. The upper shore area showed an increase in diversity, evenness and species richness during May or November, whilst the lower shore area showed an increase during July. The biomass data showed a less clear picture due to the large sampling error of the larger bodied species, which greatly influenced the biomass measures. Although significant models were created for the change in community using the water quality variables of the effluent and the other pollution sources, it was concluded that these were coincidental, in that they showed the same seasonal pattern that can be adequately explained by natural events. There was also no conclusive evidence to suggest that the climate was responsible for the seasonal fluctuations. The sediment characteristics of the two areas showed no change over the two-year study period, therefore indicating that the movement of the outfall did not change the hydrodynamics of the two areas and therefore the sediment deposition.

It is therefore concluded that there is no evidence to suggest that the movement of the chemicals outfall to its lower shore site had any effect on the abundance or biomass at either upper or lower shore areas. Seasonal trends can be detected, which can be explained by recruitment and post settlement mortality. Sampling variability

and patchiness of the larger bodied species was important in explaining the biomass in the lower shore area.

The lack of effect to the lower shore area indicates that the movement of the chemical outfall down shore has met its design target not to cause any impact to the area around the new site. It was however expected that the movement would allow the upper shore area to recover from the impact of the effluent. This however was not the case but there are four possible hypotheses that can explain why a recovery was not seen: -

- The upper shore area is still impacted from the refinery effluent and therefore can not recover until this impact is also reduced or removed.
- The upper shore area is still impacted from historical inputs that remain within the sediments and therefore the area needs more time to be able to recover.
- The upper shore area is impacted by the environmental conditions, such as shore height, which inhibits any further recovery of the area.
- The chemical effluent was having little effect on the upper shore area before the outfall was moved and therefore the removal of the effluent did not cause any change in the benthic community.

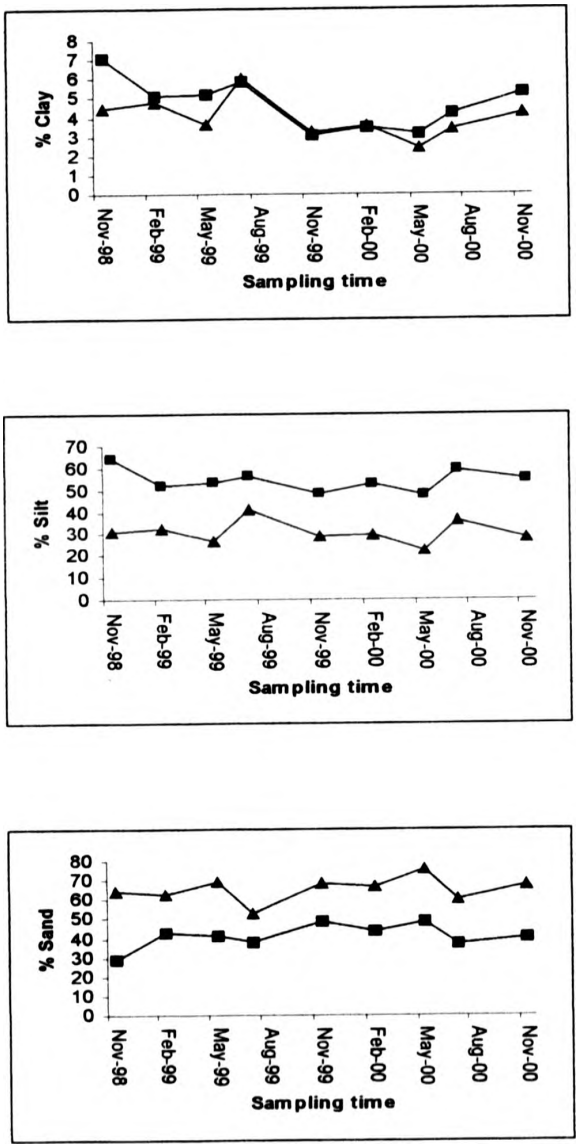
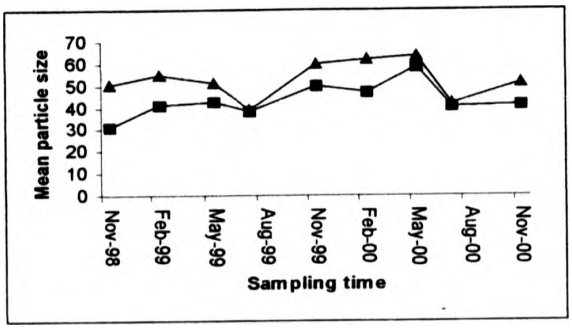


Figure 6.1. Change in the % clay (Top), % silt (middle) and % Sand (bottom) over time for upper shore (triangle) and lower shore (square) areas.



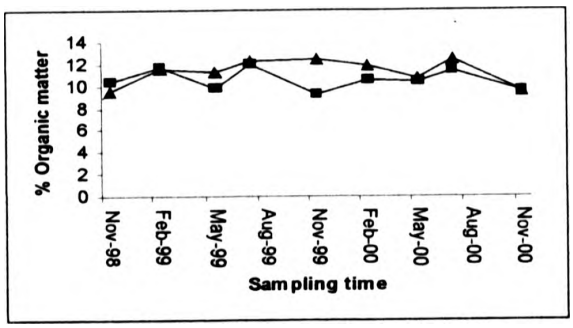


Figure 6.2. Change in the mean particle size (Top), and % Organic matter (bottom) over time for the upper shore (triangle) and lower shore (square) areas.

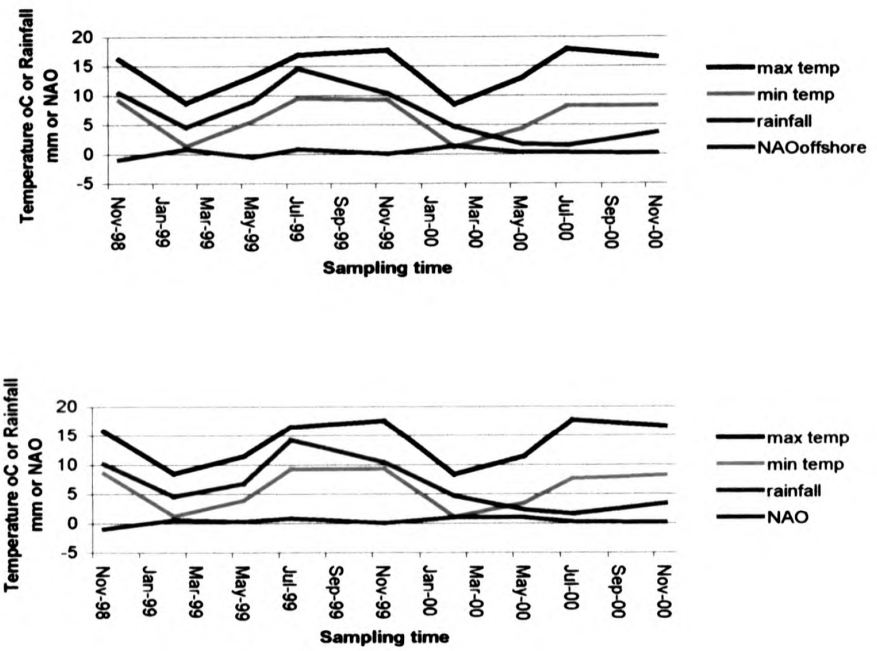


Figure 6.3. The change over time in the climate data in the lower shore area (Top) and the upper shore area (Bottom), with the maximum and minimum temperatures, Rainfall and NAO index.

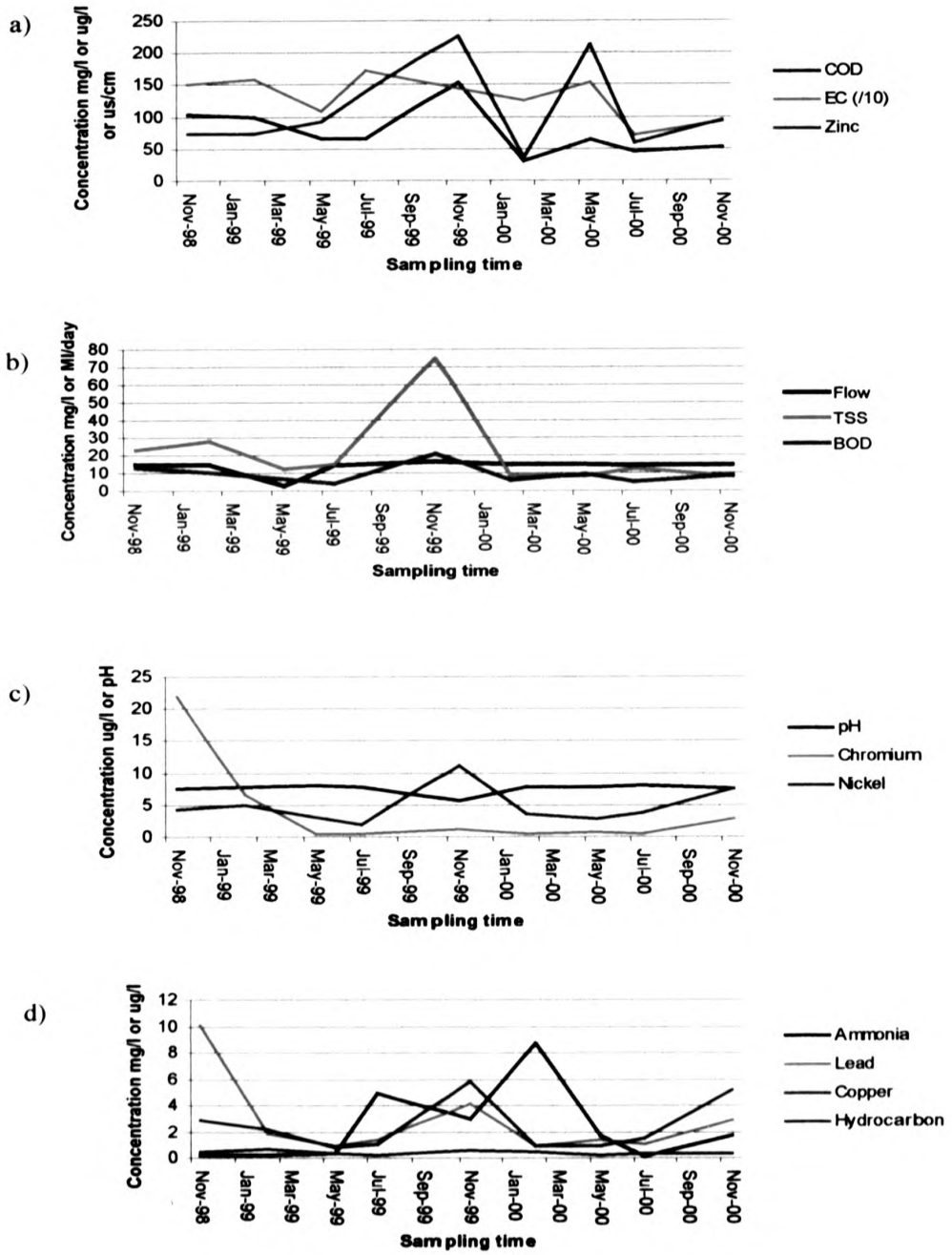


Figure 6.4. Change over time in the variables measured in the refinery effluent for the lower shore area. EC (/10) (us/cm), Zinc (ug/l), Flow (Ml/day), Chromium (ug/l), Nickel (ug/l), Lead (ug/l), Copper (ug/l). All other variables measured in mg/l.

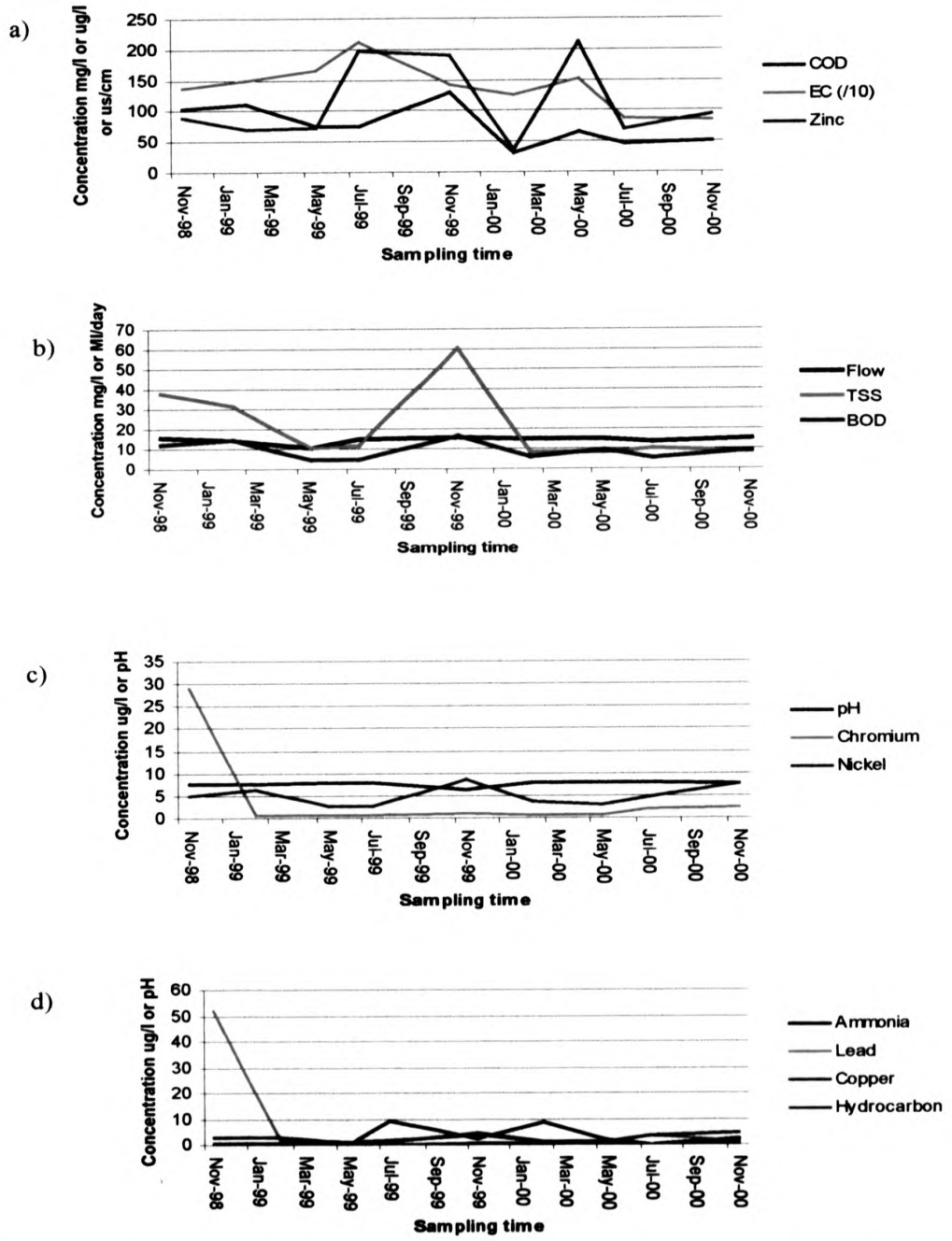


Figure 6.5. Change over time in the variables measured in the refinery effluent for the upper shore area. EC (/10) (us/cm), Zinc (ug/l), Flow (Ml/day), Chromium (ug/l), Nickel (ug/l), Lead (ug/l), Copper (ug/l). All other variables measured in mg/l.

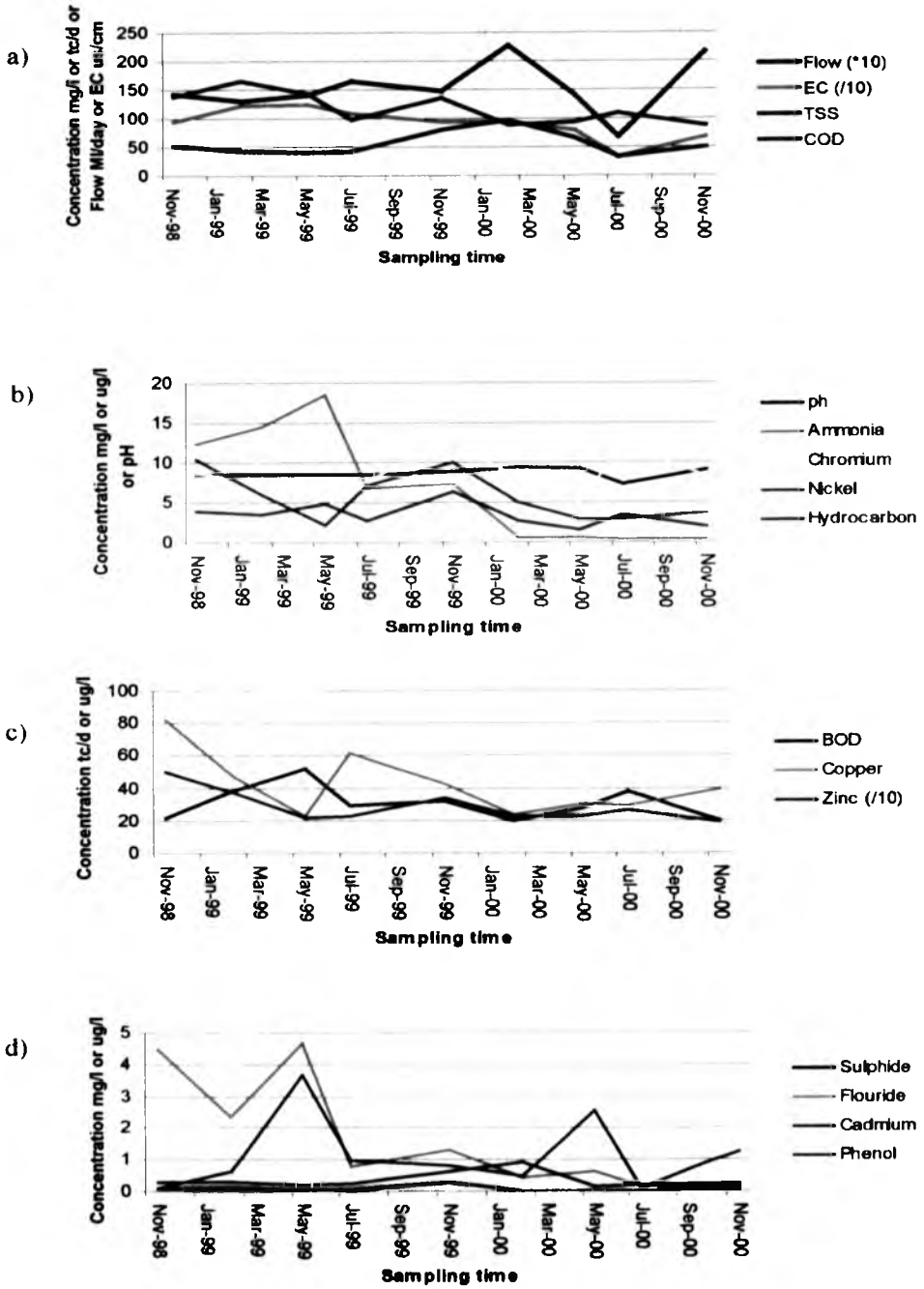
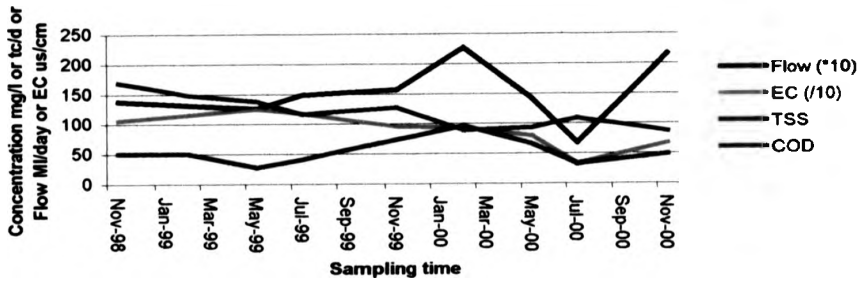
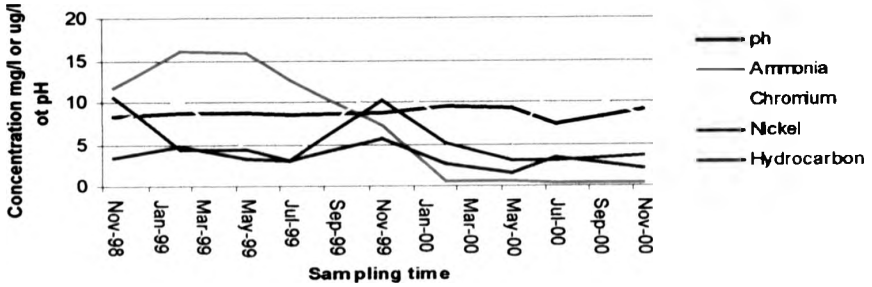


Figure 6.6. Change over time in the variables measure in the chemical effluent for the lower shore area. Flow (x10) (Ml/day), EC (/10) (us/cm), COD (tc/d), Chromium (ug/l), Nickel (ug/l), BOD (tc/d), Copper (ug/l) and Zinc (/10) (ug/l), Cadmium (ug/l) All other variables measured in mg/l.

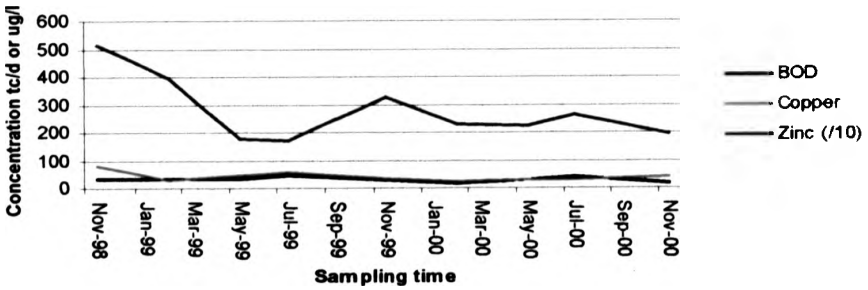
a)



b)



c)



d)

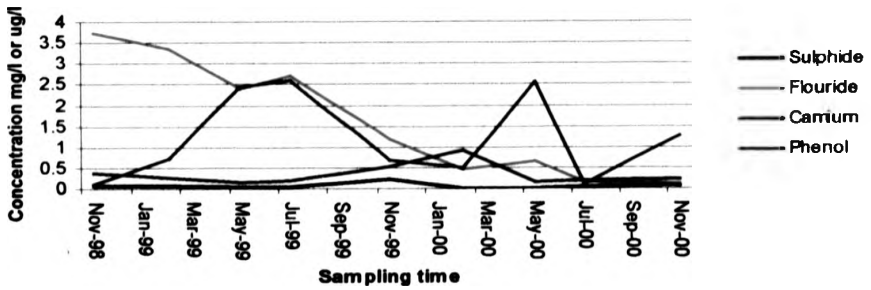


Figure 6.7. Change over time of the variables measured in the chemical effluent for the upper shore area. Flow (x10) (Ml/day), EC (/10) (us/cm), COD (tc/d), Chromium (ug/l), Nickel (ug/l), BOD (tc/d), Copper (ug/l) and Zinc (/10) (ug/l), Cadmium (ug/l) All other variables measured in mg/l.

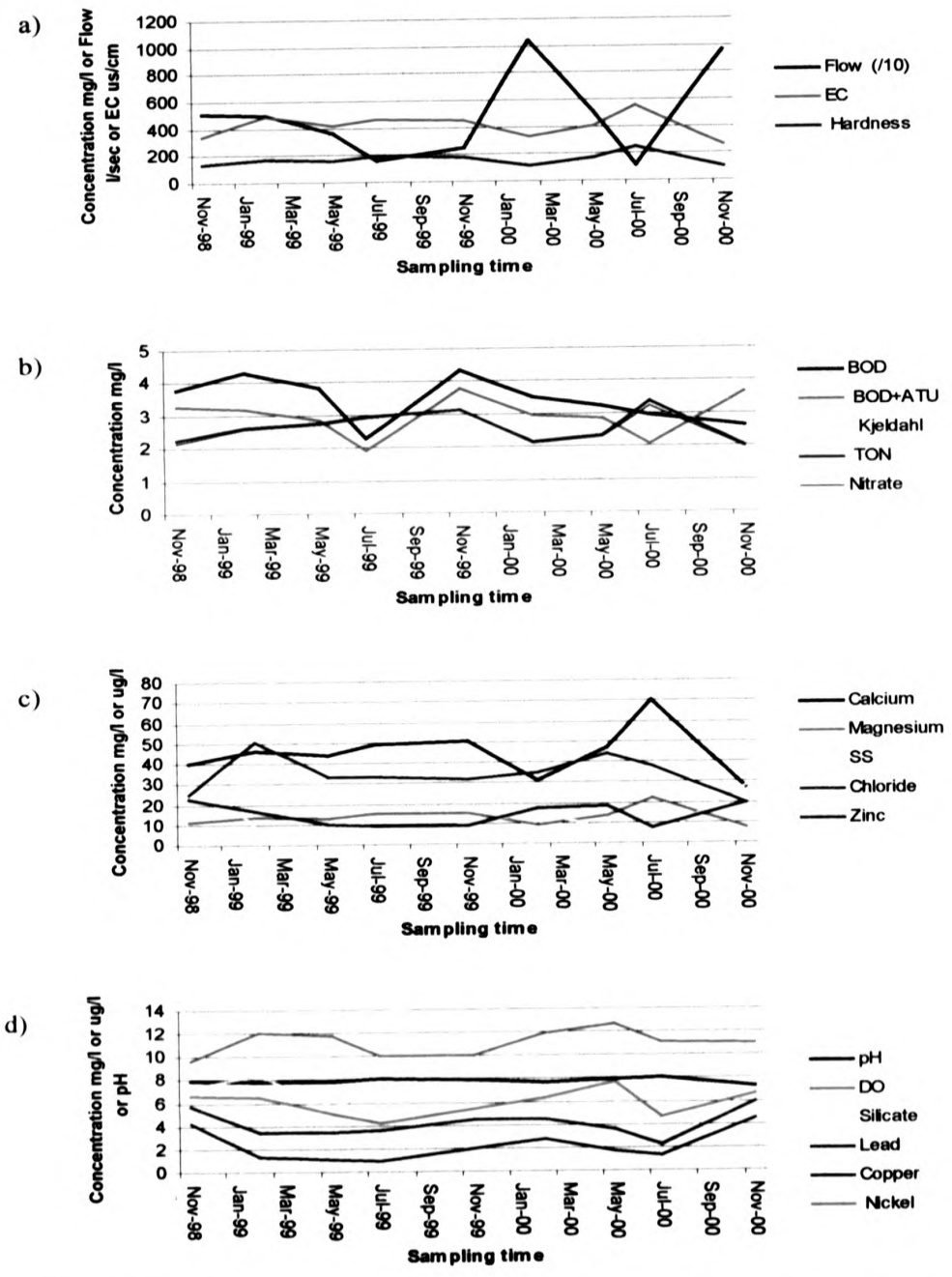


Figure 6.8. The change over time of the water quality variables measured within the Avon for the lower shore area. Flow (/10) (l/sec), EC (us/cm), Zinc (ug/l), Lead (ug/l), Copper (ug/l), Nickel (ug/l). All other variables measured in mg/l.

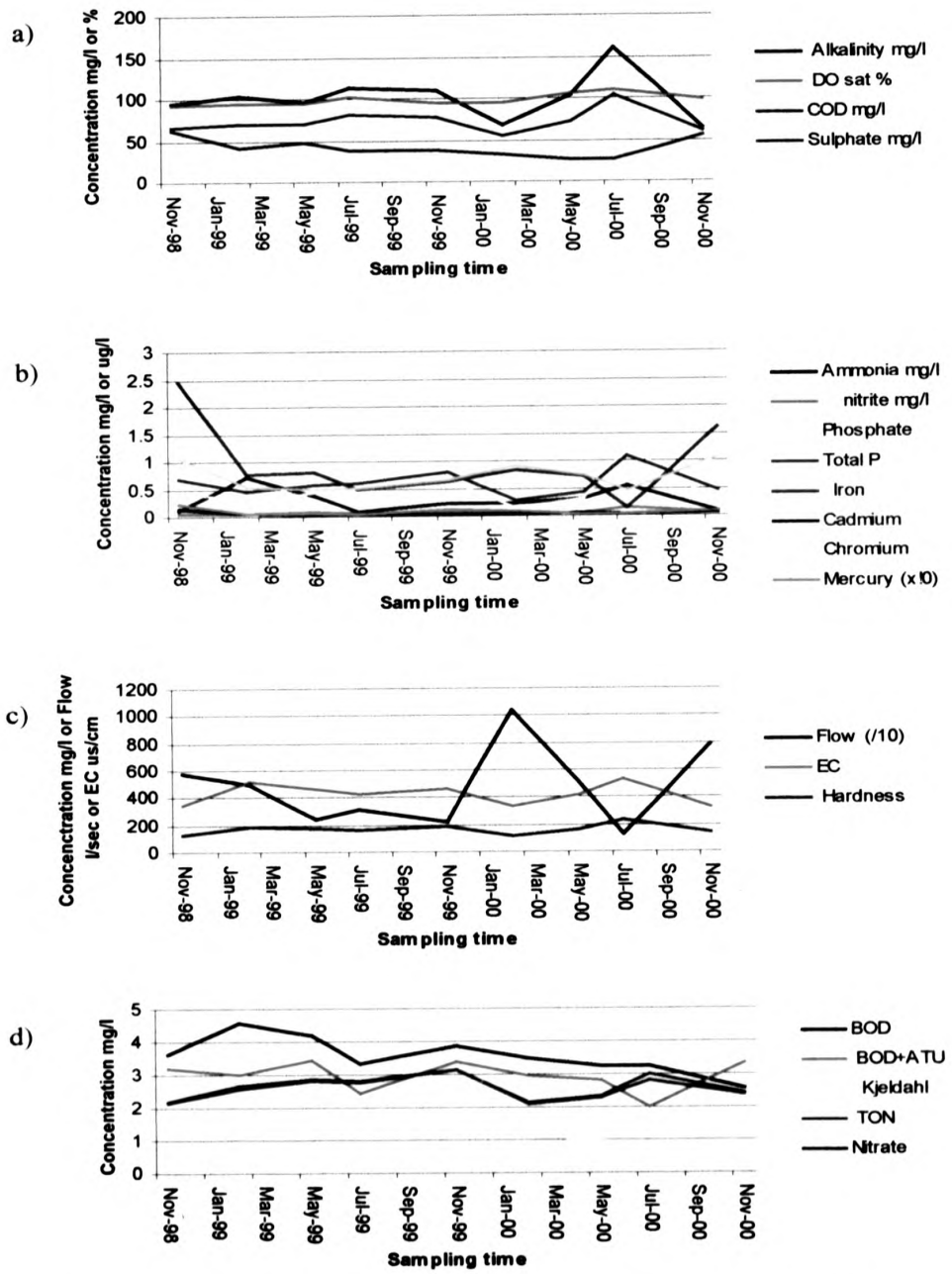


Figure 6.9. Change over time in the water quality variables for the Avon for the lower shore area (a and b) - Dosat (%), Cadmium (ug/l), Chromium (ug/l), Mercury (ug/l). For the upper shore area (c and d) - Flow (/10) (l/sec), EC (us/cm). All other variables measured in mg/l.

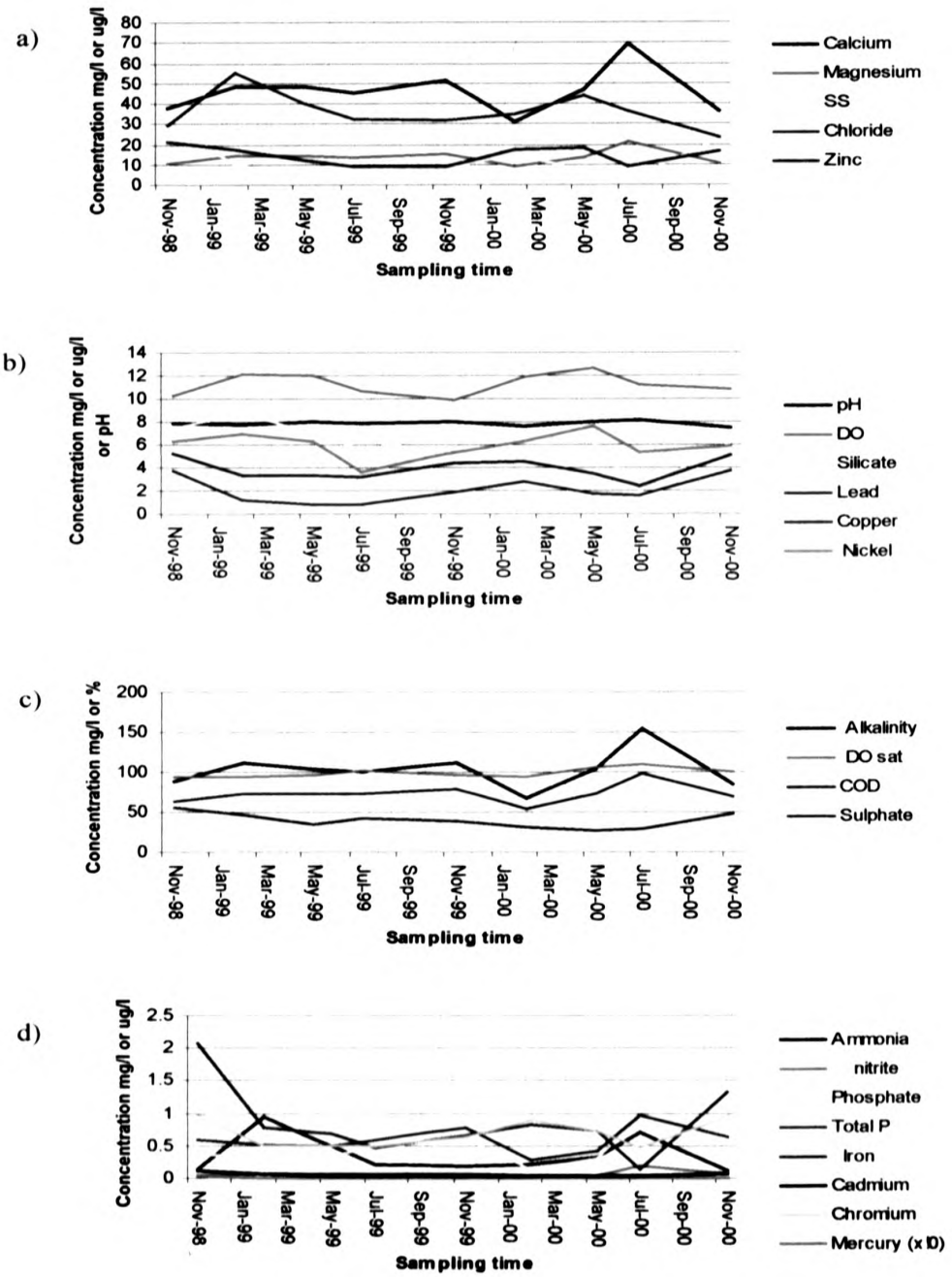


Figure 6.10. Change over time in the water quality variable for the Avon for the upper shore area. Zinc (ug/l), Lead (ug/l), Copper (ug/l) Nickel (ug/l), Dosat (%), Cadmium (ug/l), Chromium (ug/l), Mercury (ug/l). All other variables measured in mg/l.

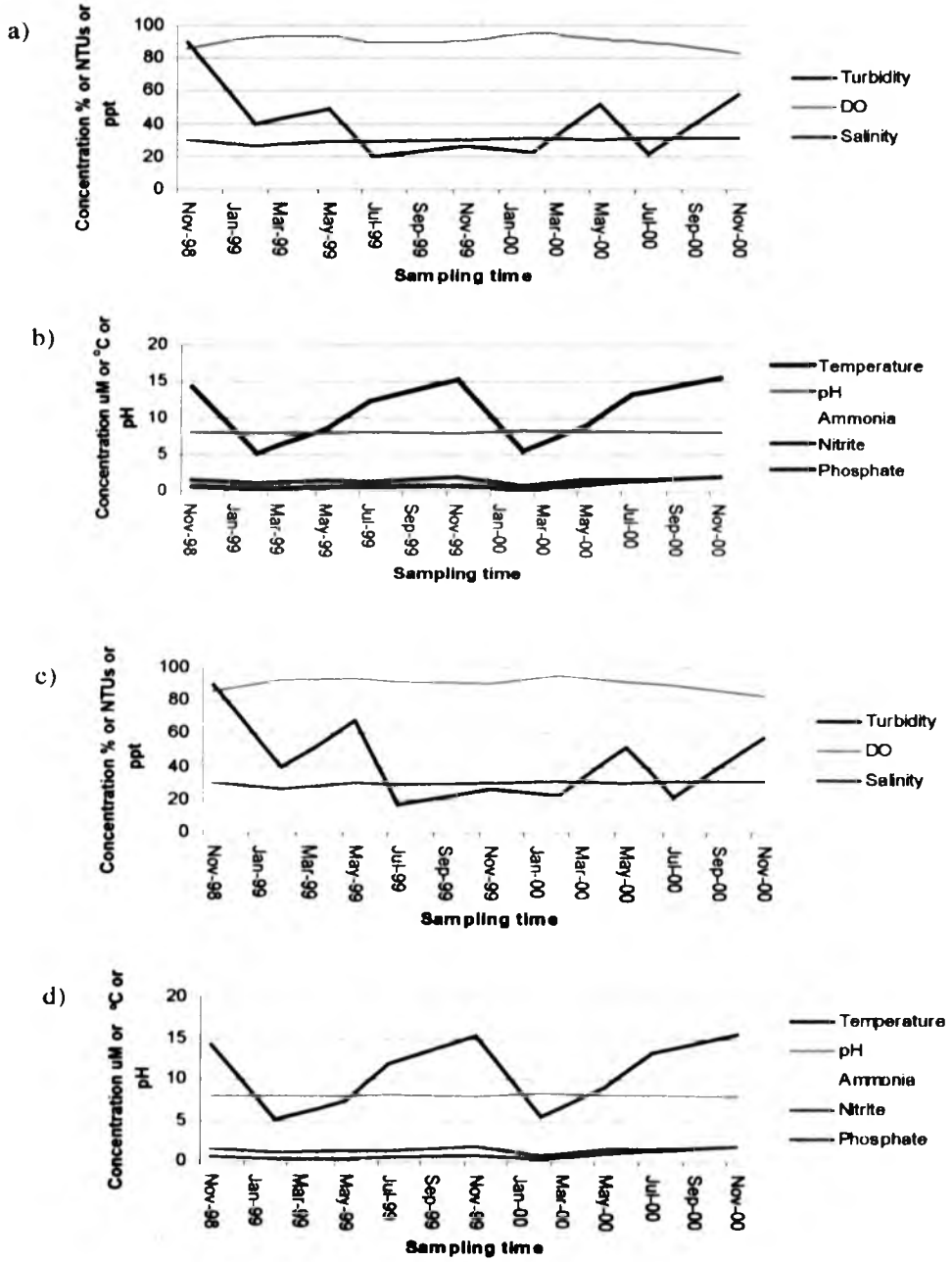


Figure 6.11. Change over time in the water quality variables measure in the Kinneil channel for the lower shore area (a and b), upper shore area (c and d). Turbidity (NTUs), DO (%), Salinity (ppt), Temperature (°C), Ammonia (μM), Nitrite (μ M), Phosphate (μM).

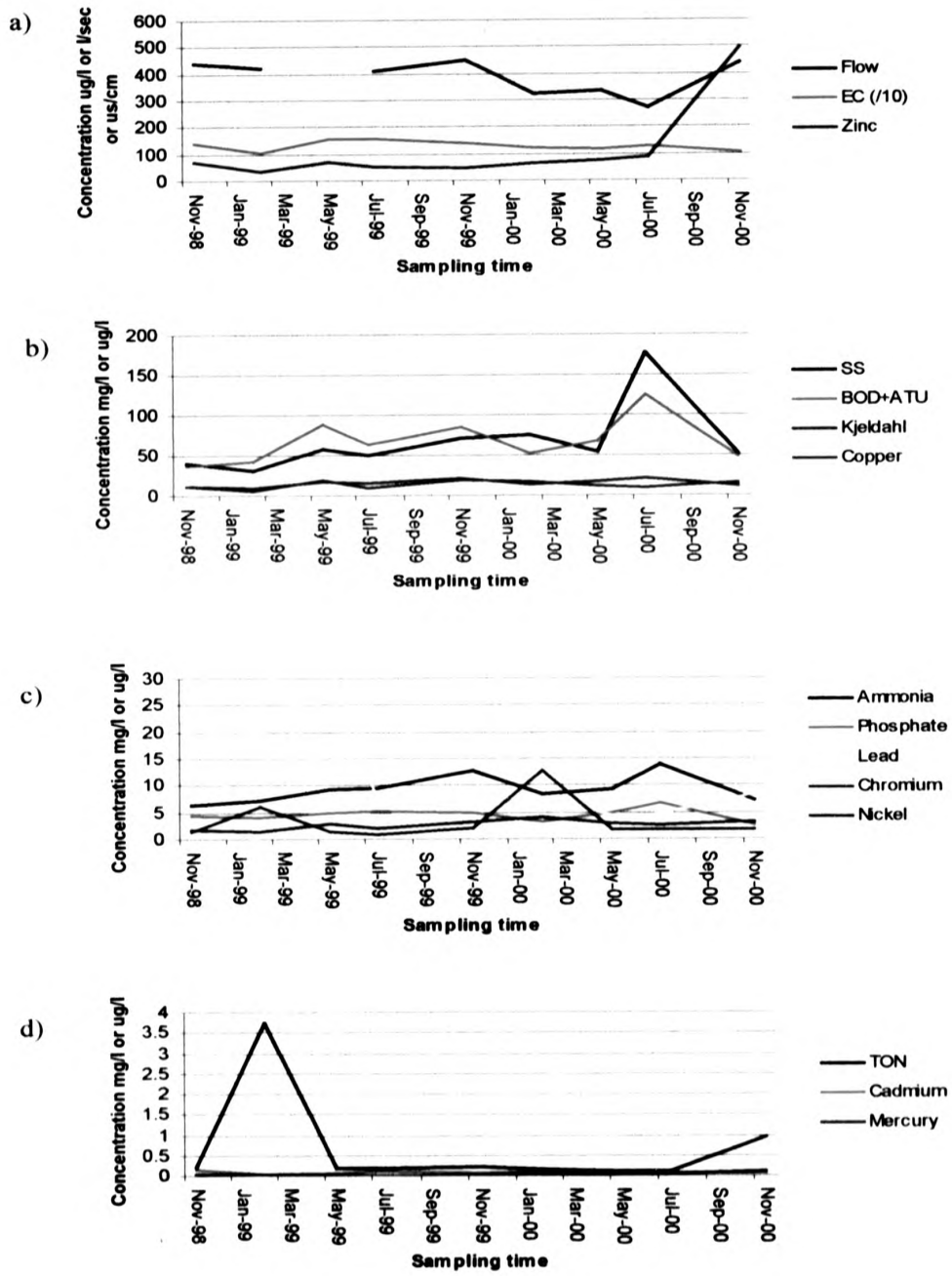


Figure 6.12. Change over time of the variables measured at the sewage works for the lower shore area. Flow (l/sec), EC (/10) (us/cm), Zinc (ug/l), Copper (ug/l), Lead (ug/l), Chromium (ug/l), Nickel (ug/l), Cadmium (ug/l), Mercury (ug/l). All other variables measured in mg/l.

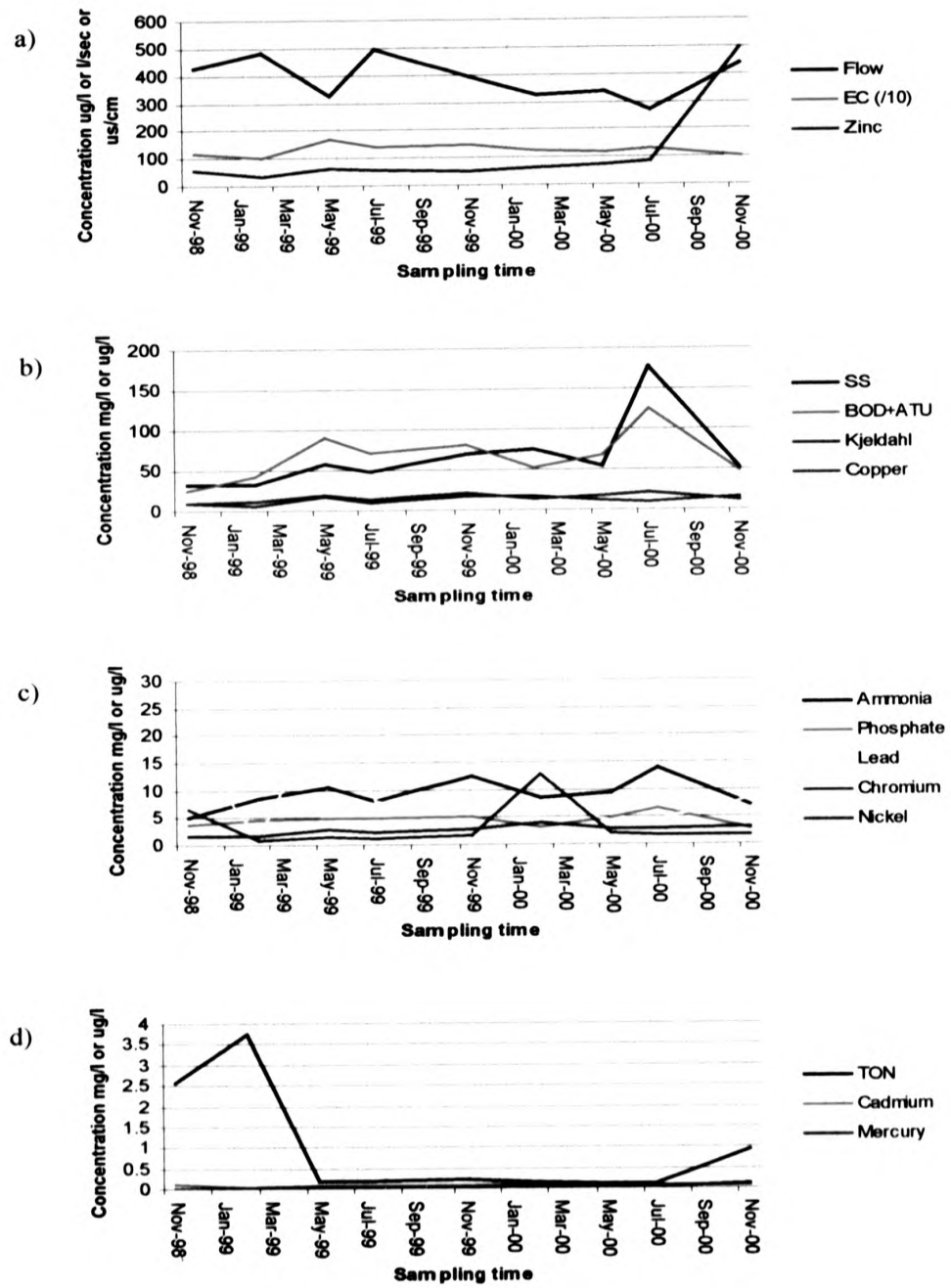
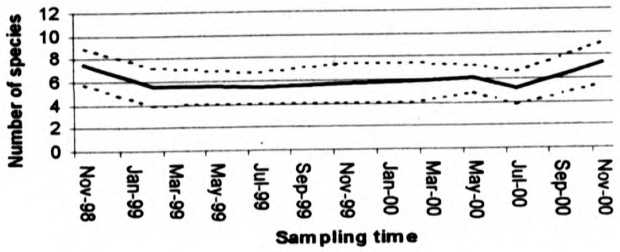
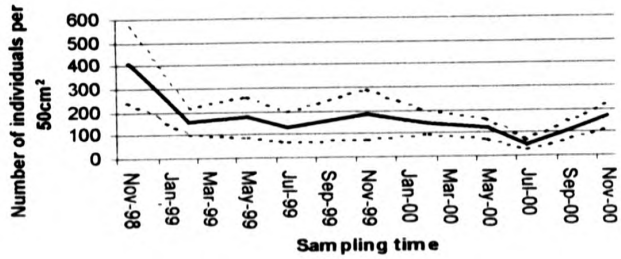


Figure 6.13. Change over time in the variables measured at the sewage works for the upper shore area. Flow (l/sec), EC (/10) (us/cm), Zinc (ug/l), Copper (ug/l), Lead (ug/l), Chromium (ug/l), Nickel (ug/l), Cadmium (ug/l), Mercury (ug/l). All other variables measured in mg/l.

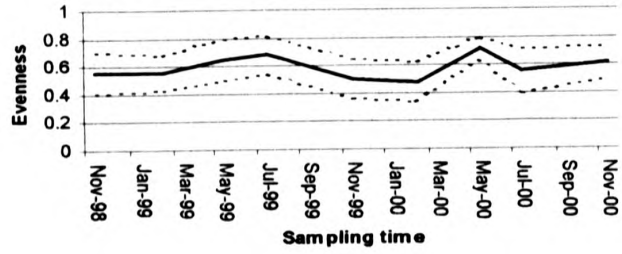
a)



b)



c)



d)

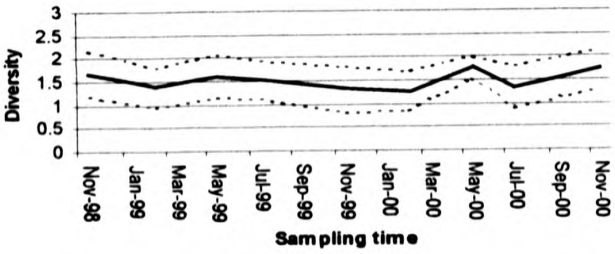
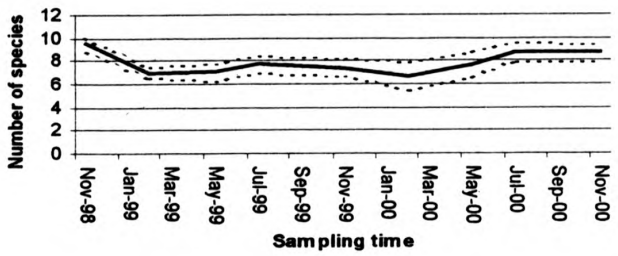
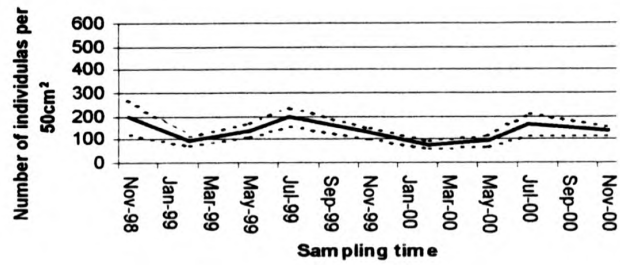


Figure 6.14. The change over time of the average number of species (a), number of individuals (b), Evenness (c) and Diversity (d) including 95% confidence limits, for the upper shore area.

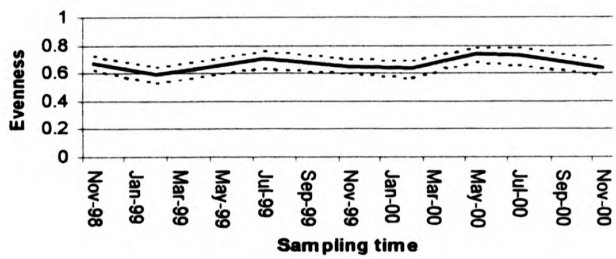
a)



b)



c)



d)

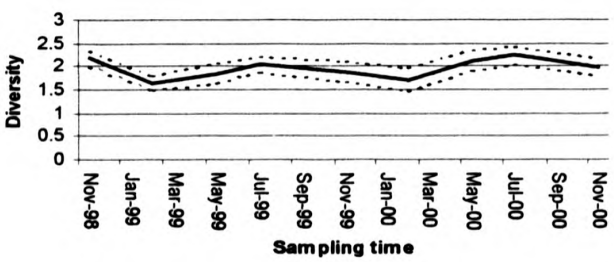
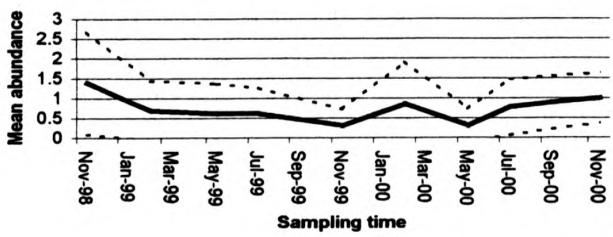
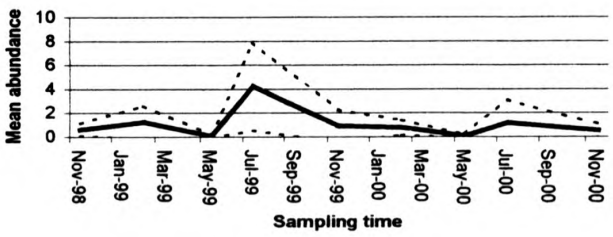


Figure 6.15.The change over time of the mean (—) number of species (a), number of individuals (b), Evenness (c) and Diversity (d) including 95% confidence limits (---), for the lower shore area.

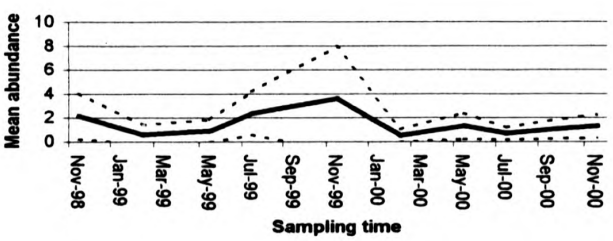
a)



b)



c)



d)

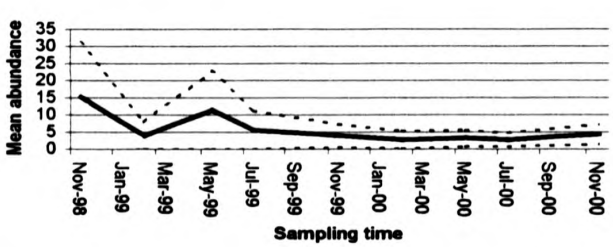
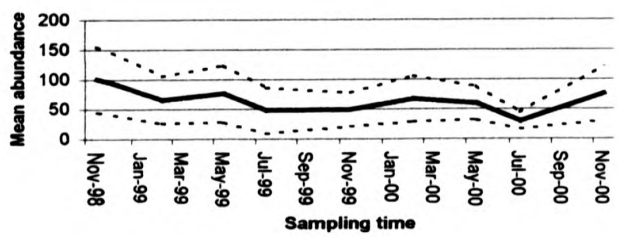
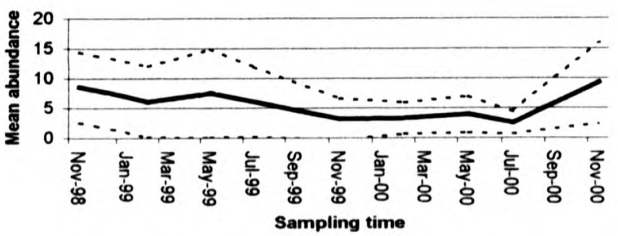


Figure 6.16. Change in the mean abundance 50cm^{-2} over time with 95% confidence limits for a) *Macoma balthica*, b) *Hydrobia ulvae*, c) *Nereis diversicolor* and d) *Pygospio elegans* for the upper shore area.

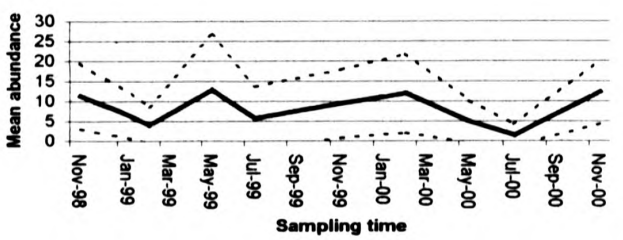
a)



b)



c)



d)

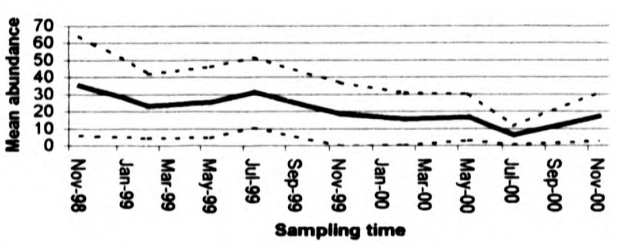


Figure 6.17. Change in the mean abundance 50cm^{-2} over time with 95% confidence limits for a) *Manayunkia aestuarina*, b) *Streblospio shrubsolii*, c) *Heterochaeta costata* and d) *Tubificoides benedii* for the upper shore area.

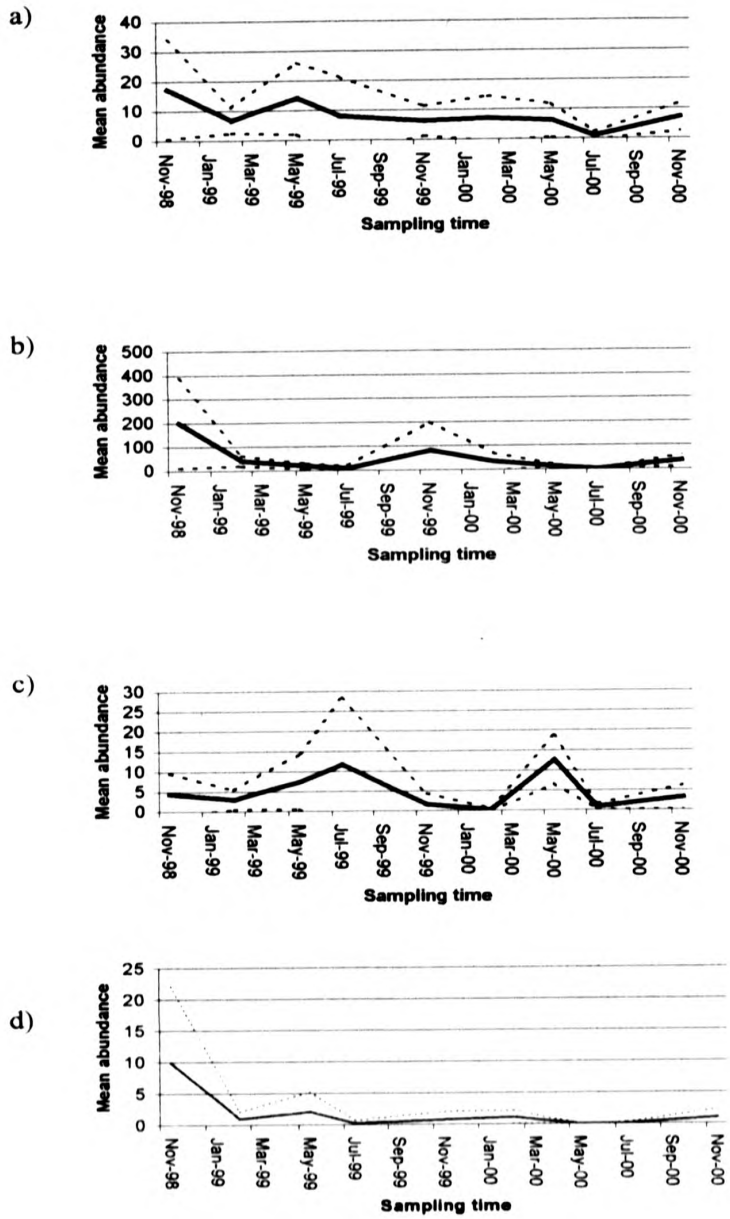


Figure 6.18. Change in the mean abundance 50cm^{-2} over time with 95% confidence limits for a) *Tubificidae sp.* b) *Lumbricillus sp.* c) *Paranais litoralis* and d) *Clitellio arenarius* for the upper shore area.

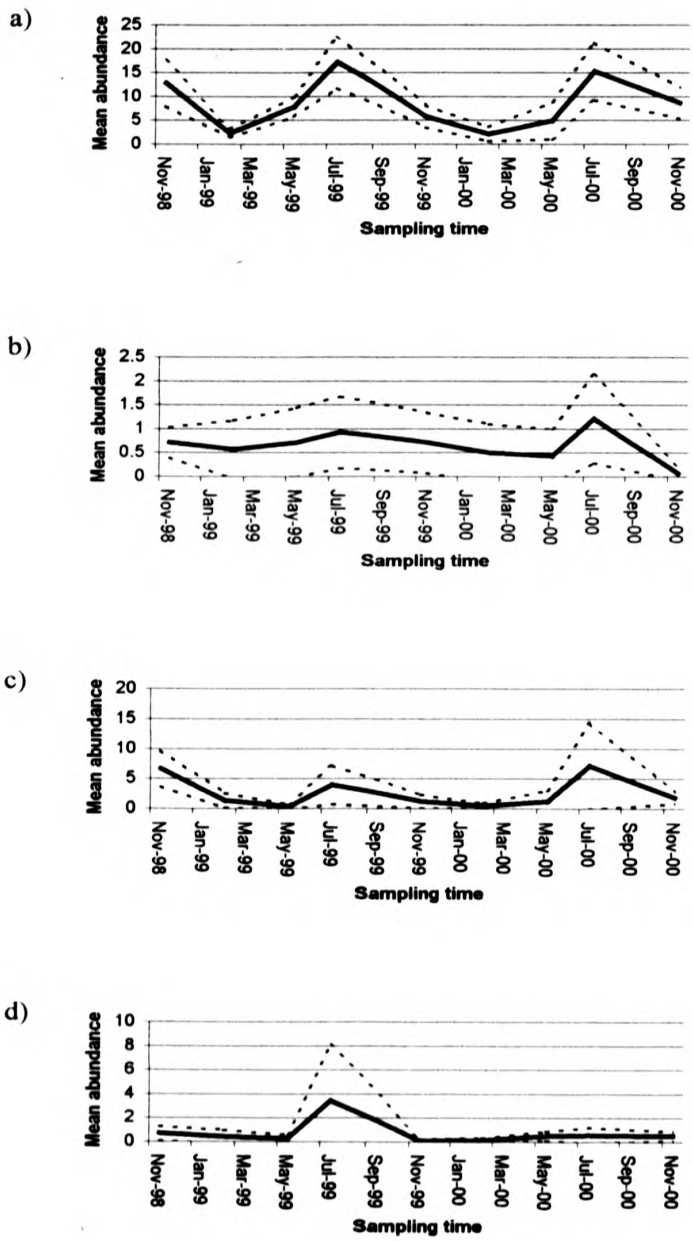
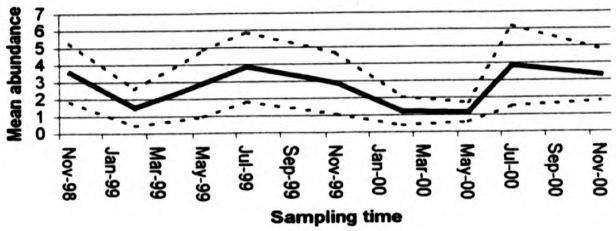
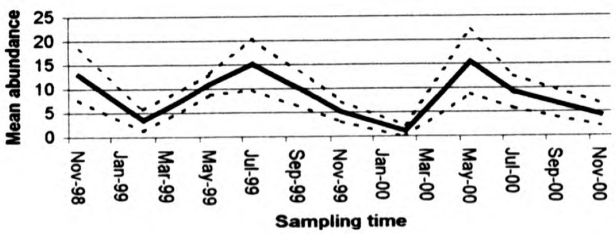


Figure 6.19. Change in the mean abundance 50cm^{-2} over time with 95% confidence limits for a) *Macoma balthica*, b) *Cerastoderma edule*, c) *Hydrobia ulvae* and d) *Nereis diversicolor* for the lower shore area.

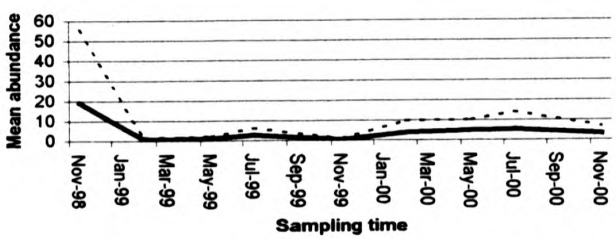
a)



b)



c)



d)

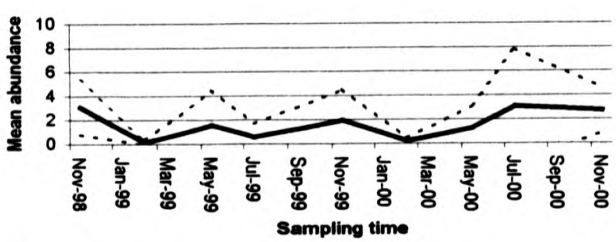
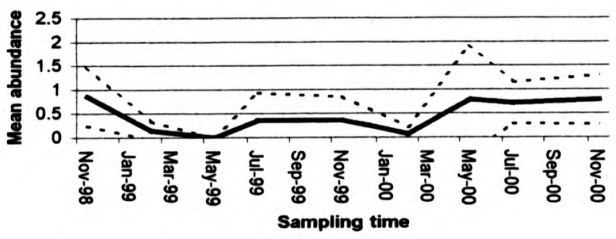
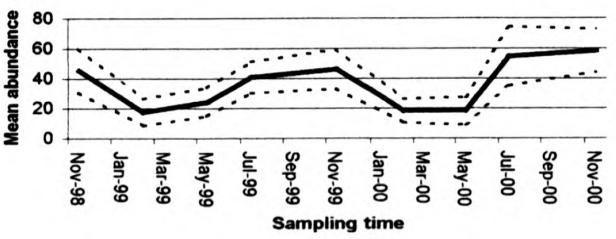


Figure 6.20. Change in the mean abundance 50cm^{-2} over time with 95% confidence limits for a) *Nephtys hombergii*, b) *Pygospio elegans*, c) *Manayunkia aestuarina* and d) *Corophium volutator* for the lower shore area.

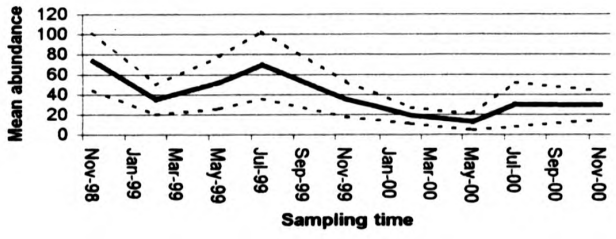
a)



b)



c)



d)

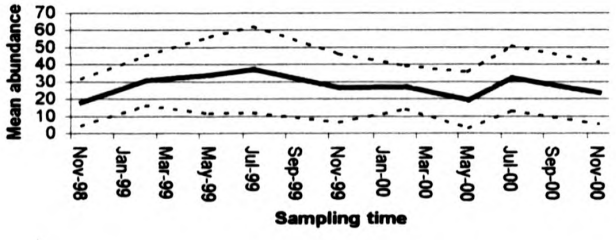


Figure 6.21. Change in the mean abundance 50cm^{-2} over time with 95% confidence limits for a) *Eteone longa*, b) *Streblospio shrubsolii*, c) *Tubificoides benedii* and d) *Tubificoides swirenocoides* for the lower shore area.

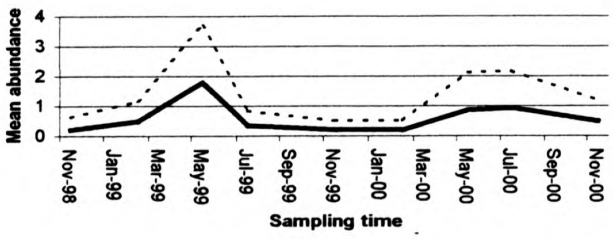


Figure 6.22 Change in the mean abundance $50cm^{-2}$ over time with 95% confidence limits for *Tubificidae spp* for the lower shore area.

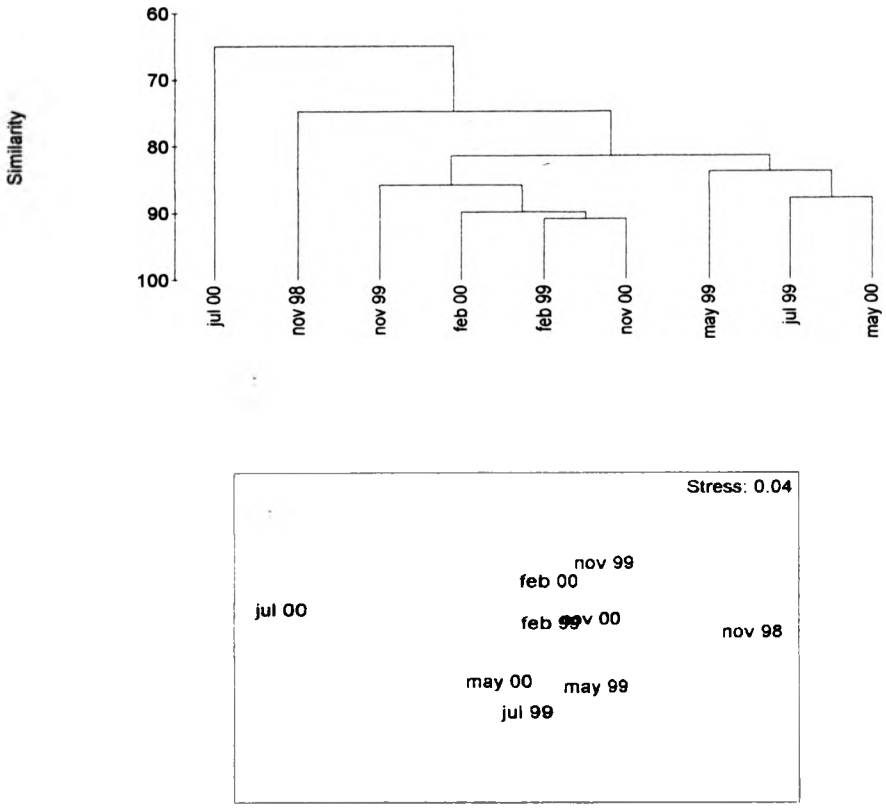


Figure 6.23. Cluster dendrogram (top) and MDS plot (bottom) showing the change over time during the survey for the upper shore stations.

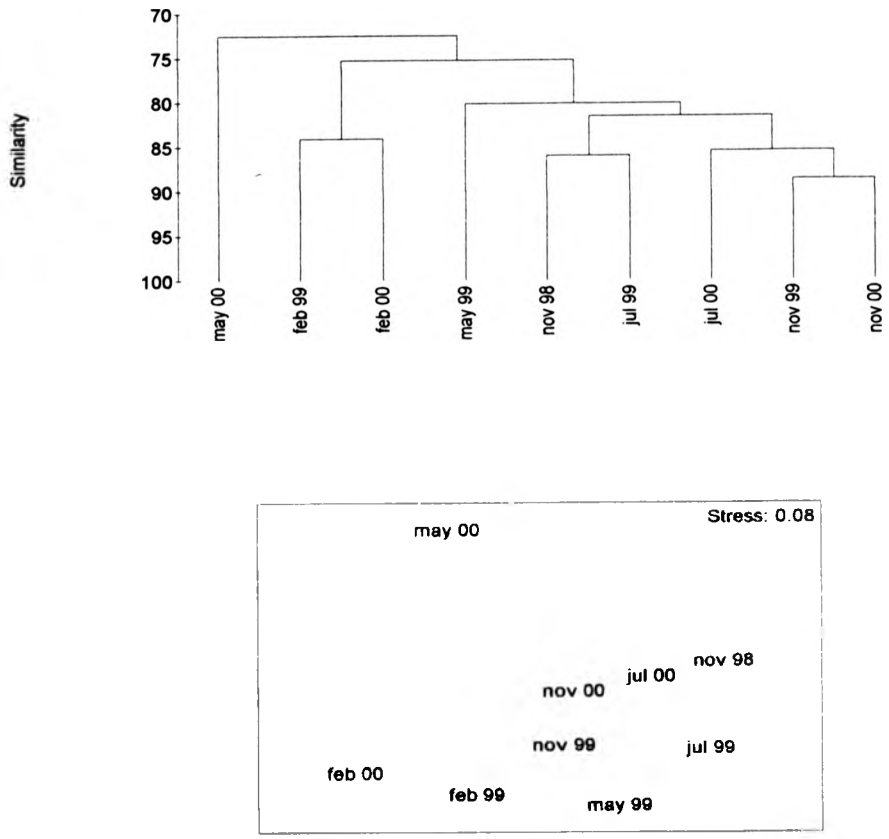


Figure 6.24. Cluster dendrogram (top) and MDS plot (bottom) showing the change over time during the survey for the lower shore stations.

Table 6.1. SIMPER results showing the species differences during different times for the upper shore area. Group 1 = November 1998, Group 2 = February 1999 – May 2000 + November 2000, Group 3 = July 2000 (* = good discriminating species).

Average dissimilarity = 46.61					
Species	**Group 1 Average abundance**	**Group 2 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Lumbricillus spp	198.77	32.95	5.67*	63.73	63.73
Manayunkia aestuarina	100.46	63.19	3.14*	14.33	78.06
Tubificoides benedii	35.08	21.07	2.38*	5.36	83.42
Pygospio elegans	15.15	5.01	3.3*	3.9	87.32
Tubificidae spp	17.31	7.96	3.28*	3.6	90.92
Average dissimilarity = 78.71					
Species	**Group 1 Average abundance**	**Group 3 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Lumbricillus spp	198.77	1.31	-	55.11	55.11
Manayunkia aestuarina	100.46	29.92	-	19.69	74.8
Tubificoides benedii	35.08	6.23	-	8.05	82.85
Tubificidae spp	17.31	0.92	-	4.57	87.42
Pygospio elegans	15.15	2.77	-	3.46	90.88
Average dissimilarity = 52.53					
Species	**Group 2 Average abundance**	**Group 3 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Lumbricillus spp	63.19	29.92	3.24*	30.96	30.96
Manayunkia aestuarina	32.95	1.31	1.43*	28.27	59.23
Tubificoides benedii	21.07	6.23	2.31*	14.01	73.24
Heterochaeta costata	8.75	1.46	2.09*	6.65	79.89
Tubificidae spp	7.96	0.92	2.98*	6.53	86.42
Paranais litoralis	5.68	0.85	0.97	5.04	91.46

Table 6.2. SIMPER results showing the species differences during different times for the lower shore area. Group 1 = May 2000, Group 2 = February 1999 + 2000, Group 3 = November 1998 + May 1999 – November 1999 + July 2000 + November 2000 (* = good discriminating species).

Average dissimilarity = 34.61					
Species	**Group 3 Average abundance**	**Group 2 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Streblospio shrubsolii	44.93	17.96	2.16*	32.02	32.02
Tubificoides benedii	48.21	27.21	1.35	25.72	57.74
Macoma balthica	11.27	2.21	2.53*	10.28	68.02
Pygospio elegans	9.62	2.29	1.89*	8.33	76.36
Tubificoides swirenocoides	28.52	28.86	1.83*	6.7	83.06
Manayunkia aestuarina	5.29	2.25	0.79	4.75	87.81
Hydrobia ulvae	3.52	0.93	1.13	3.1	90.91
Average dissimilarity = 42.1					
Species	**Group 3 Average abundance**	**Group 1 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Tubificoides benedii	48.21	12.86	2.13*	31.93	31.93
Streblospio shrubsolii	44.93	18.29	2.05*	25.1	57.04
Tubificoides swirenocoides	28.52	19.36	1.59*	8.99	66.03
Lumbricillus spp	0.12	7.29	6.93*	6.77	72.8
Pygospio elegans	9.62	15.43	1.27	5.82	78.62
Macoma balthica	11.27	4.93	1.54*	5.61	84.22
Manayunkia aestuarina	5.29	4.93	1.01	4.03	88.26
Paranais litoralis	0.11	3.64	7.48*	3.34	91.59

Table 6.3. SIMPER results showing the species differences during different times for the lower shore area. Group 1 = May 2000, Group 2 = February 1999 + 2000 (* = good discriminating species).

Average dissimilarity = 32.63					
Species	**Group 2 Average abundance**	**Group 1 Average abundance**	**Diss/SD**	**Contribute%**	**Cum. %**
Tubificoides benedii	27.21	12.86	1.36	23.81	23.81
Pygospio elegans	2.29	15.43	4.99*	22.53	46.34
Tubificoides swirenocoides	28.86	19.36	4.57*	16.05	62.39
Lumbricillus spp	0.04	7.29	15.69*	12.37	74.76
Paranais litoralis	0	3.64	14.15*	6.22	80.97
Macoma balthica	2.21	4.93	9.28*	4.64	85.61
Manayunkia aestuarina	2.25	4.93	1.31	4.44	90.05

Table 6.4. Temporal BIO-ENV results showing the correlation coefficient (p_s) and the subset of variables selected for upper shore and lower shore areas. Those with p_s above 0.7 are highlighted in bold.

Source	Lower shore p_s, Variables	Upper shore p_s, Variables
Sediment	0.428, Mean particle size	0.320, Mean particle size, % Silt
Avon	0.398, DO	**0.890, BOD+ATU, Nitrate, Mercury**
Kinneil	0.601, Temperature, pH, Ammonia	0.227, Tubidity
Sewage	0.364, Chromium	0.649, Suspended soilds, BOD+ATU, Ammonia
Temperature/NAO	0.607, Minimum Temperature	0.293, Rainfall, NAO
Chemcial effluent	0.321, TSS, COD, Ammonia, Cadmium, Chromium, Phenol	**0.862, pH, EC, Copper Nickel, Phenol**
Refinery effluent	0.269, Ammonical nitrogen, Hydrocarbon	0.526, Lead

Table 6.5. Results of the temporal stepwise regression analysis for the upper shore and lower shore areas. High Rsq(adj) values, above 70%, are highlighted in bold.

Upper shore

Source	Diversity p, Rsq(adj)	Evenness p, Rsq(adj)	No. Species p, Rsq(adj)	No. Individuals p, Rsq(adj)
Sediment	0.009, 56.6%	None selected	**0.000, 82.8%**	None selected
Avon	None selected	None selected	**0.000, 98.0%**	**0.000, 99.9%**
Kinneil	0.025, 47.8%	None selected	0.010, 57.9%	0.018, 51.7%
Sewage	None selected	None selected	0.027, 46.0%	**0.001, 93.4%**
Temperature/NAO	None selected	None selected	None selected	0.012, 56.1%
Chemical effluent	None selected	**0.000, 96.2%**	None selected	**0.000, 99.8%**
Refinery effluent	None selected	None selected	**0.001, 96.1%**	Not normal

Lower shore

Source	Diversity p, Rsq(adj)	Evenness p, Rsq(adj)	No. Species p, Rsq(adj)	No. Individuals p, Rsq(adj)
Sediment	None selected	None selected	0.024, 47.4%	0.013, 55.9%
Avon	**0.001, 87.4%**	**0.000, 99.3%**	**0.011, 70.8%**	**0.003, 87.9%**
Kinneil	0.031, 43.6%	None selected	**0.003, 70.2%**	0.047, 37.4%
Sewage	None selected	None selected	0.016, 66.4%	**0.011, 70.4%**
Temperature/NAO	0.020, 50.2%	None selected	0.039, 40.4%	0.004, 67.1%
Chemical effluent	None selected	None selected	None selected	0.048, 37.3%
Refinery effluent	0.034, 42.6%	0.007, 62.0%	None selected	None selected

Table 6.6. The significant regression models with an Rsq(adj) above 70% for upper shore area.

Source	Reggression Model
Sediment	No. Species = 13.3 - 0.641 Organic matter, p=0.000, F=39.51, Rsq(Adj)=82.8%
Refinery	No. Speices =15.5 + 1.34 Chromium - 0.714 Lead - 1.19 pH - 0.248 Copper, p=0.001, F=50.56
	Rsq(adj)=96.1%
Avon	No. Species = -385 + 0.0900 SS + 0.0707 Dosat + 0.619 BOD+ATU, p=0.000, F=131.04, Rsq(adj)=98.0%
	No. Individuals = -1104 + 177 Iron + 211 pH - 4.23 Dosat - 16.1 Nickel - 166 Nitrite, p=0.000, F=3068.79, Rsq(adj)=99.9%
Sewage	No. Individuals = 77.6 - 3.01 BOD+ATU = 4214 Cadmium - 14.9 Chromium, p=0.001, F=38.46, Rsq(adj)=93.4%
Chemical	No. Individuals = -49.1 + 4.35 Copper - 6.02 BOD + 1.57 COD + 0.773 TSS, p=0.000, F=840.17 Rsq(adj)=99.8%
Effluent	Evenness = 0.644 + 0.041 Phenol - 0.146 Cadmium - 0.0170 Hydrocarbon, p=0.000, F=69.02 Rsq(Adj)=96.2%

Table 6.7. The significant regression models with an Rsq(adj) above 70% for lower shore area.

Source	Reggression Model
Avon	Evenness = 0.919 + 0.00516 Dosat - 0.0461 Ammonia + 0.161 pH - 0.000301 EC - 0.00593 Silicate, p=0.000 F=222.03, Rsq(adj)=99.3%
	No. Individuals = 481 - 25.5 DO - 43.4 BOD+ATU = 1.65 COD, p=0.003, F=20.44, Rsq(Adj)=87.9%
	Diversity = -2.28 + 0.411 DO + 26.3 Mercury, p=0.001, F=28.78, Rsq(adj)=84.4%
	No. Species = 6.98 + 2.67 Kjeldahl - 0.752 BOD, p=0.011, F=10.69, Rsq(adj)=70.8%
Kinneil	No. Species = 27.3 - 0.216 DO, p=0.003, F=19.83, Rsq(adj)=70.2%
Sewage	No. Individuals = 84.9 - 7.31 Chromium, + 878 Cadmium, p=0.011, F=10.54, Rsq(adj)=70.4%

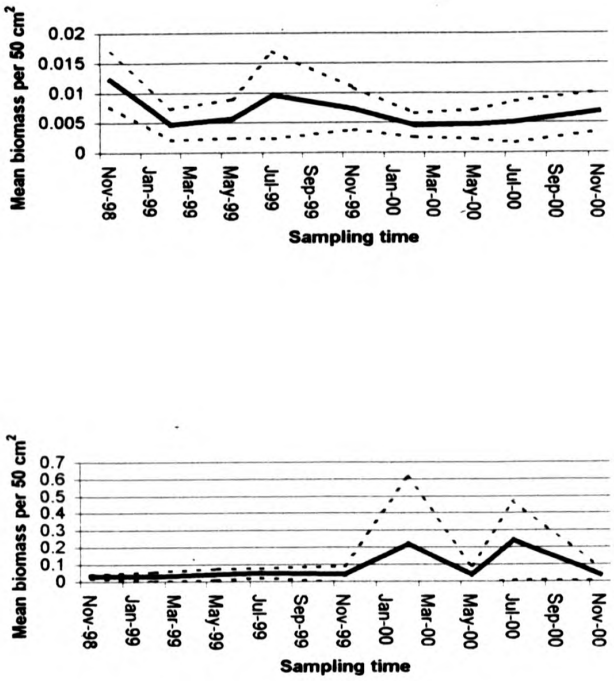
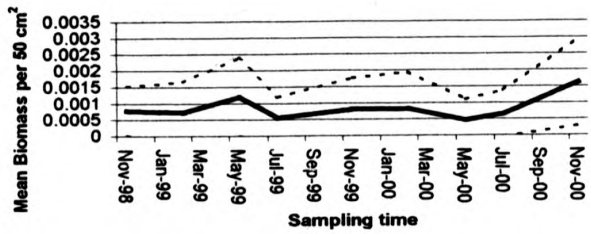
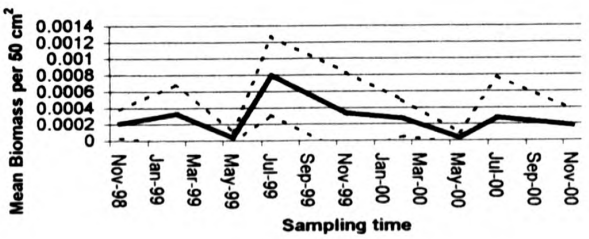


Figure 6.25. Change over time in the mean biomass including the 95 percent confidence limits (dashed lines) for the upper shore stations (Top) and the lower shore stations (Bottom).

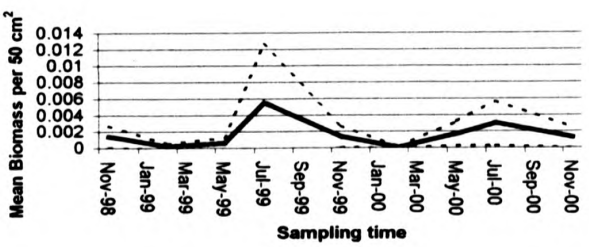
a)



b)



c)



d)

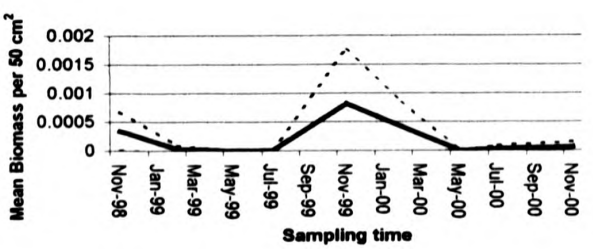
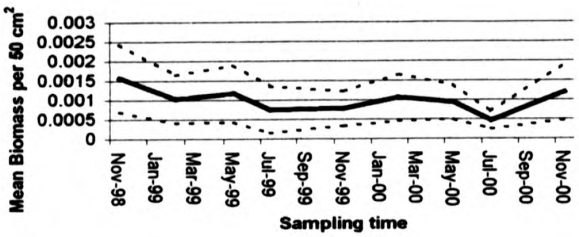
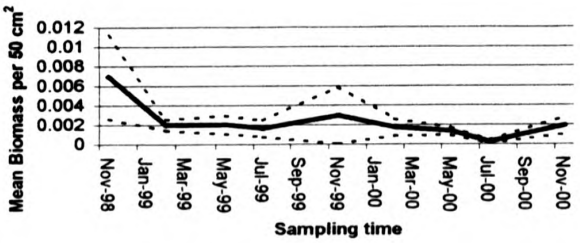


Figure 6.26. Change in the mean biomass over time with 95% confidence limits for the upper shore area for a) *Macoma balthica*, b) *Hydrobia ulvae*, c) *Nereis diversicolor* and d) *Corophium volutator*.

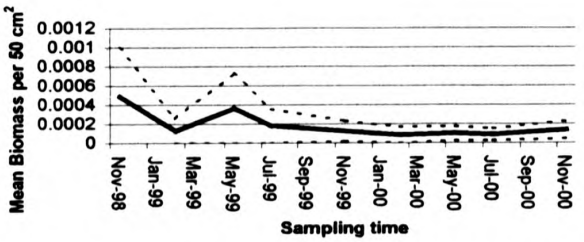
a)



b)



c)



d)

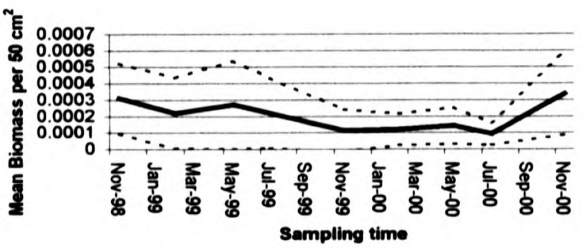


Figure 6.27. Change in the mean biomass over time with 95% confidence limits for the upper shore area for a) *Manayunkia aestuarina*, b) *Oligochaetes*, c) *Pygospio elegans* and d) *Streblospio shrubsolii*.

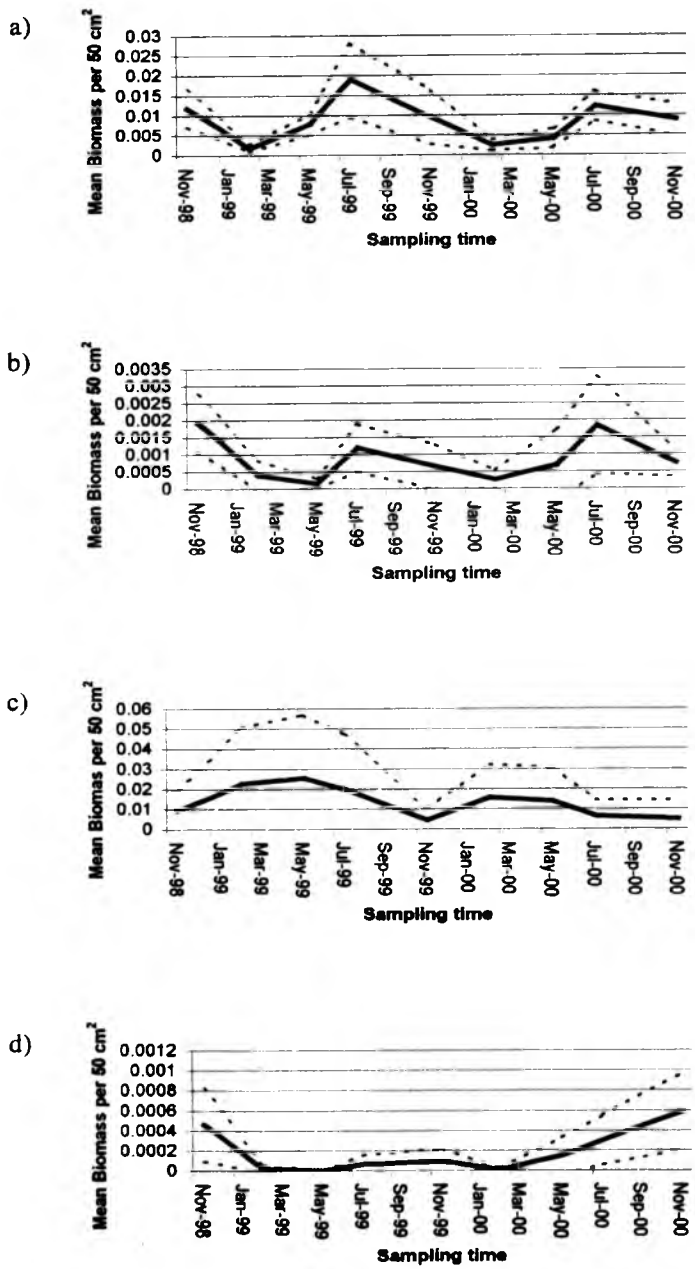
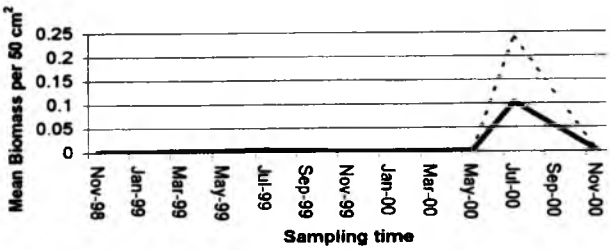
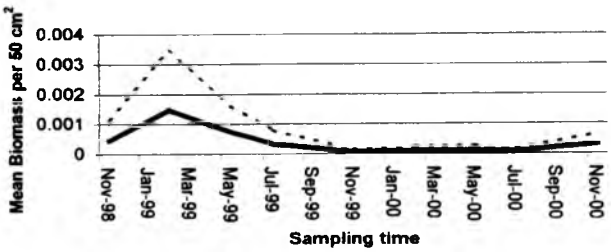


Figure 6.28. Change in the mean biomass over time with 95% confidence limits for the lower shore area for a) *Macoma balthica*, b) *Hydrobia ulvae*, c) *Cerastoderma edule* and d) *Eteone longa*.

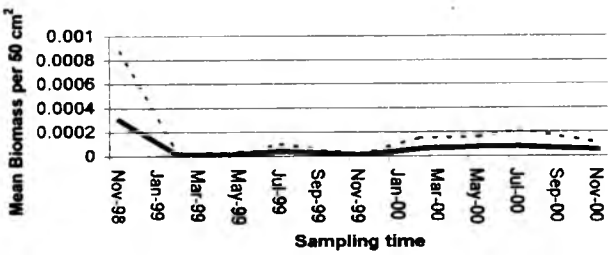
a)



b)



c)



d)

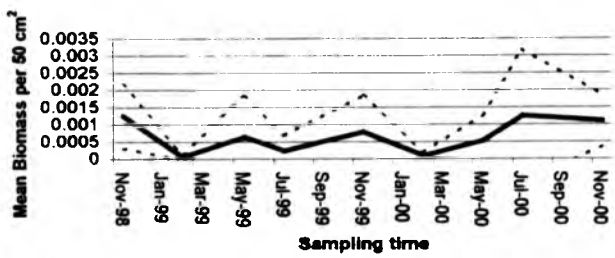
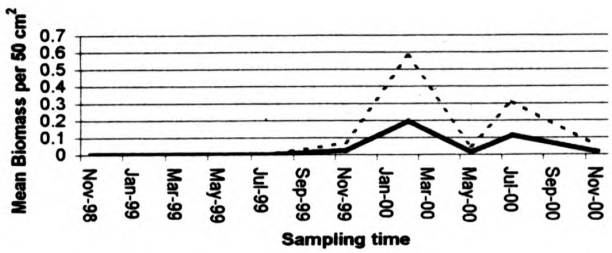
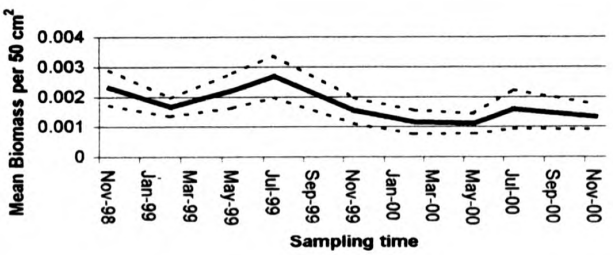


Figure 6.29. Change in the mean biomass over time with 95% confidence limits for the lower shore area for a) *Nephtys hombergii*, b) *Nereis diversicolor*, c) and *Manayunkia aestuarina* d) *Corophium volutator*.

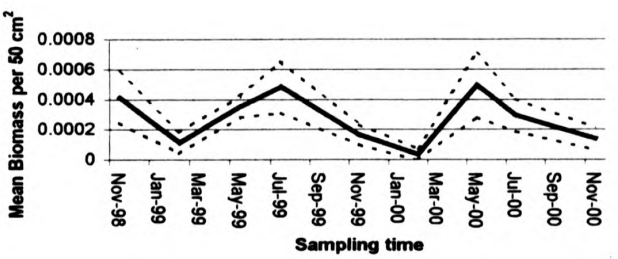
a)



b)



c)



d)

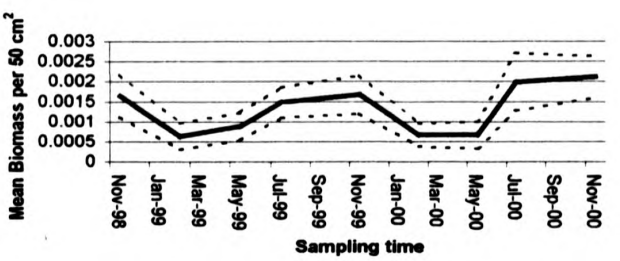


Figure 6.30. Change in the mean biomass over time with 95% confidence limits for the lower shore area for a) *Mytilus edulis*, b) *Oligochaetes*, c) and *Pygospio elegans* d) *Streblospio shrubsolii.*

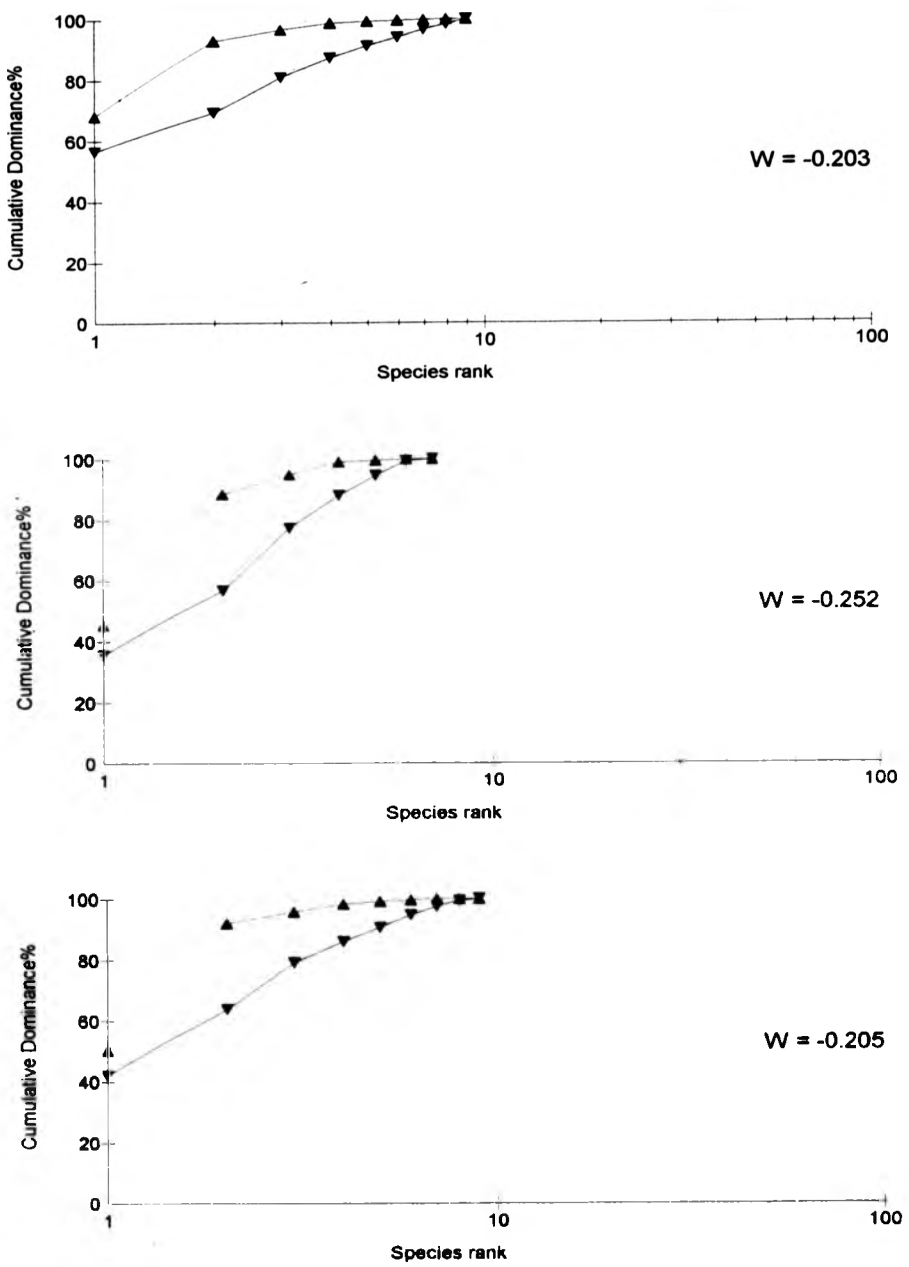


Figure 6.31. ABC plots showing the Abundance (Grey) and Biomass (Black) curves for the upper shore area in November 1998 (Top), February 1999 (Middle) and May 1999 (Bottom).

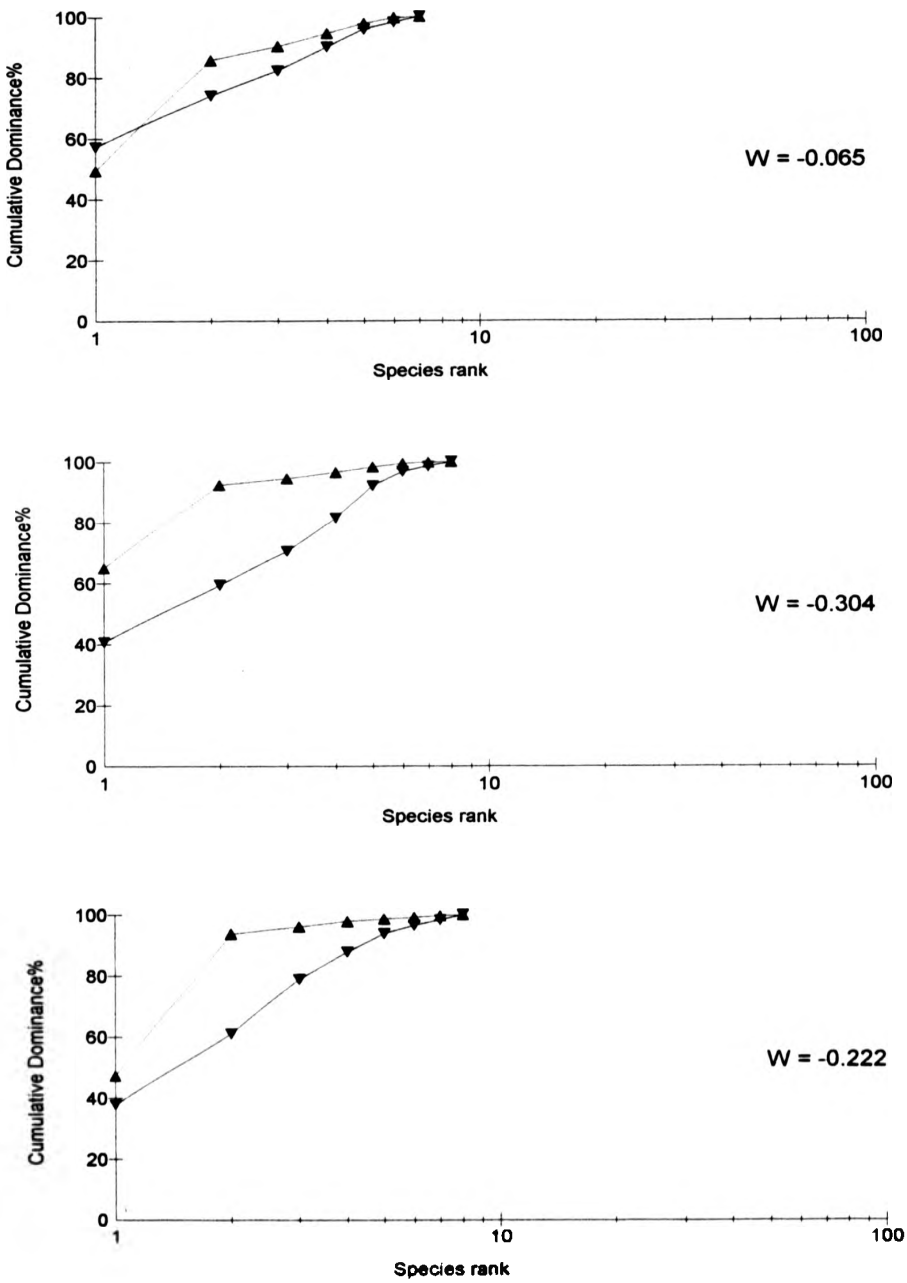


Figure 6.32. ABC plots showing the Abundance (Grey) and Biomass (Black) curves for the upper shore area in July 1999 (Top), November 1999 (Middle) and February 2000 (Bottom).

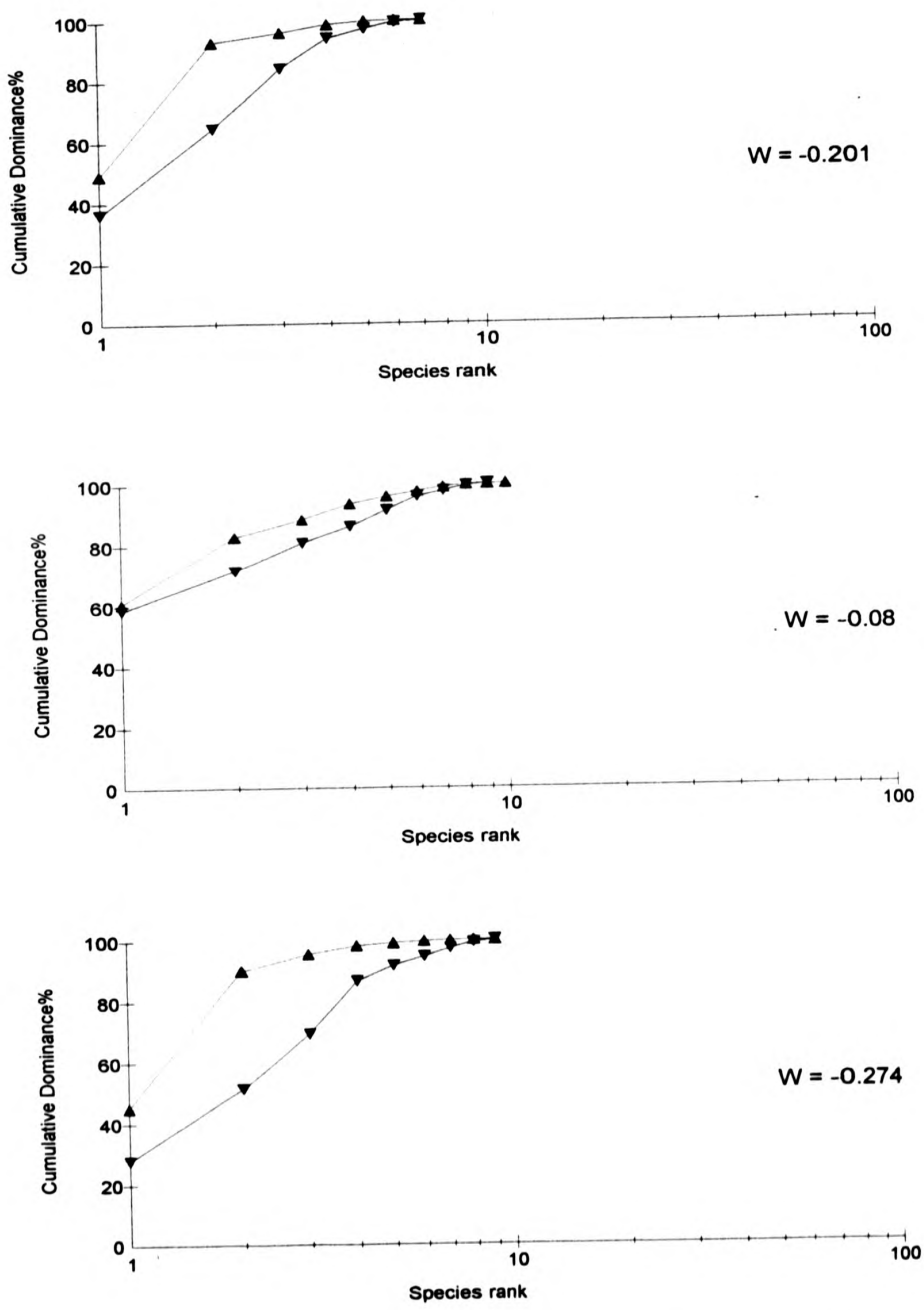


Figure 6.33. ABC plots showing the Abundance (Grey) and Biomass (Black) curves for the upper shore area in May 2000 (Top), July 2000 (Middle) and November 2000 (Bottom).

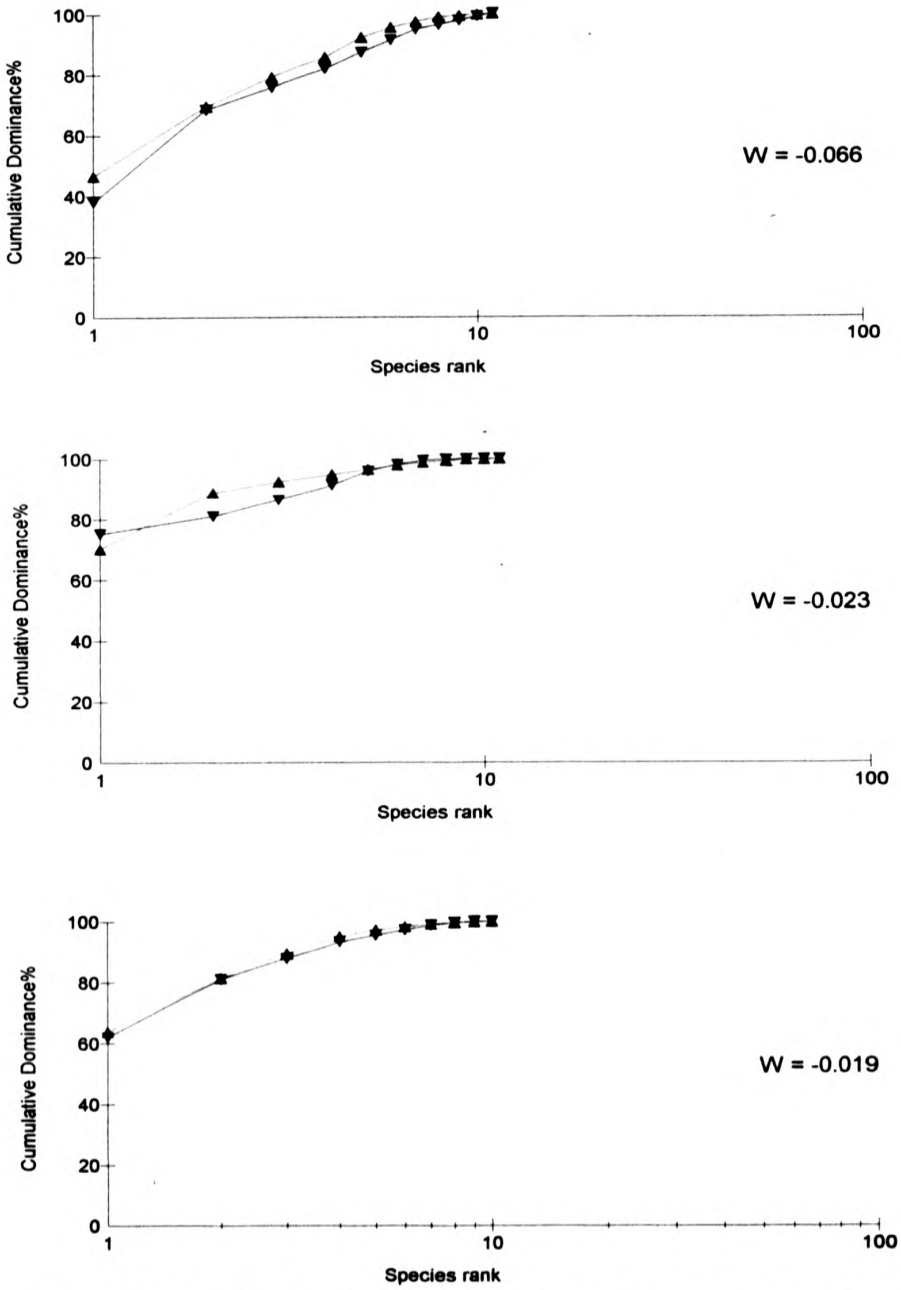


Figure 6.34. ABC plots showing the Abundance (Grey) and Biomass (Black) curves for the lower shore area in November 1998 (Top), February 1999 (Middle) and May 1999 (Bottom).

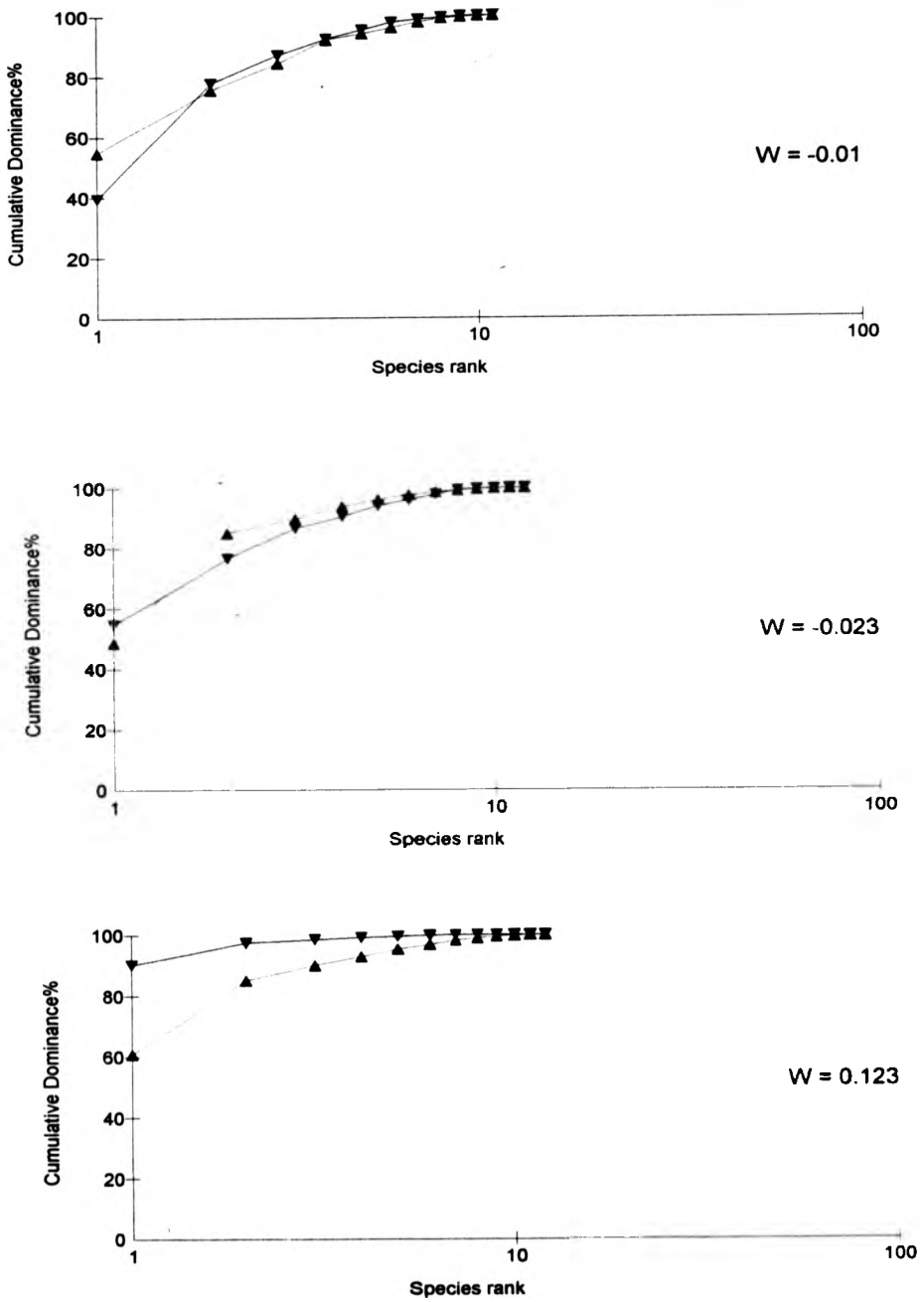


Figure 6.35. ABC plots showing the Abundance (Grey) and Biomass (Black) curves for the lower shore area in July 1999 (Top), November 1999 (Middle) and February 2000 (Bottom).

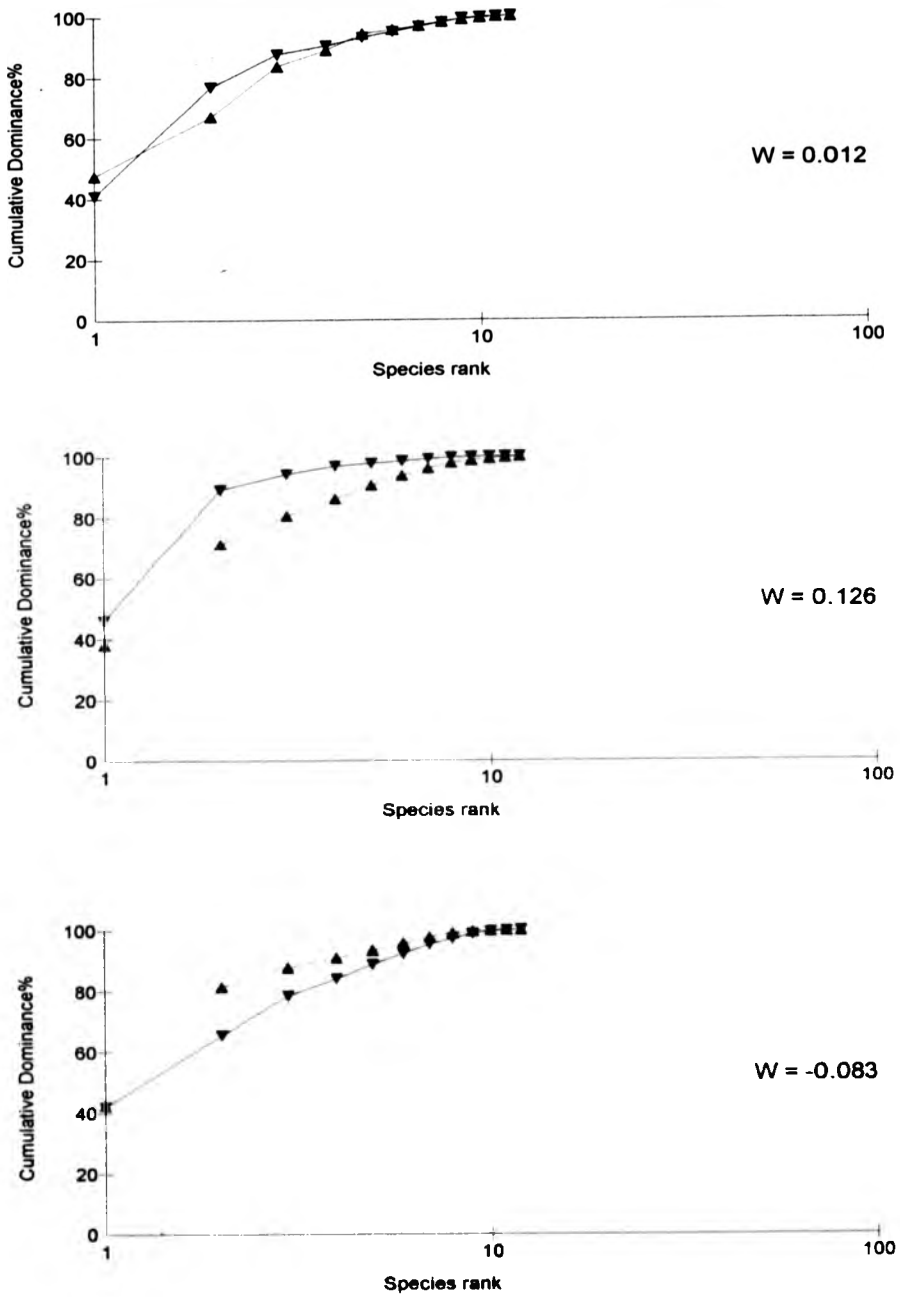


Figure 6.36. ABC plots showing the Abundance (Grey) and Biomass (Black) curves for the lower shore area in May 2000 (Top), July 2000 (Middle) and November 2000 (Bottom).

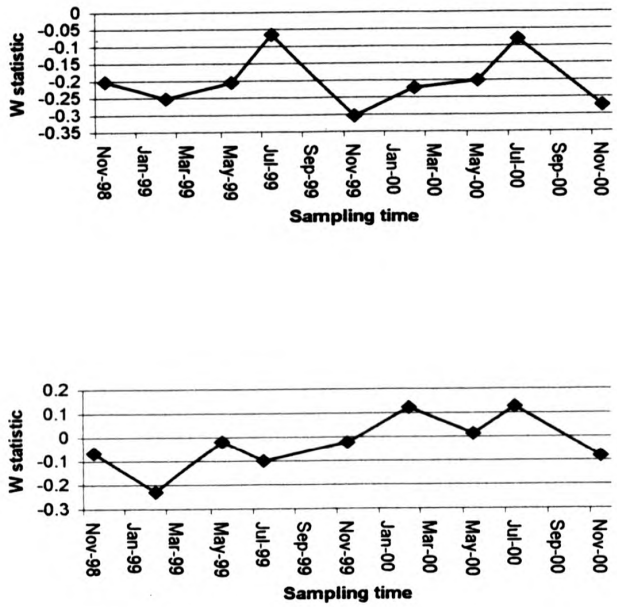


Figure 6.37. Change over time in the W statistic for the upper shore (Top) and the lower shore (Bottom) areas.

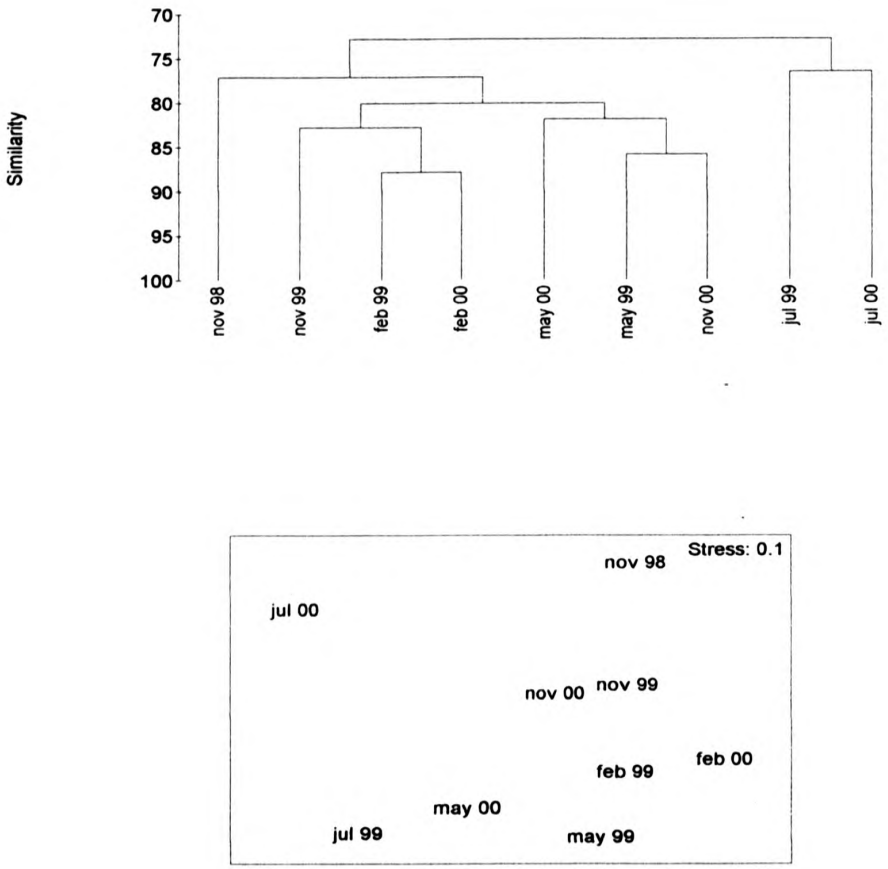


Figure 6.38. Cluster analysis dendrogram (Top) and MDS plot (Bottom) showing the change over time in the mean biomass for the upper shore stations.

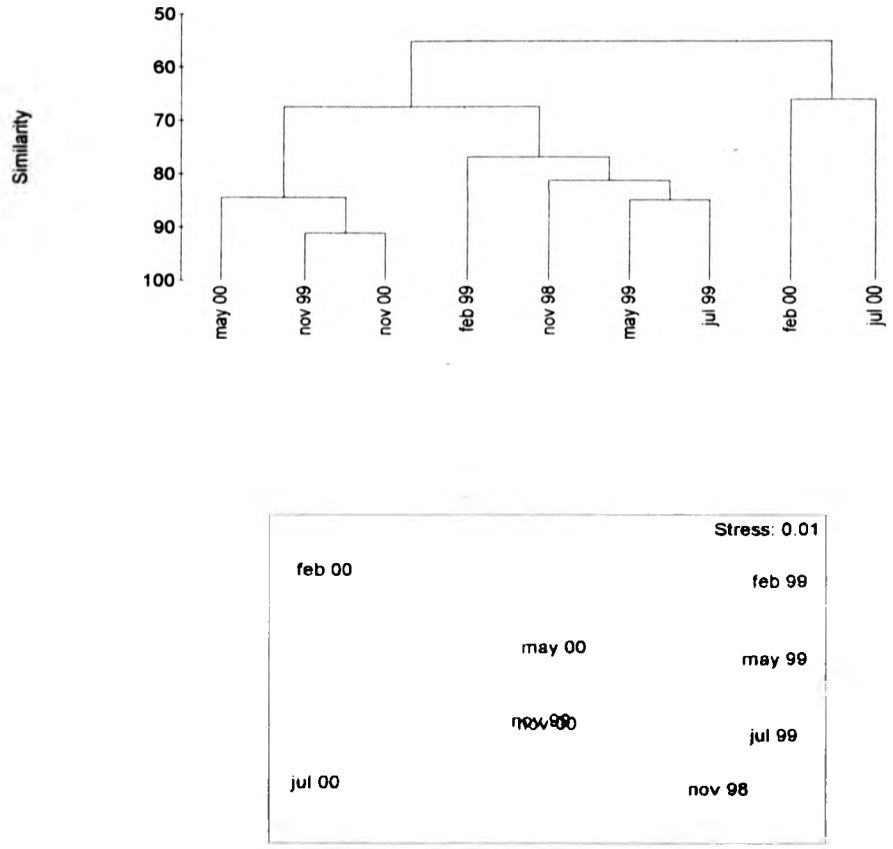


Figure 6.39. Cluster analysis dendrogram (Top) and MDS plot (Bottom) showing the change over time in the mean biomass for the lower shore stations.

Table 6.8. SIMPER results for the upper shore area based on the biomass where Group 1 = July 1999, Group 2 = February 1999, May 1999, November 1999 – May 2000 and November 2000, Group 3 = July 2000 and Group 4 = November 1998 (* = Good discriminating species).

Average dissimilarity = 41.93					
Species	**Group 4 Mean biomass ($x10^4$)**	**Group 2 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Oligochaetes	6.92	1.99	6.25*	66.24	66.24
Nereis diversicolor	1.42	0.86	1.14	9.02	75.26
Manayunkia aestuarina	1.57	1.03	3.8*	7.32	82.59
Pygospio elegans	0.48	0.16	3*	4.4	86.99
Corophium volutator	0.34	0.22	2.38*	3.97	90.96
Average dissimilarity = 54.64					
Species	**Group 4 Mean biomass ($x10^4$)**	**Group 1 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Oligochaetes	6.92	1.63	-	44.17	44.17
Nereis diversicolor	1.42	5.51	-	34.21	78.38
Manayunkia aestuarina	1.57	0.75	-	6.87	85.25
Hydrobia ulvae	0.21	0.8	-	4.89	90.14
Average dissimilarity = 44.72					
Species	**Group 2 Mean biomass ($x10^4$)**	**Group 1 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Nereis diversicolor	0.86	5.51	5.13*	8.85	68.67
Hydrobia ulvae	0.19	0.8	3.97*	6.47	77.52
Oligochaetes	1.99	1.63	1.14	5.86	84
Macoma balthica	0.94	0.55	1.17	4.04	89.86
Manayunkia aestuarina	1.03	0.75	1.87*	5.04	93.9

Table 6.9. SIMPER results for the upper shore area based on the biomass where Group 1 = July 1999, Group 2 = February 1999, May 1999, November 1999 – May 2000 and November 2000, Group 3 = July 2000 and Group 4 = November 1998 (* = good discriminating species).

Average dissimilarity = 60.08					
Species	**Group 4 Mean biomass ($x10^4$)**	**Group 3 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Oligochaetes	6.92	0.27	-	63.78	63.78
Nereis diversicolor	1.42	2.98	-	14.99	78.77
Manayunkia aestuarina	1.57	0.47	-	10.61	89.38
Pygospio elegans	0.48	0.09	-	3.8	93.19
Average dissimilarity = 50.54					
Species	**Group 2 Mean biomass ($x10^4$)**	**Group 3 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Nereis diversicolor	0.86	2.98	2.59*	39.97	39.97
Oligochaetes	1.99	0.27	4.26*	31.33	71.31
Manayunkia aestuarina	1.03	0.47	3.56*	10.41	81.71
Macoma balthica	0.94	0.66	1.06	6.01	87.72
Corophium volutator	0.22	0.03	0.7	3.64	91.36
Average dissimilarity = 35.84					
Species	**Group 1 Mean biomass ($x10^4$)**	**Group 3 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Nereis diversicolor	5.51	2.98	-	47.94	47.94
Oligochaetes	1.63	0.27	-	24.75	73.7
Hydrobia ulvae	0.8	0.27	-	9.91	83.61
Manayunkia aestuarina	0.75	0.47	-	5.36	88.97
Eteone longa	0	0.22	-	4.2	93.17

Table 6.10. SIMPER results for the lower shore area based on the biomass where Group 1 = November 1998 – July 1999, Group 2 = February 2000, Group 3 = November 1999, May 2000 and November 2000 and Group 4 = July 2000 (* = good discriminating species).

Average dissimilarity = 53.79					
Species	**Group 1 Mean biomass ($x10^4$)**	**Group 2 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Mytilus edulis	0	18.35	4.94*	44.11	44.11
Cerastoderma edule	19.31	7.65	1.93*	29.5	73.61
Macoma balthica	10.08	7.47	1.52*	13.87	87.49
Nephtys hombergii	2.48	1.17	1.19	3.23	90.72
Average dissimilarity = 84.71					
Species	**Group 1 Mean biomass ($x10^4$)**	**Group 3 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Mytilus edulis	0	196.59	28.16*	90.73	90.73
Average dissimilarity = 75.45					
Species	**Group 2 Mean biomass ($x10^4$)**	**Group 3 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Mytilus edulis	18.35	196.59	26.9*	91.7	91.7

Table 6.11. SIMPER results for the lower shore area based on the biomass where Group 1 = November 1998 – July 1999, Group 2 = February 2000, Group 3 = November 1999, May 2000 and November 2000 and Group 4 = July 2000 (* = good discriminating species).

Average dissimilarity = 84.31					
Species	**Group 1 Mean biomass ($x10^4$)**	**Group 4 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Mytilus edulis	0	110.09	30.43*	47.2	47.2
Nephtys hombergii	2.48	102.89	20.59*	43.06	90.26
Average dissimilarity = 73.53					
Species	**Group 2 Mean biomass ($x10^4$)**	**Group 4 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Nephtys hombergii	1.17	102.89	124.65	49.66	49.66
Mytilus edulis	18.35	110.09	16.63	44.81	94.48
Average dissimilarity = 46.55					
Species	**Group 3 Mean biomass ($x10^4$)**	**Group 4 Mean biomass ($x10^4$)**	**Diss/SD**	**Contribute%**	**Cum. %**
Nephtys hombergii	0.89	102.89	-	47.96	47.96
Mytilus edulis	196.59	110.09	-	40.67	88.62
Macoma balthica	2.47	12.21	-	4.58	93.2

Table 6.12. BIO-ENV results for the upper shore and lower shore areas, for the different environmental sources. Highly significant correlations are highlighted in bold

Source	Lower shore p_s, Variables	Upper shore p_s, Variables
Sediment	0.068, % Clay	0.586, Mean particle size, % Silt
Avon	0.556, Akalinity, Total phosphate	**0.803, Dosat, BOD+ATU, Total phosphate, Nickel, Mercury**
Kinneil	0.533, Salinity, Phosphate	0.281, Turbidity, Ammonia
Sewage	**0.775, Suspendid solids, Chromium, Nickel**	0.459, pH, Suspendid solids, Total phosphate
Temperature/NAO	0.331, Maximum Temperature	0.399, Maximum Temperature, Rainfall, NAO
Chemcial effluent	**0.789, Ec, TSS**	0.665, pH, EC, Copper, Phenol
Refinery effluent	0.436, EC, Ammonia	0.513, EC, Ammonia, Chromium, Zinc, Hydrocarbon

Table 6.13. Top - Stepwise regression results showing the p and Rsq(adj) values for the upper shore and lower shore areas, for each of the environmental sources. High Rsq(adj) values are highlighted in bold. Bottom – The two highly significant regression models for the upper shore area.

Source	Upper shore p, Rsq(adj)	Lower shore p, Rsq(adj)
Sediment	None selected	None selected
Avon	0.006, 64.3%	0.046, 37.9%
Kinneil	0.036, 41.5%	0.012, 69.1%
Sewage	None selected	0.020, 55.7%
Temperature/NAO	**0.003, 80.7%**	None selected
Chemical effluent	**0.000, 86.7%**	None selected
Refinery effluent	0.016, 53.2%	None selected

Source	Reggression Model
Temperature/NAO	1/Biomass = 251 - 8.35 Min Temp - 5.86 Rainfall p=0.003, F = 17.86, Rdq(Adj) = 80.7%
Chemical effluent	Biomass = 0.000892 + 0.000141 Copper, p=0.000, F = 6.68, Rsq(Adj) = 86.7%

7. FIELD SURVEY – SIZE DATA ANALYSIS

7.1 INTRODUCTION

7.1.1 Sublethal effects on growth

Although it has been shown that there was no effect on the faunal biomass and abundance from moving the position of the chemical outfall, there is still the possibility of a sublethal effect, and there is also the possibility that the refinery effluent may be having a sublethal effect. Sublethal toxicity tests on refinery effluent and the individual chemical components have shown that they can reduce the growth of an organism (Buikema *et al.*, 1981; Southward, 1982; Rowe *et al.*, 1983b; Sherry *et al.*, 1994). It may therefore be hypothesised that the petrochemical effluents at Kinneil could potentially cause the organisms to be smaller close to the outfalls. Yule (1996) found that the size of *Macoma balthica* and *Hydrobia ulvae* at Kinneil was smaller in the inner corner, close to the refinery outfall, than elsewhere on the mudflat. Growth can also be limited by other factors such as lack of food (Wolff, 1983), predation (Nakaoka, 2000) and competition (Jensen, 1992a), which would have the same effect. There is however some evidence to suggest that certain chemicals could promote growth in some species (Saiz-Salina & Frances-Zubillaga, 1997). If this were the case then the individuals would increase in size faster and would reach a larger size than normal.

7.1.2 Recruitment patterns

Naturally a population will show seasonal variation in its size distribution. During the recruitment season the small juveniles will settle and then undergo a period of growth, although often many juveniles do not survive due to high post-settlement mortality rates (Udalov *et al*, 2000). There can also be natural yearly variations in growth and year class structure which are caused by differences in salinity, dissolved oxygen or other factors (Holland, 1987). It has been shown that some chemical pollutants can effect the reproductive success of certain species and therefore cause poor recruitment (Rowe *et al.*, 1983a; Saha & Konar, 1984a; Southward, 1982). Recruitment could also be affected by the mortality of the larvae

and juveniles. Larvae and juveniles are often more sensitive to the toxicity of pollutants than adults (Lewis, 1982). If recruitment is inhibited, larger individuals would dominate the population and eventually the population may even become extinct if recruitment was very low.

7.1.3 Aims

The size distributions of the larger species (*Macoma balthica, Hydrobia ulvae, Cerastoderma edule, Nereis diversicolor, Nephtys hombergii* and *Eteone longa*) during each sampling period were examined with the following aims:-

- To determine if there was any difference in the size distribution of the species between the upper shore and lower shore areas based on the hypothesis that the individuals would be smaller in the upper shore area.
- To determine if there was any change in the size distribution of each species over time.

7.2 RESULTS

7.2.1 Length to width relationship

The length and width of the species *Eteone longa, Nephtys hombergii, Nereis diversicolor, Hydrobia ulvae, Cerastoderma edule* and *Macoma balthica* were measured. For these species the length to width relationship was calculated (Figures 7.1 to 7.3). All of the species show a significant linear relationship between width and length. This relationship can now be used to make comparisons with other populations to see if the size distribution of the Kinneil population is similar or very different to those found in other locations.

7.2.2 Spatial and temporal change in the population size distributions

Size frequency histograms for each sampling time for the six species in both the lower and upper shore areas are considered and the differences between the two areas and the changes over time are examined.

7.2.2.1 Nereis diversicolor

The size frequency histograms for *Nereis diversicolor* in the upper and lower shore areas (Figure 7.4 and 7.5) suggest that there may have been a change in the size distribution between the two areas and over time. The GLM results (Table 7.1) however indicate that irrespective of time there was no difference between the two sites ($p = 0.532$). Irrespective of the site there was however a significant difference between the different sampling periods (time, $p= 0.000$). The main effects plot (Figure 7.6) indicates that the greatest change in the mean size was a decrease from May 1999 to July 1999 and from considering the pairwise comparisons (Appendix 2a) this decrease in mean was found to be significant. The interaction between site and time was also found to be significant ($p = 0.000$), which means that there was a difference over time of the mean size for the upper and lower shore areas. The interaction plot (Figure 7.6) suggests that the differences between the two areas occurred during the sampling periods February 1999, November 1999, February 2000, May 2000 and July 2000. During May and July 2000 the lower shore area had a smaller mean size than the upper shore area, whilst the opposite was seen for the other sampling periods. The pairwise comparisons (Appendix 2a) indicate that only the differences between May and July 2000 are significant ($p = 0.0001$, $p = 0.0043$). The interaction plot also suggests that the two areas showed different temporal changes. The lower shore area showed a decrease in the mean size between May and July 1999 and February and May 2000. Whilst the upper shore area showed a similar decrease in mean size between May and July 1999 but showed an increase between February and May 2000. The pairwise comparisons (Appendix 2a) only found the decrease between February and May 2000 for the lower shore area to be significant at the 10% level ($p = 0.0577$).

7.2.2.2 Nephtys hombergii

Nephtys hombergii was only found in the lower shore area and therefore only the temporal change can be considered. The size frequency histograms (Figure 7.7) suggest that there was a larger number of small individuals during November 1998, July and November 1999 and July 2000. The temporal change in the size

distribution was found to be significant ($p = 0.000$) (Table 7.2). The main effects plot (Figure 7.8) indicates that the mean size for each sampling time was lowest during November 1998, 1999 and July 2000 and was highest during May 1999 and 2000. This suggests that the temporal change in the mean size had a seasonal fluctuation, with a greater number of larger individuals present during the spring and smaller individuals during the winter. The pairwise comparisons (Appendix 2b) indicate that there was a significant difference in the mean size between May and July 1999 ($p = 0.0605$), between May and July 2000 ($p = 0.0056$) and finally between July and November 2000 ($p = 0.0081$). Between both May and July 1999 and May and July 2000 there was a decrease in the mean size, whilst between July and November 2000 there was an increase in mean size.

7.2.2.3 Macoma balthica

The size frequency histograms for *Macoma balthica* (Figures 7.9 and 7.10) indicate that the lower shore area had a greater density of individuals than the upper shore area. It appears that there was little variation in size between the sampling times in the upper shore area. In the lower shore area there did seem to be a change in the size distribution over time with there being a large number of smaller individuals during November 1998, July 1999 and July 2000. The GLM results (Table 7.3) however indicated that there was no difference between the two sites ($p = 0.158$) but there was a significant difference over time ($p = 0.000$). The main effects plot (Figure 7.10) suggests that the mean size was lowest during November 1998, February 1999, May 2000 and July 2000 and highest in July 1999. The major changes between sampling periods occurred as a large increase in mean size between February and May 1999 and a decrease in mean size between February and May 2000 followed by another increase between July and November 2000. The pairwise comparisons (Appendix 2c) indicated that only the increases in the mean size (February to May 1999 and July to November 2000) are significant ($p = 0.0409$, $p = 0.0020$). The interaction between site and time was also found to be significant ($p = 0.000$) indicating that there was a difference between the two areas during certain sampling times. The interaction plot (Figure 7.11) suggests that the upper shore area had a larger mean size than the lower shore area during all

sampling times except November 1998, July 1999 and July 2000. The only difference between the two areas that was found to be significant was during July 1999 ($p = 0.0128$) (Appendix 2c). The temporal change within each of the areas showed different patterns. The upper shore area showed a seasonal pattern with large decreases in mean size during July 1999 and 2000, although only the change between May and July 1999 was significant ($p = 0.0374$). The lower shore area on the other hand did not show a clear pattern, with significant increases in mean size, at the 10% level, observed between February and May 1999 ($p = 0.0518$) and between July and November 2000 ($p = 0.0558$).

7.2.2.4 Hydrobia ulvae

The frequency histograms for *Hydrobia ulvae* (Figures 7.12 and 7.13) indicated that the lower shore area tended to have a higher overall density and a greater abundance of larger individuals than the upper shore area. In both areas there did seem to be an increase in the number of small individuals during July periods in both years. There was both a significant difference in the mean size between the two sites ($p = 0.016$) and between the different sampling periods ($p = 0.000$) (Table 7.4). There was however no significant difference in the mean size between the two sites during the different sampling periods ($p = 0.111$). This indicates that the two sites showed very similar changes over time in their mean size, which can be clearly seen in the interactions plot (Figure 7.14) and therefore they can be considered together. The main effects plot (Figure 7.14) clearly shows that the lower shore site had a larger mean size that the upper shore site. It also indicates that the largest variation between sampling time was a decrease in size between May and July 1999 followed by an increase in size between July and November 1999 and lastly a further decrease between May and July 2000. The pairwise comparisons (Appendix 2d) indicates that all three of these changes in mean size are significant ($p = 0.0234$, $p = 0.0000$, $p = 0.0289$).

7.2.2.5 Eteone longa

Few individuals of this species were found over the study period (Figure 7.15 and 7.16). Very few of these were in the upper shore area and therefore it shows no clear pattern, although the individuals seem to be relatively large. For the lower shore area small individuals (> 0.3 mm) were found during most of the sampling times although no individuals were found during May 1999. Due to the lack of data for this species the interaction between site and time could not be assessed and therefore only the main effects have been considered. The effects of both time and site were found to be significant ($p = 0.001$, $p = 0.001$) (Table 7.5). The main effect plot (Figure 7.17) indicates that the upper shore area had a larger mean size than the lower shore area. The change over time showed a decrease in the mean size from February 1999 till February 2000 when it was at its lowest and then an increase again till November 2000. The change between successive time periods however was not found to be significant (Appendix 2e) and therefore the change in mean size has been gradual over the two-year survey period.

7.2.2.6 Cerastoderma edule

This species was only found in the lower shore area so only the change over time is considered. The size frequency histograms (Figure 7.18) indicate that both large and small individuals were present during most time periods except February and November 2000 when only larger individuals were present. Individuals up to 25 mm in length were collected during the sampling period. There was a significant change in the size distribution of the population over time ($p = 0.007$) (Table 7.6). The main effects plot (Figure 7.19) indicates that the major changes in the mean size for successive time periods were between November 1999 and February 2000, between May and July 2000 and finally between July and November 2000. The pairwise comparisons (Appendix 2f) however indicate that although these changes were large they are not significant.

7.3 DISCUSSION

7.3.1 Sublethal effect of petrochemical effluents

A sublethal effect from the petrochemical effluent could potentially have a negative effect on growth (Buikema *et al.*, 1981; Southward, 1982; Rowe *et al.*, 1983b; Sherry *et al.*, 1994) and/or reproductive success (Rowe *et al.*, 1983a; Saha & Konar, 1984a; Southward, 1982). This would have an impact on the distribution of a population and it would be expected that areas that were subjected to greater pollution would contain smaller individuals.

7.3.1.1 Difference between upper and lower shore

At Kinneil it has already been shown by the two-year survey analysis that the upper shore area had a lower abundance and biomass and was therefore considered to be more impacted than the lower shore (Chapter 5). It could therefore be hypothesised that the upper shore area would have smaller individuals than the lower shore area. Yule (1996) found this to be the case for two species, *Macoma balthica* and *Hydrobia ulvae*, who had smaller individuals in the inner corner area. The present study has shown that out of the four species that were present in both areas only two showed a significant difference between the upper and lower shore area irrespective of time, these were *H. ulvae* and *Eteone longa*. The population of *E. longa* however showed the opposite pattern to that hypothesised, with the upper shore area having a larger mean size than the lower shore area. Although this difference was significant it was based on only a limited number of individuals and therefore further testing is needed to confirm that this distributional pattern is accurate. The population of *H. ulvae* in the upper shore did have a significantly smaller mean size than the lower shore area, which is consistent with a sublethal effect from the petrochemical effluents. There are however other possible explanations for this difference, it could be due to predation (Nakaoka, 2000), food availability (Wolff, 1983) or physical conditions, such as shore height (Montaudouin & Bachelet, 1996) which have all been shown to affect the growth of benthic invertebrates.

Although no significant difference between the two sites overall was detected for *Nereis diversicolor* and *Macoma balthica*, significant differences between the sites during specific sampling times was detected. For *N. diversicolor* the differences were detected during May and July 2000 when the lower shore population had a smaller mean size than the upper shore population. For *M. balthica* the lower also had a larger mean size than the upper shore during November 1998, 2000 and July 1999, although the difference was only significant during July 1999. Therefore these two species show the opposite spatial size distribution pattern to that hypothesised. As the differences in the size distributions for these two species between the two areas is also dependent on time then it seems more likely that the differences in the mean size is due to recruitment (see 7.3.2) rather than a sublethal effect from the effluents.

7.3.1.2 Movement of the chemical outfall

If the chemical effluent were causing a sublethal effect then it would be expected that when it was moved to the lower shore site its impact would also move. It could therefore be hypothesised that any sublethal effect seen in the upper shore area in November 1998 would move to the lower shore area in February 1999. The refinery effluent may also have had a sublethal effect on both the upper and lower shore areas after January 1999. No obvious changes in the size distributions were seen between November 1998 and February 1999 in either of the two areas for any of the six species. It therefore seems that either the chemical effluent was not having a sublethal effect or that it was not detectable using the methods used in this study.

7.3.1.3 Comparison of Kinneil size distribution with other locations

To determine whether the size distribution of the individual species population measured at Kinneil are 'normal' they have been compared to the results of studies from other locations both from within the UK and abroad.

7.3.1.3.1 *Nereis diversicolor*

The maximal size class of *Nereis diversicolor* found at Kinneil is 2.5 mm width and 6.66 cm in length. At the Stiffkey saltmarsh the maximal width was found to be 3.0

mm (Nithart, 1998) and in the Canal de Mira the mean length before extinction here was 9.2 – 10.6 cm (Abrante *et al.*, 1999). Therefore the population of *N. diversicolor* at Kinneil is slightly smaller that in other areas. This could mean that Kinneil is affected by a sublethal effect from pollution, which is restricting the growth of this species or alternatively it could mean that survivorship is reduced, or that other environmental factors are responsible.

7.3.1.3.2 *Nephtys hombergii*

The largest individual that was found during all of the sampling periods was in February 1999 and had a width of 1.67 mm. This is small compared to the maximum body width that was found by Rainer (1991) of 7 mm. The regression equation that was calculated for *Nephtys hombergii* was compared to that determined by Olive (1977) from a population in the Tyne estuary and to a population in the North Sea (Rainer, 1991) (Figure 7.20). The comparisons of the body width conversions suggest that compared to the Tyne estuary (Olive, 1977) the individuals at Kinneil are 19 mm shorter in length, for the same width, but are longer than those found by Rainer of the same width (1991). Both Olive and Rainer's equations were mainly based on larger individuals than those found at Kinneil. The increased lengths of the Tyne estuary *N. hombergii* may be due to differences in the method as Olive (1977) used a relaxing agent before measuring size. The slightly smaller length to width relationship for individuals found by Rainer (1991) also may be due to the method, as the width was measured to include the parapodia. The lack of large individuals at Kinneil is however still apparent. This may indicate a sublethal effect from pollution which is reducing the growth rate (Buikema *et al.*, 1981) or possibly the lack of food (Wolff, 1983). It could however be that growth is not affected but it is survivorship. As age was not determined it may be that individuals do not survive for more than one or two years at Kinneil, whereas in other areas *N. hombergii* has been noted to have a life span of 5 to 7 years (Olive, 1977; Rainer, 1991). If survivorship is low this is likely to be caused by increased disturbance, either natural or pollution induced, by high predation pressure or by competition (Connell, 1978; Pearson & Rosenberg, 1978; Warnes, 1981).

7.3.1.3.3 *Macoma balthica*

The size of the individuals that have been found are fairly small compared to those found by Kamermans *et al.* (1999). This could be an indication that survival or growth is impaired at Kinneil. It could however be due to the sampling method as samples to a depth of 5 cm only were taken at Kinneil and Kamermans *et al.* (1999) took cores of 40 cm depth. It is known that smaller individuals remain close to the surface all year but that the larger individuals move deeper in the sediment during the winter (Ratcliffe *et al.*, 1981). It is likely that 5 cm is not deep enough to capture the larger individuals (Warnes, 1981). This is also true for other species such as *Nereis diversicolor*.

7.3.1.3.4 *Hydrobia ulvae* and *Eteone longa*

For both *Hydrobia ulvae* and *Eteone longa* size distributional data from other locations was not available so comparisons could not be made.

7.3.1.3.5 *Cerastoderma edule*

The large individuals are present throughout the year in both 1999 and 2000, with sizes up to 25 mm in length. This size is comparable to other populations of *Cerastoderma edule* studied by Jensen (1992b), Guillou & Tartu (1994) and Masski & Guillou (1999). This indicates that there may be no effect of the effluents on the growth or survival of *C. edule*.

7.3.2 Recruitment

During the recruitment period the mean size of the population is likely to be at its smallest. Some species have specific periods during the year when they undergo recruitment, whilst others undergo recruitment continually throughout the year. It is expected that the density of recruits will vary as recruitment is often patchy and dependent on many different factors (David *et al.*, 1997).

7.3.2.1. Nereis diversicolor

Nereis diversicolor has been found to have differing reproductive cycles in different geographic locations. Davey & George (1986) show that in the Thames *N. diversicolor* spawns only once in February whilst in Cherbourg this species spawns all year round. In the Canal de Mira the recruitment of this species occurred twice a year once during February/March and again in April/May (Abrante *et al.*, 1999). Nithart (1998) however found that populations in Norfolk saltmarshes only showed one recruitment period during July. At Kinneil the mean size of the *N. diversicolor* population showed a significant decrease between May and July 1999. This decrease occurred in both the upper and lower shore areas suggesting that recruitment may have occurred at this time in both areas. A decrease in the mean size of the population was also detected in the lower shore area between February and May 2000. This suggests that the recruitment for *N. diversicolor* is probably during May/July each year.

7.3.2.2 Nephtys hombergii

Again like *Nereis diversicolor* there are geographical differences in reproductive and recruitment patterns of *Nephtys hombergii*. Oyenekan (1986) found that in Southampton Water breeding occurred at a low level all year but peaks were seen in July to September and November to January. On the other hand Mathivat-Lallier & Cazaux (1991) discovered that recruitment occurred twice a year in March and in May/June in Arcachon Bay, France. At Kinneil the lower shore area showed a seasonal pattern with the mean population size being at its lowest during November 1998, 1999 and July 2000 and at its highest during May 1999 and 2000. Significant decreases in the mean size of the *N. hombergii* population took place between May and July 1999 and 2000. This indicates that recruitment is taking place between May and November each year, which is similar to that found for other locations.

7.3.2.3 Macoma balthica

At Kinneil there was found to be a significant difference in the size distribution of the *Macoma balthica* population over time. The two areas showed different

temporal patterns, with the upper shore area showing a clear seasonal pattern and the lower shore area showing no clear pattern. The upper shore area showed a decrease in the mean size between May and July 1999 and 2000, indicating that recruitment may be taking place at this time. The lower shore area shows no large decrease in mean size. This suggests that recruitment in the lower shore area could be continual throughout the year. Other studies indicate that peak settlement occurs during May (Armonies & Hellwig-armonies, 1992).

7.3.2.4 Hydrobia ulvae

Lillebo *et al.* (1999) found that for *Hydrobia ulvae* in the Mondego estuary settlement took place in March and June to September. Whilst in the Bidasoa estuary, Spain, recruitment also took place in February/March and again starting in July (Sola, 1996). At Kinneil both the upper and lower shore areas show the same pattern of change in the size distribution of the population over time. Decreases in the mean size were seen between May and July 1999 and 2000, suggesting that recruitment takes place at this time each year. This is slightly different to the other locations as only one recruitment period could be identified at Kinneil.

7.3.2.5 Eteone longa

The life cycle and population dynamics of *Eteone longa* are not well documented. At Kinneil this species was not very abundant which means that getting a clear picture of what is happening is very difficult. The size distributions for both areas were very sporadic. No clear indication can be seen as to when this species undergoes recruitment. The only real conclusion that can be made is that densities were higher in 2000 than 1999 suggesting that 2000 was a better year for the recruitment or survival of *E. longa*.

7.3.2.6 Cerastoderma edule

Again differences in the settlement time can be seen, with populations in the Bay of Morlaix showing peak settlement during September/October (Guillou & Tartu, 1994). Whilst in the in the Danish Wadden Sea high settlement during June/July has

been noted (Jensen, 1992b). At Kinneil there were changes in the size distribution of the population over time but there were no significant changes between successive time periods, indicating that there was only a gradual change in the size over time. This could be because this species shows recruitment throughout the year and therefore shows no large changes in the size distribution of the population. It is however also possible that the recruitment period was not detected due to the small number of individuals that were measured. It is probable however that recruitment was not successful during the two year survey period. Many studies indicate that recruitment success for *C. edule* is governed by temperature. Beukema *et al.* (2000) found that recruitment densities were high after severe winters and low after mild winters. During the two-year survey period the temperature for both winters periods were very similar and were relatively mild so this could explain the lack of any substantial recruitment pattern. The long-term data indicates recruitment in only a few years explaining why *C. edule* has shown large fluctuations in abundance with no apparent trend over the long-term (Chapter 3).

7.3.3 Migration

Some species are known to undergo migrations from one area of the shore to another after settlement, which will therefore alter spatial distribution of the different sized individuals. Dispersal is potentially more important than mortality for the population dynamics of juvenile bivalves over small and meso-spatial time scales (Norkko *et al.*, 2001). Of the six species considered in this study both *Nephtys hombergii* and *Macoma balthica* are known to undergo significant migrations from one area of the shore to another. Davey & George (1986) found that the larvae of *N. hombergii* settled in the sublittoral zone and then migrated up shore between October and April. No obvious migration pattern was seen at Kinneil only a distinct seasonal pattern which is likely to be due to recruitment rather than migration. It has been identified that *M. balthica* undergoes two migrations during its first year of life (Armonies & Hellwig-armonies, 1992; Beukema, 1993). After the initial settlement the juveniles migrate to the upper intertidal area by byssus drift. In the subsequent winter the grown spat move back down the shore. It is thought that the spat migrate to the most favourable habitat. At Kinneil the upper and lower shore areas did show different changes in the mean size over time. The

lower shore area showed a decrease in mean size between November and February 1999, whilst the upper shore area showed a decrease in mean size between May and July 1999. It could therefore be hypothesised that the larvae settled in the lower shore area first between November and February and then migrated to the upper shore area, causing the decrease in mean size between May and July 1999. The fact that the same pattern does not occur in 2000, only a decrease in the mean size in the upper shore area suggests that the pattern may be entirely due to recruitment. The migration hypothesis can therefore not be proved or disproved by this.

7.3.4 Predation

Predation can affect the size distribution of a benthic invertebrate population through mortality (Masski & Guillou, 1999) which is often size selective, and through reduces the growth rates of individuals (Nakoaka, 2000). Previous studies have revealed that both *Hydrobia ulvae* and *Cerastoderma edule* tend to have high post-settlement mortality rates (Guillou & Tartu, 1994; Udalov *et al.*, 2000). Masski & Guillou (1999) discovered that selective predation on juveniles below 11 mm in length was responsible for more than 85% of the mortality to *C. edule*. The green crab (*Carcinus maenas*) was found to be the most important predator. Jensen (1992b) also found that crabs were the main predators on *C. edule* but only till September, during the winter birds became important predators. At Kinneil *H. ulvae*, *Nephtys hombergii* and *Macoma balthica* all show a large increase in mean size, after a large decrease that can be attributed to recruitment. This suggests that these species have a high post-settlement mortality rate but whether this is caused by predation can not be determined. Birds are present in large numbers at Kinneil over winter and are known to consume large numbers of polychaetes and molluscs (Warnes, 1981; Bryant & McLusky, 1995). Shrimps and fish were however found to have a greater impact (Warnes, 1981; McLusky, 1989), consuming up to 75% of the invertebrate abundance, and are present all year round. Infaunal predators have also been found to have significant effects on infaunal densities in other areas, although this is probably limited to smaller polychaetes, oligochaetes and juvenile bivalves (Ambrose, 1991). It therefore seems likely that predation will have some impact on the density and size distribution of the benthic invertebrate populations, but further investigation is needed to determine its relative importance.

7.3.5 Competition and density effects

Competition, whether intraspecific or interspecific, can cause a reduction in growth or even cause mortality of some benthic invertebrates, therefore affecting the size distribution of a population. Jensen (1992a) and Jensen (1993) found that intraspecific competition was the major cause of low growth rates for *Cerastoderma edule*. Masski & Guillu (1999) however found that intraspecific competition did not influence either the survival or growth of *C. edule*. For *Hydrobia ulvae*, *Macoma balthica* and *C. edule* there has been evidence to suggest that increased population density may inhibit growth and even cause mortality in some cases. Morrisey (1987) discovered that *H. ulvae* had a reduction in growth rate and increased mortality at densities found in natural populations. Population density was also found to affect shell growth in *M. balthica* (Vincent *et al.*, 1994) and *C. edule* (Montaudouin & Bachelet, 1996). Montaudouin & Bachelet (1996) found that density could also affect the survival of *C. edule*, but only for recruits, not for adults. At Kinneil both *H. ulvae* and *M. balthica* had higher densities in the upper shore area than in the lower shore area. It may therefore be expected that due to the lower densities individuals may be larger in the upper shore area. This was not found to be the case for *H. ulvae* which although it showed a significant difference in the mean size between the two area, had a larger mean size in the lower shore area. For *M. balthica* the upper shore area did have a larger mean size but during most of the sampling periods but not during November 1998, July 1999 and July 2000. It is possible that the decreases in mean size during these periods are due to recruitment. It can therefore be concluded that for *H. ulvae* there was no obvious effect of density on growth, whilst for *M. balthica* there was some evidence to indicate that increased density could reduce growth. From this study it can not be determined whether intraspecific competition was causing mortality of individuals but this could potentially explain the high post settlement mortality in some species.

7.4 CONCLUSIONS

There is some evidence to suggest that the petrochemical effluents may have had a sublethal effect on growth or reproduction of the six benthic invertebrate species. *Nereis diversicolor*, *Nephtys hombergii* and *Macoma balthica* consisted of relatively small individuals compared to the sizes documented at other locations. This may be an indication that at Kinneil there is a sublethal effect on growth and/or reproduction or that survivorship is impaired. Only the smaller size of *Hydrobia ulvae* in the upper shore area suggests that there may be spatial size differences within the Kinneil intertidal area. The reduced sizes of individuals could however have been caused by other factors such as availability of food, predation and physical conditions. The fact that there was no change in the size distributions after the movement of the chemical outfall in January 1999 indicates that it is likely that this effluent has not had a detectable sublethal effect on the benthos.

The temporal changes in the size distributions of the six species that were detected can all be explained by natural recruitment, migration and post settlement mortality patterns. A likely recruitment pattern was detected for all species except *Eteone longa* and *Cerastoderma edule*. *Nereis diversicolor*, *Macoma balthica* and *H. ulvae* underwent recruitment between May and July each year, whilst *Nephtys hombergii* had a more variable recruitment period between May and November. Significant post-settlement mortality was detected for *H. ulvae*, *N. hombergii* and *M. balthica*, which was possibly caused by size selective predation from birds, crabs, shrimps and/or fish.

It is therefore concluded that natural temporal and spatial differences in the population size distributions of the six species were detected and there is some indication that the individuals at Kinneil may be stressed. Further investigation is needed to determine the relative importance of pollution, competition, predation, and migration on the population size structure of these species at Kinneil.

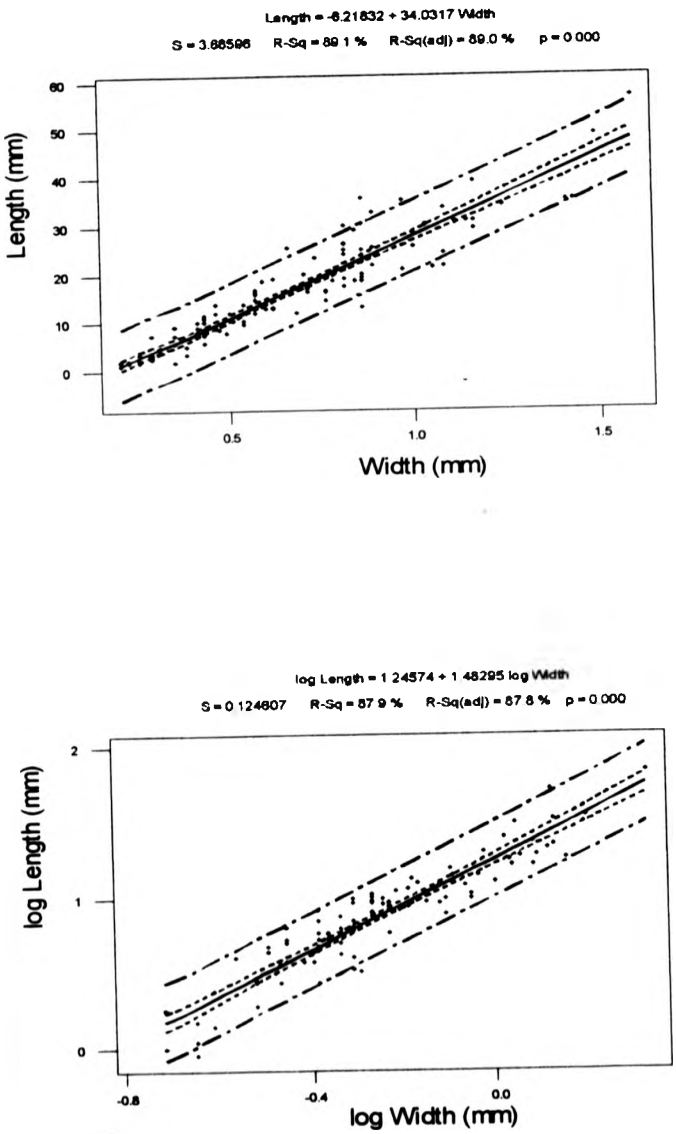


Figure 7.1. Regression plots showing the relationship between Length and Width for *Nephtys hombergii* (Top), *Nereis diversicolor* (Bottom) with the 95% confidence limits (---) and prediction limits (– • –).

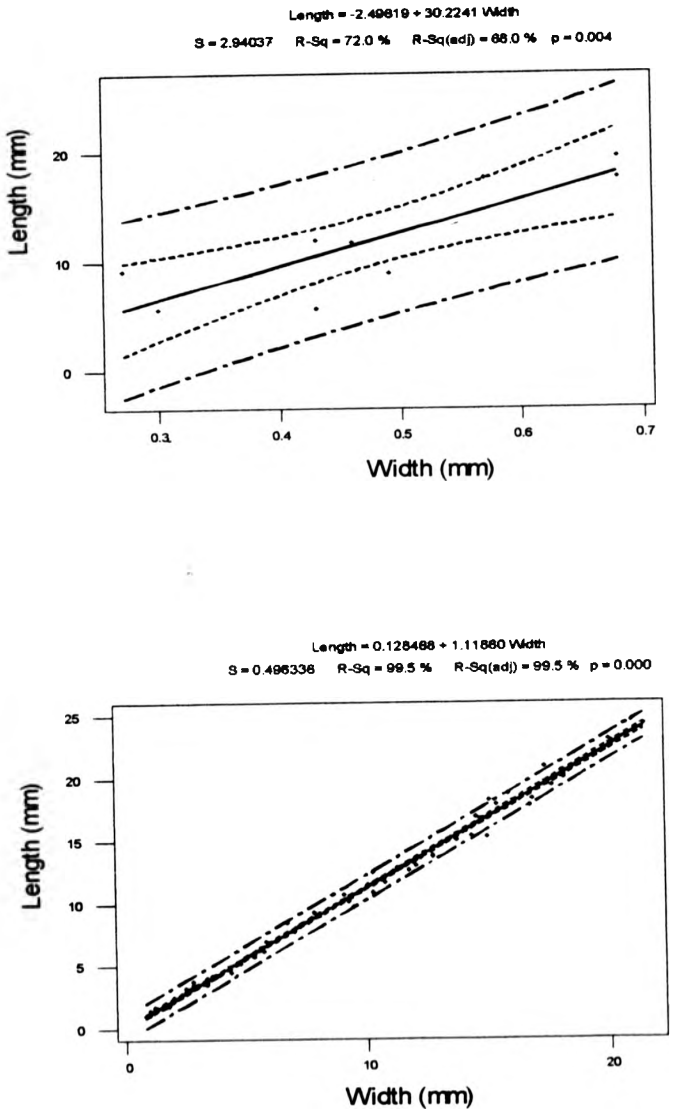


Figure 7.2. Regression plots showing the relationship between Length and Width for *Eteone longa* (Top), *Cerastoderma edule* (Bottom) with the 95% confidence limits (---) and prediction limits (– • –).

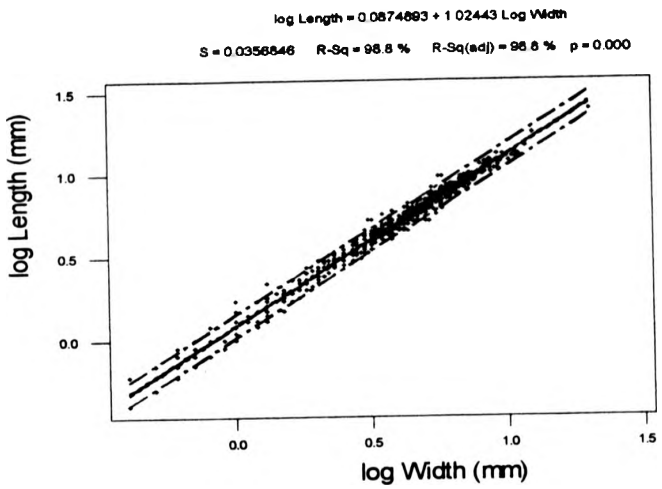


Figure 7.3. Regression plot showing the relationship between length and width for *Macoma balthica* with the 95% confidence limits (---) and prediction limits (– • –).

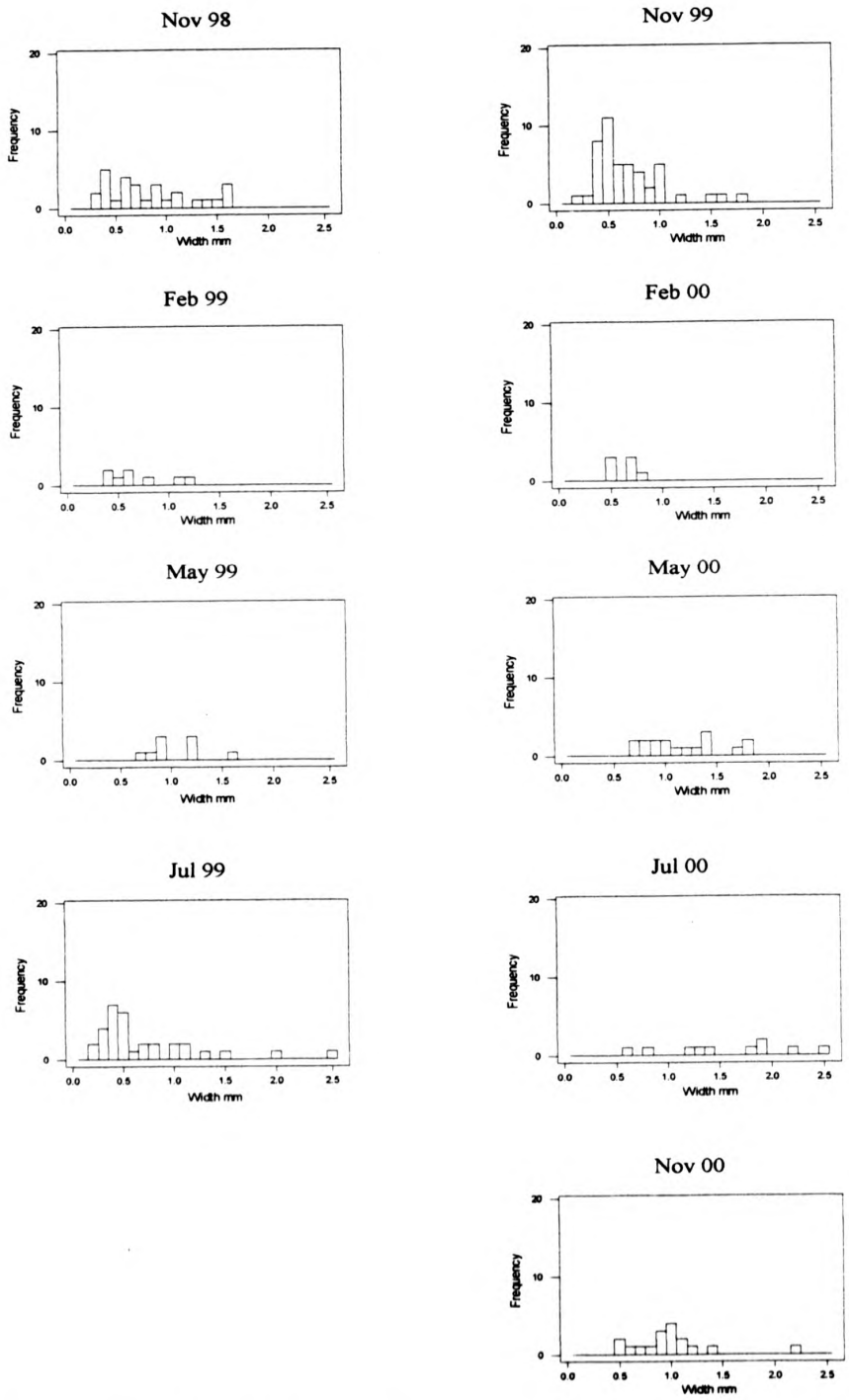


Figure 7.4. Histograms showing the width frequency for the different sampling times for *Nereis diversicolor* in the upper shore area.

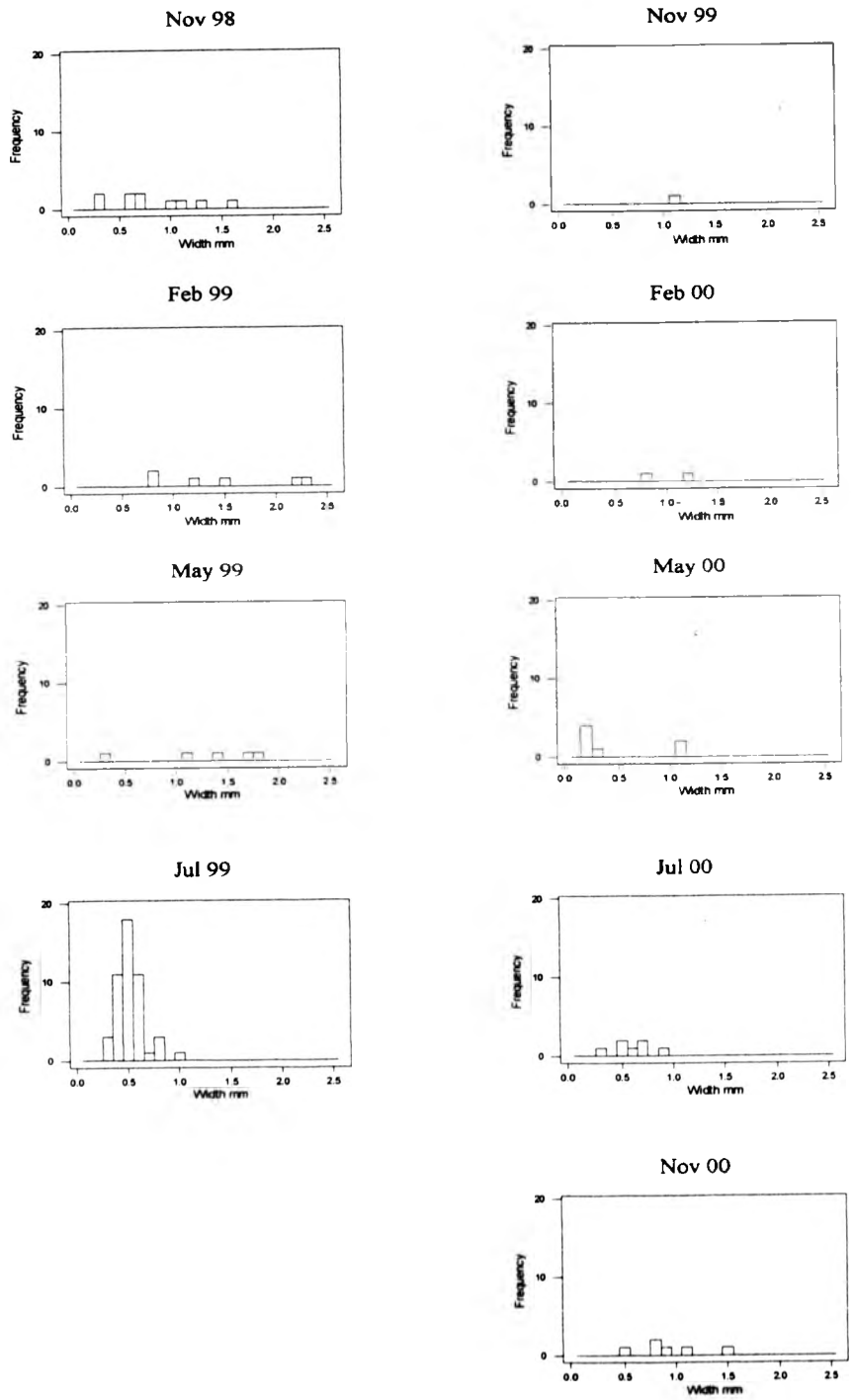


Figure 7.5. Histograms showing the width frequency for the different sampling times for *Nereis diversicolor* in the lower shore area.

Table 7.1. Results of GLM (log width = site | time) for *Nereis diversicolor*.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
site	1	0.49975	0.01553	0.01553	0.39	0.532
time	8	2.10285	1.85652	0.23207	5.86	0.000
site*time	8	2.12417	2.12417	0.26552	6.70	0.000
Error	248	9.82783	9.82783	0.03963		
Total	265	14.55459				

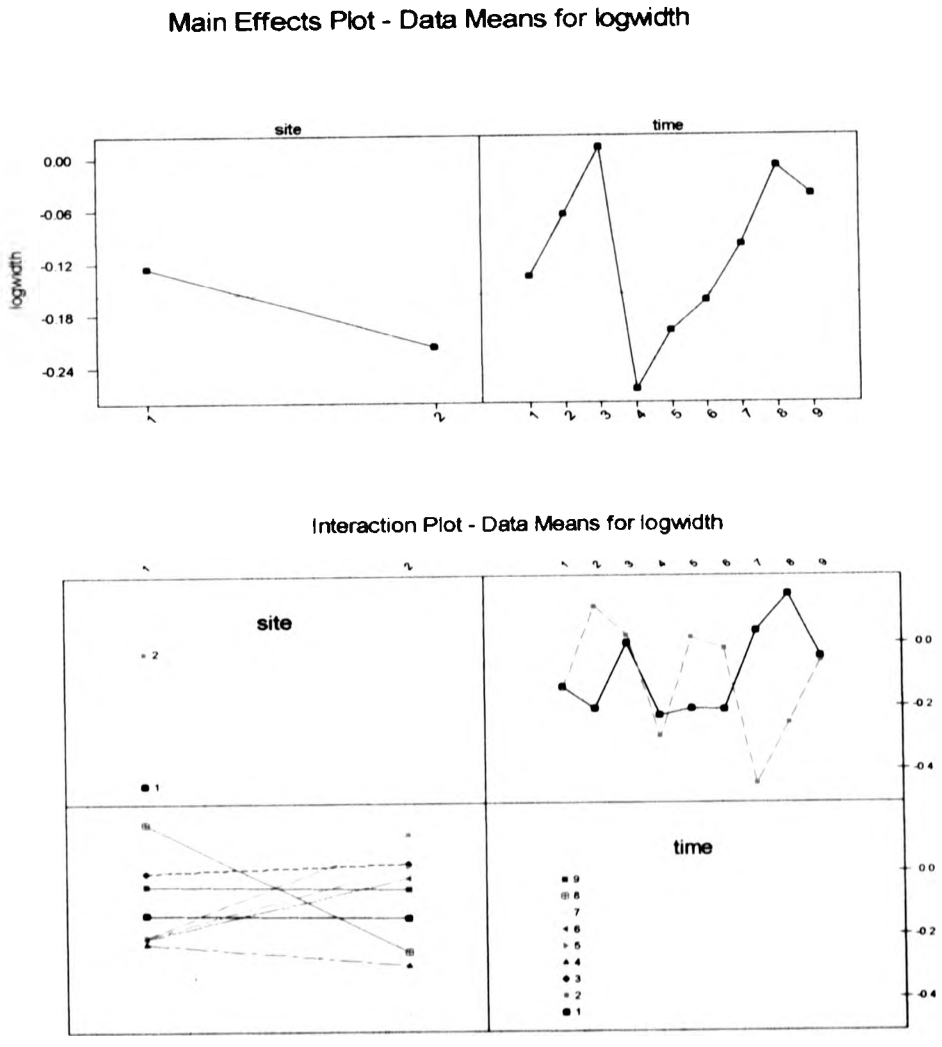


Figure 7.6. Main effects plot for site and time (top) and interaction plot for site*time (bottom) for *Nereis diversicolor* where site 1 = upper shore, site 2 = lower shore, time 1 = November 1998, time 2 = February 1999, time 3 = May 1999, time 4 = July 1999, time 5 = November 2999, time 6 = February 2000, time 7 = May 2000, time 8 = July 2000, time 9 = November 2000.

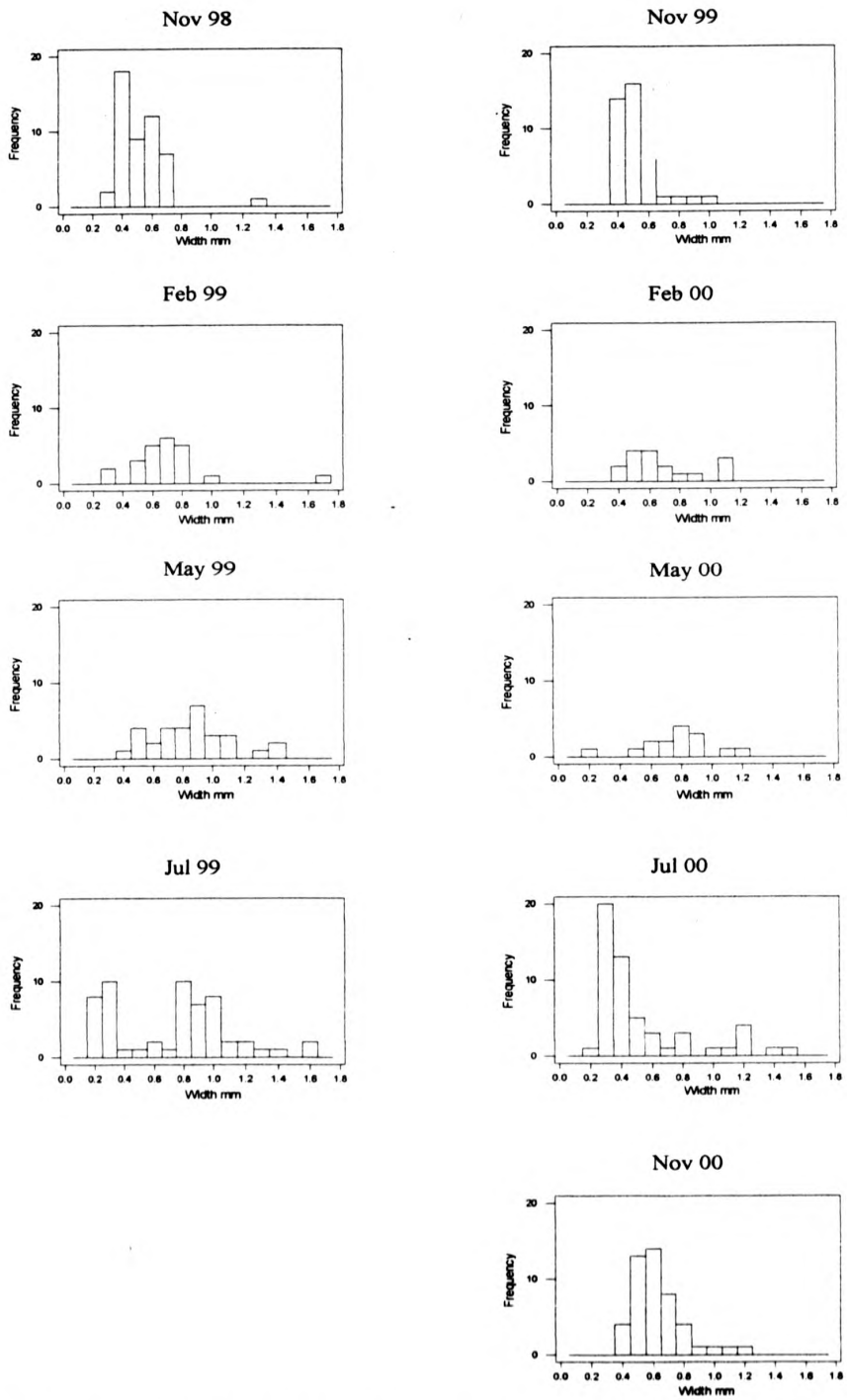


Figure 7.7. Histograms showing the width frequency for the different sampling times for *Nephtys hombergii* in the lower shore area.

Table 7.2. Results of GLM (log width = time) for *Nephtys hombergii*.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	8	1.76442	1.76442	0.22055	7.08	0.000
Error	325	10.11827	10.11827	0.03113		
Total	333	11.88269				

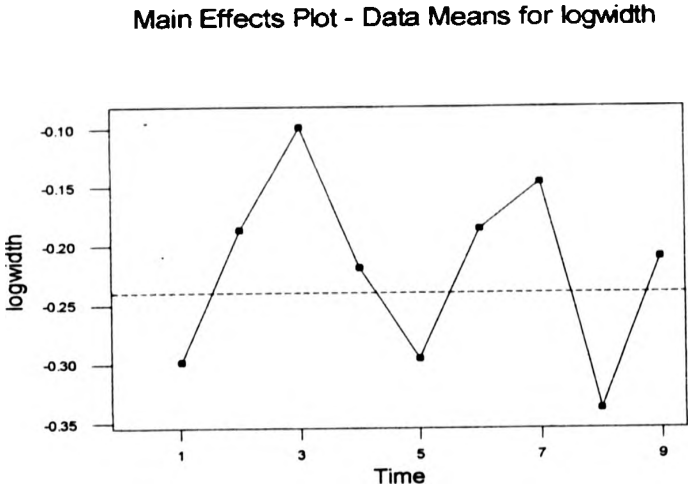


Figure 7.8. Main effects plot for time for *Nephtys hombergii* where time 1 = November 1998, time 2 = February 1999, time 3 = May 1999, time 4 = July 1999, time 5 = November 2999, time 6 = February 2000, time 7 = May 2000, time 8 = July 2000, time 9 = November 2000.

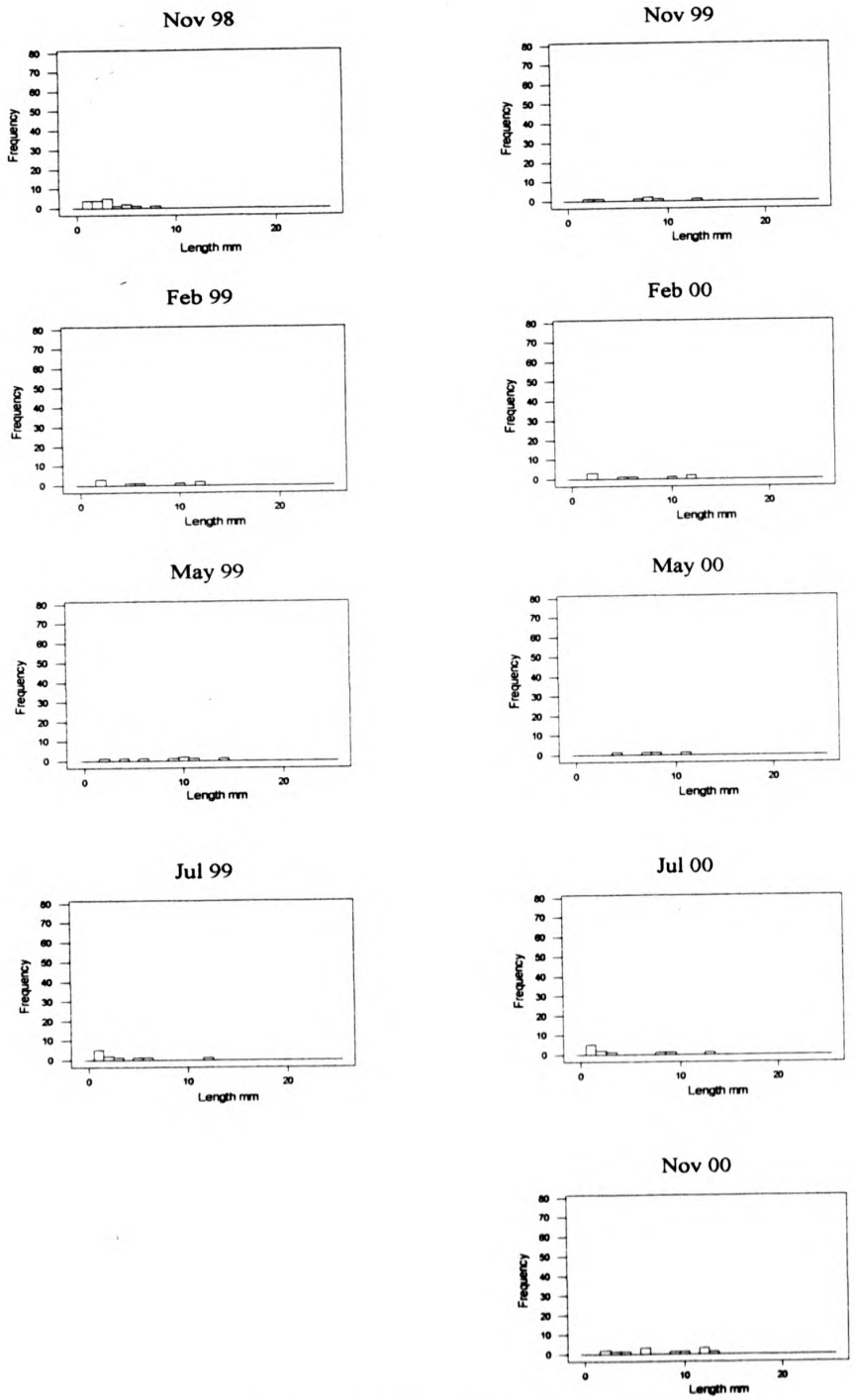


Figure 7.9. Histograms showing the length frequency for the different sampling times for *Macoma balthica* in the upper shore area.

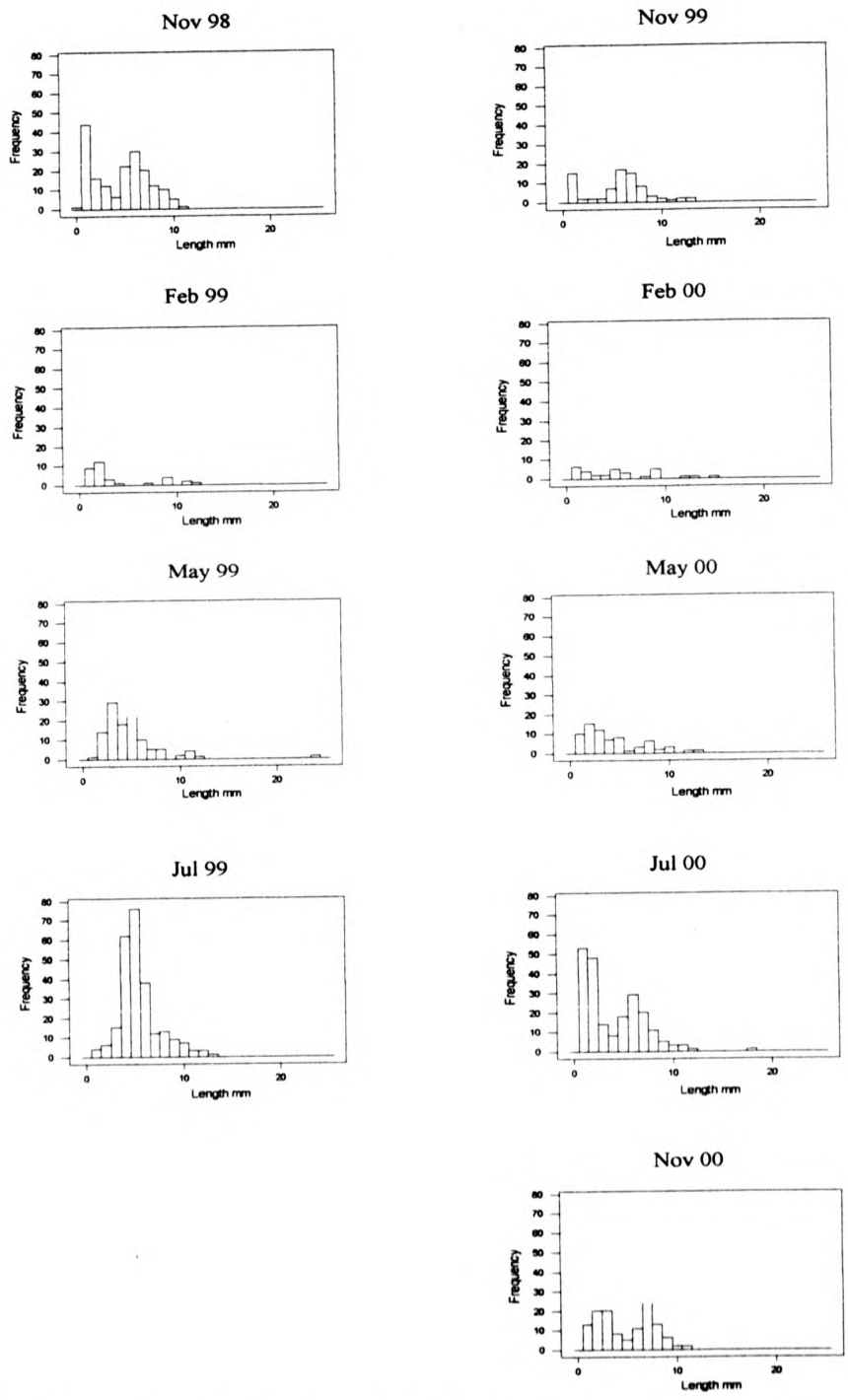


Figure 7.10. Histograms showing the length frequency for the different sampling times for *Macoma balthica* in the lower shore area.

Table 7.3. Results of GLM (log width = site | time) for *Macoma balthica*.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
site	1	0.0044	0.2041	0.2041	2.00	0.158
time	8	10.2640	4.3856	0.5482	5.37	0.000
site*time	8	3.4322	3.4322	0.4290	4.20	0.000
Error	1160	118.5050	118.5050	0.1022		
Total	1177	132.2057				

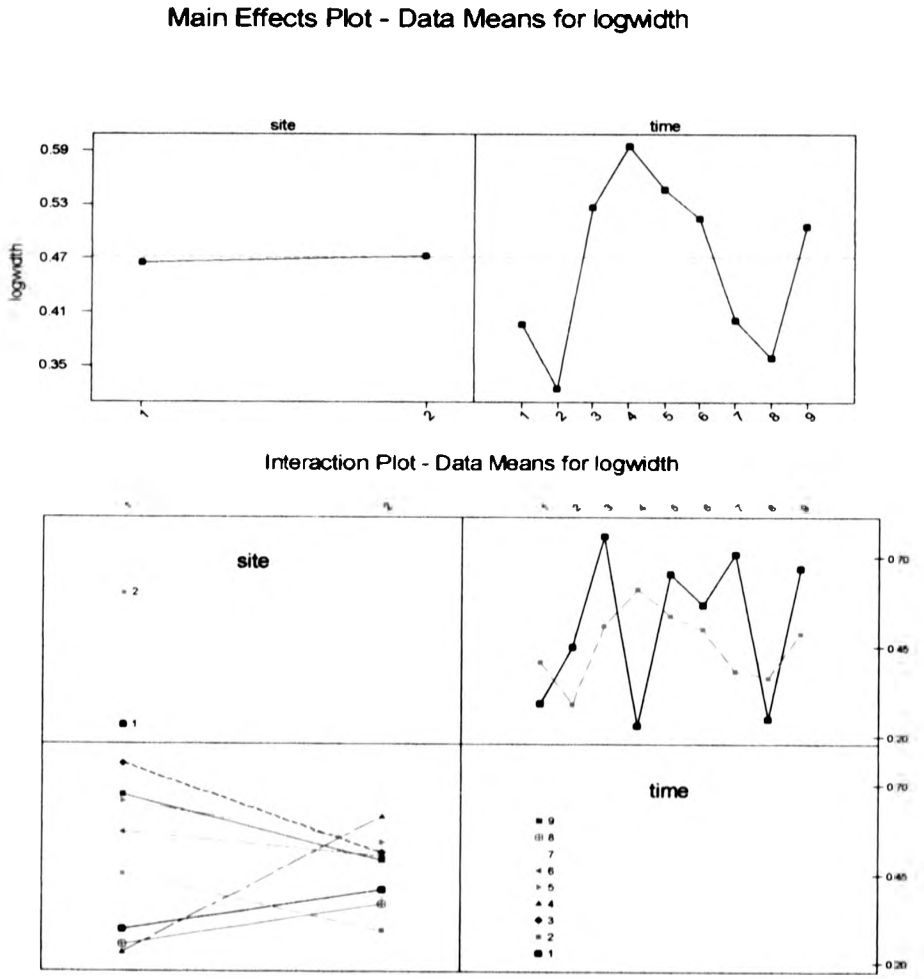


Figure 7.11. Main effects plot for site and time (top) and interaction plot for site*time (bottom) for *Macoma balthica* where site 1 = upper shore, site 2 = lower shore, time 1 = November 1998, time 2 = February 1999, time 3 = May 1999, time 4 = July 1999, time 5 = November 2999, time 6 = February 2000, time 7 = May 2000, time 8 = July 2000, time 9 = November 2000.

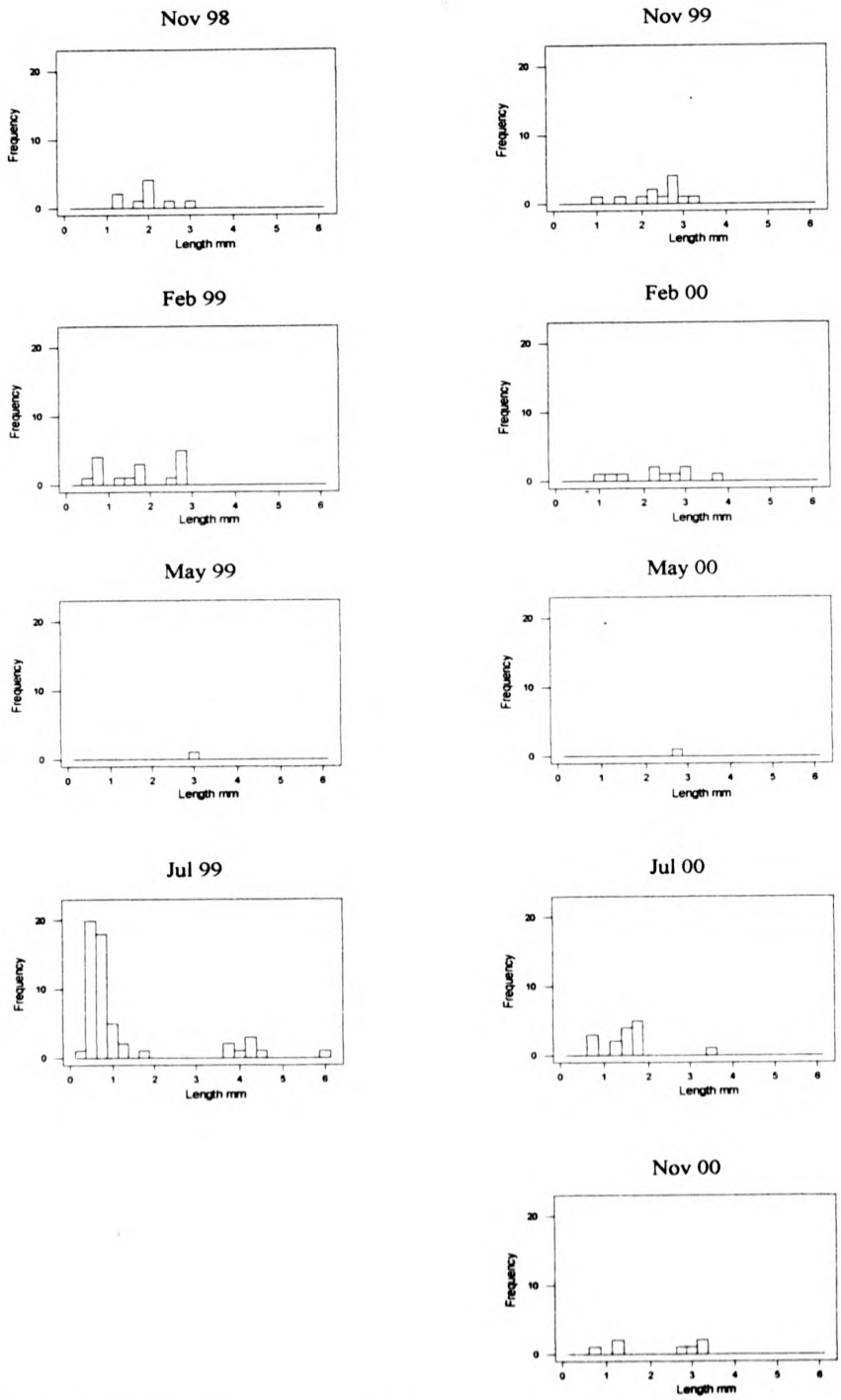


Figure 7.12. Histograms showing the length frequency for the different sampling times for *Hydrobia ulvae* in the upper shore area.

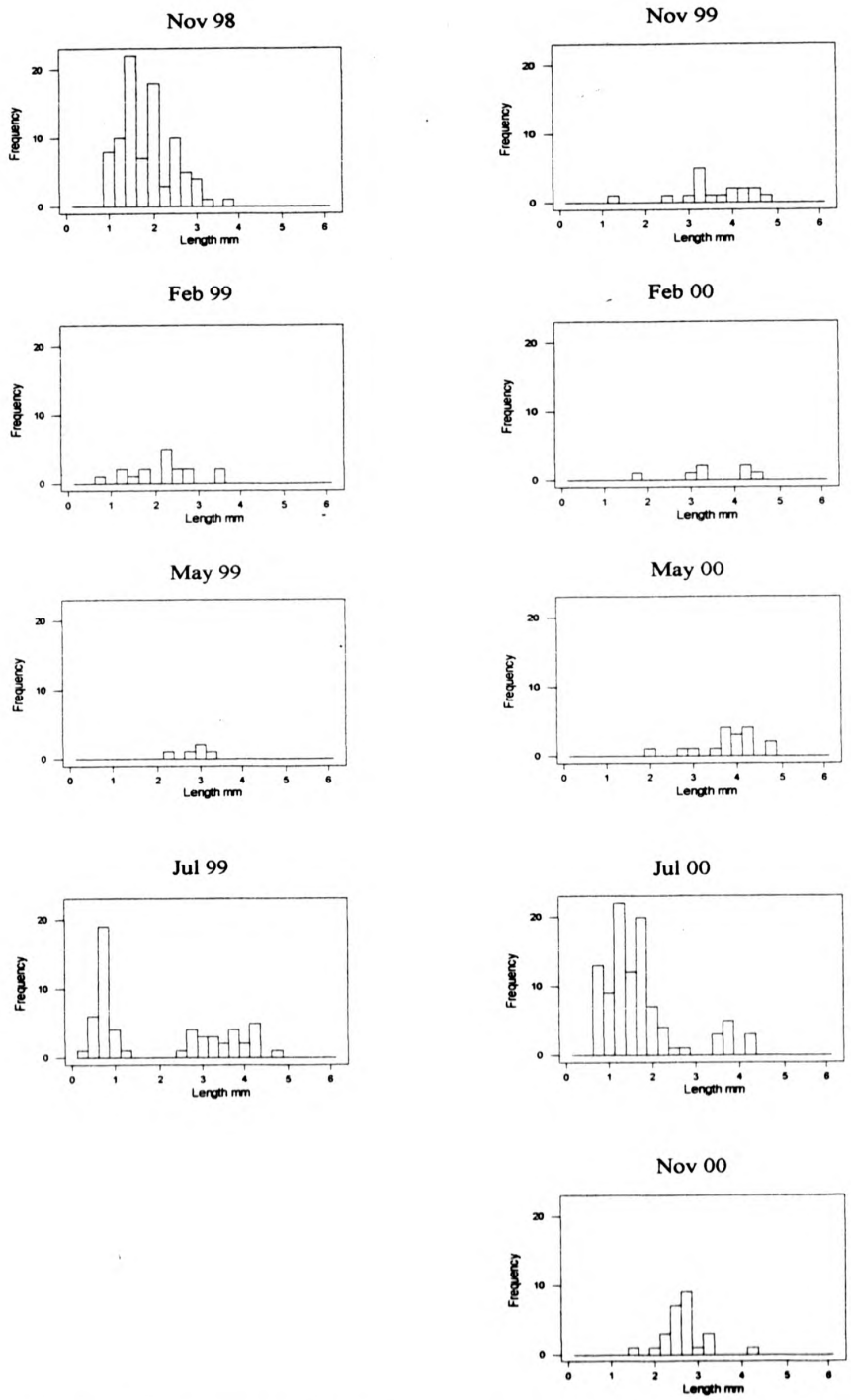


Figure 7.13. Histograms showing the length frequency for the different sampling times for *Hydrobia ulvae* in the lower shore area.

Table 7.4. Results of GLM (log length = site | time) for *Hydrobia ulvae*.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
site	1	2.03771	0.28472	0.28472	5.84	0.016
time	8	8.41320	6.33350	0.79169	16.23	0.000
site*time	8	0.64071	0.64071	0.08009	1.64	0.111
Error	442	21.55598	21.55598	0.04877		
Total	459	32.64760				

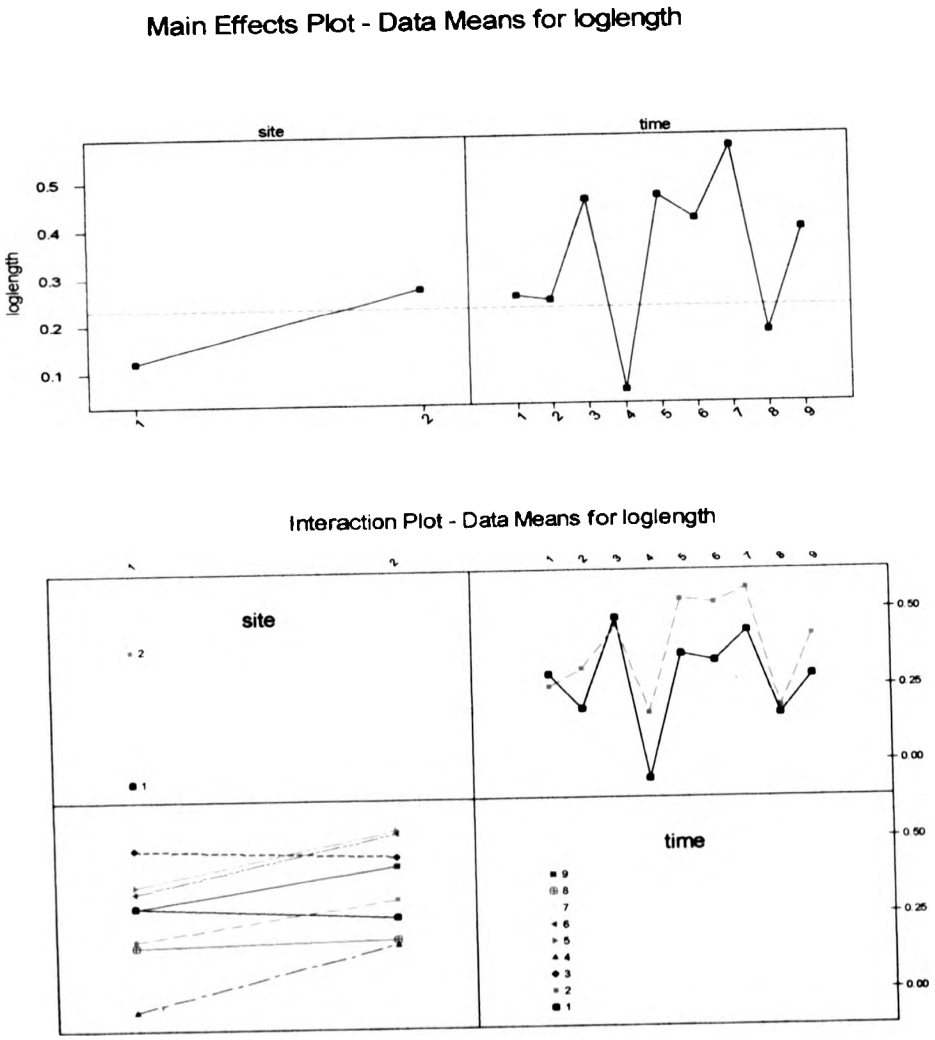


Figure 7.14. Main effects plot for site and time (top) and interaction plot for site*time (bottom) for *Hydrobia ulvae* where site 1 = upper shore, site 2 = lower shore, time 1 = November 1998, time 2 = February 1999, time 3 = May 1999, time 4 = July 1999, time 5 = November 2999, time 6 = February 2000, time 7 = May 2000, time 8 = July 2000, time 9 = November 2000.

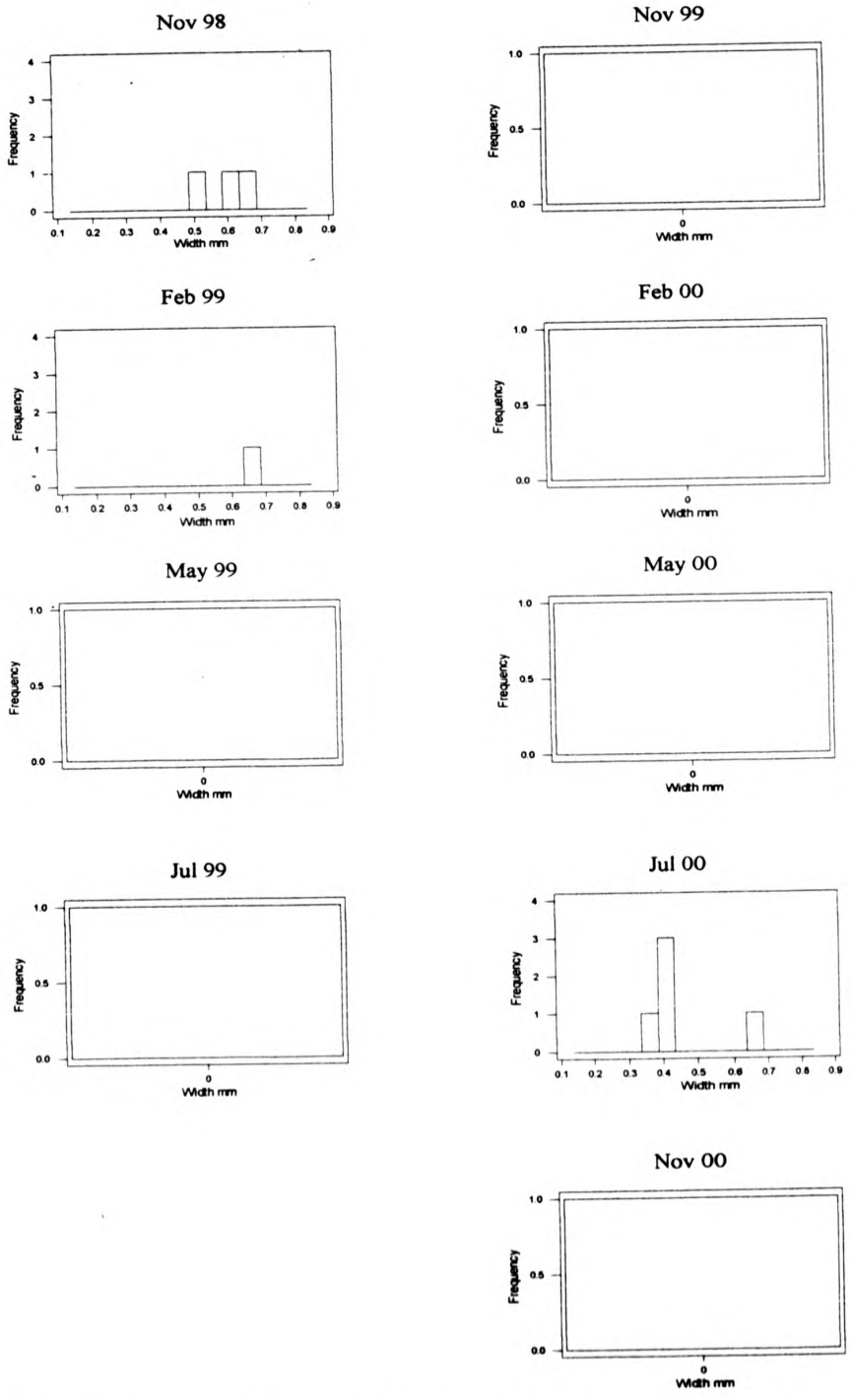


Figure 7.15. Histograms showing the width frequency for *Eteone longa* for the different sampling times in the upper shore area.

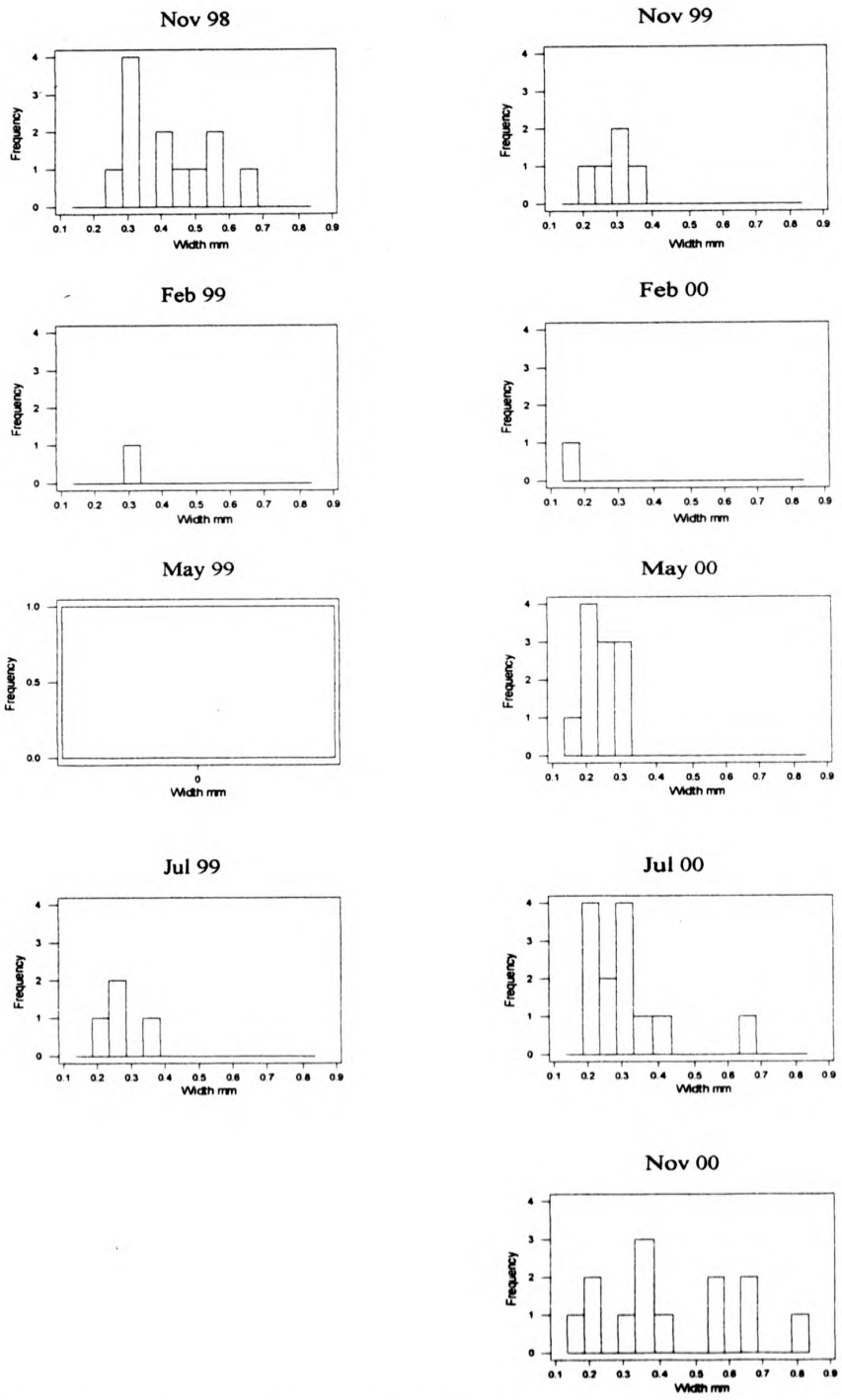


Figure 7.16. Histograms showing the width frequency for *Eteone longa* for the different sampling times in the lower shore area.

Table 7.5. Results of GLM (log width = site time) for *Eteone longa*.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
site	1	0.46280	0.25994	0.25994	12.56	0.001
time	7	0.60388	0.60388	0.08627	4.17	0.001
Error	63	1.30364	1.30364	0.02069		
Total	71	2.37033				

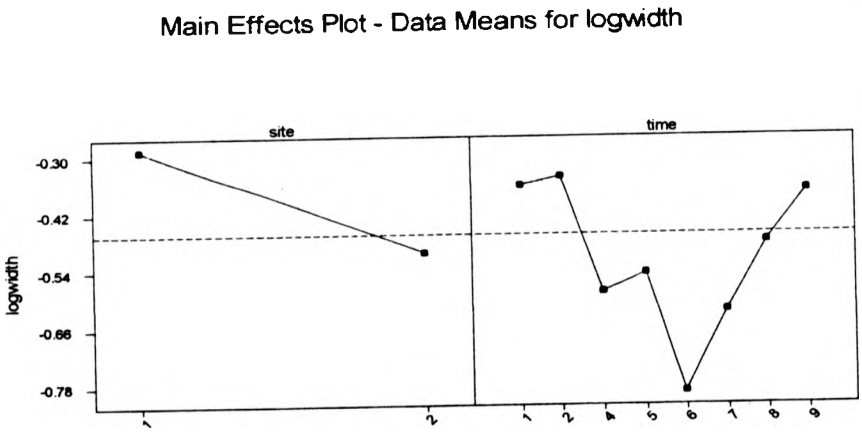


Figure 7.17. Main effects plot for site and time for *Eteone longa* where site 1 = upper shore, site 2 = lower shore, time 1 = November 1998, time 2 = February 1999, time 3 = May 1999, time 4 = July 1999, time 5 = November 2999, time 6 = February 2000, time 7 = May 2000, time 8 = July 2000, time 9 = November 2000.

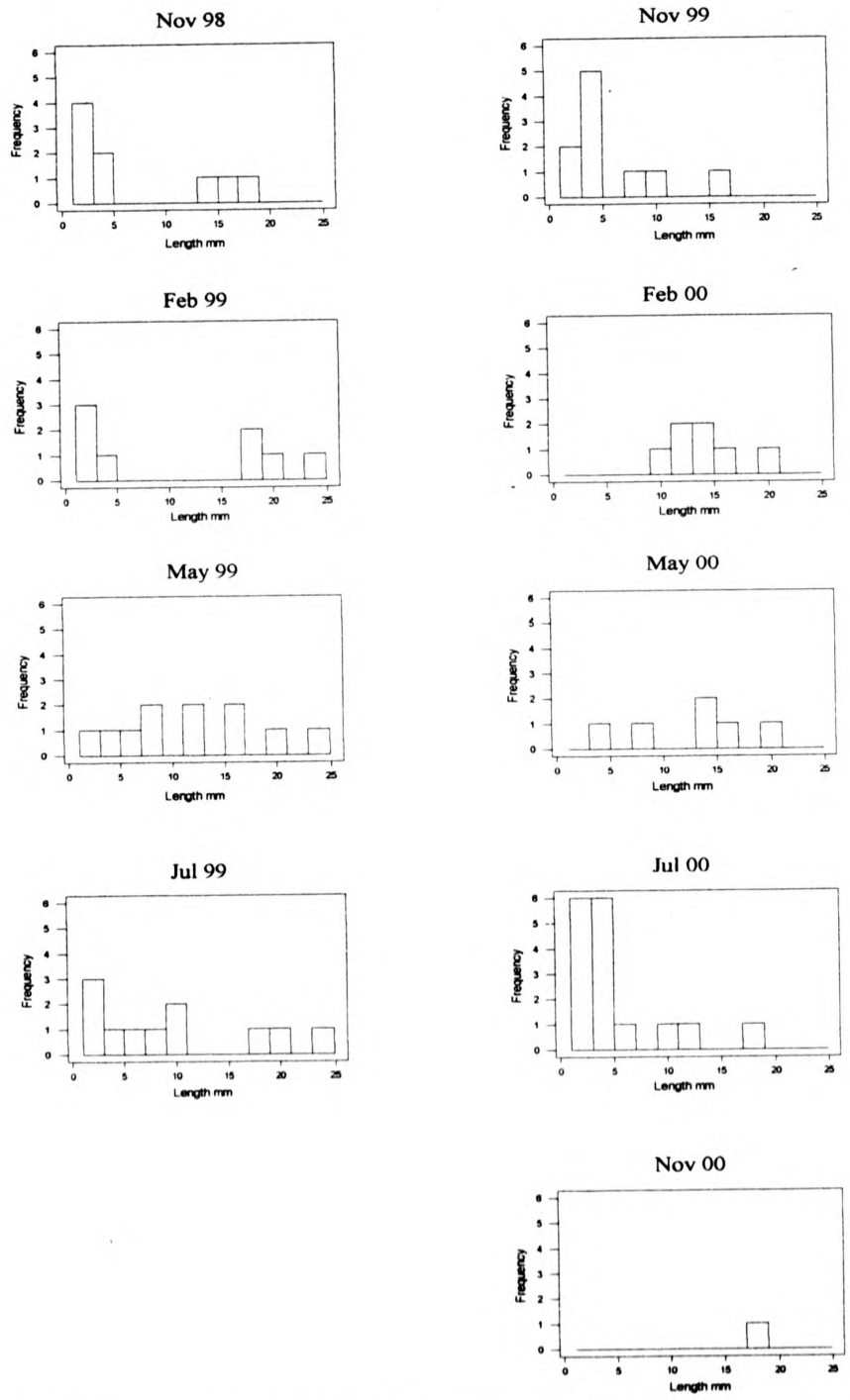


Figure 7.18. Histograms showing the length frequencies for the different sampling times for *Cerastoderma edule* in the lower shore area.

Table 7.6. Results of GLM (log width = time) for *Cerastoderma edule.*

Source	DF	Seq SS	Adj SS	Adj MS	F	P
time	8	3.5216	3.5216	0.4402	2.94	0.007
Error	70	10.4781	10.4781	0.1497		
Total	78	13.9997				

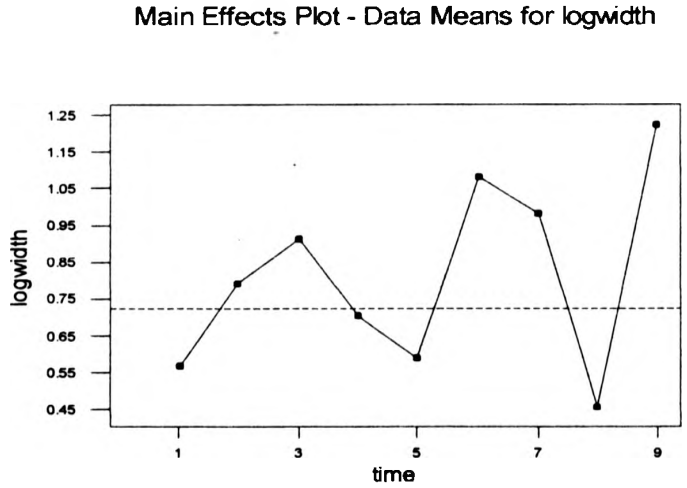


Figure 7.19. Main effects plot for time for *Cerastoderma edule* where site 1 = upper shore, site 2 = lower shore, time 1 = November 1998, time 2 = February 1999, time 3 = May 1999, time 4 = July 1999, time 5 = November 2999, time 6 = February 2000, time 7 = May 2000, time 8 = July 2000, time 9 = November 2000.

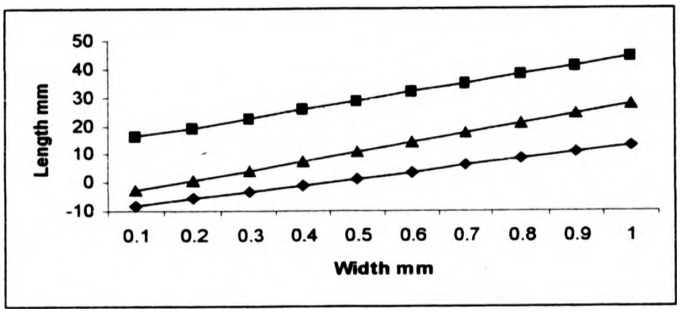


Figure 7.20. The predicted regression lines for *Nephtys hombergii* from this study - Length = -6.21832 + 34.0317 width (Triangle), from Olive (1977) - Length = 13.18 + 31.15 width (Square) and from Rainer (1991) - Length = -0.444 + 0.0423/width (Diamond).

8. GENERAL DISCUSSION

8.1 DISCUSSION

There are many different factors that can affect spatial and temporal community distributions. Previous studies at Kinneil have found that shore height and pollution from the petrochemical complex (McLusky, 1982a; Moore *et al.*, 1987; McLusky & McCrory, 1989, McLusky & Martins, 1998) were important factors affecting the distribution of the macrofauna and meiofauna. Other factors which could also have an effect are sediment type (Gray, 1974), competition (Wilson, 1991), predation (Thrush, 1991), the availability of food (Pearson & Rosenberg, 1987), other pollution sources, the climate (De Jong, 1999) and recruitment (Hughes, 1990).

8.1.1 Effect of pollution

Kinneil is subjected to pollution from many different sources including petrochemical effluents, two rivers, the main estuary channel and a sewage works. Pollution can be considered as a form of physical disturbance and therefore could potentially be an important factor controlling the spatial and temporal community composition (Connell, 1978). The intermediate disturbance hypothesis (Connell, 1978) states that diversity is highest at intermediate levels of disturbance and diversity is low at low and high levels of disturbance. Therefore if the pollution sources at Kinneil were causing a disturbance effect then the diversity would be low close to the source, it would increase with distance from the source but then would show a subsequent decrease. If the severity of the disturbance was changed then a similar temporal pattern would been seen, with a decrease in the severity the diversity would increase over time. Pearson & Rosenberg (1978) described the successional change of a community subjected to organic pollution, in terms of the relationship between the species, abundance and biomass. This species abundance biomass (SAB) relationship shows an increase in the number of species, abundance and biomass with increasing distance from the source or increasing time from the disturbance. It has four distinct phases, which are related to the level of disturbance and therefore the successional state that is reached. Firstly there is a peak of

opportunists, where the abundance reaches its maximum, caused by a large number of small opportunistic species. After this is the ectone point, where there has been a decrease in the abundance of the opportunistic species and new larger non-opportunist species appear. This causes an increase in the number of species, very little change in the biomass but a decrease in abundance. This develops into a transitory community that has a greater number of species and larger individuals. Finally a 'mature community' develops that has an intermediate abundance, biomass and number of species. Therefore if the pollution sources at Kinneil were causing an enrichment effect a peak of opportunists would probably be seen along with an initial increase in abundance, species richness and biomass.

8.1.1.1 Spatial effect of the petrochemical effluents

8.1.1.1.1 Refinery effluent

Many studies looking at the effects of refinery effluents have shown that they have an adverse effect on the marine environment, by reducing the diversity and species richness (Wharfe, 1975; Petiproon & Dicks, 1982; Saha & Konar, 1984a; Talsi, 1987; Dicks & Levell, 1989). Often it is a small area close to the refinery outfall that is found to be grossly impacted, with a gradual reduction in impact with increasing distance from the source (Wharfe, 1975; Petiproon & Dicks, 1982; Houston *et al.*, 1983; Saha & Konar, 1984a). This suggests that the intermediate disturbance hypothesis and/or the SAB relationship can explain the impact from refinery effluents. Previous studies at Kinneil have detected this same spatial distribution in the community in relation to the distance from the refinery outfall (McLusky, 1982a). The present study detected the same pattern, with the lowest diversity, abundance and biomass identified close to the refinery outfall in the inner corner area and an increase in species abundance and biomass with distance away from that area. The area of the Kinneil mudflat that was furthest away from the refinery outfall was the least impacted area. Large numbers of opportunistic oligochaetes were detected in the inner corner during certain months, which may be an indication of an enrichment effect of the refinery effluent. Scott (1982) also found that Oligochaetes, Annelids and *Nereis diversicolor* seemed to thrive in the area subjected to effluent discharge, whilst other species such as *Macoma balthica*,

Hydrobia ulvae and *Cerastoderma edule* had a lower than average biomass at Kinneil. It can therefore be concluded that the effect of the refinery effluent does conform to the intermediate disturbance hypothesis like most areas elsewhere subjected to these effluents. It suggests that the refinery effluent may have an enrichment effect that is enhancing the number of individuals, as well as a toxic effect that is reducing the diversity close to the outfall.

8.1.1.1.2 Chemical effluent

The chemical effluent contains many of the same chemicals as the refinery effluent and therefore also has the potential to cause both toxic and/or enrichment effects. Earlier toxicity tests have shown the chemical effluent to be more toxic than the refinery effluent to the benthic invertebrates at Kinneil (Smith, 1987). The volume of the chemical effluent that is discharged at Kinneil is however smaller than the refinery effluent. The present study has shown that the chemical effluent did have an impact on the spatial distribution of the benthic community but only during the beginning of the long-term analysis period. The present findings were consistent with the findings of McLusky (1982a) and McLusky & McCrory (1989), in that the area around the chemical effluent had a low diversity. A peak of opportunists was also observed that could be attributed to the effects of the chemical effluent. This indicates that the chemical effluent was important in structuring the community during the beginning of the long-term survey period but has not been important in more recent years. The effects of the chemical effluent conformed to the successional pattern proposed by Person & Rosenberg (1978), suggesting that it may have had an enrichment effect.

8.1.1.1.3 Sediment hydrocarbon content

The sediments act as a sink for hydrocarbons and other chemicals such as heavy metals that are discharged in the petrochemical effluents (Knap & Willams, 1982; Davies, 1987; Elliott & Griffiths, 1987; Talsi, 1987; Harrison, 1996). Hydrocarbons are known to be toxic to many marine benthic invertebrates, although the different hydrocarbon compounds have different toxicities with different species (Cote, 1976; Shaw *et al.*, 1976; Hall *et al.*, 1978; Tatem *et al.*, 1978; Reece & Burks, 1985; Das & Konar, 1988; Kennish, 1991; Harrison, 1996). Anderson (1979) and Straughan (1977) identified crustaceans as being the most sensitive organisms to petroleum

hydrocarbons. Studies that have investigated the hydrocarbon concentrations around refinery outfalls have found that the highest concentrations are close to the outfall and decrease with increasing distance (Knap *et al.*, 1982; Houston *et al.*, 1983; Armannsson *et al.*, 1985; Talsi, 1987; le Dreau *et al.*, 1997). Previous sediment analysis for hydrocarbon concentrations at Kinneil have found high levels close to the refinery outfall, which originated from a petrogenic source (Ajayi & Poxton, 1987; Mohd-Long, 1987; Cranthorne *et al.*, 1989). The spatial distribution of the hydrocarbons within the sediment may therefore affect the community composition through individual species distributions (Houston *et al.*, 1983). The present study found that the concentration of petrogenic hydrocarbons was greatest nearest to the refinery outfall and that there was a fresh petrogenic source. Compared to other areas of the Forth estuary the concentrations that were detected in the sediments at Kinneil were high (Elliott & Griffiths, 1987). Cranthorne *et al.* (1989) found high levels of hydrocarbons that originated from plants which the present study also detected in certain areas at Kinneil. The sediment hydrocarbon concentration at Kinneil was found to be related to the diversity, which suggests that it may have a toxic effect that is determining the species that are able to survive at the high concentrations close to the refinery outfall. It can therefore be concluded that although the concentration of the hydrocarbons that are discharged within the refinery effluent have been reduced substantially, they are still present at high levels within the sediment close to the refinery outfall. It seems probable that the high concentrations of hydrocarbons within this area will have had an effect on the community composition, limiting the species that can survive in this area. The impact from hydrocarbons within the sediments is likely to remain for many years as microbial degradation is much slower in sediments than in the water column (Carlberg, 1980).

8.1.1.2 Effect of the reduction in the toxicity of the petrochemical effluents

Dicks (1976a), Leppakoski & Lindstrom (1978) and Dicks & Levell (1989) have documented the recovery of areas that have been subjected to oil refinery effluents. In these areas either a reduction in the toxicity of the effluent or the removal of the effluent discharge was observed. Recolonisation of denuded areas and an increase in the species diversity were seen. An overall increase in diversity, as well as the

recolonisation of the previously afaunal inner corner area, at Kinneil was documented by McLusky and Martins (1998) for the period 1976 to 1997. At Kinneil there have been several reductions in the toxicity of both the refinery and chemical effluents since the area was first monitored in 1976. During the 1980s the chemical effluents toxicity was gradually reduced as old plants were closed and new more efficient plants were opened. The toxicity of the refinery effluent was dramatically reduced in 1994 when a biological treatment plant was installed. The present analysis of the community data at Kinneil since 1976 indicated that the increase in diversity that was detected by McLusky & Martins (1998) continued until the end of the monitoring in 1999. The reductions in the toxicity of both the chemical and refinery effluents were identified as the likely causes for the increase in the diversity. This increase was widespread and occurred across the whole of the mudflat, therefore indicating that the petrochemical effluents were affecting the whole area. In recent years the level of impact has therefore reduced and the majority of Kinneil now has a similar community composition. Only the inner corner area, close to the refinery outfall still has a low diversity and number of species when compared to the rest of Kinneil. It can therefore be concluded that the reduction in the toxicity of the effluents at Kinneil has allowed the recovery of the majority of the mudflat in a manner consistent with other similar areas.

8.1.1.3 Effect of the movement of the chemical outfall

The chemical outfall has been moved twice since 1976. It was first moved in 1979 from the River Avon site to another upper shore site closer to the refinery outfall. The impact of the movement of this outfall on the benthic community at Kinneil was assessed by McLusky (1982a) and it was found that the area around the old outfall showed signs of recovery, whilst around the new outfall there was a decline in the diversity. This was also detected in the long-term analysis in the present study. It was also noticed that a new species, *Manayunkia aestuarina* was first found during 1979 co-incident to the movement of the outfall at that time. The second movement of the chemical outfall occurred in January 1999. Unlike the first movement, the present two-year survey data did not detect any change to either the area around the new or the old outfalls that could be attributed to the petrochemical effluents. The new lower shore outfall was designed not to cause any impact to the

area around the new outfall, but it was expected that the area around the old outfall would show signs of recovery. There are however several possible reasons why no improvement to this area was seen (See 6.4).

8.1.1.4 Sublethal effects of the petrochemical effluents

Sublethal effects of refinery effluents and its components have often been detected, including effects on growth (Buikema *et al.*, 1981; Rowe *et al.*, 1983b; Sherry *et al.*, 1994), reproduction (Buikema *et al.*, 1981; Rowe *et al.*, 1983a) and respiration (Saha & Konar, 1984a). Previously Yule (1996) found that both *Macoma balthica* and *Hydrobia ulvae* had smaller individuals in the inner corner area, which was attributed to the effects of the petrochemical effluents. Although differences between the upper and lower shore areas and over time were detected in the present study these differences could be explained through natural variations in recruitment, migration and predation. There was however some evidence to suggest that *Nereis diversicolor*, *Nephtys hombergii* and *Macoma balthica* may be stressed, due to their relatively small size at Kinneil compared to other locations.

8.1.1.5 Spatial and temporal effects of the other pollution sources

The two rivers, the main channel and the sewage works could potentially affect the spatial distribution of the community through pollution effects. The rivers and main channel receive sewage wastes that are organically rich. All these pollution sources may therefore be expected to produce enrichment effects such as those seen around other organic effluent discharges (Dauer & Conner, 1980; Anderlini & Wear, 1992; Simboura *et al.*, 1995). These enrichment effects generally take the pattern of the SAB relationship described by Pearson & Rosenberg (1978). The impact of these other potential pollution sources at Kinneil had not previously been considered. The present study has shown that generally these sources are not important factors in controlling the distribution of the species over the entire mudflat. Changes in the community composition did occur close to the River Avon and Grange Burn during certain years which may have been caused by changes in the water quality of these pollution sources, but this could not be proved. It can therefore be concluded that there is some evidence to suggest that the two rivers may have had localised effects on the community composition but only during certain time periods.

8.1.1.6 Present level of pollution impact at Kinneil

Although an improvement in the health of the benthic community at Kinneil has been identified, by the increase in diversity, evenness and species richness, it is not known how the present condition of Kinneil compares to that of other intertidal estuarine mudflats. To determine the level of impact of pollution Kinneil is presently subjected to, the area has been compared to a variety of different estuarine mudflats that experience a range of different pollution levels (Table 8.1). The water quality levels were based on the Environment Agency's estuarine water quality Classification Scheme of bad, poor, fair and good, where very low is comparable to bad, low to poor, moderate to fair and high to good. The classification of each site was determined using data from the Environment Agency (Environment Agency, 2002) and from the quoted data sources (Table 8.1). The full list of species abundance data for each location is given in Appendix 3. It should be noted that the number of species at each site are not necessary directly comparable, as they may include a small error due to the differences in the size of area sampled at each location.

The MDS plot indicates that the areas with good water quality such as the Tamar, Eden and Loughor estuaries have similar community compositions (Figure 8.1). The bubble plots (Figure 8.2) indicate that all these locations have a relatively high diversity and richness but a low number of individuals. The majority of the locations that are subjected to bad water quality conditions have similar community compositions to each other, including Kinneil in 1978. The bubble plots for diversity, evenness, species richness and number of individuals indicate that these locations tend to have low values in each case. The exceptions are the two Clyde Estuary locations that have very high numbers of individuals, which is probably indicative of local enrichment at these sites. The Solway Firth locations both tend to have different community compositions to all the other locations. They have a low diversity, species richness and number of individuals, like the impacted areas, but have a different species composition. This difference could be attributed to the sampling method, in that a larger sieve mesh size was used which would mean that the smaller species, such as oligochaetes, would be considerably underestimated.

Kinneil in 1999 had a similar community composition to that of the Montrose Basin and other mudflats from the Forth estuary; Torry Bay and Skinflats. These all tend to have a relatively high diversity and species richness and an intermediate number of individuals, although Kinneil had the largest number apart from the two Clyde locations. The difference between these locations and those from the "good water quality" conditions is in the specific species involved. The locations from the good water quality areas tended to have lower numbers of oligochaetes and/ or *Manayunkia aestuarina*. This suggests that the Forth and Montrose intertidal areas are impacted to some level when compared to other estuarine intertidal areas. It can therefore be concluded that the Kinneil intertidal area, although having shown a clear decrease in the level of impact, is still impacted to some degree.

Compared to the other mudflats on the Forth, Kinneil has a large number of individuals. This could be an indication of a slight enrichment effect, however it is probably due to the fact that Kinneil was sampled at a different time of year. Torry bay and Skinflats were both sampled during November when species abundances will be at there lowest, whilst Kinneil was sampled during July when abundances will be high due to recruitment. The present short-term study did indicate that the number of individuals at Kinneil did show a seasonal fluctuation, with a decrease during the winter and an increase during the summer. The values that were found for the winter period are around 20,000 individuals per m^{-2} which is comparable to the values for Torry Bay (28780 individuals per m^{-2}) and Skinflats (19531 individuals per m^{-2}). The fact that all the mudflats on the Forth estuary, including Kinneil, now have a similar species composition suggests that the estuary as a whole can be considered as moderately impacted compared to other estuaries, and that local influences from the petrochemical effluents at Kinneil are not detectable.

8.1.2 Effect of shore height

The effect of shore height is related to the immersion time and therefore the stress induced by desiccation effects. Stress will be greater in the upper shore area than in the lower shore area and therefore only those species that can tolerate the higher stress in the upper shore area will be able to survive there. The species diversity is

therefore likely to be lower in the upper shore area due to the smaller number of species which have the physiological adaptations, behavioural characteristics or spatiotemporal recruitment patterns necessary to overcome the stressful conditions (Dauer & Ranasinghe, 1992). The species that are found in such areas are often opportunistic species, which are species whose reproductive and growth characteristics allow them to take advantage of changes in the environment (Pearson & Rosenberg, 1978). If shore height was an important factor determining the spatial community distribution at Kinneil then it would be expected that the diversity would be lowest in the upper shore area and that this area would be dominated by opportunistic species. The analysis of the long term and two-year survey data both indicated that the upper shore area had a low diversity compared to other areas of the mudflat, including the lower shore. The community of the upper shore also contained mainly opportunistic species, whilst the lower shore community consisted of both opportunistic and other non-opportunistic species. When tested, the shore height was found to be highly correlated with the spatial community distribution. Moore *et al.* (1987) also found that the shore height was an important factor in explaining the compositional differences of the meiofauna at Kinneil, although the community gradient within each shore height was related to pollution. It therefore seems that the shore height is an important factor influencing the spatial distribution of both the macrofauna and the meiofauna at Kinneil.

8.1.3 Effect of food availability

Person & Rosenberg (1987) suggest that it is the availability of food that is the fundamental variable underlying the structure of a community. Areas of high food availability will be dominated by opportunistic species, areas with low but predictable food will have the greatest diversity with species that have low growth rates. Unpredictable and low food availability areas will have slow growing species but a maximum biomass so that the individuals can overcome long starvation periods. Lastly, areas with very low spasmodic food availability will have mobile rather than sessile individuals (Pearson & Rosenberg, 1987). If this hypothesis applies at Kinneil then it would mean that the upper shore area was an area of high food availability whereas the lower shore area had a lower abundance of food. There have been indications that the effluents have an enrichment effect and many

of the chemicals that they contain are potential sources of food, such as the hydrocarbons. The concentrations of these are likely to be greater closer to the outfalls. The organic matter content values measured at Kinneil in the present study were very high (9-12%) but can be attributed to the combustion method over estimating the values due to the presence of coal dust in the sediment at Kinneil. Other methods such as hydrogen peroxide have produced values of 1-6% organic matter for Kinneil (Bullman, *Pers comm.*). A comparison of the methods showed that the loss on ignition method was consistently higher but that the differences in organic matter content between samples showed the same pattern for both methods (Bullman, *Pers comm.*). The organic matter content detected in the present study was found to be only slightly higher at the upper shore stations than at the lower shore stations and the organic matter content was only found to be important in structuring the community when combined with other factors. This suggests that the availability of food is probably not the reason for the differences in the community structure between the upper and lower shore areas.

8.1.4 Effect of the sediment composition

Settlement is thought to be one of the major factors influencing the spatial distribution of species (Lewin, 1986; Holland, 1987; Thrush, 1991; David *et al.*, 1997). Settlement may be affected by hydrodynamic conditions (Armonies & Hellwig-armonies, 1992; Udalov *et al.*, 2000), for larvae that undergo passive settlement rely on the hydrodynamics to transport them to suitable areas. Many larvae do actively select where they settle and physical conditions such as sediment type, organic matter content and salinity may play a role in determining suitable settlement areas (Gray, 1974; Wolff, 1983). The distribution of some species has been shown to be influenced by sediment properties along with other physical factors such as salinity (Barnes, 1981; Holland, 1985; Oyenekan, 1986; Newell *et al.*, 1998; Bolan & Fernandes, *pers. comm.*). Neither the particle size nor the organic matter content were found to be the major factor governing the community composition at Kinneil. There was evidence from the BIO-ENV analysis to suggest that they may play a small role along with the more major influences of pollution and/or shore height. The percentage of clay did indicate a difference within the shore area, with the highly impacted stations in the inner corner area having a high

percentage of clay, whilst the other upper shore stations had a very low percentage of clay.

8.1.5 Effect of competition

There are several different kinds of competition (Yodiz, 1986) which can be over different resources such as space and food. Competition can occur both between and within species and can be both direct and mediated (Wilson, 1991). The spatial distribution of some benthic species has been found to be related to competition. A competitive species often will show uniform spacing between individuals whilst a non-competitive species would exhibit a spatial distribution not significantly different from random (Thrush, 1991). Lawrie *et al.* (2000) noted that the small-scale pattern of the distribution of *Corophium volutator* was determined by the interactions between individuals. Competition is often regulated by density, as at low density there is no need for competition. This has been shown to be evident for *Cerastoderma edule*, which shows increased effects on both growth and mortality at large densities, which have been attributed to intraspecific competition (Jensen, 1992a; Jensen, 1993; Montaudouin & Bachelet, 1996). Suspension feeders such as *C. edule* and *Macoma balthica* are thought to affect the recruitment success of other species. Flach (1996) found that the presence of *C. edule* significantly reduced the densities of juvenile *C. edule, M. balthica, Pygospio elegans, Eteone longa* and *Nephtys hombergii*. It has been argued though that in soft-substratum communities there is little evidence of competition or of competitive dominance (Dayton, 1984). There was evidence of large post settlement mortality at Kinneil in many of the benthic species, which could have been caused by competition effects. It can not however be proven whether competition was involved, although it seems unlikely because of the relatively low densities of most species.

8.1.6 Effect of predation

There is some debate over the extent to which predation can alter prey density (top-down control). Many studies have found that epifaunal predators such as crabs, shrimps and birds do not have a significant effect on benthic invertebrate densities (Raffaelli & Milne, 1987; Raffaelli *et al.*, 1989), however many studies have shown

that they can (Gee *et al.*, 1985; Masski & Guillou, 1999). Kinneil is subject to predation from fish and particularly from shrimps (Jayamanne & McLusky, 1997) and also from birds during the winter months (Bryant & McLusky, 1997). Beukema *et al.* (2000) found that predator abundance was frequently determined by weather conditions and therefore the impact of predators on the benthic invertebrates will vary between and within a year. Warnes (1981) noted that at Skinflats, another mudflat on the Forth, shrimps and fish tended to have a greater impact consuming up to 75% of the invertebrate abundance, whilst birds only consumed 25%. Infaunal predators have also been found to cause significant prey mortality at other locations (Ambrose, 1991; Desroy *et al.*, 1998; Beukema *et al*, 2000). At Kinneil there is therefore the potential for predation on the benthic invertebrates from fish, shrimps, crabs, birds and other infauna. Beukema *et al.* (2000) found that prey abundance was governed by predator abundance and food supply and only indirectly by temperature. It is therefore probable that the mortality that was detected at Kinneil was at least in part due to predation, probably from fish and crustaceans and to a lesser extent birds and other infauna.

There is also the potential for bottom-up control, whereby the abundance and distribution of the benthic invertebrates will affect the abundance and distribution of the higher trophic levels. Kinneil is a Site of Special Scientific Interest (SSSI) due to the overwintering birds that use the mudflat as feeding grounds (Leatherland, 1987). Higher densities of birds have been shown to occur where food is most abundant at Kinneil (Bryant & McLusky, 1997). The increase in invertebrate abundance could also affect the shrimp population and therefore, through the food chain, the fish populations of the Forth estuary (Jayamanne & McLusky, 1997). It is possible therefore that the changes in the benthic invertebrate community may have had an impact on the productivity of the whole estuary. The overall increase in the abundance and diversity of the benthic community at Kinneil will have increased the productivity of the benthos and will therefore be able to sustain a larger abundance of predators.

8.1.7 Effect of recruitment

Recruitment is a major source of variation in abundance and biomass both between and within years (Holland, 1985). Within a year each species will undergo normal changes in abundance and biomass that are related to its recruitment patterns. During recruitment the abundance of the population will increase and the population will become dominated by smaller individuals. Recruitment patterns differ from species to species, some species have a single recruitment period whilst others have two or three and some even show continuos recruitment throughout the year. The timing and number of the recruitment periods for the same species can vary between locations. For example, Davey & George (1986) found that in the Thames *Nereis diversicolor* spawns only once in February whilst in Cherbourg this species spawns all year round. In the Canal de Mira the recruitment of *N. diversicolor* occurred twice a year (Abrante *et al.*, 1999), whilst in the Norfolk saltmarshes there was only one recruitment period (Nithart, 19998). It would therefore be expected that at Kinneil there would be a seasonal change in the abundance and biomass patterns that would be related to the recruitment patterns of the individual species. The two-year survey did detect seasonal cycles in abundance for both the upper and shore areas, which could be attributed to recruitment. The two areas did however show different seasonal patterns but this was probably caused by the different species that are dominant in the two areas having different recruitment patterns. Between years, it is often the success of recruitment coupled with the level of post settlement mortality, which controls the size of a population and therefore the community composition (Hughes, 1990, Van der Meer *et al.*, 2001). The success of recruitment can be affected by many different factors including temperature (Jensen, 1992b, Honkoop *et al.*, 1998; Beukema *et al.*, 2001), larval supply (Lewin, 1986) and predation (Flach, 1996) and therefore natural yearly variation in abundance is normal. Yearly variation in abundance over the 24-year monitoring period was detected for most species, as well as for the diversity indices, number of species and evenness. Although there was yearly variation which can at least in part be attributed to the recruitment success, a significant increase in diversity, evenness and species richness was also seen. It can be concluded that the major changes observed within a year during the two-year survey period were due to the recruitment patterns of the individual species at Kinneil, whilst the yearly variation

between years seen in the long-term monitoring data can be attributed to the natural variation caused by recruitment success and mortality. Therefore at Kinneil recruitment has a major influence on the individual species populations and therefore the overall community composition.

8.1.8 Effect of climate

Changes in the climate can cause mortality of individuals and therefore can affect the community composition. Changes in temperature especially are known to affect the abundance of certain species such as *Nephtys hombergii* (Beukema, 1991; Beukema *et al*, 2000) and *Cerastoderma edule* (Jensen, 1992). In contrast, certain species are known to be tolerant to large changes in temperature, such as *Macoma balthica* (Ratcliffe *et al.*, 1981). Studies have shown that *C. edule* has whole population extinctions during years with severe winters (Jensen, 1992b). Jensen (1992b) hypothesised that this was due to either a low freezing resistance, low tolerance of oxygen deficiency and hydrogen sulphide in stagnant water below ice or mechanical damage due to ice souring. In the years following harsh winters recruitment success was high causing a large increase in the abundance of *C. edule* (Jensen, 1992b, Beukema *et al.*, 2001). If winter temperatures at Kinneil were influencing the *C. edule* population then large variations in abundance would be seen after cold winters. The analysis of the size data from the two-year survey analysis indicated that the population had remained relatively stable and had shown no large recruitment periods. This is consistent with the temperature hypothesis in that winter temperatures were relatively mild during the survey period. The long-term data indicated that *C. edule* did show large fluctuations in abundance between certain years, which could have been related to temperature. Previously McLusky & McCrory (1989) found that the abundance of *C. edule* at Kinneil from 1976 to 1986 was related to the winter temperature. The present study however found that the change in the overall community was not related to the change in the air temperature or North Atlantic Oscillation (NAO). This therefore suggests that the community at Kinneil, in general, was not greatly influenced directly by temperature, although it may have had indirect effect through recruitment and/or predation, but it may be important for certain individual species populations, such as *C. edule*.

8.2 CONCLUSIONS AND PREDICTIONS

The following conclusions can be drawn in relation to the initial hypotheses of this study: -

- An increase in the diversity, evenness and species richness of the whole mudflat was found between 1976 and 1999, which can be attributed to the improved quality of the refinery and chemical effluents along with the increased water quality of the River Avon.
- Specific changes in the species composition occurred over most of the Kinneil area around 1979 and 1994. These involved the addition of new species to the area, namely *Manayunkia aestuarina* in 1979 and *Streblospio shrubsolii* in 1994. These coincided with the first movement of the chemical outfall in 1979 and the addition of the biological treatment plant for the refinery effluent in 1994. The movement of the chemical outfall and its related effects or the reduced toxicity of the refinery effluent caused changes to the benthic community and allowed the invasion of new species into the area.
- In 1976 the whole of Kinneil was impacted, although the area to the east of the Avon was the least impacted area. This is consistent with the successional pattern seen with increasing distance from the petrochemical effluents. The whole area was impacted, as both the effluents were moderately toxic at this time.
- In 1999 the inner corner Group 1 was still impacted although to a lesser extent than in 1976. Groups 2 and 4 were also still impacted but not as heavily as Group 1. All other areas had reached the same level of diversity but were still impacted and slightly enriched when compared to other estuarine mudflats. The reduction in the size of the highly impact area has been caused by the improved quality of the effluents, as most areas have undergone succession and have now reached a similar community state.
- There are differences in the community composition between the upper shore and lower shore areas. The spatial distribution of species can be best explained by station height, the distance from the refinery effluent and/or the hydrocarbon concentration.

- No change in the community composition was observed after the movement of the chemical outfall in January 1999, in either the upper or lower shore area. This indicates that the movement of the chemical outfall downshore has met its design target not to cause any impact to the area around the new site. There are four possible hypotheses that can explain why a recovery was not seen in the upper shore area: -

1. The upper shore area was still impacted from the prevailing refinery effluent and therefore cannot recover futher until this impact is also reduced or removed.
2. The upper shore area was still impacted from historical inputs that remain within the sediments and therefore the area needs more time to be able to recover.
3. The upper shore area was impacted by the physical environmental conditions, such as shore height, which inhibit further recovery of the area.
4. The chemical effluent was having little effect on the upper shore area before the outfall was moved and therefore the removal of the effluent did not cause any change in the benthic community.

- Seasonal changes in the community composition were detected during the two-year survey, which can be attributed to recruitment and post settlement mortality from predation.

The Kinneil mudflat appears to have reached a maximum diversity in most areas, which is comparable to that detected at the other two intertidal mudflats on the Forth estuary. It is therefore predicted that if the water quality of the estuary remains the same then the community composition will remain largely constant from now on except for natural variations. The inner corner will probably remain impacted due to the height of these stations, which is the most likely reason for the lack of recovery in this area.

8.3 FUTURE WORK

Further monitoring of the Kinneil intertidal area, especially those areas that are still showing signs of impact (Groups 1, 2 and 4) would be useful, to see if they persist and so that the causes of these impacts may be determined. Further sediment analysis for hydrocarbons at other stations or of heavy metals (Davies, 1987) may

be useful in helping to determine if the residual chemicals within the sediments are a potential cause of the increased impacts. Temporal analysis may also be useful to determine if the hydrocarbon levels decrease now that the levels in the effluents are low and especially if they are reduced further in later years.

It has been obvious from this study that abundance data is more useful than biomass data in detecting changes in the benthic macroinvertebrate community, which was also found by Camargo (1996). Therefore in further monitoring work the abundance data should be collected rather than biomass data, or if biomass data are to be used the size and number of replicates should be increased so that the larger bodied species are more accurately sampled. The ABC method was not reliable using the current method of sampling the biomass but this may not be the case if the method of sampling the biomass is changed. This would require further sampling and data analysis to test this method. Most of the changes in the community have been from the opportunistic species particularly the oligochaetes and *Manayunkia aestuarina*. It is therefore important that these species are sampled in further surveys, which requires using a 250µm sieve. Lastly it may be beneficial to identify the oligochaetes to species level as it has been shown in this study that there are several different species which are dominant in different areas of the mudflat. Although changes in the total oligochaete abundance were detected during the long-term survey this could not indicate whether there had been any changes in the species composition. The method of sub-sampling the oligochaetes to identify them species level that was designed for the present study did prove useful as it allowed for the species composition to be taken into consideration, but was not as time consuming as identifying every individual.

No sublethal effects could be detected from this study, although this does not mean that sublethal effects are not occurring. Elliott & Griffiths (1987) found sublethal effects at Kinneil on invertebrate egg development and behaviour. Further testing would be needed using both toxicity tests and field studies to assess if the refinery or the chemical effluent are having any effect on growth or reproduction of the benthic invertebrates. For those field studies looking at growth or population structure the method of sampling, in particular the core depth, needs to be considered carefully.

Table 8.1. Comparison of the diversity, evenness, number of species and number of individuals from different estuaries with similar salinity and sediment characteristics, includes details of the sampling time and techniques and the water quality for each site.

Source	Location	Year	Month	Seive size	Water quality	No. Species	Number of individuals (m^{-2})	Evenness	Diversity
Present study	Forth - Kinneil	1999	July	0.25	?	12	62156	0.68	2.43
Present study	Forth - Kinneil	1978	August	0.25	very low	10	23058	0.29	0.96
Bullman (Pers. Comm.)	Forth - Torry Bay	2000	November	0.25	mod	10	28780	0.74	2.46
Bullman (Pers. Comm.)	Forth - Skinflats	2000	November	0.25	low	9	19531	0.61	1.95
Ratcliffe (1979)	Humber - Skeffling	1974-1975	Jan - April	0.125	high	14	22457	0.64	2.44
Stobie et al (1976)	Clyde - D	1975	July	0.5 + 0.25	very low	6	163428	0.34	0.87
Stobie et al (1976)	Clyde - E	1975	August	0.5 + 0.25	very low	9	153879	0.69	2.2
CRPB (1986)	Solway Firth - Whinnyrig	1986	June	1.6	mod	4	25888	0.25	0.5
CRPB (1986)	Solway Firth - Kenneth Bank	1986	June	1.6	high	7	52734	0.09	0.27
Spooner & Moore (1940)	Tamar - Thanckes Lake	1937	August	0.8	high	7	958	0.73	2.04
McLusky & Roddie(1982)	Montrose basin	1982	September	0.25	mod	20	40362	0.57	2.48
SEPA (1999)	Eden - T2	1998	July	0.5	high	11	45296	0.43	1.51
Ysebaert (2000)	Schelde - Paulina	1993-1994	March - Aug	1	low	17	23914	0.71	2.9
Poopetch (1980)	Loughor	1976	Summer	1	high	22	23377	0.61	2.71
JNCC (Pers. Comm.)	medway	1993	June	0.5	mod	16	1512.5	0.65	2.58
Brazier & Murray (1994)	Tees	1992	Aug - Oct	0.5	very low	15	34102	0.24	0.95
Brazier & Murray (1994)	Tyne	1992	Aug - Oct	0.5	very low	2	52	1	1
Ghase (1980)	Mersey - Speke	1976	July	0.125	very low	7	15674	0.29	0.82

Figure 8.1. MDS plot showing the difference in the community composition between Kinneil and intertidal mudflats subjected to different water qualities, where 0 = water quality not known, 1 = Very low, 2 = Low, 3 = Moderate, 4 = High

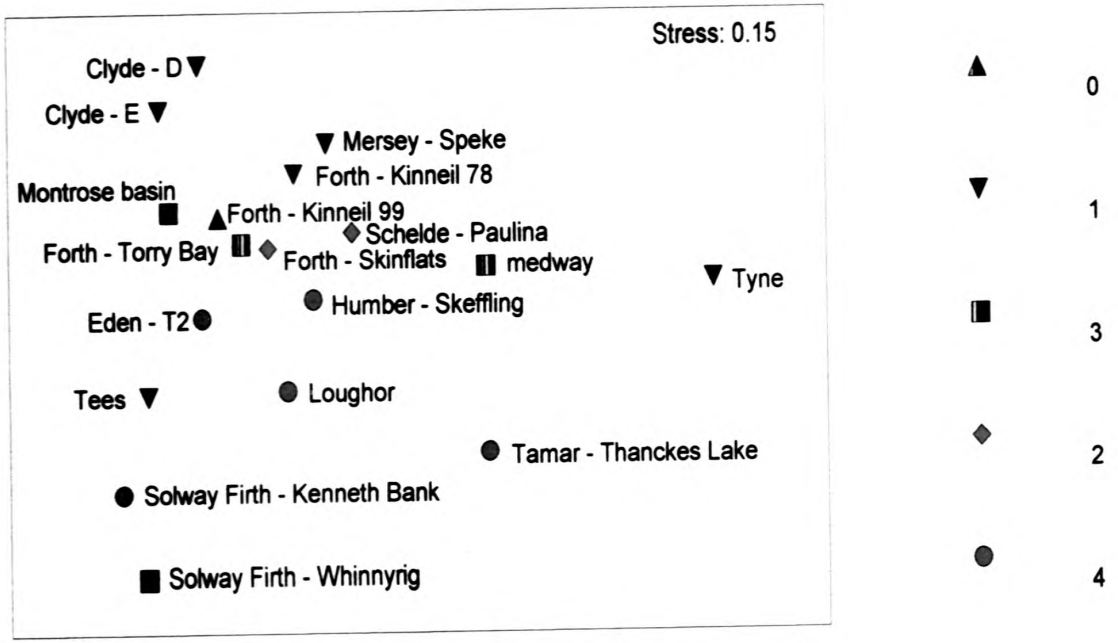
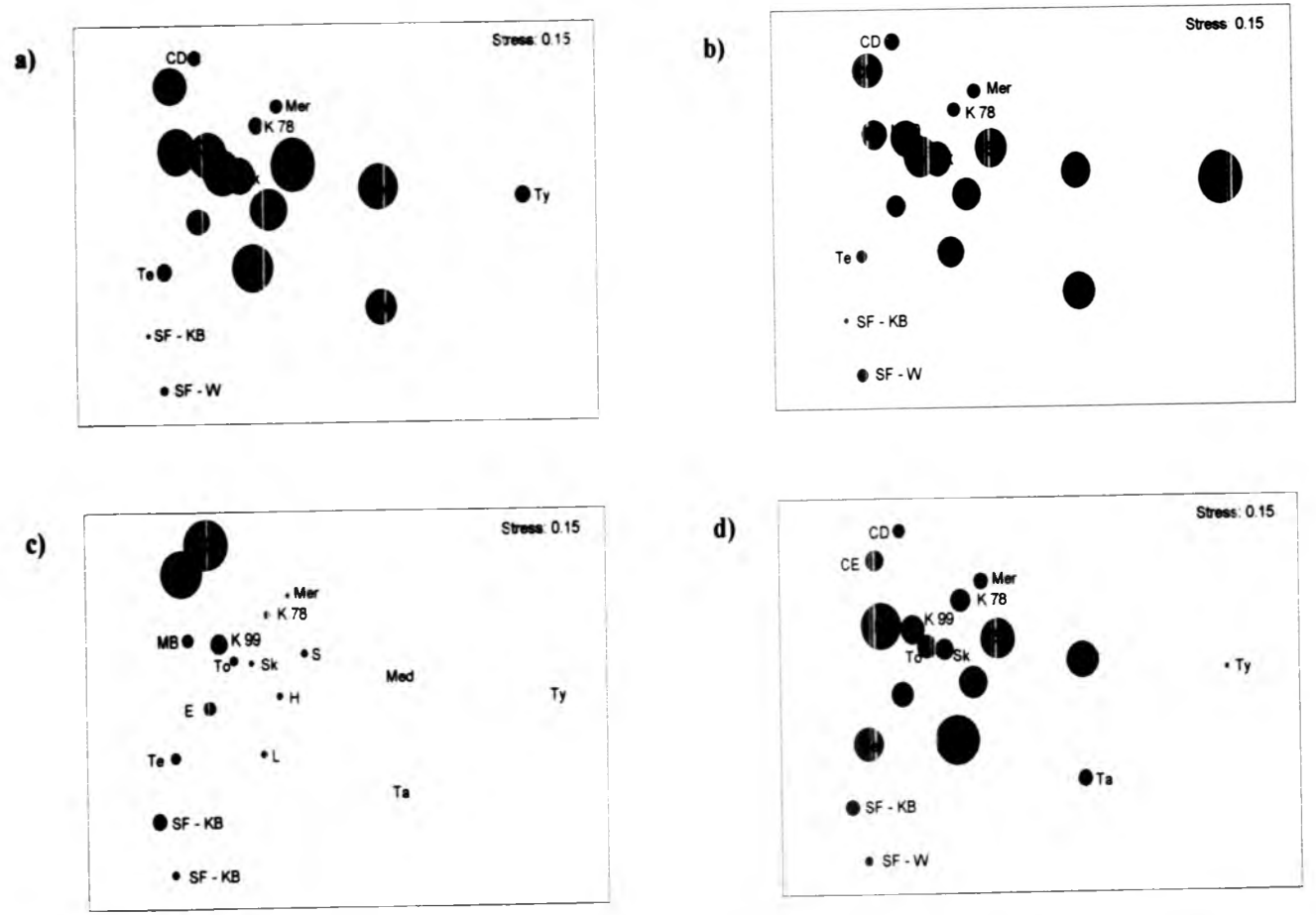


Figure 8.2. Bubble MDS plots showing the a) diversity, b) evenness, c) number of individuals, d) number of species for each of the different intertidal estuarine mudflats. CD = Clyde D, CE = Clyde E, E – Eden, H = Humber, K 78 = Kinneil 78, K 99 = Kinneil 99, L = Loughor, Med = Medway, Mer = Mersey, MB = Montrose Basin, S = Schelde, Sk = Skinflats, SF – KB = Solway Forth Kenneth Bank, SF – W = Solway Firth – Whinnyrig, Te = Tees, To = Torry Bay, Ty = Tyne.



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APPENDIX 1

Sediment Hydrocarbon analysis method (From M-Scan, 2001).

Extraction and Fractionation of Samples for hydrocarbon analysis

Sediment samples were stored at -18°C prior to extraction and analysis. In laboratory, the samples were defrosted, homogenised and sub-samples (ca. 10g) weighed out for analysis. Known amounts of heptamethylnonane (HMN), chlorooctadecane (COD), squalane (Sq), d8-naphthalene (d8-N), d10-phenanthrene (d10-Ph) and d10-pyrene (d10-P) were added to each sediment as internal standards prior to extraction, and the sample re-homogenised. After the addition of 100ml of isopropanol/hexane (4:1), the sediments were extracted using ultrasonication (2 x 5 min, stirring in between) and the centrifuged at 2000rpm for 10 minutes. The supernatant extract was then decanted and partitioned between water and pentane. Organic fractions were collected in pre-cleaned 500ml round bottom flasks. The extraction procedure was then repeated with a further 100ml of isopropanol/hexane (4:1), omitting addition of internal standards. Organic fractions were combined and re-washed, giving a total organic extract (TOE), a suitable aliquot of which was removed for analysis by UVF. The TOE was then concentrated to ca. 5ml under vacuum (<30°C, Buchi Rotovap R110) and saponified by adding ca. 1ml of aqueous potassium hydroxide (60% W/V), ca. 5ml of ethanol and incubating at room temperature for 24 hours.

After saponification, the resulting mixtures were backwashed by water partitioning. Organic layers were separated and reduced by rotary evaporation to ca. 2ml. These total neutral fractions were then chromatographed on a silica column (silica, 60-230 mesh) to provide aliphatic and aromatic fractions as follows. Pentane (40ml) was used to elute the aliphatic fraction, followed by 50ml dicloromthane to elute the aromatic fraction. Each fraction was collected in a pre-cleaned 100ml round bottom flask and reduced under vacuum (<30°C) to ca. 1ml, transferred to a clean glass vial and further concentrated using dry nitrogen blow-down. A procedural blank was carried out in a parallel with the samples.

GC of Aliphatic hydrocarbons

A 1µl aliquot of the aliphatic fraction was analysed by GC under the following conditions:

Instrument	Hewlett Packard 6890
Injector	Split/splitless at 325°C (splitless mode)
Column	30m x 0.32mm i.d. DB-1 Fused Silica WCOT
Temperature programme	40°C(1min)-325°C at 8°C/mon, 325°C (10min)
Detector	Flame ionisation at 325°C
Carrier Gas	Helium
Data handling	Hewlett Packard Chemstation Rev. A.07.01

Quantification of total aliphatic hydrocarbons (TAH) was carried out against an average of internal standards heptamethylnonane and squalane.

Ultra-violet Fluorescence (UVF) of total Oil

Determined by Ultra Violet Fluorescence (UVF) using a Perkin Elmer LS30 Luminesence Spectrometer. A suitable aliquot of the TOE was analysed by UVF, the readings from which were translated to Forties crude equivalents using pre-plotted calibration curve. To quantify crude oil equivalents, readings were taken at 310nm excitation/ 360nm emission. Synchronous scans were carried out over the range 230-450nm excitation ($\Delta\lambda$ 25nm).

APPENDIX 2

The temporal and spatial pairwise comparisons of the mean sizes for the different species populations.

Where: -

site 1 = upper shore
site 2 = lower shore

time 1 = November 1998
time 2 = February 1999
time 3 = May 1999
time 4 = July 1999
time 5 = November 1999
time 6 = February 2000
time 7 = May 2000
time 8 = July 2000
time 9 = November 2000

* = Significant at 5% level
** = Significant at 10% level

a) Pairwise comparisons for *Nereis diversicolor*

All Pairwise Comparisons among Levels of time

time = 1 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	0.0966	0.06507	1.485	0.8628
3	0.1527	0.06653	2.294	0.3454
4	-0.1210	0.04313	-2.805	0.1138
5	0.0468	0.10708	0.437	1.0000
6	0.0260	0.08783	0.296	1.0000
7	-0.0631	0.05782	-1.092	0.9756
8	0.0911	0.06124	1.488	0.8615
9	0.0916	0.05982	1.531	0.8412

time = 2 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	0.0560	0.07728	0.725	0.9985
4	-0.2176	0.05836	-3.729	0.0060*
5	-0.0499	0.11407	-0.437	1.0000
6	-0.0706	0.09622	-0.734	0.9983
7	-0.1598	0.06991	-2.285	0.3509
8	-0.0055	0.07277	-0.076	1.0000
9	-0.0051	0.07158	-0.071	1.0000

time = 3 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	-0.2736	0.05998	-4.562	0.0002*
5	-0.1059	0.11491	-0.921	0.9918
6	-0.1266	0.09722	-1.303	0.9309

7	-0.2158	0.07128	-3.027	0.0621**
8	-0.0615	0.07408	-0.830	0.9960
9	-0.0611	0.07291	-0.838	0.9957

time = 4 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
5	0.16776	0.10314	1.627	0.7904
6	0.14699	0.08297	1.772	0.7012
7	0.05785	0.05014	1.154	0.9657
8	0.21212	0.05406	3.924	0.0028*
9	0.21257	0.05244	4.054	0.0017*

time = 5 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
6	-0.0208	0.1284	-0.1617	1.0000
7	-0.1099	0.1101	-0.9983	0.9862
8	0.0444	0.1119	0.3962	1.0000
9	0.0448	0.1112	0.4031	1.0000

time = 6 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	-0.08914	0.09147	-0.9745	0.9882
8	0.06512	0.09367	0.6952	0.9989
9	0.06558	0.09275	0.7070	0.9987

time = 7 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
8	0.1543	0.06636	2.325	0.3271
9	0.1547	0.06505	2.378	0.2959

time = 8 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
9	0.000453	0.06812	0.006654	1.000

All Pairwise Comparisons among Levels of site*time

site = 1
time = 1 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1 2	-0.0693	0.07981	-0.869	1.0000
1 3	0.1349	0.07628	1.769	0.9524
1 4	-0.0902	0.05151	-1.751	0.9565
1 5	-0.0697	0.04772	-1.460	0.9932
1 6	-0.0738	0.08412	-0.878	1.0000
1 7	0.1764	0.06121	2.881	0.2630
1 8	0.2906	0.07334	3.963	0.0092*
1 9	0.0937	0.06121	1.532	0.9885
2 1	-0.0085	0.07334	-0.116	1.0000
2 2	0.2541	0.08955	2.838	0.2891
2 3	0.1619	0.09665	1.675	0.9713
2 4	-0.1603	0.04734	-3.386	0.0692**
2 5	0.1547	0.20259	0.764	1.0000
2 6	0.1174	0.14570	0.805	1.0000
2 7	-0.3111	0.08412	-3.698	0.0247*
2 8	-0.1169	0.08412	-1.389	0.9961

2	9	0.0809	0.08955	0.904	1.0000

site = 1
time = 2 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	3	0.2042	0.09673	2.111	0.8101
1	4	-0.0209	0.07869	-0.265	1.0000
1	5	-0.0003	0.07626	-0.004	1.0000
1	6	-0.0045	0.10303	-0.044	1.0000
1	7	0.2457	0.08535	2.879	0.2646
1	8	0.3599	0.09443	3.812	0.0164*
1	9	0.1631	0.08535	1.911	0.9083
2	1	0.0608	0.09443	0.644	1.0000
2	2	0.3234	0.10751	3.008	0.1959
2	3	0.2312	0.11349	2.038	0.8511
2	4	-0.0909	0.07602	-1.196	0.9994
2	5	0.2240	0.21114	1.061	0.9999
2	6	0.1867	0.15738	1.186	0.9994
2	7	-0.2418	0.10303	-2.347	0.6486
2	8	-0.0475	0.10303	-0.461	1.0000
2	9	0.1503	0.10751	1.398	0.9958

site = 1
time = 3 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	4	-0.2251	0.07511	-2.997	0.2013
1	5	-0.2046	0.07256	-2.819	0.3004
1	6	-0.2087	0.10032	-2.081	0.8278
1	7	0.0415	0.08206	0.505	1.0000
1	8	0.1557	0.09147	1.702	0.9665
1	9	-0.0412	0.08206	-0.502	1.0000
2	1	-0.1434	0.09147	-1.568	0.9853
2	2	0.1192	0.10492	1.136	0.9997
2	3	0.0270	0.11104	0.243	1.0000
2	4	-0.2952	0.07231	-4.082	0.0058*
2	5	0.0198	0.20984	0.094	1.0000
2	6	-0.0175	0.15562	-0.113	1.0000
2	7	-0.4460	0.10032	-4.446	0.0012*
2	8	-0.2518	0.10032	-2.510	0.5229
2	9	-0.0540	0.10492	-0.514	1.0000

site = 1
time = 4 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	5	0.0205	0.04582	0.448	1.0000
1	6	0.0164	0.08306	0.197	1.0000
1	7	0.2666	0.05974	4.462	0.0012*
1	8	0.3808	0.07212	5.280	0.0001*
1	9	0.1839	0.05974	3.079	0.1643
2	1	0.0817	0.07212	1.133	0.9997
2	2	0.3443	0.08856	3.888	0.0123*
2	3	0.2521	0.09573	2.634	0.4284
2	4	-0.0701	0.04543	-1.542	0.9876
2	5	0.2449	0.20216	1.212	0.9993
2	6	0.2076	0.14509	1.430	0.9946
2	7	-0.2209	0.08306	-2.660	0.4092
2	8	-0.0267	0.08306	-0.321	1.0000
2	9	0.1711	0.08856	1.932	0.8998

site = 1
time = 5 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1 6	-0.0042	0.08076	-0.052	1.0000
1 7	0.2460	0.05650	4.354	0.0019*
1 8	0.3603	0.06946	5.187	0.0001*
1 9	0.1634	0.05650	2.892	0.2568
2 1	0.0612	0.06946	0.881	1.0000
2 2	0.3238	0.08641	3.747	0.0207*
2 3	0.2316	0.09374	2.470	0.5534
2 4	-0.0906	0.04107	-2.206	0.7501
2 5	0.2244	0.20122	1.115	0.9998
2 6	0.1870	0.14379	1.301	0.9982
2 7	-0.2415	0.08076	-2.990	0.2050
2 8	-0.0472	0.08076	-0.584	1.0000
2 9	0.1506	0.08641	1.743	0.9584

site = 1
time = 6 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1 7	0.2502	0.08940	2.799	0.3136
1 8	0.3645	0.09810	3.715	0.0232*
1 9	0.1676	0.08940	1.875	0.9215
2 1	0.0653	0.09810	0.666	1.0000
2 2	0.3280	0.11075	2.961	0.2193
2 3	0.2357	0.11656	2.022	0.8588
2 4	-0.0864	0.08054	-1.073	0.9999
2 5	0.2286	0.21281	1.074	0.9999
2 6	0.1912	0.15961	1.198	0.9994
2 7	-0.2373	0.10641	-2.230	0.7337
2 8	-0.0430	0.10641	-0.404	1.0000
2 9	0.1548	0.11075	1.397	0.9959

site = 1
time = 7 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1 8	0.1143	0.07933	1.440	0.9941
1 9	-0.0826	0.06828	-1.210	0.9993
2 1	-0.1849	0.07933	-2.330	0.6613
2 2	0.0778	0.09453	0.823	1.0000
2 3	-0.0145	0.10128	-0.143	1.0000
2 4	-0.3366	0.05618	-5.991	0.0000*
2 5	-0.0216	0.20484	-0.106	1.0000
2 6	-0.0590	0.14881	-0.396	1.0000
2 7	-0.4875	0.08940	-5.453	0.0001*
2 8	-0.2932	0.08940	-3.280	0.0949**
2 9	-0.0954	0.09453	-1.010	0.9999

site = 1
time = 8 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1 9	-0.1969	0.07933	-2.482	0.5445
2 1	-0.2991	0.08903	-3.360	0.0749**
2 2	-0.0365	0.10280	-0.355	1.0000
2 3	-0.1287	0.10903	-1.181	0.9995
2 4	-0.4509	0.06920	-6.516	0.0000*
2 5	-0.1359	0.20878	-0.651	1.0000
2 6	-0.1733	0.15420	-1.124	0.9997
2 7	-0.6017	0.09810	-6.134	0.0000*
2 8	-0.4075	0.09810	-4.154	0.0043*

2	9	-0.2097	0.10280	-2.040	0.8499

site = 1
time = 9 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	1	-0.1022	0.07933	-1.289	0.9984
2	2	0.1604	0.09453	1.696	0.9676
2	3	0.0682	0.10128	0.673	1.0000
2	4	-0.2540	0.05618	-4.521	0.0009*
2	5	0.0610	0.20484	0.298	1.0000
2	6	0.0236	0.14881	0.159	1.0000
2	7	-0.4049	0.08940	-4.529	0.0009*
2	8	-0.2106	0.08940	-2.356	0.6418
2	9	-0.0128	0.09453	-0.136	1.0000

site = 2
time = 1 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	2	0.2626	0.10280	2.555	0.4880
2	3	0.1704	0.10903	1.563	0.9857
2	4	-0.1518	0.06920	-2.193	0.7586
2	5	0.1632	0.20878	0.782	1.0000
2	6	0.1259	0.15420	0.816	1.0000
2	7	-0.3026	0.09810	-3.085	0.1618
2	8	-0.1084	0.09810	-1.105	0.9998
2	9	0.0894	0.10280	0.870	1.0000

site = 2
time = 2 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	3	-0.0922	0.12054	-0.765	1.0000
2	4	-0.4144	0.08620	-4.807	0.0003*
2	5	-0.0994	0.21502	-0.462	1.0000
2	6	-0.1368	0.16254	-0.841	1.0000
2	7	-0.5652	0.11075	-5.104	0.0001*
2	8	-0.3710	0.11075	-3.350	0.0772**
2	9	-0.1732	0.11493	-1.507	0.9903

site = 2
time = 3 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	4	-0.3222	0.09355	-3.444	0.0577**
2	5	-0.0072	0.21807	-0.033	1.0000
2	6	-0.0445	0.16655	-0.267	1.0000
2	7	-0.4730	0.11656	-4.058	0.0064*
2	8	-0.2788	0.11656	-2.392	0.6145
2	9	-0.0810	0.12054	-0.672	1.0000

site = 2
time = 4 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	5	0.3150	0.20113	1.566	0.9854
2	6	0.2776	0.14367	1.932	0.8997
2	7	-0.1508	0.08054	-1.873	0.9220
2	8	0.0434	0.08054	0.539	1.0000
2	9	0.2412	0.08620	2.798	0.3139

site = 2
time = 5 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 6	-0.0374	0.2438	-0.153	1.0000
2 7	-0.4658	0.2128	-2.189	0.7614
2 8	-0.2716	0.2128	-1.276	0.9986
2 9	-0.0738	0.2150	-0.343	1.0000

site = 2
time = 6 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 7	-0.4285	0.1596	-2.684	0.3913
2 8	-0.2342	0.1596	-1.467	0.9928
2 9	-0.0364	0.1625	-0.224	1.0000

site = 2
time = 7 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 8	0.1943	0.1064	1.826	0.9371
2 9	0.3920	0.1108	3.540	0.0424*

site = 2
time = 8 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 9	0.1978	0.1108	1.786	0.9481

b) Pairwise comparisons for *Nephtys hombergii*

All Pairwise Comparisons among Levels of Time

Time = 1 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	0.11196	0.04460	2.510	0.2268
3	0.19993	0.04049	4.937	0.0000*
4	0.07998	0.03452	2.317	0.3316
5	0.00277	0.03760	0.074	1.0000
6	0.11281	0.04967	2.271	0.3597
7	0.15298	0.05207	2.938	0.0799**
8	-0.03930	0.03452	-1.139	0.9684
9	0.08810	0.03602	2.446	0.2592

Time = 2 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	0.0880	0.04856	1.812	0.6744
4	-0.0320	0.04370	-0.732	0.9983
5	-0.1092	0.04617	-2.365	0.3036
6	0.0009	0.05644	0.015	1.0000
7	0.0410	0.05856	0.701	0.9988
8	-0.1513	0.04370	-3.461	0.0158*
9	-0.0239	0.04490	-0.531	0.9998

Time = 3 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	-0.1200	0.03950	-3.037	0.0605**
5	-0.1972	0.04222	-4.670	0.0001*
6	-0.0871	0.05325	-1.636	0.7849
7	-0.0469	0.05550	-0.846	0.9954
8	-0.2392	0.03950	-6.056	0.0000*
9	-0.1118	0.04083	-2.739	0.1342

Time = 4 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
5	-0.0772	0.03653	-2.114	0.4638
6	0.0328	0.04886	0.672	0.9991
7	0.0730	0.05130	1.423	0.8892
8	-0.1193	0.03335	-3.577	0.0105*
9	0.0081	0.03490	0.233	1.0000

Time = 5 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
6	0.11004	0.05109	2.154	0.4363
7	0.15021	0.05342	2.812	0.1118
8	-0.04207	0.03653	-1.152	0.9661
9	0.08533	0.03796	2.248	0.3743

Time = 6 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	0.0402	0.06251	0.643	0.9994
8	-0.1521	0.04886	-3.113	0.0484*
9	-0.0247	0.04994	-0.495	0.9999

Time = 7 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
8	-0.1923	0.05130	-3.748	0.0056*
9	-0.0649	0.05233	-1.240	0.9477

Time = 8 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
9	0.1274	0.03490	3.650	0.0081*

c) Pairwise comparisons for *Macoma balthica*

All Pairwise Comparisons among Levels of time

time = 1 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	0.01940	0.07193	0.2697	1.0000
3	0.28509	0.07058	4.0390	0.0018*
4	0.06966	0.06313	1.1033	0.9739
5	0.24566	0.07441	3.3012	0.0269*

6	0.18380	0.07469	2.4610	0.2512
7	0.19649	0.09120	2.1546	0.4358
8	-0.04283	0.06327	-0.6769	0.9991
9	0.22985	0.06109	3.7624	0.0053*

time = 2 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	0.26569	0.08386	3.1683	0.0409*
4	0.05026	0.07769	0.6469	0.9993
5	0.22626	0.08711	2.5975	0.1874
6	0.16440	0.08734	1.8824	0.6261
7	0.17710	0.10182	1.7394	0.7221
8	-0.06222	0.07780	-0.7998	0.9969
9	0.21045	0.07604	2.7676	0.1251

time = 3 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	-0.2154	0.07645	-2.818	0.1101
5	-0.0394	0.08600	-0.458	0.9999
6	-0.1013	0.08624	-1.175	0.9619
7	-0.0886	0.10087	-0.878	0.9941
8	-0.3279	0.07656	-4.283	0.0006*
9	-0.0552	0.07477	-0.739	0.9982

time = 4 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
5	0.1760	0.08000	2.200	0.4056
6	0.1141	0.08025	1.422	0.8896
7	0.1268	0.09581	1.324	0.9245
8	-0.1125	0.06975	-1.613	0.7982
9	0.1602	0.06779	2.363	0.3045

time = 5 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
6	-0.0619	0.08940	-0.692	0.9989
7	-0.0492	0.10359	-0.475	0.9999
8	-0.2885	0.08011	-3.601	0.0096*
9	-0.0158	0.07840	-0.202	1.0000

time = 6 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	0.0127	0.10379	0.122	1.0000
8	-0.2266	0.08036	-2.820	0.1094
9	0.0460	0.07866	0.585	0.9997

time = 7 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
8	-0.2393	0.09590	-2.496	0.2339
9	0.0334	0.09448	0.353	1.0000

time = 8 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
9	0.2727	0.06791	4.015	0.0020*

All Pairwise Comparisons among Levels of site*time

site = 1
time = 1 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	2	0.15540	0.13049	1.1909	0.9994
1	3	0.46658	0.13581	3.4355	0.0593**
1	4	-0.06475	0.12232	-0.5293	1.0000
1	5	0.36054	0.14237	2.5324	0.5051
1	6	0.27425	0.13581	2.0193	0.8604
1	7	0.41612	0.17668	2.3552	0.6422
1	8	-0.04347	0.12232	-0.3554	1.0000
1	9	0.37727	0.11634	3.2429	0.1055
2	1	0.11299	0.07903	1.4297	0.9946
2	2	-0.00361	0.09366	-0.0386	1.0000
2	3	0.21658	0.08116	2.6685	0.4028
2	4	0.31706	0.07801	4.0642	0.0062*
2	5	0.24376	0.08358	2.9166	0.2431
2	6	0.20635	0.09472	2.1786	0.7682
2	7	0.08986	0.08459	1.0623	0.9999
2	8	0.07081	0.07844	0.9027	1.0000
2	9	0.19542	0.08062	2.4240	0.5894

site = 1
time = 2 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	3	0.3112	0.1553	2.004	0.8681
1	4	-0.2201	0.1437	-1.532	0.9884
1	5	0.2051	0.1611	1.274	0.9986
1	6	0.1188	0.1553	0.765	1.0000
1	7	0.2607	0.1921	1.357	0.9970
1	8	-0.1989	0.1437	-1.384	0.9963
1	9	0.2219	0.1386	1.601	0.9817
2	1	-0.0424	0.1092	-0.388	1.0000
2	2	-0.1590	0.1202	-1.323	0.9978
2	3	0.0612	0.1107	0.553	1.0000
2	4	0.1617	0.1084	1.491	0.9914
2	5	0.0884	0.1125	0.785	1.0000
2	6	0.0510	0.1210	0.421	1.0000
2	7	-0.0655	0.1133	-0.579	1.0000
2	8	-0.0846	0.1088	-0.778	1.0000
2	9	0.0400	0.1103	0.363	1.0000

site = 1
time = 3 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	4	-0.5313	0.1485	-3.578	0.0374*
1	5	-0.1060	0.1654	-0.641	1.0000
1	6	-0.1923	0.1598	-1.204	0.9993
1	7	-0.0505	0.1957	-0.258	1.0000
1	8	-0.5101	0.1485	-3.434	0.0595**
1	9	-0.0893	0.1436	-0.622	1.0000
2	1	-0.3536	0.1155	-3.061	0.1717
2	2	-0.4702	0.1260	-3.733	0.0218*
2	3	-0.2500	0.1170	-2.137	0.7944
2	4	-0.1495	0.1148	-1.302	0.9982
2	5	-0.2228	0.1187	-1.878	0.9203
2	6	-0.2602	0.1267	-2.053	0.8429
2	7	-0.3767	0.1194	-3.156	0.1342
2	8	-0.3958	0.1151	-3.439	0.0587**

2	9	-0.2712	0.1166	-2.326	0.6645

site = 1
time = 4 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	5	0.42529	0.15454	2.7520	0.3443
1	6	0.33900	0.14852	2.2825	0.6963
1	7	0.48087	0.18662	2.5767	0.4711
1	8	0.02128	0.13629	0.1561	1.0000
1	9	0.44202	0.13094	3.3757	0.0714**
2	1	0.17774	0.09929	1.7902	0.9470
2	2	0.06114	0.11128	0.5494	1.0000
2	3	0.28133	0.10099	2.7857	0.3219
2	4	0.38181	0.09848	3.8772	0.0128*
2	5	0.30851	0.10294	2.9970	0.2014
2	6	0.27110	0.11217	2.4168	0.5950
2	7	0.15461	0.10377	1.4900	0.9914
2	8	0.13556	0.09882	1.3718	0.9967
2	9	0.26017	0.10055	2.5873	0.4630

site = 1
time = 5 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	6	-0.0863	0.1654	-0.522	1.0000
1	7	0.0556	0.2003	0.277	1.0000
1	8	-0.4040	0.1545	-2.614	0.4427
1	9	0.0167	0.1498	0.112	1.0000
2	1	-0.2475	0.1231	-2.010	0.8649
2	2	-0.3642	0.1330	-2.738	0.3538
2	3	-0.1440	0.1245	-1.156	0.9996
2	4	-0.0435	0.1225	-0.355	1.0000
2	5	-0.1168	0.1261	-0.926	1.0000
2	6	-0.1542	0.1338	-1.153	0.9996
2	7	-0.2707	0.1268	-2.135	0.7959
2	8	-0.2897	0.1228	-2.360	0.6386
2	9	-0.1651	0.1242	-1.330	0.9977

site = 1
time = 6 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	7	0.1419	0.1957	0.725	1.0000
1	8	-0.3177	0.1485	-2.139	0.7932
1	9	0.1030	0.1436	0.717	1.0000
2	1	-0.1613	0.1155	-1.396	0.9959
2	2	-0.2779	0.1260	-2.206	0.7501
2	3	-0.0577	0.1170	-0.493	1.0000
2	4	0.0428	0.1148	0.373	1.0000
2	5	-0.0305	0.1187	-0.257	1.0000
2	6	-0.0679	0.1267	-0.536	1.0000
2	7	-0.1844	0.1194	-1.545	0.9874
2	8	-0.2034	0.1151	-1.768	0.9527
2	9	-0.0788	0.1166	-0.676	1.0000

site = 1
time = 7 subtracted from:

Level site*time		Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1	8	-0.4596	0.1866	-2.463	0.5593
1	9	-0.0389	0.1828	-0.213	1.0000
2	1	-0.3031	0.1616	-1.876	0.9210
2	2	-0.4197	0.1692	-2.480	0.5455

2	3	-0.1995	0.1626	-1.227	0.9991
2	4	-0.0991	0.1611	-0.615	1.0000
2	5	-0.1724	0.1639	-1.052	0.9999
2	6	-0.2098	0.1698	-1.235	0.9991
2	7	-0.3263	0.1644	-1.985	0.8770
2	8	-0.3453	0.1613	-2.141	0.7922
2	9	-0.2207	0.1624	-1.359	0.9970

site = 1
time = 8 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
1 9	0.42074	0.13094	3.2132	0.1147
2 1	0.15646	0.09929	1.5759	0.9844
2 2	0.03986	0.11128	0.3582	1.0000
2 3	0.26005	0.10099	2.5750	0.4724
2 4	0.36053	0.09848	3.6611	0.0281*
2 5	0.28723	0.10294	2.7902	0.3190
2 6	0.24982	0.11217	2.2271	0.7356
2 7	0.13333	0.10377	1.2849	0.9985
2 8	0.11428	0.09882	1.1564	0.9996
2 9	0.23889	0.10055	2.3757	0.6266

site = 1
time = 9 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 1	-0.2643	0.09181	-2.878	0.2647
2 2	-0.3809	0.10466	-3.639	0.0303*
2 3	-0.1607	0.09365	-1.716	0.9640
2 4	-0.0602	0.09093	-0.662	1.0000
2 5	-0.1335	0.09575	-1.394	0.9960
2 6	-0.1709	0.10561	-1.618	0.9796
2 7	-0.2874	0.09664	-2.974	0.2128
2 8	-0.3065	0.09130	-3.357	0.0756**
2 9	-0.1819	0.09318	-1.952	0.8917

site = 2
time = 1 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 2	-0.1166	0.06055	-1.926	0.9024
2 3	0.1036	0.03851	2.690	0.3872
2 4	0.2041	0.03132	6.515	0.0000*
2 5	0.1308	0.04336	3.016	0.1925
2 6	0.0934	0.06218	1.501	0.9907
2 7	-0.0231	0.04529	-0.511	1.0000
2 8	-0.0422	0.03237	-1.303	0.9982
2 9	0.0824	0.03734	2.207	0.7492

site = 2
time = 2 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 3	0.22020	0.06331	3.478	0.0518**
2 4	0.32067	0.05921	5.416	0.0001
2 5	0.24737	0.06637	3.727	0.0223
2 6	0.20996	0.07995	2.626	0.4337
2 7	0.09347	0.06765	1.382	0.9964
2 8	0.07442	0.05978	1.245	0.9990
2 9	0.19903	0.06261	3.179	0.1260

site = 2
time = 3 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 4	0.1005	0.03637	2.763	0.3369
2 5	0.0272	0.04714	0.577	1.0000
2 6	-0.0102	0.06487	-0.158	1.0000
2 7	-0.1267	0.04892	-2.591	0.4605
2 8	-0.1458	0.03728	-3.911	0.0113*
2 9	-0.0212	0.04167	-0.508	1.0000

site = 2
time = 4 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 5	-0.0733	0.04147	-1.767	0.9527
2 6	-0.1107	0.06087	-1.819	0.9392
2 7	-0.2272	0.04348	-5.225	0.0001*
2 8	-0.2463	0.02979	-8.265	0.0000*
2 9	-0.1216	0.03513	-3.463	0.0544**

site = 2
time = 5 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 6	-0.0374	0.06786	-0.551	1.0000
2 7	-0.1539	0.05282	-2.914	0.2448
2 8	-0.1730	0.04227	-4.091	0.0056*
2 9	-0.0483	0.04619	-1.047	0.9999

site = 2
time = 6 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 7	-0.1165	0.06911	-1.686	0.9696
2 8	-0.1355	0.06142	-2.207	0.7496
2 9	-0.0109	0.06418	-0.170	1.0000

site = 2
time = 7 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 8	-0.01906	0.04425	-0.4307	1.0000
2 9	0.10556	0.04800	2.1989	0.7548

site = 2
time = 8 subtracted from:

Level site*time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2 9	0.1246	0.03607	3.454	0.0558**

d) Pairwise comparisons for *Hydrobia ulvae*

All Pairwise Comparisons among Levels of time

time = 1 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	-0.0274	0.05451	-0.503	0.9999
3	0.1933	0.12697	1.522	0.8453
4	-0.2172	0.04394	-4.943	0.0000*
5	0.1765	0.05679	3.109	0.0490*
6	0.1602	0.06673	2.401	0.2834
7	0.2310	0.12000	1.925	0.5961
8	-0.1020	0.04926	-2.070	0.4943
9	0.0819	0.06085	1.347	0.9171

time = 2 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	0.2207	0.12693	1.739	0.7223
4	-0.1898	0.04380	-4.333	0.0005*
5	0.2039	0.05668	3.598	0.0097*
6	0.1876	0.06663	2.815	0.1108
7	0.2584	0.11995	2.155	0.4359
8	-0.0745	0.04913	-1.517	0.8479
9	0.1094	0.06074	1.800	0.6822

time = 3 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	-0.4105	0.1228	-3.344	0.0234*
5	-0.0168	0.1279	-0.131	1.0000
6	-0.0331	0.1326	-0.250	1.0000
7	0.0377	0.1660	0.227	1.0000
8	-0.2953	0.1248	-2.367	0.3025
9	-0.1114	0.1298	-0.858	0.9950

time = 4 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
5	0.3937	0.04661	8.447	0.0000*
6	0.3774	0.05831	6.472	0.0000*
7	0.4482	0.11554	3.880	0.0034*
8	0.1153	0.03707	3.109	0.0489
9	0.2992	0.05148	5.811	0.0000*

time = 5 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
6	-0.0163	0.06851	-0.239	1.0000
7	0.0545	0.12101	0.450	1.0000
8	-0.2785	0.05165	-5.391	0.0000*
9	-0.0946	0.06280	-1.506	0.8532

time = 6 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	0.0708	0.12598	0.562	0.9998
8	-0.2621	0.06242	-4.200	0.0009*

9	-0.0782	0.07191	-1.088	0.9761

time = 7 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
8	-0.3330	0.1177	-2.830	0.1067
9	-0.1491	0.1230	-1.212	0.9541

time = 8 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
9	0.1839	0.05608	3.279	0.0289*

e) Pairwise comparisons for *Eteone longa*

All Pairwise Comparisons among Levels of time

time = 1 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	-0.0343	0.10924	-0.314	1.0000
4	-0.1897	0.08152	-2.327	0.2957
5	-0.1494	0.07490	-1.995	0.4933
6	-0.3998	0.14888	-2.685	0.1465
7	-0.2288	0.05791	-3.951	0.0047*
8	-0.1320	0.05043	-2.618	0.1688
9	-0.0097	0.05170	-0.188	1.0000

time = 2 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	-0.1554	0.1269	-1.225	0.9214
5	-0.1152	0.1227	-0.938	0.9810
6	-0.3655	0.1778	-2.055	0.4544
7	-0.1945	0.1132	-1.719	0.6750
8	-0.0978	0.1078	-0.907	0.9843
9	0.0245	0.1089	0.225	1.0000

time = 4 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
5	0.0403	0.09650	0.417	0.9999
6	-0.2100	0.16083	-1.306	0.8932
7	-0.0391	0.08399	-0.466	0.9998
8	0.0577	0.08063	0.715	0.9962
9	0.1800	0.08092	2.224	0.3519

time = 5 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
6	-0.2503	0.15758	-1.588	0.7553
7	-0.0794	0.07759	-1.023	0.9692
8	0.0174	0.07394	0.235	1.0000
9	0.1397	0.07425	1.882	0.5683

time = 6 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	0.1709	0.1502	1.138	0.9458
8	0.2677	0.1484	1.804	0.6197
9	0.3900	0.1486	2.626	0.1663

time = 7 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
8	0.09678	0.05665	1.708	0.6819
9	0.21908	0.05706	3.840	0.0066*

time = 8 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
9	0.1223	0.04962	2.465	0.2296

f) Pairwise comparisons for *Cerastoderma edule*

All Pairwise Comparisons among Levels of time

time = 1 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	0.2253	0.1880	1.1985	0.9542
3	0.3445	0.1739	1.9813	0.5612
4	0.1385	0.1739	0.7966	0.9966
5	0.0215	0.1778	0.1208	1.0000
6	0.5147	0.1950	2.6396	0.1895
7	0.4165	0.2039	2.0424	0.5201
8	-0.1096	0.1612	-0.6800	0.9989
9	0.6583	0.4078	1.6143	0.7938

time = 2 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	0.1192	0.1798	0.663	0.9991
4	-0.0868	0.1798	-0.483	0.9999
5	-0.2038	0.1835	-1.111	0.9707
6	0.2893	0.2002	1.445	0.8761
7	0.1911	0.2089	0.915	0.9914
8	-0.3349	0.1675	-1.999	0.5491
9	0.4330	0.4104	1.055	0.9786

time = 3 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	-0.2060	0.1650	-1.249	0.9423
5	-0.3231	0.1690	-1.911	0.6086
6	0.1701	0.1871	0.909	0.9917
7	0.0719	0.1964	0.366	1.0000
8	-0.4542	0.1515	-2.997	0.0842**
9	0.3138	0.4041	0.777	0.9972

time = 4 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
5	-0.1171	0.1690	-0.692	0.9987
6	0.3761	0.1871	2.011	0.5414
7	0.2779	0.1964	1.415	0.8881
8	-0.2482	0.1515	-1.638	0.7809
9	0.5198	0.4041	1.286	0.9321

time = 5 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
6	0.4932	0.1907	2.5867	0.2111
7	0.3950	0.1998	1.9770	0.5642
8	-0.1311	0.1560	-0.8406	0.9951
9	0.6369	0.4058	1.5695	0.8177

time = 6 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
7	-0.0982	0.2152	-0.456	0.9999
8	-0.6243	0.1753	-3.561	0.0182*
9	0.1437	0.4136	0.347	1.0000

time = 7 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
8	-0.5261	0.1852	-2.840	0.1222
9	0.2419	0.4179	0.579	0.9997

time = 8 subtracted from:

Level time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
9	0.7680	0.3988	1.926	0.5988

APPENDIX 3

Table 3a. Species abundance data (m^{-2}) for different intertidal estuarine mudflats.

Source Location	Present study Forth - Kinneil	Present study Forth - Kinneil	Bullman (Pers. Comm.) Forth - Torry Bay	Bullman (Pers. Comm.) Forth - Skinflats	Ratcliffe (1979) Humber - Skeffling
Macoma balthica	6440	1120	2300	1206	7239
Cerastoderma edule	160	20	160	0	88
Hydrobia ulvae	8680	2500	3520	497	31
Nereis diversicolor	986	280	290	285	294
Oligochaeta	25120	18920	11190	1903	2299
Nephtys hombergii	400	80	220	29	654
Spionds	9980	80	2690	6177	6388
Manayunkia aestuarina	8500	0	6250	9126	167
Corophium volutator	900	40	2030	297	18
Eteone longa	920	14	130	11	312
Mya arenaria	0	0	0	0	0
Audouinia tentaculata	0	0	0	0	61
Carcinus maenas	0	0	0	0	18
Retusa obtusa	68	0	0	0	715
Prothydra leuckarti	0	0	0	0	4173
Capitella capitata	0	0	0	0	0
Fabricia sabella	0	0	0	0	0
Loittorina saxatilis	0	0	0	0	0
Nemertean	0	0	0	0	0
Neomysis interger	0	0	0	0	0
Corphium arenarium	0	0	0	0	0
Ampharete grubei	0	0	0	0	0
Gamerarus locusta	0	0	0	0	0
Scrobicularia plana	0	0	0	0	0
Mytilus edulis	2	4	0	0	0
Bathyporeia pilosa	0	0	0	0	0
Etone flara	0	0	0	0	0
Ophelia sp	0	0	0	0	0
Nereis pelagica	0	0	0	0	0
Scololepis sp	0	0	0	0	0
Phyllodoce sp	0	0	0	0	0
Arenicola marina	0	0	0	0	0
Paraonis fulgens	0	0	0	0	0
Heteromastus filiformis	0	0	0	0	0
Sabellidae sp	0	0	0	0	0
Malacoceros fuliginosus	0	0	0	0	0
Tharyx marioni	0	0	0	0	0
Abra tenuis	0	0	0	0	0
Spio spp	0	0	0	0	0
Nereis succinea	0	0	0	0	0
Polydora ligni	0	0	0	0	0
Bathyporeia sarsi	0	0	0	0	0
Bathyporeia pelagica	0	0	0	0	0
Urothoe grimaldii	0	0	0	0	0
Eurydice pulchra	0	0	0	0	0
Nephtys cirrosa	0	0	0	0	0
Polydora cornuta	0	0	0	0	0
Tharyx killariensis	0	0	0	0	0
Mysidae	0	0	0	0	0
Melita palmata	0	0	0	0	0
Mysella bidentata	0	0	0	0	0
Mysta picta	0	0	0	0	0
Anaitides maculata	0	0	0	0	0
Polydora ciliata	0	0	0	0	0
Mediomastus fragilis	0	0	0	0	0
Enchytraeidae	0	0	0	0	0

Table 3a. Continued

Source Location	Stobie et al (1976) Clyde - D	Stobie et al (1976) Clyde - E	CRPB (1986) Solway Firth - Whinnyrig	CRPB (1986) Solway Firth - Kenneth Bank
Macoma balthica	0	25	0	293
Cerastoderma edule	0	0	0	0
Hydrobia ulvae	888	3232	32	0
Nereis diversicolor	3626	25	0	133
Oligochaeta	25671	35545	0	0
Nephtys hombergii	0	0	2656	0
Spionds	0	21722	0	442
Manayunkia aestuarina	132306	3949	0	0
Corophium volutator	888	518	23168	51045
Eteone longa	0	0	32	0
Mya arenaria	0	0	0	21
Audouinia tentaculata	0	0	0	0
Carcinus maenas	0	0	0	0
Retusa obtusa	0	0	0	0
Prothydra leuckarti	0	0	0	0
Capitella capitata	0	37520	0	0
Fabricia sabella	0	51343	0	0
Loittorina saxatilis	49	0	0	0
Nemertean	0	0	0	0
Neomysis interger	0	0	0	80
Corphium arenarium	0	0	0	720
Ampharete grubei	0	0	0	0
Gamerarus locusta	0	0	0	0
Scrobicularia plana	0	0	0	0
Mytilus edulis	0	0	0	0
Bathyporeia pilosa	0	0	0	0
Etone flara	0	0	0	0
Ophelia sp	0	0	0	0
Nereis pelagica	0	0	0	0
Scololepis sp	0	0	0	0
Phyllodoce sp	0	0	0	0
Arenicola marina	0	0	0	0
Paraonis fulgens	0	0	0	0
Heteromastus filiformis	0	0	0	0
Sabellidae sp	0	0	0	0
Malacoceros fuliginosus	0	0	0	0
Tharyx marioni	0	0	0	0
Abra tenuis	0	0	0	0
Spio spp	0	0	0	0
Nereis succinea	0	0	0	0
Poldora ligni	0	0	0	0
Bathyporeia sarsi	0	0	0	0
Bathyporeia pelagica	0	0	0	0
Urothoe grimaldii	0	0	0	0
Eurydice pulchra	0	0	0	0
Nephtys cirrosa	0	0	0	0
Polydora cornuta	0	0	0	0
Tharyx killariensis	0	0	0	0
Mysidae	0	0	0	0
Melita palmata	0	0	0	0
Myselia bidentata	0	0	0	0
Mysta picta	0	0	0	0
Anaitides maculata	0	0	0	0
Polydora ciliata	0	0	0	0
Mediomastus fragilis	0	0	0	0
Enchytraeidae	0	0	0	0

Table 3a. Continued

Source Location	Spooner & Moore (1940) Tamar - Thanckes Lake	McLusky & Roddie(1982) Montrose basin	SEPA (1999) Eden - T2	Ysebaert (2000) Schelde - Paulina	Poopetch (1990) Loughor
Macoma balthica	44	304	59	2060	1250
Cerastoderma edule	0	62	0	520	75
Hydrobia ulvae	351	7442	176.5	234	5308
Nereis diversicolor	130	5	825	696	167
Oligochaeta	0	15392	18671.5	4393	0
Nephtys hombergii	360	0	0	0	325
Spionds	0	720	2945	7080	8475
Manayunkia aestuarina	0	7944	0	0	0
Corophium volutator	0	3477	22264.5	3	700
Eteone longa	0	37	118	874	150
Mya arenaria	0	0	0	7	0
Audouinia tentaculata	0	0	0	0	0
Carcinus maenas	0	0	0.5	0	33
Retusa obtusa	0	0	0	202	0
Prothydra leuckarti	0	0	0	0	0
Capitella capitata	0	816	0	0	0
Fabricia sabella	0	3592	0	0	0
Loittorina saxatilis	0	0	0	0	0
Nemertean	0	0	0	0	17
Neomysis interger	0	0	0	0	0
Corphium arenarium	0	0	0	0	4050
Ampharete grubei	7	0	0	0	0
Gamerarus locusta	30	0	0	0	0
Scrobicularia plana	36	0	0	642	267
Mytilus edulis	0	201	0	0	0
Bathyporeia pilosa	0	34	0	0	570
Etone flara	0	138	0	0	0
Ophelia sp	0	85	0	0	0
Nereis pelagica	0	56	0	0	0
Scololepis sp	0	8	0	0	75
Phyllodoce sp	0	26	0	0	0
Arenicola marina	0	12	0	0	125
Parsonis fulgens	0	11	0	0	0
Heteromastus filiformis	0	0	59	2800	0
Sabellidae sp	0	0	118	0	0
Malacoceros fuliginosus	0	0	59	0	0
Tharyx marioni	0	0	0	4031	0
Abra tenuis	0	0	0	68	25
Spio spp	0	0	0	97	67
Nereis succinea	0	0	0	3	0
Poldora ligni	0	0	0	204	0
Bathyporeia sarsi	0	0	0	0	1340
Bathyporeia pelagica	0	0	0	0	8
Urothoe grimaldii	0	0	0	0	300
Eurydice pulchra	0	0	0	0	25
Nephtys cirrosa	0	0	0	0	25
Polydora cornuta	0	0	0	0	0
Tharyx killariensis	0	0	0	0	0
Mysidae	0	0	0	0	0
Melita palmata	0	0	0	0	0
Mysella bidentata	0	0	0	0	0
Mysta picta	0	0	0	0	0
Anaitides maculata	0	0	0	0	0
Polydora ciliata	0	0	0	0	0
Mediomastus fragilis	0	0	0	0	0
Enchytraeidae	0	0	0	0	0

Table 3a. Continued

Source Location	JNCC (Pers. Comm.,) medway	Brazier & Murray (1994) Tees	Brazier & Murray (1994) Tyne	Gibbs (1980) Mersey - Speke
Macoma balthica	50	0	0	842
Cerastoderma edule	37.5	132	0	0
Hydrobia ulvae	50	0	0	673
Nereis diversicolor	25	579	26	188
Oligochaeta	650	312	26	13640
Nephtys hombergii	50	0	0	0
Spionds	37.5	2290	0	84
Manayunkia aestuarina	0	132	0	168
Corophium volutator	0	29316	0	0
Eteone longa	12.5	0	0	0
Mya arenaria	0	53	0	0
Audouinia tentaculata	0	0	0	0
Carcinus maenas	0	0	0	0
Retusa obtusa	0	0	0	0
Prothydra leuckarti	0	0	0	0
Capitella capitata	50	263	0	0
Fabricia sabella	0	0	0	0
Loittorina saxatilis	0	0	0	0
Nemertean	0	26	0	0
Neomysis interger	0	0	0	0
Corphium arenarium	0	0	0	0
Ampharete grubei	0	0	0	0
Gamerarus locusta	0	0	0	0
Scrobicularia plana	62.5	0	0	0
Mytilus edulis	12.5	0	0	0
Bathyporeia pilosa	0	0	0	0
Etone flara	0	0	0	0
Ophelia sp	0	0	0	0
Nereis pelagica	0	0	0	0
Scololepis sp	0	0	0	0
Phyllodoce sp	0	0	0	0
Arenicola marina	0	0	0	0
Paraonis fulgens	0	0	0	0
Heteromastus filiformis	0	0	0	0
Sabellidae sp	0	0	0	0
Malacoceros fuliginosus	0	0	0	0
Tharyx marioni	0	0	0	0
Abra tenuis	0	0	0	0
Spio spp	0	105	0	0
Nereis succinea	0	0	0	0
Poldora ligni	0	0	0	0
Bathyporeia sarsi	0	0	0	0
Bathyporeia pelagica	0	0	0	0
Urothoe grimaldii	0	0	0	0
Eurydice pulchra	0	0	0	0
Nephtys cirrosa	0	0	0	0
Polydora cornuta	12.5	0	0	0
Tharyx killariensis	425	0	0	0
Mysidae	12.5	0	0	0
Melita palmata	12.5	0	0	0
Mysella bidentata	12.5	0	0	0
Mysta picta	0	26	0	0
Anaitides maculata	0	79	0	0
Polydora ciliata	0	605	0	0
Mediomastus fragilis	0	105	0	0
Enchytraeidae	0	79	0	0

TARGET C

For material accompanying this thesis please apply direct to the issuing university/awarding body.

Video cassette ☐

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