THE UNIVERSITY OF HULL

The influence of a petrochemical discharge on the bioturbation and erosion potential of an intertidal estuarine mudflat (Humber estuary, UK).

being a Thesis submitted for the Degree of Doctor of Philosophy in the University of Hull

by

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ABSTRACT

The influence of sedimentary variables on the structure and function of infaunal estuarine and marine communities is well studied but less is known of the influence of biota on sediment properties. Feeding and burrowing activity, locomotion, the production of faecal pellets and biological secretions (bioturbation) have important implications for sediment structure, chemistry, transport characteristics and the flux of nutrients and contaminants. Although spatial and temporal patterns in bioturbation have been studied to some extent, little attention has been given to the effects of pollution. The present study examines the effects of an intertidal petrochemical discharge into the Humber estuary (UK), from BP chemicals (Saltend) Ltd on the structure and function of the communities.

Field and laboratory techniques were used to determine the effects of community change on bioturbation potential. In addition, a laboratory flume was constructed to measure sediment erosion potential with field measurements being taken using a Cohesive Strength Meter (CSM). The physico-chemical properties of the sediment, changes to the infaunal community structure, bioturbation potential and the interaction of these variables were used to explain differences between the erosion potential of sediments subject to varying levels of contamination. The main study was carried out on the Saltend mudflats near Hull, with sites at various distances from the outfall being used. A further set of control sites on the adjacent, and largely unaffected, mudflat at Paull were also used.

In terms of the sediment properties, sites closest to the outfall showed the greatest degree of anoxia and the highest chlorophyll-*a* and carbohydrate concentrations, with all three parameters being seasonally influenced. No consistent spatial or temporal patterns were found for any of the other parameters (water and organic content, particle size). Whilst the infaunal communities were characteristic of estuarine areas, macrobenthic community response followed the Pearson & Rosenberg (1978) model for organic discharges with high abundance and low species diversity being associated with the more polluted sediments. Close to the discharge, there was an impoverished community consisting predominantly of highly abundant oligochate worms. With increasing distance from the outfall, species diversity and biomass increased with *Hediste diversicolor* becoming increasingly dominant and the appearance of *Corophium volutator, Streblospio shrubsolii* and *Macoma balthica.*

Bioturbation potential was significantly reduced (in terms of depth and burrow volume and density) by increasing effluent concentrations and with proximity to the discharge. The diversity of both feeding and sediment modification guilds was also reduced as a result of the discharge.

Both field and laboratory studies indicated a stabilising effect of this type of pollution. Using the CSM, critical shear stress values were found to be significantly lower from unpolluted sites, indicating higher erosion potential, than those from sites close to the discharge. As a result of this, the total mass of sediment eroded from unpolluted sites was significantly higher than that from polluted areas. A similar trend was observed in the laboratory with sediments treated with an effluent concentration of 32% being considerably more stable than untreated sediments. Flume studies also indicated the stabilising effect of pollution with suspended particulate matter (SPM) concentrations and mass of sediment being transported as bedload being significantly higher for unpolluted sediments.

These differences in erosion potential were attributed to the direct effects of the effluent on the physico-chemical properties of the sediment, the effects of the effluent and sediment type on macrofaunal community structure and function and the differences in bioturbation potential between sites. The implications of these findings in the wider context of coastal management are discussed.

CHAPTER 1

GENERAL INTRODUCTION

1.1. BACKGROUND AND RATIONALE.

1.1.1. Estuarine environments and communities

The communities present in estuarine environments are continually subjected to the physiological stresses imposed by widely fluctuating conditions of salinity, temperature, dissolved oxygen and turbidity (McLusky, 1989). Intertidal organisms are faced with the added stress of exposure to the air, sometimes for long periods of time. Nutrients and organic matter enter estuaries either as dissolved or particulate matter and become incorporated into the sediments either by direct settlement of particles or through precipitation and subsequent settling as the salinity of the water increases (Meadows & Campbell, 1988). Estuarine sediments are therefore extremely rich in nutrients. The sediments of estuaries are generally fine grained, rich in organic matter and often anoxic, conditions which are generally unfavourable to living organisms. The inability of most living organisms to overcome the physiological difficulties presented by these conditions, together with the rich food supply means that estuarine communities are generally highly productive, consisting of large numbers of individuals of relatively few species (Meadows & Campbell, 1988; McLusky, 1989). Adaptations to these physiological stresses range from the modification of organs and biochemical processes to the evolution of new races of species (McLusky, 1989).

The effects of sediment characteristics on the distribution of benthic invertebrates have been extensively studied (Gray, 1974; Gray 1981). Correlations between grain size and species distribution have been made (Petersen, 1913, in Snelgrove & Butman, 1994; Jones, 1950; Sanders, 1958), leading to generalisations of distinct associations between animals and specific sediment types (Snelgrove & Butman, 1994). Heterotrophic marine macro-organisms are predominantly suspension or deposit feeders with deposit feeders tending to dominate in muddy habitats (Rhoads, 1974). Sanders (1958) was the first to quantify this relationship between particle size and species composition and found a general pattern of around 66% of the organisms in sandy habitats being suspension feeders with over 80% of the organisms in muddy habitats being deposit feeders.

Rhoads & Young (1970) suggested that suspension feeders were largely excluded from fine, unstable sediments due to the clogging of their delicate respiratory structures by suspended

material, whilst deposit feeders were found at comparatively low densities in coarser sediments as a result of their lower organic content. However, this general pattern of segregation between the two feeding guilds is not always observed and Snelgrove & Butman (1994) highlighted several studies which did not support some of the theories given (e.g., Gray, 1974; Rhoads, 1974; Jumars & Nowell, 1984a). It was concluded that animal-sediment relationships are considerably more variable than initially thought and that the mechanisms determining organism distribution are clearly poorly understood (Snelgrove & Butman, 1994). It is evident that the occurrence of a species in a particular habitat is due not to one factor alone but to the complex interaction between particle size, organic and microbial content, hydrodynamic and chemical conditions, and biological interactions which makes a habitat suitable for a particular species. Conditions such as these are variable on a spatial and temporal scale and it is therefore not surprising that correlations between fauna and grain size have often been weak.

One adaptation of benthic infauna is to escape physico-chemical fluctuations by burrowing into the sediment where environmental variations are less pronounced. Although the effects of the sediment characteristics on benthic fauna have been relatively well studied, much less appears to be known about the effect of the organisms on the sediment properties, particularly with reference to sediment transport and biogeochemial cycling (Jones & Jago, 1993). This burrowing activity, together with feeding activity and the production of faeces not only has profound impacts on the physical and chemical characteristics of the sediment but also affects the biology (Meadows, 1991; de Wilde, 1991; Wheatcroft *et al.*, 1994). The processes leading to such changes are collectively termed biomodification, a general term which encompasses the individual processes of bioturbation, biodeposition, biostabilisation and bioirrigation.

Bioturbation or biogenic re-working is defined as the result of sediment ingestion, manipulation (e.g. tube construction) and displacement as organisms pass through or over the sediment (Rhoads, 1967; Rhoads & Boyer, 1982; Reichelt, 1991). The resultant changes in water content, particle size distribution, cohesive and adhesive properties, bed roughness and pH all have some impact on sediment stability, erosion potential and deposition (Winston & Anderson, 1971; Rhoads, 1974; Rhoads & Boyer, 1982; Jumars & 1984a; 1984b; Meadows & Meadows, 1991; Jones & Jago, 1993; Widdows *et al.* 1998a; 1998b). In general, bioturbation results in direct destabilisation of the bed, through bioresuspension, or indirect destabilisation following biomodification of the physical properties of the sediment as described above (Graf & Rosenberg, 1997). For example, species such as *Macoma balthica, Yoldia limatula* and *Cerastoderma edule* and may inject watery plumes of faecal and pseudofaecal material into

the water column resulting in direct bioresuspension (Bender & Davis, 1984; Davis, 1993; Retraubun *et al.*, 1996; Widdows 1998a; 1998b). Species feeding below the surface (subsurface deposit feeders such as *Arenicola marina*) create voids within the sediment and may also inject water into the sediment in order to facilitate movement (Rhoads, 1974). This can reduce sediment shear strength, resulting in sediment destabilisation through indirect bioresuspension.

In contrast, suspension feeding and the subsequent production of faecal pellets together with the production of tests and shells can result in direct biodeposition (Rhoads, 1974; Meadows & Meadows, 1991; Reichelt, 1991; Graf & Rosenberg, 1997; Widdows 1998a; 1998b). Organisms associated with direct biodeposition include *Mytilus edulis* and *Cerastoderma edule*. Other groups of organisms, such as tubiculous Polychaetes, may occur at densities high enough so that their tubes protect the bed from erosion (biostabilisation) and indirectly encourage biodeposition (Eckman *et al.*, 1981; Jumars & Nowell, 1984a; Meadows and Horiri, 1991; Graf & Rosenberg, 1997). Adhesive mucillage produced by benthic organisms (both fauna and microflora) has also been found to encourage biodeposition and biostabilisation (Paterson, 1989; Graf & Rosenberg, 1997).

Bioirrigation, or burrow ventilation, by benthic infauna, together with the transportation of reduced sediment to the surface as a result of feeding, increases the depth of oxygen penetration, therefore increasing the depth of the Redox potential discontinuity (RPD) (Rhoads, 1974; Meadows & Meadows, 1991; Reichelt, 1991; Woodin & Marinelli, 1991; Graf & Rosenberg, 1997). In addition to alteration of the chemical properties of the sediment, this also has important implications for the flux of nutrients and contaminants (Wheatcroft *et al.*, 1994). The impact of animal activity on chemical and nutrient fluxes has also been termed 'biodiffusion' (Meadows & Meadows, 1991).

As the majority of the particles in muddy substrata are of a size which may be ingested or otherwise manipulated by benthic organisms, the degree of bioturbation tends to be greater than that in coarser sediments (Rhoads, 1974). Rhoads (1967) stated that surface sediments, wherever deposit feeders are abundant, may be completely reworked several times before being isolated from further biological activity by additional sedimentation.
Sediment transport studies, in relation to animal / microbial activity, have generally involved the use of various types of flume in order to measure critical erosion velocities. These studies have involved both field (e.g., Widdows *et al.*, 1998 a; 1998b) and laboratory (e.g., Meadows & Tait 1989; Davis, 1993) measurements. In addition to this, attempts have also been made to quantify the level of bioturbation caused by various species and faunal compositions.

Methods used include the deliberate addition of inert particles (e.g., glass beads) to the sediment (Mahut & Graf, 1987; Wheatcroft, 1992); the use of labelled particles to act as tracers (Olmez *et al.*, 1994; Wheatcroft *et al.*, 1994; Krezoski *et al.*, 1995); the measurement of natural radionuclide deficits within the sediment (Smethie *et al.*, 1981, Wheatcroft & Martin, 1996) and various techniques to directly measure sediment displacement (Rhoads, 1967; Hickson, 1994; Retraubun *et al.*, 1996). The degree of bioturbation is species specific and is closely related to the feeding mode and depth of the organism concerned, together with its motility, size and abundance as well as community composition (Rhoads, 1974; Rhoads & Boyer, 1982).

1.1.2. Estuarine pollution

Estuarine and coastal waters have, for many centuries, served as a resource base to be used by humans for food, minerals, power generation, transport and recreation. They also receive large volumes of domestic and industrial waste, the environmental effects of which have long been cause for concern. The presence of fine grained and cohesive sediments, together with the affinity of pollutants for being adsorbed onto particles leads to estuaries becoming a sink for contaminants. Diagenetic processes within sediments lead to contaminants being sequestered within the sediments, especially under anaerobic conditions (Elderfield & Hepworth, 1975; Libes, 1992; Knezovich, 1994). As stated by Rhoads (1974), oxygen penetration into the sediment may be increased as a result of burrow construction and ventilation. Changes in the aerobic / anaerobic balance in the sediments may affect the chemical state of the pollutants in question and their affinity for binding to sediment particles. For example, sulphide, present in anoxic, organic rich sediments, is known to form complexes with a number of metal ions. However, disturbance of contaminated, sulphidic sediments can result in an increase in redox potential, due to exposure to oxygenated water, and may result in the breakdown of sulphide-metal complexes causing the release of metals into the water column (Calmano *et al.*, 1996). In contrast, anoxia has been found to cause the release of metals such as arsenic, cobalt and chromium into the water column and the introduction of oxygen would lead to their precipitation by hydrous ferrous oxides (Petersen *et al.*, 1996). A knowledge of the impact of animal activity on sediment dynamics is therefore of great relevance to the study of the fate of contaminants. It is also needed in coastal and estuarine management, navigation, channel dredging and waste disposal and may influence decisions on where to place various marine structures.

It is generally accepted that increased levels of contamination adversely affect organism survival, growth and biomass and that communities in organically polluted areas do not progress beyond the pioneering stages (dominated by opportunistic species) of development

(Figure 1.1) (Pearson & Rosenberg, 1978). These types of communities, with low species richness, a high abundance of *r*-strategists and low biomass, have been found to have less of an impact on the sediment than the more developed equilibrium communities (Meadows, 1991). Whilst it is accepted that the high abundance of opportunistic species (e.g. oligochate worms) may result in higher rates of bioturabation, it is hypothesised that bioturbation potential should increase, in terms of depth and burrow volume, as the level of contamination declines (Figure 1.1). With this, changes in the sediment properties and, ultimately, sediment stability might be expected although the relationship between animal activity and sediment stability is not simple.

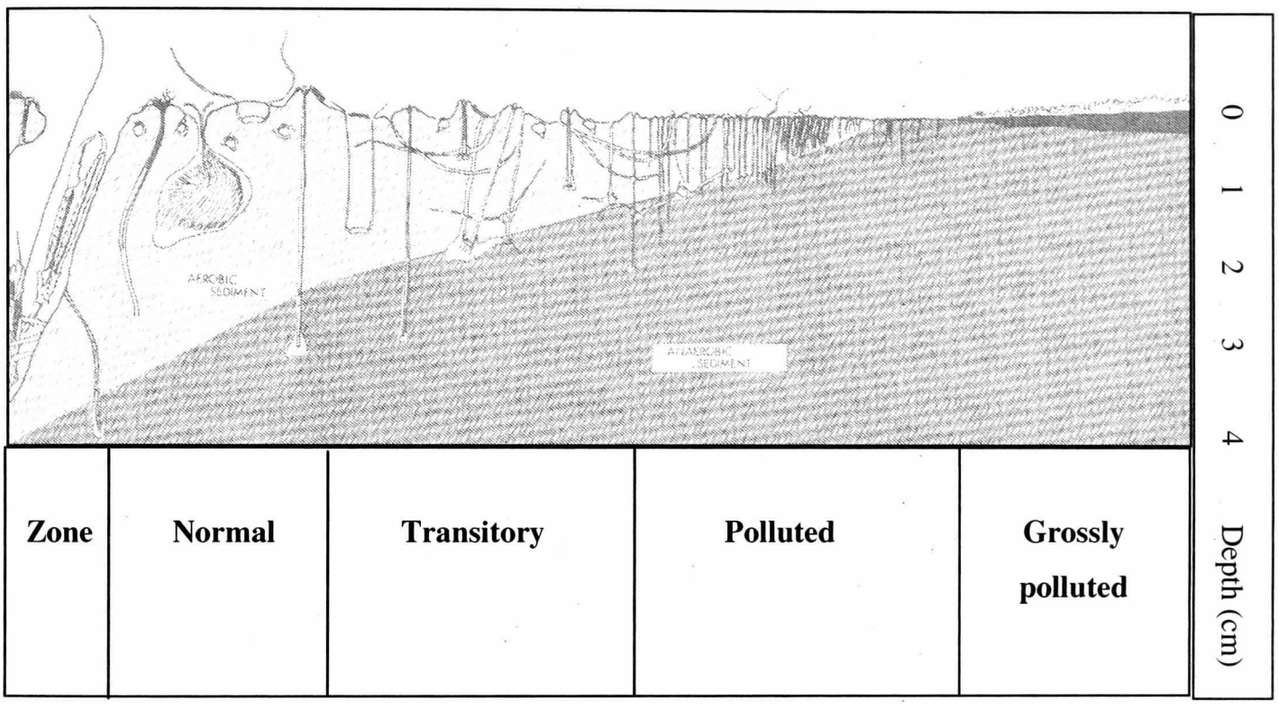


Figure 1.1. Typical changes in fauna and sediment structure along a gradient of organic enrichment (redrawn from Pearson & Rosenberg, 1978).

1.1.3. The role of intertidal sediments in coastal protection.

In areas prone to flooding or where land has been claimed from the sea (e.g. much of the land surrounding the Humber estuary, the Tees estuary and a number of estuaries in south eastern England), coastal defence is required. Hard sea defences (such as sea walls and groynes) often result in accelerated erosion at their edges and are often more effective at deflecting wave energy than attenuating it (CPSL, 2001). Natural intertidal areas such as mudfalts and saltmarshes attenuate wave action therefore buffering coastal areas against erosion and protecting or enhancing the protection offered by sea walls. According to Dyer (1998), the high suspended sediment concentrations in estuarine waters cause viscous attenuation which, in combination with bed friction, can result in the loss of 93-96% of the energy of non breaking waves. The resulting oscillatory currents are strongest in a shoreward direction

which causes net transport of sediment towards the upper flats (Wells & Coleman, 1981, in Dyer, 1998).

In recent times, the rate of global warming is thought to have increased and there is now great concern over the associated sea level rise and the expected increased frequency of storms and tidal surges (CPSL, 2001). Win *et al.* (2003) stated that the magnitude and timing of this increase in sea level rise are uncertain but MAFF recommendations suggest that an average annual rate of 6 mm should be assumed. This is more than twice the rate that has occurred over the last 50 years and implies that sea levels will rise by 300 mm, relative to land levels, over the next 50 years (Win *et al.*, 2003). This, together with subsidence of the land due to isostatic rebound, will significantly increase the risk of flooding in coastal areas and the role of mudflats in coastal defence is therefore becoming increasingly important. In areas where large scale land claim has taken place, such as the Humber, the Tees and much of the Essex coastline (Möller & Spencer, 2002), coastal defence is particularly important and natural sea defences are often a more effective and economically viable method of coastal protection than hard engineering structures such as sea walls (Hughes, 1999; CPSL, 2001). In a number of areas (e.g., Paull Holme Strays in the Humber estuary and Tollsbury in Essex), managed realignment schemes, where sea walls are neglected or deliberately breached, are being carried out. This allows some of the claimed land to be returned to the sea to allow the development of intertidal mudflats and saltmarsh, thus not only increasing natural defence from the sea, but also leading to habitat creation (Hughes, 1999).

Given their potential to modify sediment properties, sediment dwelling organisms may play an important role in maintaining conditions (e.g. maintenance of surface roughness) within these intertidal areas which enhance their ability to absorb wave energy. They also have a strong influence over the establishment of saltmarsh vegetation which also provides effective coastal protection (Hughes, 1999). Therefore, the study of their behaviour within and influence over mudflat properties and sediment dynamics is of great relevance.

1.2. AIMS

The previous section highlights the link between the hydrodynamic regime of estuaries, the sedimentological properties and the benthic communities and the fact that they may all influence each other. The present study aims to examine the effects of petrochemical pollution on the interaction between these processes and parameters, determining the way in which petrochemical pollutants can modify benthic communities and animal behaviour and determining the subsequent impacts of these changes on the sediment properties. The study was carried out on the intertidal mudflat at Saltend and the adjacent mudflat at Paull (Figure 1.2) which are influenced, to varying degrees, by effluent discharged from BP Chemicals (Saltend) Ltd into the intertidal area at Saltend. These mudflats are situated in the middle region of the Humber estuary where the environmental conditions are unfavourable to most organisms due to salinity stress and highly turbid conditions. Species diversity is low and additional work was therefore carried out on the Skeffling mudflats at the more seaward end of the estuary.

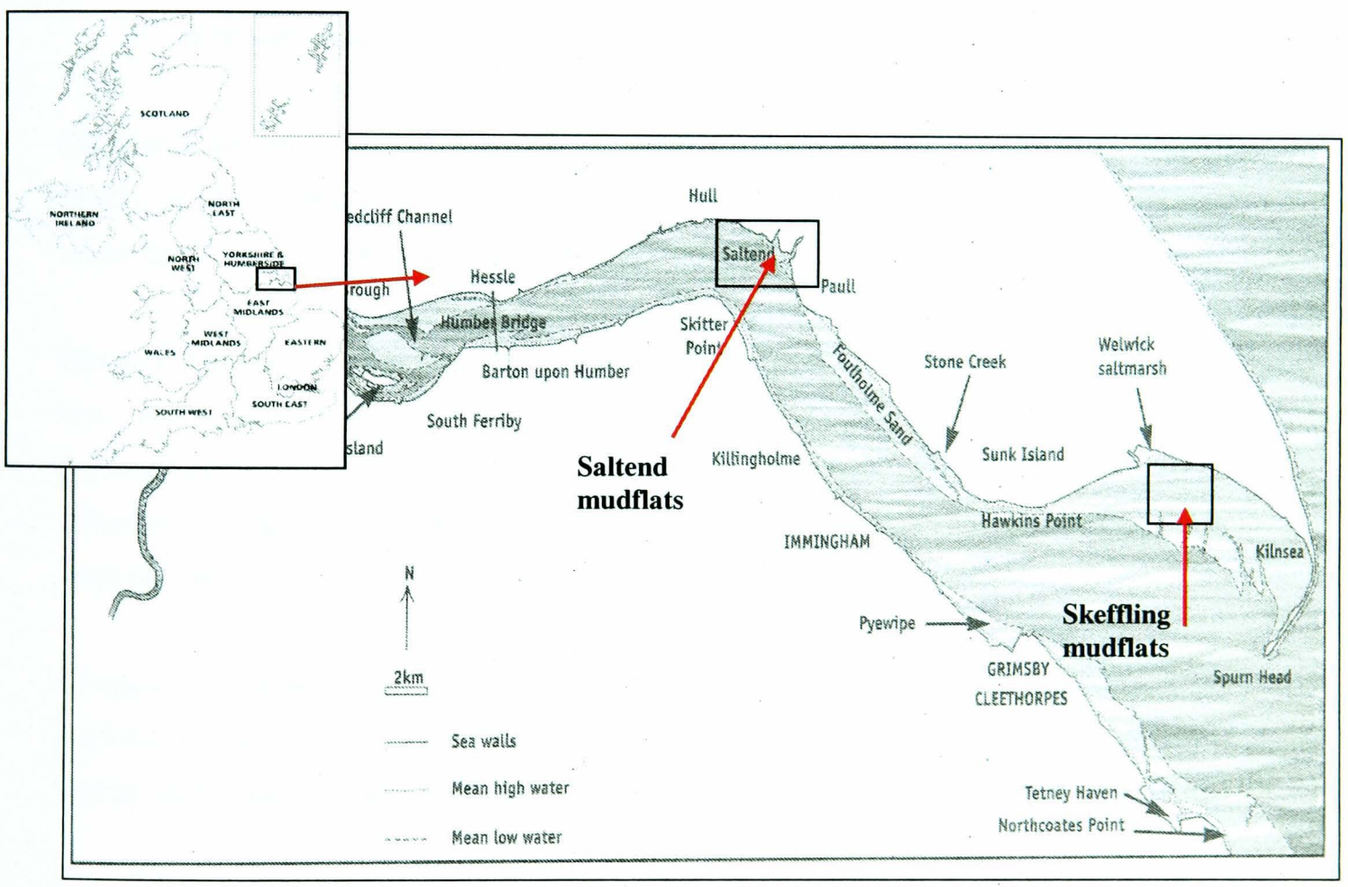


Figure 1.2. The location of the Humber estuary and the mudflats at Paull, Saltend and Skeffling

According to the findings of Pearson & Rosenberg (1978), benthic community changes are expected to occur in the form of reduced number of species and community biomass together

with increased abundance in the vicinity of the discharge. Individual animal size and biomass is also expected to decrease with increasing levels of contamination. It is therefore hypothesised that a reduction in bioturbation potential will be observed as a result of pollution induced benthic community changes, both in terms of sediment mixing and the depth and volume of burrow structures created by the communities. Following this, an increase in the stability of the more polluted sediments might be expected. The objectives of the present study are therefore as follows:

- To determine the impact of the discharge from BP Chemicals (Saltend) Ltd on the benthic communities at Saltend and Paull in terms of their species, composition, abundance, biomass and size / biomass spectra.
- To examine the bioturbation potential, in relation to pollution, of different species and community compositions.
- To determine whether the overall activity of both benthic communities (including macrofauna and benthic microalgae) and individual species is sediment stabilising or destabilising.

Chapter 1 provides a general introduction to the Humber estuary and study sites including details of the ecology of the key species present since their ecology has important implications for bioturbation and therefore their effect on sediment properties.

Chapter 2 examines the physical and chemical properties of the sediment including particle size, water and organic content, redox potential (Eh), pore water pH and salinity, sedimentation rate, metal content, carbohydrate and microalgal content. These parameters influence both the benthic communities present, and therefore their potential to modify the sediment properties, and the erosion characteristics of the sediment.

Chapter 3 examines the toxicity of both the effluent being discharged from BP Chemicals (Saltend) Ltd and of the receiving sediment. This information, together with that in Chapter 2 will be used to aid the interpretation of the faunal community data presented in Chapter 4.

Chapter 4 describes changes in the faunal community characteristics at Saltend and Paull, both in terms of seasonality and with respect to proximity to the discharge. Temporal and spatial patterns of species composition, abundance, biomass and diversity are examined together with changes in the size and biomass frequency distribution with respect to pollution.

Chapter 5 describes the impact of biological activity on sediment properties. Various field and laboratory methods of quantifying bioturbation are used and the impact of pollution, and the resultant benthic community changes, on bioturbation potential are examined.

Chapter 6 introduces the processes of sediment erosion and the various methods of quantifying it. Details of the construction of a laboratory flume are given and initial calibration data are presented.

Chapter 7 describes the measurement of sediment erosion properties using sediment samples taken from areas under the influence of different levels of pollution with different community characteristics. Laboratory flume studies are carried out together with field and laboratory measurements using a Cohesive Strength Meter.

Chapter 8 links the forgoing chapters and provides a general discussion of the overall effects of pollution and biological communities on the properties of estuarine sediments.

1.3. STUDY AREA

1.3.1. Humber estuary

The Humber is the UK's largest estuary with a tidal excursion of 120Km on the River Trent and 140Km on the River Ouse (Pethick, 1990). The catchment covers one fifth of England (over 24000 km^2), extending from Birmingham in the south, Swaledale in the north and the Pennine watershed in the west (Pethick, 1990; Environment Agency, 1995). At high water, the channel depth is 18m at its deepest and the estuary has a tidal range of 6.5 m at the mouth, rising to a maximum of 7.2 m at Saltend (Environment Agency, 1995). The duration of the ebb is approximately eight hours and current velocities are considerably lower than on the flood tide which only lasts for four and a half hours. The estuary is generally well mixed although a horizontal halocline exists across the estuary as a result of the Coriolis force (Pethick, 1990; Allen *et al.*, 1996). This forces the saline intrusion along the northern bank whilst the dominant freshwater flow is along the southern bank. Variations in salinity are between 29 in the south and 32 in the north (Pethick, 1990). Since the current velocity on the flood tide is greater than that on the ebb, the currents along the north bank are stronger. An important implication of this is that industrial waste discharged into freshwaters passes along the southern bank whilst the cleaner North sea water flows along the northern bank (Edwards *et al.*, 1987, in Pethick, 1990). The saline intrusion reaches 45Km inland and turbidity reaches over 5g l^{-1} (Allen *et al.*, 1996). The catchment area is inhabited by 20% of the UK population (Uncles *et al.*, 1998) which equates to almost 12 million (http://www.statistics.gov.uk/) people from whom the estuary receives domestic, industrial and agricultural waste (Environment Agency, 1995).

The chemical and biological quality of the Humber estuary is routinely monitored by the Environment Agency and, in the case of the chemistry, concentrations are compared with Environmental Quality Standards (EQSs). This includes analysis of pH, ammonia, BOD, metals, synthetic organic compounds and pesticides. The Environment Agency (1995), suggested that with the exception of copper, the concentrations of all substances complied with their respective EQS at all sites, including Saltend. In addition, concentrations show a general trend of decline in comparison to previous years (Environment Agency, 1995). With the exception of iron (on the north bank), levels of metals in the intertidal sediments were all below the five year mean in 1995.

1.3.2. Sampling sites

The study was carried out primarily on the Saltend mudflats, where effluent from BP Chemicals (Saltend) Ltd. is discharged intertidally into a natural drain (Old Fleet Drain) running through the mudflat (Figure 1.3). The adjacent mudflat at Paull appears to be largely

unaffected by the discharge and since the sediment characteristics are similar to those at Saltend, this site serves as a good control (Allen, 2000a; Nikitik, University of Hull, Pers. comm). Both mudflats consist of fine grained, cohesive sediments with a high organic content and dense populations of invertebrate species. They are therefore valuable feeding grounds for birds. The Humber estuary is a designated European Marine Site and a proposed SAC (Special Area of Conservation) (English Nature, 2002). In particular, the mudflats at Saltend and Paull are collectively classed as a SSSI (Site of Special Scientific Interest), are a designated SPA (Special Protection Area) under the Wild Birds Directive (79/409/EEC), as a result of the bird populations, and are also protected under the Species and Habitats Directive (92/43/EEC) (English Nature, 2002). Species of national importance include Teal and Golden Plover (Allen, 2000a; Cutts, 2001). Eighty three species of fish are also present in the Humber including cod (*Gadus morhua*), flounder (*Pleuronectes flesus*), four species of goby (*Pomatoschistus minutus, P. pictus, P. microps* and *Aphia minuta*), whiting (*Merlangus merlangus*) and sprat (*Sprattus sprattus*) (Elliott & Marshall, 2000). The estuary also serves as an important spawning and nursery area for plaice (*Pleuronectes platessa*) and sole (*Solea solea*).

Following an initial pilot survey, seven sites along the banks of Old Fleet Drain were chosen to represent spatial changes in the community types present along a pollution gradient (Figure 1.3). Due to the soft, unconsolidated nature and depth of the mud, it was not possible to walk safely any further down the shore and the range of community types is therefore more restricted than it perhaps could have been. Since Old Fleet Drain runs from the high shore to the low shore, a further five control sites were chosen at Paull to account for community changes attributable to tidal height rather than pollution (Figure 1.3). The salinity in this part of the estuary ranges from 11-21 on a spring tide and 14.6-23.4 on a neap tide (Ratcliffe, 1979). These conditions are generally unfavourable to most benthic organisms and although the animals present in this part of the estuary are highly abundant, the number of species is limited. Therefore, an invertebrate survey along a 500 m long transect of the more seaward Skeffling mudflat was carried out with the aim of examining the effects of bioturbation by a wider range of species (Figure 1.4). Grid references for all sampling sites are given in Table 1.1.

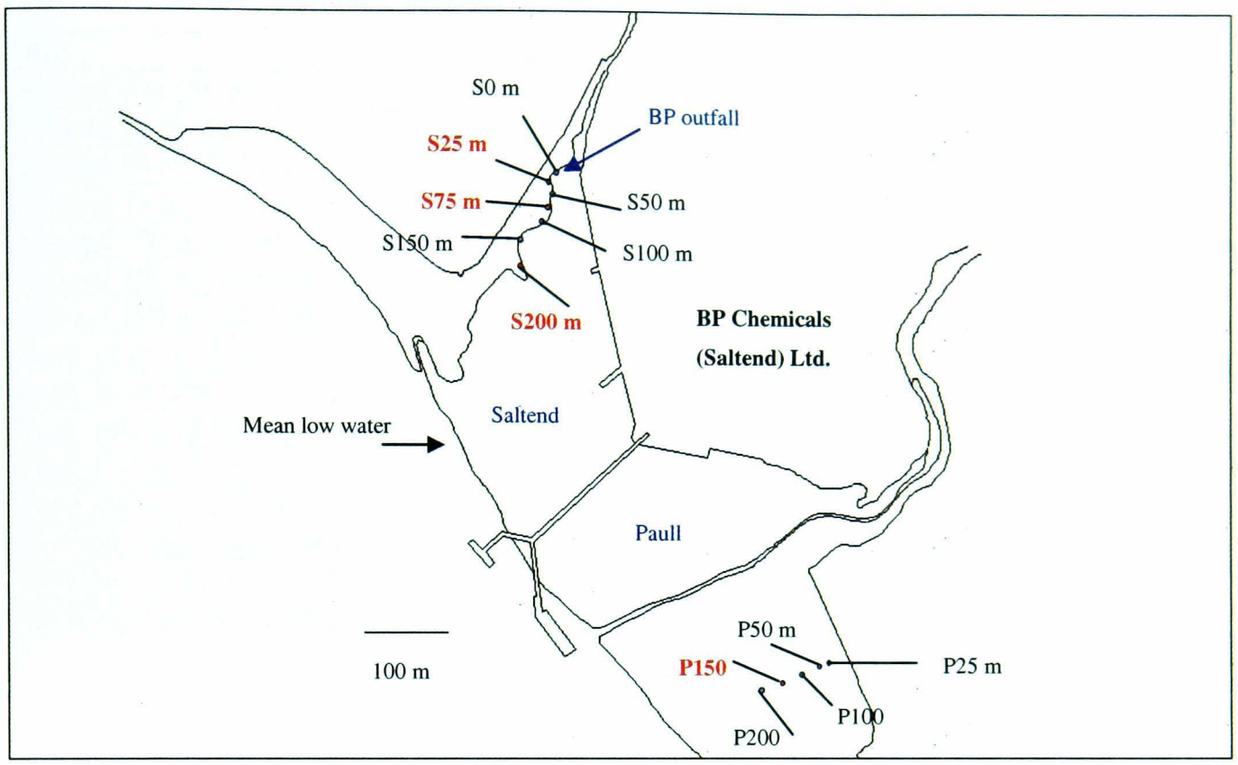


Figure 1.3. Locations of the sampling sites at Paull and Saltend. Key sites are in red.

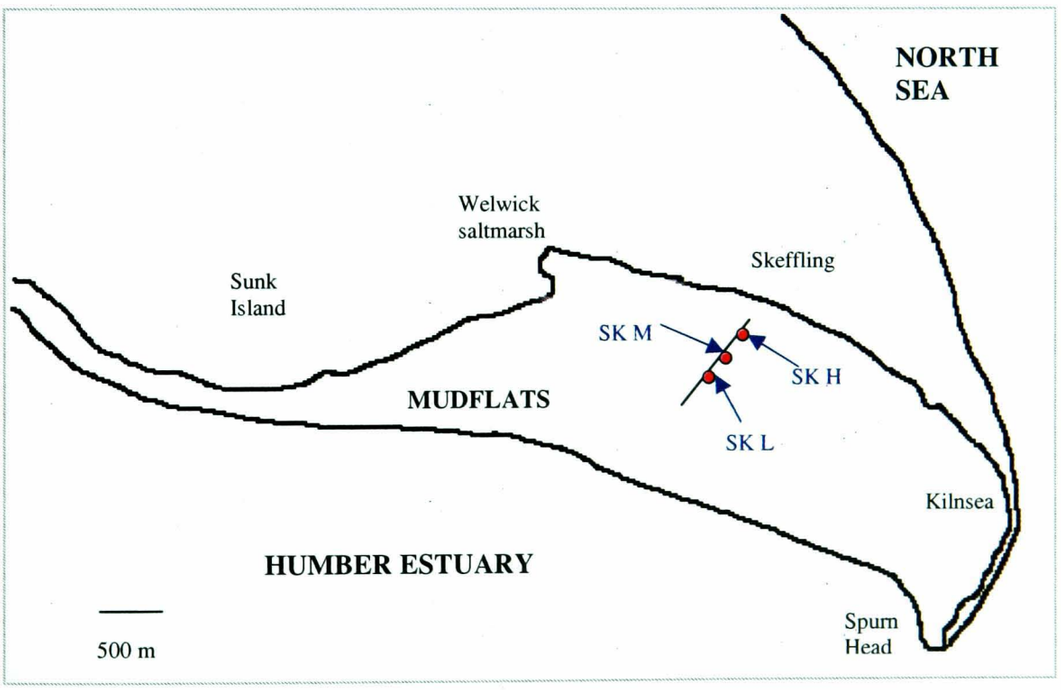


Figure 1.4. Locations of the Skeffling sampling sites.

Table 1.1. Positions and tidal heights of sampling sites

Site	Tidal height (m)	OS Grid reference
Saltend 0 m (S0 m)	1.15	TA 158 279
Saltend 25 m (S25 m)	1.12	TA 157 279
Saltend 50 m (S50 m)	0.96	TA 158 279
Saltend 75 m (S75 m)	0.89	TA 158 278
Saltend 100 m (S100 m)	0.79	TA 157 279
Saltend 150 m (S150 m)	0.70	TA 157 278
Saltend 200 m (S200 m)	0.62	TA 157 277
Paull 25 m (P25 m)	1.26	TA 163 267
Paull 50 m (P50 m)	1.16	TA 163 268
Paull 100 m (P100 m)	0.96	TA 164 267
Paull 150 m (P150 m)	0.85	TA 164 268
Paull 200 m (P200 m)	0.81	TA 165 268
Skeffling high shore (SKH)	5.21	TA 368 181
Skeffling mid shore (SKM)	5.09	TA 366 177
Skeffling low shore (SKL)	4.97	TA 365 175

1.3.3. BP Chemicals

The BP plant, which was commissioned in 1927 as an alcohol distillery, is now Europes largest acetic acid plant (M. Joslin, BP Chemicals (Saltend) Ltd. Pers. comm.), producing over 600 000 tonnes yr^{-1} with smaller amounts of formic acid, acetone and acetyl aldehyde. The effluent, which has been continually discharged since 1982, is made up of a number of internal discharges which are collectively known as EPH10. Each separate point of discharge has an individual consent with IPPC (Integrated Pollution Prevention and Control) authentication. The effluent, 95% of which is water (M. Joslin, BP Chemicals (Saltend) Ltd, pers. comm.), is largely organic, acidic, biodegradable and has a high BOD. It is discharged at 30°C and therefore can be seen floating (as an oily sheen) on the surface of the water. This means that at high tide, the effluent can potentially spread and as the tide recedes and settle over the whole mudflat. At low water, the only source of dilution is a small freshwater stream entering the drain on the upper shore. Limited treatment, including neutralisation, recycling, natural treatment in a pond (oxidation and photooxidation) and some oil separation, is carried out although the company has a policy of waste minimisation rather than treatment prior to disposal. Improvements to plant processes have led to a significant improvement in effluent quality. For example, between 1989 and 1997, the metals content of the effluent was reduced from 9 to 1.5 tonnes / month (M. Joslin, BP Chemicals Ltd. Pers. comm.). The invertebrate communities in the vicinity of the discharge are impoverished consisting mainly of Oligochaetes and Nematodes (Allen *et al.,* 1996; Scott, 1996; Nikitik, University of Hull, Pers. comm).

The relative concentrations of the components of the effluent are variable and can change on an hourly basis. In addition to water quality monitoring, the effluent is monitored approximately once a month (by the Environment Agency) to ensure that the consent limits of the discharge are not breached. These limits are based on toxicity data, together with mixing and dilution within the receiving body of water and are set to ensure that the EQS is not breached (J. Wilson, BP Chemicals (Saltend) Ltd. Pers. comm.). Effluent quality data for the years 1999 and 2000 are presented in Appendix 1 with a summary of the overall means for this time period being given in Table 1.2.

Table 1.2. Characteristics of the BP effluent (means for 1999 and 2000) provided by the Environment Agency (Willerby office). Total DDT includes DDT-op and pp, DDE-pp and TDE-pp. Total drins includes aldrin, dieldrin, endrin and isodrin.

Determinand	Units	Mean concentration	Determinand	Units	Mean concentration
pH	pH units	5.9	Trichloroethene	$\mu g\ l^{-1}$	3.4
BOD ATU	$mg\ l^{-1}$	1483.8	Tetrachloroethene	$\mu g\ l^{-1}$	2.87
COD	$mg\ l^{-1}$	2650.0	Trichloromethane	$\mu g\ l^{-1}$	4.78
CONDUCTIVITY @25C	$uScm^{-1}$	1911.7	Tetrachloromethane	$\mu g\ l^{-1}$	4.47
Microtox	(5 min)	0.1	Trichlorobenzene (total)	$\mu g\ l^{-1}$	0.29
Microtox	(15 min)	0.1	Hexachlorobenzene	$\mu g\ l^{-1}$	0.05
Suspended solids @105C	$mg\ l^{-1}$	48.3	Total PCB	$\mu g\ l^{-1}$	0.53
Ammoniacal nitrogen	$mg\ l^{-1}$	3.1	Hexachloro 1,3 Butadiene	$\mu g\ l^{-1}$	0.07
Nitrate	$mg\ l^{-1}$	2.3	Pentachlorophenol	$\mu g\ l^{-1}$	1.2
Nitrite	$mg\ l^{-1}$	2.1	Azinphos (ethyl+methyl)	$\mu g\ l^{-1}$	0.06
Total oxidised nitrogen	$mg\ l^{-1}$	4.5	Chlorfenvinphos	$\mu g\ l^{-1}$	0.02
Orthophosphate	$mg\ l^{-1}$	1.4	Diazinon	$\mu g\ l^{-1}$	0.02
Total phosphate	$mg\ l^{-1}$	1.7	Fenitrothion	$\mu g\ l^{-1}$	0.02
Arsenic	$\mu g\ l^{-1}$	4.7	Fenthion	$\mu g\ l^{-1}$	0.03
Cadmium	$\mu g\ l^{-1}$	1.6	Propetamphos	$\mu g\ l^{-1}$	0.02
Chromium	$\mu g\ l^{-1}$	77.1	Simazine	$\mu g\ l^{-1}$	0.05
Copper	$\mu g\ l^{-1}$	260.3	Total Drins	$\mu g\ l^{-1}$	0.24
Iron	$mg\ l^{-1}$	1.27	Atrazine	$\mu g\ l^{-1}$	0.05
Lead	$\mu g\ l^{-1}$	6.1	Total DDT	$\mu g\ l^{-1}$	0.16
Molybdenum	$\mu g\ l^{-1}$	578.2	Dichlorvos	$\mu g\ l^{-1}$	0.02
Nickel	$\mu g\ l^{-1}$	171.7	Endosulphan (α,β)	$\mu g\ l^{-1}$	0.14
Zinc	$\mu g\ l^{-1}$	184.0	Total HCH (α,β,γ)	$\mu g\ l^{-1}$	0.16
Tributyl tin	$\mu g\ l^{-1}$	0.0	Malathion	$\mu g\ l^{-1}$	0.02
Triphenyltin	$\mu g\ l^{-1}$	0.0	Parathion (ethyl, methyl)	$\mu g\ l^{-1}$	0.03
1,2-Dichloroethane	$\mu g\ l^{-1}$	5.1	Trifluralin	$\mu g\ l^{-1}$	0.11
1,1,1-Trichloroethane	$\mu g\ l^{-1}$	3.3			

1.4. ECOLOGY OF THE HUMBER SPECIES

Despite the history of industrial discharge to and pollution of the Humber, the estuary is biologically highly productive and the mudflats support rich invertebrate communities. These, in turn, provide a source of food for the internationally important populations of over wintering birds which use the estuary (Cutts, 2001; English Nature, 2002).

Communities occurring in the upper shore areas of the Saltend site, in the main study area, are generally dominated by tubificid and enchytraeid worms with densities in the vicinity of the discharge being between 16, 000 - 28, 000 m^{-2} (Allen, 2000a). Other important species in this area include *Hediste diversicolor* at densities of around 5000 m^{-2}; *Manayunkia aestuarina* at densities of around 3000 m^{-2}; *Streblospio shrubsolii* (approximately 900 m^{-2}); *Corophium volutator* (<100 m^{-2}); *Macoma balthica* (<100 m^{-2}) and Nematodes (approximately 5000 m^{-2}) (Allen, 2000a; Nikitik, University of Hull, Pers. comm.). At Paull, the upper shore communities are dominated by the polychaetes *Hediste diversicolor* (approximately 1500 - 4000 m^{-2}) and *Streblospio shrubsolii* (approximately 3000 m^{-2}). *Corophium volutator* and *Macoma balthica* are more abundant than at Saltend with densities of around 500 - 45, 000 m^{-2} and 250 - 400 m^{-2} respectively (Mortimer *et al,* 1999; Allen, 2000a). *Manayunkia aestuarina,* oligochaetes (Tubificidae and Enchytraeidae) and Nematodes are also present at densities of approximately 2000, <200 and 1000 m^{-2} respectively. Details of the biology (where information exists) are given below for these species.

Infaunal species occurring in the more seaward part of the estuary at Skeffling include those listed above together with *Eteone longa, Nephtys hombergii, Cerastoderma edule, Pygospio elegans, Hydrobia ulvae, Retusa obtusa* (Davey & Partridge, 1998; Widdows, 1998a; 1998b; Mortimer *et al,.* 1999) *Mya arenaria, Scrobicularia plana* and *Arenicola marina* (personal observations).

1.4.1. *Hediste diversicolor*

Hediste (*Nereis*) *diversicolor*, the estuary ragworm, is a widely distributed inhabitant of the intertidal zone of brackish and marine waters throughout Europe and is found at its greatest densities in the middle and innermost parts of estuaries during summer (Green, 1968, Kristensen, 1984, Ólafsson & Persson, 1986). Together with *Corophium volutator,* it is one of the most common and abundant species in sheltered intertidal and shallow bottoms (Ólafsson & Persson, 1986). *H. diversicolor* is an euryhaline species and is able to tolerate salinities as low as 2-3 and as high as 200 (Nielsen *et al.*, 1995) but is unable to breed in areas where the salinity is less than 10 (Green, 1968, Neuhoff, 1979, in Scaps, 2002). It is the ability to tolerate conditions of hypoxia (Wells & Dales, 1951, in Scaps, 2002; Kristensen,

1983) and variations in temperature (Ivela, 1970, in Scaps, 2002; Wolff, 1973) and salinity (Wolff, 1973; Neuhoff, 1979, in Scaps, 2002) which has allowed this species to thrive in the naturally fluctuating conditions prevalent in estuaries.

H. diversicolor is an aggressive, territorial species which distributes itself regularly across the substratum (Lambert, 1986, in Scaps, 2002). Whilst it is generally a poor competitor (Scaps, 2002), it is an important structuring factor in estuarine ecosystems (Kristensen, 1984; Rönn *et al.*, 1988). Interspecific interactions can cause death, migration or deter the recruitment of species such as *Corophium volutator* and *Macoma balthica,* either directly by predation or indirectly through mechanical disturbance of the substratum (Ólafsson & Persson 1986; Rönn *et al.* 1988). In addition, large populations of *C. volutator* are able to negatively affect recruitment of *H. diversicolor* by the burial and accidental ingestion of the larvae (Ólafsson & Persson, 1986). Occasionally acting as a predator itself, *H. diversicolor* is also an important food source for wading birds, fish and other predatory invertebrate species.

According to Scaps (2002), individuals of this species reach maturity after 1-2 years although this time-scale varies geographically, according to environmental conditions and the size of the worms (Mettham *et al.*, 1982). Olive & Garwood (1981) reported a three year life span for worms in the Blyth estuary. Immature worms are a reddish brown colour with males and females being externally indistinguishable until they mature and become ripe (Dales, 1950). During this time, the males are completely green, due to the white mass of sperm which packs the coelom and the females are a darker green, usually retaining some of the reddish brown pigment characteristic of their colour for the rest of the year (Dales, 1950; Scaps, 2002). Prior to spawning, histolysis begins with the gradual digestion of the muscle layers by the phagocytes. This allows the females to release their oocytes, into their burrows, by rupture of the body wall which then stimulates the males to release their sperm. Sperm are generally released into the water column overlying burrow entrances, and are then drawn into the burrow to fertilise the eggs by ventilation currents (Bartels-Hardege & Zeeck, 1990). As in the females, there is also considerable rupture of the body wall when males release their sperm making the worms abnormally fragile during spawning (Green, 1968). Due to this rupture of the body wall, spawning is shortly followed by death and individuals of this species are therefore only capable of reproducing once (Dales, 1950; Scaps, 2002).

The time of spawning varies between populations and is thought to be controlled by temperature and lunar periodicity (Dales, 1950; Scaps, 2002). Bartels-Hardege & Zeeck (1990) reported spawning to coincide with a new or full moon, at temperatures above 6°C. According to Scaps (2002), the temperature at which spawning is induced varies between

studies but is generally between 5 and 11°C. In the Humber, spawning has been reported to take place in March, at the mouth and in June or July in the upper reaches of the estuary (Grant *et al.*, 1990, in Scaps, 2002). Following fertilisation, the eggs, which are incubated in the burrow by the dying female, hatch within about a week (Green, 1968). Although the larvae are able to swim, they develop in the burrows or on the surface of the mud and lack a true pelagic stage (Dales, 1950).

H. diversicolor is classed as an omnivore and has been reported to utilise a wide range of foraging strategies (Wolff, 1973). Diatoms and bacteria are consumed through surface deposit feeding (Green, 1968; Trevor, 1977) although this species may also act as a facultative filter feeder by secreting a mucous net to trap food particles in the water column and then consuming the net and its contents (Riisgård,1991). According to Vedel *et al.* (1994), *H. diversicolor* can survive purely on a diet of phytoplankton and Nielsen *et al.* (1995) reported a threshold phytoplankton concentration of 1-3 μg chlorophyll a l^{-1} to induce a shift from deposit to suspension feeding. Meiofauna and other invertebrates are taken and Rönn *et al.* (1988) found parts of *Corophium volutator* and juveniles of *Macoma balthica* in the guts of *H. diversicolor.* According to Green (1968), feeding on the dying juveniles of grey mullet has also been observed.

H. diversicolor generally inhabits muddy substrata, where it constructs mucous lined 'Y' shaped burrows (Trevor, 1977; Davey, 1994) but may also be found in coarse sandy or gravelly sediment (Clay, 1967, in Scaps, 2002). A detailed account of the way in which *H. diversicolor* constructs its burrow is given by Trevor (1977). During burrow construction, the proboscis is rapidly everted to create a hole into which the worm can crawl. Initial entrance to the sediment is by means of lateral movements of the anterior segments with digging beginning once the first 3-4 segments are buried. During digging, the proboscis is everted at the peak of a coincident pressure pulse in the anterior coelom. The magnitude of this pulse increases as burrowing proceeds and is related to the resistance of the substratum. Despite the fact that *H. diversicolor* is an important species in terms of bioturbation through its burrowing activity, burrow ventilation, feeding and faecal production (Davey, 1994; Scaps, 2002) it is thought to construct new burrows only infrequently (Trevor, 1977).

1.4.2. *Corophium volutator*

The amphipod, *Corophium volutator* (Pallas) is an euryhaline species (Hart, 1930; McLusky, 1967) present along European coasts from southern Norway, throughout the North Sea to the Bay of Biscay, the Adriatic, the Baltic Sea, the Black Sea and the Azov Sea (Hart, 1930;

Crawford, 1937; Watkin, 1941; Stock, 1952; Segerstråle, 1959; Stock & de Vos, 1960, in Meadows & Reid, 1966; McLusky, 1968a). Along North American coasts, the species is found between Nova Scotia and southern Maine (Hart, 1930; Muus, 1967; Larsen & Doggett, 1991 in Wilson & Parker, 1996). In Britain, it generally occurs intertidally, the maximum density being at the mid tide level, although subtidal populations, to a depth of 10 m, have been reported in Norway (Sars, 1895, in Meadows & Reid, 1966; Zenkevitch, 1963, in McLusky, 1967). Although restricted in distribution, populations of *C. volutator* may reach extremely high densities on estuarine mudflats (Meadows & Reid, 1966, McLusky, 1967) and together with *Hediste diversicolor,* it is one of the most common and abundant species in sheltered intertidal and shallow subtidal areas (Ólafsson & Persson, 1986). According to Hart (1930) and Watkin (1941), this species is found in its greatest abundance in shallow pools or puddles in the mud.

C. volutator frequently occurs with *Hediste diversicolor* and *Pygospio elegans* and, where abundant, may be the dominant species within the community (Hart, 1930; Meadows & Reid, 1966). Ecologically, it is an important species involved in the breakdown of organic matter and detritus and forming a major constituent of the diet of a number of demersal fish species, wading birds and invertebrate species (McLusky, 1968a; Wilson & Parker, 1996).

Both salinity and the nature of the substratum are thought to be key factors determining the distribution of this species although given the wide salinity tolerance range, the latter is thought to be of greater importance (Hart, 1930; Meadows, 1964a; 1964b; 1964c; McLusky, 1968a). *C. volutator* is found in mud or muddy sand (Hart, 1930; Meadow, 1964a, b, c; s & Reid, 1966; McLusky, 1967) with a high water content (Green, 1968) and, in the Thames estuary, was found to be most abundant in sediments containing approximately 37- 38% silt or clay (Gee, 1961, in Meadows & Reid, 1966; McLusky 1967). *C. volutator* is not found in sulphurous anoxic muds, adversely affected by pollution or containing large amounts of decaying organic matter (Hart, 1930, Meadows & Reid, 1966). However, Remane & Schlieper (1958, in McLusky, 1968a) found *C. volutator* to be tolerant of low oxygen concentrations and studies by Meadows (1964a) revealed that the species actually shows a preference for sediments with some degree of anaerobiosis over aerobic sediments of the same particle size. McLusky (1968a) stated that suitable substrata for this species consist of muds and fine muddy sands with a plentiful supply of detritus but without excessive amounts of organic matter and which are not adversely affected by pollution. Experiments by Meadows (1964a) showed that *C. volutator* actively selects the sediment type in which it will burrow and it was concluded that the nature of the microbiological films on the surface of the

sediment particles was a particularly important factor influencing the sediment type in which *C. volutator* will burrow.

C. volutator is capable of withstanding great variations in salinity (Hart, 1930; Green, 1968) and is found from the fully marine end of estuaries almost to the freshwater end (McLusky 1968a). It has been found to survive for up to 16 days in completely freshwater (Green, 1968) although McLusky (1967; 1968a) stated that the actual tolerance range is 2-50 psu, abundance is reduced between 2 and 5 and that animals are absent from areas with salinities of less than 2. In addition, the species is unable to breed at salinities below 7.5 (McLusky, 1968a; 1968b).

Juvenile amphipods are hatched as small versions of the adults and like *H. diversicolor*, they lack a planktonic larval stage and do not undergo any form of metamorphosis prior to reaching adulthood (Hart, 1930; Meadows, 1964a; Ólafsson & Persson, 1986, Schram, 1986). Fecundity in gammarids is subject to seasonal variation being generally higher in the spring and summer months than during the winter (Cheng, 1942). Breeding patterns also vary geographically (McLusky, 1968a; Ólafsson & Persson, 1986; Wilson & Parker, 1996) with some populations producing two generations in a year whilst others only produce one. According to Wilson & Parker (1996), the females usually die after the release of one or two broods and generally do not live to reproduce the following season. Therefore, populations of *C. volutator* have barely overlapping generations. McLusky (1968a) studied the breeding behaviour of *C. volutator* in the Ythan estuary, Scotland where the first berried (egg bearing) females were noted to appear in May. A dramatic increase in abundance of animals, following hatching, was noted in June and July, ending in August. As is reported for many species, the onset of reproduction was found to be related to temperature and salinity and McLusky (1968a) observed a decrease in both these factors to coincide with the end of the breeding season. According to Segerstråle (1940, in Mclusky, 1968a), the minimum temperature at which *C. volutator* can reproduce is 7 °C breeding does not occur at salinities below 7.5.

Wilson & Parker (1996) studied the breeding patterns of *C. volutator* in the Bay of Fundy where, in the Lower Bay, populations of produced one generation per year whereas, in the Upper Bay, two generations were produced. This difference in reproductive behaviour was attributed to the fact that the waters of the Lower Bay are 6-10 °C cooler than in the Upper Bay which results in a slower growth rate and, therefore, delayed reproduction. This difference in reproductive behaviour in relation to temperature has also been observed in European waters with populations producing two generations per year generally being found in the more southerly regions. Populations in the Dovey estuary (Watkin, 1941; Fish & Mills,

1979) and Whitby Harbour, north east England (Hart, 1930) were found to produce two generations per year whereas more northerly populations in the Ythan estuary (McLusky, 1968a) and the inner Baltic Sea (Segerståle, 1940, in Wilson & Parker, 1996) were found to produce only one generation per year.

C. volutator is primarily a selective deposit feeder (Hart, 1930; Agrawal, 1963; McLusky, 1967; Ólafsson & Persson, 1986), feeding by ingestion of the mud and consuming detritus and the bacteria and diatom films associated with the sediment particles (McLusky, 1967; Green, 1968; Gerdol & Hughes, 1994). *C. volutator* is also capable of filter feeding (Hart, 1930; Segerstråle, 1959, in Green, 1968; Meadows & Reid, 1966; Ólafsson & Persson, 1986; Gerdol & Hughes, 1994) although according to Meadows & Reid (1966), this is only of importance to the juveniles which do not leave their burrows.

C. volutator inhabits a 'U'shaped burrow within the top 5-10 cm of the sediment (Green, 1968; Meadows, 1964a; Meadows & Reid, 1966). A description of the method by which *C. volutator* creates it burrows is given by Meadows & Reid (1966). The animal creates a furrow, by beating of the pleopods, into which the body sinks. The second antennae is then inserted into the mud and the body anchored using the telson, uropods and pereiopods whilst the gnathopods are used to dig. Beating of the pleopods allows expulsion of debris from the burrow. After completion of the burrow, the antennae are used to scrape detritus, with which to line the burrow walls, from around the burrow entrance. This lining extends 1.5-2 mm above the sediment surface and prevents all but the finest particles from entering the burrow and, together with mucous secretions from the animal, prevents the burrow walls from collapsing. Cementation of the burrow walls is thought to be primarily by the biofilms present on the sediment particles and detritus with which the animal lines the burrow although secretions by the animal also aid this binding.

According to Green (1968), the burrows of *C. volutator* are relatively permanent although the animals are capable of swimming and crawling over the sediment surface. At low tide, animals crawl along the surface of the mud, pushing themselves with the telson and uropods and using the second antennae to pull the body along (Hart, 1930). Under water, this species can either crawl over the mud using the pereiopods or beat the pleopods to skim across the surface of the mud. Morgan (1965) observed maximum swimming activity to occur during the early ebb of the tide and according to Meadows & Reid (1966), swimming occurs in short bursts of 5-10 seconds, during which the animal rises to the surface with a sharp flick of the abdomen and beating of the pleopods then sinks back to the bottom as the second antennae comes into contact with the surface. During sinking, beating of the pleopods ceases. The

animals generally swim on their backs which is thought to increase streamlining and facilitate swimming. Swimming is alternated with investigation of the substratum until the animals burrow. Individuals tend to burrow as quickly as possible which may partly be due to predator evasion but also due to the high energy expenditure involved in swimming. Underwater, photopositive behaviour in *C. volutator* was observed whereas out of water, photonegative behaviour was observed. This reaction to light is thought to help maintain the animals within the intertidal zone (Meadows & Reid, 1966).

1.4.3. Spionidae

The main species of Spionid worms found within the study area include *Pygospio elegans* and *Streblospio shrubsoli* (Nikitik, University of Hull, Pers. Comm..). There have been many studies on the North American species *Streblospio benedicti* but studies on *Streblospio shrubsolii* appear to be lacking. However, both species are found in similar ecological niches, in highly disturbed deposit feeding communities, and have similar adaptations to the environment. Therefore studies on the North American species are considered to be relevant here. Spionids of the genus *Streblospio* are small worms (generally less than 2 cm long) inhabiting the upper 2-3 cm of muddy sediments in estuarine and brackish water areas (Sardà & Martin, 1993). They are classed as opportunistic organisms which colonise organically enriched sediments, particularly in low or variable salinity areas (Pearson & Rosenberg, 1978; Dauer *et al.*, 1981; Levin & Creed, 1986). *S. benedicti* constructs an unbranched tube at the sediment surface. It is a highly motile species, moving as often as every 2-3 days although this is often in the form of a tube extension to reach new food supplies (Dauer *et al.*, 1981; Levin & Creed, 1986).

Spionids feed at the sediment-water interface using a pair of grooved, ciliated, tentaculate palps (Dauer *et al.*, 1981). Food particles are either picked up from the sediment surface or from the water column (by lashing the palps or holding them rigid). The particles are then moved down the grooves, by ciliary action, to an everted pharynx (Taghon *et al.*, 1980; Dauer *et al.*, 1981). When suspension feeding, *S. benedicti* hold their palps rigidly in the water column. The feeding rate of this species as found to increase by over 60% in the presence of suspended particles (Dauer *et al.*, 1981). During deposit feeding, in the absence of suspended material, one palp was held rigidly in the water column whilst the other remained in contact with the sediment surface. However, according to Daro & Polk (1973, in Dauer, *et al.*, 1981), Fauchald & Jumars, (1979) and Dauer, 1980 (in Dauer *et al.*, 1981), spionids, including *S. shrubsolii* and *P. elegans* have also been reported to feed on sediment particles, planktonic organisms and meiobenthos. They may therefore be considered as omnivorous.

S. benedicti deposit faecal pellets in their tubes which are removed either by ciliary action and then randomly shot out of the tube entrance (silt and clay size particles) or the worms back into their tubes, beneath the faecal pellet, and push it out of the tube (Dauer *et al.*, 1981). Spionids do not reingest their faecal pellets, or those of other species although the pellets were handled more often when a build up around the tube occurred or when no suspended particles were present. Faecal mounds and pellets are deposited at the surface (Dauer *et al.*, 1981).

According to Sardà & Martin (1993), the life cycle of polychaetes of the genus *Streblospio* is short (less than 1 year). Studies on a population of the species *Streblospio shrubsolii* in Altacs Bay, Spain, showed recruitment to occur throughout the year although it was reduced in the winter. This species is characterised by rapid growth with juveniles reaching maturity within two months. Growth and reproduction are highly influenced by temperature with the highest level of reproduction occurring between 16 and 21°C. Reproduction ceased at 10°C. Densities of *Streblospio shrubsolii* in Altacs Bay increased with temperature and were around 35 311 m^{-2}. In the Humber estuary, densities of Spionid polychaetes reach around 900 individuals m^{-2} (Allen, 2000a) at Paull. Since population density is related to temperature, it is not surprising that densities are lower than those in the Spanish population although this is also likely to be related to the physico-chemical conditions in the sediment and predation by birds, fish, crabs and other polychaetes.

1.4.4. *Manayunkia aestuarina*

M. aestuarina is a meiofaunal (up to 6 mm in length) tube building polychaete which, in Europe, is widely distributed throughout the Baltic and North Seas, both intertidally and subtidally to 20 m (Bell, 1979; 1980). It is generally classed as a brackish water species (Eckman, 1967, in Harris, 1970; Kendall, 1979), commonly found in saltmarshes (Bell, 1979) although both Harris (1970) and Kendall (1979) reported the species to be abundant in areas of near full salinity. *M. aestuarina* is generally found in soft, muddy sediments (Kendall, 1979), commonly occurring with species of the genus *Streblospio*, although Harris (1970) found large populations to be present in the soft detritus covering large boulders on the Isles of Scilly. Kendall (1979) also found the species to be capable of living in fine sands.

M. aestuarina is generally classed as a surface deposit feeder but is also known to switch to suspension feeding (Lewis, 1968, in Bell, 1979; Fauchald & Jumars, 1979; Bell, 1982). According to Fauchald & Jumars (1979), this species primarily feeds on diatoms but may also feed on small invertebrates and invertebrate larvae. It is therefore classed as omnivorous. Bell (1980) studied a population in Georgetown, North Carolina and found the species to

reproduce twice within a year – once during late spring/early summer and once during autumn.

1.4.5. Oligochaetes and nematodes

Oligochaetes of the family Tubificidae are often the dominant members of the benthos (Green, 1968) and can be overwhelmingly dominant in polluted areas (Green, 1968; Pearson & Rosenberg, 1978). The species *Tubifex costatus* and *Tubificoides* (*Peloscolex*) *benedeni*, both common within the study area, are found at salinities of 2 and 36 and above 12, respectively (Green, 1968). These species live head down in fine sediments (Birtwell & Arthur, 1980), feeding on bacteria and detritus, often around the redox potential discontinuity (Hunter, 1981) and deposit faecal pellets at the surface. In the Thames estuary, *T. costatus* was found to mature in April, and lay eggs in cocoons in May and June. Large numbers of worms were present by July although a peak in breeding was also thought to occur in January (Hunter, 1981). Brinkhurst (1964) also reported breeding by this species to take place in early summer. Breeding was found to occur between May and August for *T. benedeni* (Hunter, 1981). In the Thames estuary, the worms were found to breed only once, at an age of about two years, after which they died (Birtwell & Arthur, 1980).

Most Nematodes inhabit the top 2 cm of the substratum with the highest densities occuring in muddy substrata (Green, 1968). They commonly occur in variable salinity areas although Capstick (1959) found the overall abundance to decrease (in the Blyth estuary) with decreasing salinity from the middle reaches of the estuary. In terms of feeding, Weiser (1952, in Green, 1968) divided the nematodes into the following four groups: (1) deposit feeding species feeding on the bacteria associated with fine particles; (2) deposit feeders feeding on larger particles and diatoms; (3) those feeding on diatoms only and (4) predatory species feeding on organisms such as small polychaetes.

Warwick & Price (1979) found that for most species studied in the Lynher estuary (Cornwall), juveniles were found to dominate the population for most of the year. The presence of gravid females was not restricted to any particular part of the year (i.e. no seasonality in breeding) although there was evidence to suggest that the turnover time was faster during spring and summer when juveniles comprised 70% of the total population. Swedmark (1964, in Warwick & Price, 1979) states that continuous reproduction is typical of several meiofaunal groups (especially interstitial forms) and may be an adaptation for the maintenance of populations comprising animals with small body size and low gamete production.

1.4.6. *Macoma balthica*

Macoma balthica has an extensive geographic range that reaches from temperate to arctic coastal waters in both the North Atlantic and North Pacific oceans and it is one of the most important macrobenthic species in the Baltic and the North Sea (Beukema & Meehan, 1985; Brey, 1991; Günther, 1991; Bonsdorff *et al.*, 1995; Jahn *et al.*, 1997; Jahn & Theede, 1997). It is highly tolerant to cold and is able to survive in areas where the sea freezes for several months of the year (e.g. the Gulf of Finland) (Green, 1968).

M. balthica occurs in a wide depth range between the mid shore and 190m but is most abundant at moderate depths on muddy and sandy bottoms (Ólafsson & Persson, 1986). In Britain, this species is often abundant on intertidal estuarine mudflats and is usually found at its maximum density in a broad belt around the mid tide level (Bradfield & Newell, 1961). The densest populations of *M. balthica* appear to be associated with fine substrata, possibly due to the high densities of micro-organisms associated with these sediments (Green, 1968; Wolff, 1973; Chambers & Milne, 1975). The majority of these animals are found within the top 4-5 cm of the substratum (Chambers & Milne, 1975; Davey & Partridge, 1998) and contact with the sediment surface and overlying water is maintained by means of the separate inhalant and exhalent siphons (Bradfield & Newell, 1961). Ratcliffe *et al.* (1981) reported densities in the Humber Estuary, UK, of between 5,000 m^{-2} and 40,000 m^{-2}, depending on time since a successful spatfall, and *M. balthica* is considered to be an important prey species for a variety of fish and birds (e.g. flounder, redshank, knot and oystercatcher) (Chambers & Milne, 1975). Within its preferred habitat, the abundance of the species is primarily dependant on food availability and the amount of time available for feeding. In addition, competition for space occurs between *M. balthica* and *Scrobicularia plana* with dominance of one species and very few or no specimens of the other, at high densities. However, at low densities, both species can coexist in almost equal numbers (Green, 1968).

M. balthica has been classified both as a selective surface deposit feeder and a suspension feeder (Bradfield & Newell, 1961; Wolff, 1973; Zwarts & Wanink, 1989; Brey, 1991). During deposit feeding, the animal extends it inhalant siphon over the sediment surface, drawing in superficial sediment particles, from which it utilises the associated bacteria and organic matter, detritus, diatoms and deposited phytoplankton (Bradfield & Newell, 1961; Wolff, 1973). Suspension feeding takes place during periods of tidal inundation, when phytoplankton is used as a food source (Bradfield & Newell, 1961; Wolff, 1973). Faeces and pseudofaeces are ejected through the shorter, exhalant siphon which is held vertically (Bradfield & Newell, 1961). Unlike most intertidal lamellibranchs, which can only move short distances, vertically within the sediment, *M. balthica* moves over the sediment in order

to find food once the area around its burrow has been cleared of diatoms and detritus (Bradfield & Newell, 1961). This together with its movement within the sediment and the way in which it ejects faecal and pseudofaecal material into the water column, makes it an important species in terms of bioturbation, as demonstrated by Widdows *et al.* (1998a; 1998b).

M. balthica can live for about 6 years although, prior to recruitment, the Ythan estuary population studied by Chambers & Milne (1975) was composed primarily of 1+ animals (87-93% of the total). Following spat settlement, 0+ animals accounted for 85-91% of the population. *M. balthica* has been reported to reach sexual maturity at a variety of ages ranging from less than 1 year in the Netherlands (Lammens, 1967) to 2 years in the Thames (Caddy, 1967). Harvey & Vincent (1989) suggested that sexual maturity is a function of size rather than age in *M. balthica*, maturation occurring when the shell reaches 6mm with corresponding ages of individuals from the same population varying between 10 and 22 months. Chambers & Milne (1975) reported spawning in this species to occur between late February and April.

1.4.7. Other species

In addition to *Hediste diversicolor, Pygospio elegans* and *Streblospio shrubsolii*, several other polychaete species have been recorded from the study area at the Skeffling site. *Arenicola marina* is found on all coasts around Britain and Ireland and is widely distributed throughout north-west Europe. This species reaches its highest densities at the mid-tide level in areas of muddy sand where the salinity is above 8 (Green, 1968). Populations of this species are greatest in areas where the organic content of the sediment is high (although not to the extent of being polluted) and the mean particle size is around 100 μm (Longbottom, 1970). *A. marina* is a selective, sub-surface deposit feeder, which feeds on the bacteria and detritus attached to fine sediment particles (<2 mm) (Wells, 1945; Green, 1968; Zebe & Schiedek, 1996). This active selection of fine particles leads to the concentration of coarser material at the bottom of the burrow (Zebe & Schiedek, 1996; Riisgård & Banta, 1998). This forms a filter, retaining small particles brought down into the burrow from the overlying water by irrigation currents (Krüger, 1959, in Green, 1968). The animal inhabits a 'J' or 'U' shaped burrow with a characteristic depression at one end caused by the downward movement of surface material as the worm ingests sand from the bottom of the burrow. At the tail end, faecal material is ejected at the sediment surface in the form of a cast (Wells, 1945; Green, 1968). *A. marina* is a sedentary species and generally inhabits a burrow for several months before constructing a new one (Green, 1968). Duncan (1960) studied the spawning behaviour of *A. marina* and found most populations to spawn in autumn.

Nephtys hombergii and *Eteone longa* are carnivorous (Fauchald & Jumars, 1979) species which inhabit sandy mud in intertidal and shallow subtidal areas throughout Britain and Ireland (Clark & Haderlie, 1960; Clark *et al.*, 1962). Clark & Haderlie (1960) found *N. hombergii* to be present at salinities of 16-25 in the Bristol Channel although it has been recorded from Skeffling in the Humber estuary where the salinity is higher than this. *N. hombergii* constructs horizontal burrows within the top few cm of the sediment (although large worms may burrow deeper) (Holme, 1949; Davey & Partridge, 1998). These burrows are temporary and become infilled as the worm moves through the sediment in search of food (Davey & Partridge, 1998).

Bivalve species which are known to be present in significant numbers in the study area (at Skeffling) include *Cerastoderma edule, Scrobicuaria plana* and *Mya arenaria.* Other Molluscan species found at this site include the Gastropods *Hydrobia ulvae* and *Retusa obtusa. Cerastoderma edule is* most commonly (but not exclusively) found in muddy sands in sheltered estuarine areas where the salinity is 15-35 (Green, 1968; Boyden & Russell, 1972). It is a suspension feeder with short siphons (Zwarts & Wanink, 1989) and consequently, does not burrow to depths greater than approximately 5 cm (Green, 1968; Zwarts & Wanink, 1989). *C. edule* is an important prey species for oystercatchers, flounder and *Carcinus maenas* (Möller & Rosenberg, 1983).

Scrobicularia plana is a large bivalve commonly found in estuarine, intertidal areas with a mud or muddy sand substratum (Green, 1968). *S. plana* burrows up to 20 cm deep in the sediments with burying depth increasing with increasing animal size (Zwarts *et al.*, 1994). *S. plana* can also achieve a remarkably long siphon length (Chapman & Newell 1956, in Zwarts *et al.*, 1994), potentially allowing a large feeding radius over the sediment surface. *S. plana* is primarily a surface deposit feeder but is also capable of suspension feeding (Hughes, 1969). Deposit feeding during high water occurs at the sides or mouths of the inhalant burrows, concealing the siphons from predatory crabs and fish. At low water, feeding takes place along the surface of the mud which creates the characteristic star shapes which surround the burrows of *S. plana* (Green, 1968). The inhalant siphon occupies a semi-permanent vertical burrow leading from the shell directly to the sediment surface. Faecal and pseudofaecal material are ejected, either as a forceful spurt or slow exudation, through the exhalant siphon which generally only extends half way along its burrow (Hughes, 1969).

S. plana is a sedentary species and, in contrast to *M. balthica*, does not migrate in response to depletion of its food supply. This is possibly because it can exploit sub-surface deposits, rich

in organisms such as sulphur reducing bacteria, which are less likely to become depleted (Hughes, 1969). Due to its potential burying depth, potential feeding mode and area and the rate at which faecal and pseudofaecal material can be deposited at the sediment surface, *S. plana* can potentially have a high impact on the sediment properties.

Mya arenaria is a large long-lived bivalve which may reach 12 -15 cm in length. It is found in a variety of sediments ranging from hard, stony sand to soft mud although its preferred habitat is muddy sand (Rasmussen, 1973). *M. arenaria* is common throughout Europe and is found on all British coasts (Strasser *et al.*, 1999). It is most commonly found in the littoral zone but may also occur in sublittoral areas and has been found to a depth of 192 m (Rasmussen, 1973; Strasser, 1999).
M. arenaria is a suspension feeder (Zwarts & Wanink, 1989) which has its two elongated fused siphons so that the clam can bury itself up to 60 cm deep in the sediment and extend its siphon to the surface (Green, 1968).

The prosobranch *Hydrobia ulvae* is widely distributed throughout Europe, occurring on all British coasts, and may be found in estuaries, saltmarshes, lagoons and other areas of reduced salinity (Sola, 1996). It typically occurs in the intertidal zone, with maximum densities being found at the mid tide level, and shows a preference for fine, muddy sediments. However, it has also been recorded subtidally and has been found to be associated with sandy and rocky habitats (Sola, 1996). The species can occur at densities as high as 100 000 m^{-2}, although densities of 5000 - 9000 m^{-2} are more common (Green, 1968), and forms an important link in the estuarine food web (Newell, 1965). *H. ulvae* is primarily a surface deposit feeder which grazes on diatoms, detritus and bacteria associated with surface sediment particles (Fenchel *et al.*, 1975). *H. ulvae* maintains its position on the shore by burrowing into the surface layers of the mud on the ebb tide (Green, 1968).

CHAPTER 2

SEDIMENT PROPERTIES

2.1. INTRODUCTION

2.1.1. Physical properties of estuarine sediments.

Natural sediments rarely consist of either mud or sand in isolation and the majority of marine and estuarine sediments are complex mixtures of faecal pellets of various sizes and shapes, oils, organically bound aggregates, living and dead protistans and other skeletal components and shell fragments as well as various rock and mineral fragments ranging in size from < 1μm to 1-2mm (Wheatcroft, 1992; Whitehouse *et al.*, 2000). The mineralogical make up is also varied with sands being composed primarily of quartz and clays being made up of metal silicates in various chemical forms. Therefore, sediments are complex in terms of their mineralogy and granulometry but also in terms of their biota and its impact on the sediment as a result of burrowing, secretions and the production of faeces (Whitehouse *et al.*, 2000). This process of bioturbation is discussed in detail in Chapter 5. Sediment grains are derived from weathered bed rock (clastic), formed by the precipitation of minerals (authigenic) or are composed of calcium carbonate (biogenic), being produced from shell and the calcareous parts of invertebrates (Pethick, 1984; Meadows & Campbell, 1988). Pethick (1984) stated that 90% of marine sediment is derived from rivers with input from glaciers and biogenic sources also being of importance. However, a large proportion of the deeper, relict, sediments were deposited during the sea level changes of the most recent ice ages (Pleistocene) (Pethick, 1984; Meadows & Campbell, 1988).

The sediments of estuarine mudflats tend to be dominated by fine silt and clay particles which settle during periods of slack water (Elliott *et al.* 1998). This is particularly the case for upper shore areas which are only submerged at high water when the current speeds are at a minimum (Whitehouse *et al.*, 2000). Dyer (1979) stated that fine grained material would move in suspension, and that the processes deposition and re-erosion are continual. These processes may occur daily, over the tidal cycle, fortnightly as current speeds change with spring and neap tides and seasonally with changes in flow and storm frequency. Sediment which has settled during periods of slack water gradually consolidates but as the current increases during the next stage of the tide, some of this deposited material will be re-eroded. Mud deposited at slack water has the chance to consolidate slightly before the current becomes strong enough to re-erode it. Because of the slightly enhanced resistance to shear at the base of the layer, not all particles are eroded and a small increment of deposition occurs.

Deposition is therefore the result of several slack water periods without intervening erosion (Dyer, 1986).

Muddy sediments tend to be sticky as a result of their cohesive properties and are generally dark in colour due to their organic content which is often the major constituent. Silt and clay particles are classed as those which are 4 - 62 μm and less than 4 μm respectively (Buchanan, 1984). In contrast to sand grains which are chemically stable (Whitehouse *et al.*, 2000), clays are ionic in nature and according to Dyer (1986) and Libes (1992), all clays are susceptible to ion exchange, to a varying degree. Because clay particles carry a net negative charge (Libes, 1992), they behave very differently to other mineral particles. The large proportion of very fine particles (and associated large surface area) means that the effects of surface physico-chemical forces on the behaviour of the sediment become as important as those of gravity (Whitehouse *et al.*, 2000). The rate of sedimentation (V) for single particles is dependant on the particle radius (r) and density (ds), gravity (g), and the density and viscosity of the water (df and μ respectively), and is given by Stokes' law (Meadows and Campbell, 1988).

$$V = \frac{2(ds - df)gr^2}{9\mu}$$

The rate of settlement of clay particles, however, also depends on the charge. For example, in freshwaters where the concentration of dissolved cations is low, the particles repel each other and therefore, at high current speeds, do not settle. In sea water, the concentration of dissolved ions is much higher. Attraction between the particles occurs as a result of the Van der waals force (weak force of molecular attraction which is inversely proportional to the square of the distance between two particles) and flocculation occurs (Libes, 1992). Flocculation may also occur as a result of collisions and subsequent electrostatic attraction between particles (van Leussen, 1994, in de Dekere, 2003). This effectively increases the size of the particle and settlement may occur at current speeds equivalent to those at which particles in freshwater would remain in suspension. This combination of electrostatic and molecular attraction forces leads to bonding of clay particles and gives rise to it sticky, cohesive nature. The presence of organic matter also increases the cohesive properties of sediments (Whitehouse *et al.*, 2000).

As settled particles progressively become buried deeper and deeper, they undergo a number of chemical and physical changes. The collective term for these changes is diagenisis (Libes, 1992) which eventually leads to the formation of rock due to the consolidation and

cementation of the sediment fabric (Open University, 2002). Consolidation occurs as the result of the increasing weight of the overlying sediment and water which causes the expulsion of interstitial water and cementation occurs as a result of the precipitation of dissolved substances (Open University, 2002). The rate of diagenetic reactions is not only temperature, pH and Eh controlled but is also determined by animal and microbial activity, particularly during the early stages. In general, there are three stages of diagenisis. In the pre-burial stage, biological activity causes rapid changes in the sediment chemistry (e.g., pH, Eh, clay composition). In the early burial stage, chemical changes continue as in the pre-burial stage but carbonaceous material becomes oxidised and the formation of sulphides, calcites (from aragonite) and dolomites (from calcium carbonate) begins. Compaction and cementation also begin. In the final stage (late burial), the effects of pH and Eh become less important, compaction and cementation continue and the formation of calcite and dolomite becomes complete.

2.1.2. Chemical and biological properties of estuarine sediments

2.1.2.1. Physico-chemical properties

Estuarine bottom waters tend to be highly turbid with a high oxygen demand. This together with the fine grained nature and high organic content of estuarine muds means that oxygen penetration into the sediment is minimal in comparison to that of coarser sediments (Rhoads, 1974; McLusky, 1989). Furthermore, the high organic content of estuarine sediments provides a substrate for large bacterial populations, the respiration of which is of major importance in utilising interstitial dissolved oxygen (Rhoads, 1974). The activity of burrowing organisms can increase the depth of oxygen penetration but in the absence of such activity, there is very little vertical movement of interstitial water to replenish the oxygen used. Molecular diffusion of dissolved oxygen into the sediment is therefore restricted to the top few millimeters (Rhoads, 1974). In the oxidised, surface layers of the sediment, aerobic bacteria are responsible for the decomposition, or mineralisation, of organic matter. Bacterial decomposition of animal and plant remains generally results in the liberation of hydrogen sulphide which, under aerobic conditions, is rapidly oxidised to form sulphate (Zobel, 1946, in Perkins, 1957).

As the supply of dissolved oxygen becomes depleted, bacterial breakdown of organic matter leads to the reduction of nitrate (NO_3) to nitrite (NO_2) and eventually ammonia (NH_3) and ammonium (NH_4) as the conditions become more anaerobic (Rhoads, 1974). Ferric iron species (Fe^{3+}), which give aerobic sediments their brown colour (Johnstone, 1921, in Perkins, 1957), are also reduced to ferrous iron (Fe^{2+}). In the complete absence of oxygen, this is followed by the reduction of sulphates, due to sulphate reducing bacteria, to sulphides and,

ultimately, hydrogen sulphide (Perkins, 1957; Rhoads, 1974; Meadows & Campbell, 1988). The degradation of diatoms and proteins also leads to the formation of small amounts of hydrogen sulphide. Reactions between the sulphide and ferrous iron within the sulphate reduction zone lead to the formation of greigite, or magnetic iron sulphide ($FeFe_2S_4$) and mackinawite (a form of FeS) which give the sediment its black colour (Rhoads, 1974). Below this, anaerobic methanogenesis occurs (Meadows & Campbell, 1988) and the formation of pyrite (FeS_2) turns the sediment grey (Rhoads, 1974). The chemical gradients, associated with redox reactions, within sediments are summarised in Table 2.1 and Figure 2.1.

Soft, fine grained sediments not only have a high organic content but also tend to be waterlogged with poor permeability which further impedes aeration (Rhoads, 1974). Poor permeability results from compaction and the infilling of interstitial voids by small particles (Elliott *et al.*, 1998). Poorly aerated, soft sediments, where oxidation processes (respiration, decomposition of organic matter) exceed the rate of oxygen supply, are particularly rich in sulphides and hydrogen sulphide (Theede *et al.*, 1969; Llansó, 1991; Miron & Kristensen, 1993a). It is well known that high sulphide concentrations are related to negative redox potential (Eh) values in the sediment. The point at which the sediment changes from being aerobic (with positive Eh values) to being anaerobic is known as the redox potential discontinuity (RPD). Since redox reactions are temperature driven, it is reasonable to assume that sulphide concentrations will be seasonal with the highest concentrations occurring during the warmer summer months (Perkins, 1957, Pearson & Stanley, 1979).

Table 2.1. Chemical gradients associated with sediment diagenisis (Meadows & Campbell, 1988).

Increasing depth	Chemical species released from organic material by mineralisation	Physiological groups of bacteria.
Aerobic zone	CO_2, NH_3, H_3PO_4, SO_4^{2-}	Aerobic heterotrophs
Manganese reduction zone (suboxic zone)	HCO_3^-, NH_4^+, HPO_4^{2-}, Mn_4^{2+}	Aerobic and anaerobic heterotrophs
Nitrate reduction zone (suboxic zone)	CO_2, HCO_3^-, NH_4^+, N_2, HPO_4^{2-}	Anaerobic denitrification by heterotrophs
Iron reduction zone (anoxic zone)	HCO_3^-, NH_4^+, HPO_4^{2-}, Fe^{2+}	Anaerobic sulphate reduction
Sulphate reduction zone (anoxic zone)	CO_2, HCO_3^-, NH_4^+, HPO_4^{2-}, HS^-	Anaerobic sulphate reduction (*Desulphovibrio*)
Carbonate reduction zone (anoxic zone)	CO_2, HCO_3^-, NH_4^+, HPO_4^{2-}, HS^-, CH_4	Anaerobic methanogenesis (*Methanobacterium*)

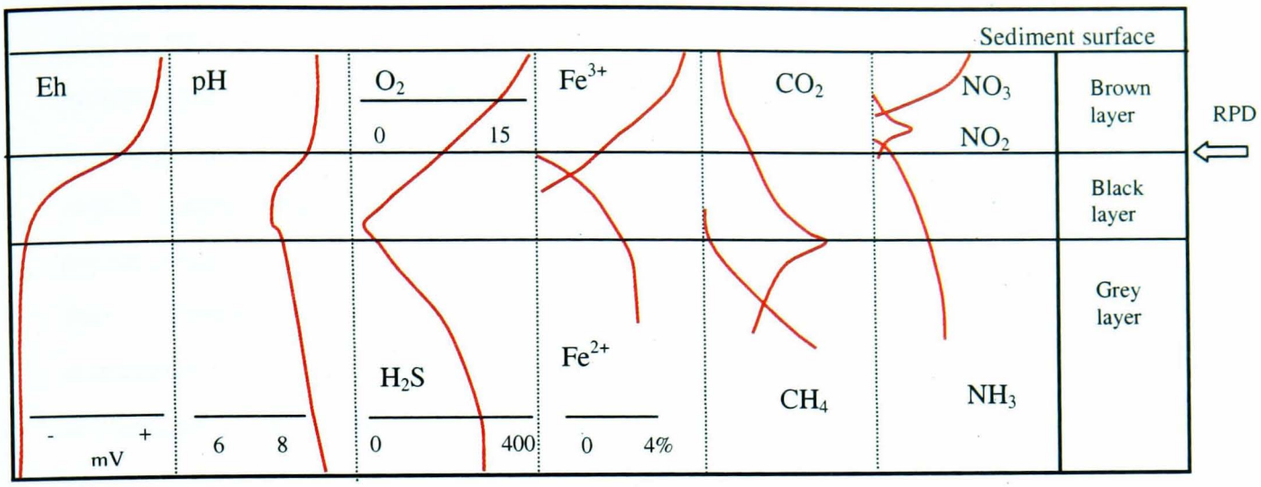


Figure 2.1. Chemical gradients within the sediment (H_2S and O_2 in mg l^{-1}). Re-drawn from Rhoads (1974).

The ionic nature of estuarine muds also makes them highly susceptible to adsorption of cations from the surrounding water making them an important sink for contaminants (Whitehouse *et al.*, 2000). The small particle size means that the available surface area for adsorption / unit mass is far greater than in coarser sediments and therefore contaminant levels can potentially become quite high in estuarine sediments (Calmano *et al.*, 1996; Petersen *et al.*, 1996). The adsorption of contaminants to sediment particles and to organic matter is dependent upon the physico-chemical conditions (e.g. pH, salinity, Eh, temperature, abundance of organic matter). In general, high pH, in combination with oxidised sediments, allows the formation of iron and manganese oxides and hydroxides and causes the co-precipitation of contaminants thus reducing the potential for contaminant uptake by organisms. In contrast, the free sulphide and low pH in anaerobic sediments does not permit this precipitation and adsorption to sediment particles is reduced so that pore water concentrations of contaminants are increased. Therefore, for some chemicals, the anoxic nature of estuarine sediments increases the bioavailability of the contaminants present. In addition, complexation with organic ligands may occur which reduces bioavailability (Knezovich, 1994; Calmano *et al.*, 1996; Petersen *et al.*, 1996;Whitehouse *et al.*, 2000). Although these substances may not be directly available to pelagic organisms, they may be readily digested and absorbed by benthic organisms (Calmano *et al.*, 1996; Petersen *et al.*, 1996; Borgmann, 2000). Although there are many examples of the effects of various types of pollutants on biological communities, there is much uncertainty about the role of chemical pollution on the dynamics of estuarine muds. However, it is likely that contaminants play a role in sediment dynamics by altering their physico-chemical properties (Whitehouse *et al.*, 2000).

2.1.2.2. Microphytobenthos

Due to their fine grained, nutrient rich characteristics, estuarine muds tend have large bacterial and microalgal populations. Diatoms are unicellular algae found wherever there is sufficient light and moisture to allow photosynthesis (Hendey, 1964). According to Wolff (1987), epipelic diatoms (i.e., those which are free living and mobile within the sediment) are the most dominant microalgae living in mud and are usually present in large numbers on tidal flats. Sediment inhabiting diatoms are an important component of intertidal estuarine mudflat communities (Admiraal, 1984) and are considered to be the most important primary producer in intertidal mudflats (Blanchard *et al.*, 2000). The microphytobenthos not only supplies the benthic food web with organic matter but, through its resuspension, also fuels the planktonic food web (de Jonge & von Beusekom, 1992; 1995). Diatoms provide a rich source of carbohydrate and are of greater importance to deposit feeding organisms (and ultimately their predators) than organic detritus (Hendey, 1964).

Two major groups of diatoms exist which are separated by the structure of their valves (Hendey, 1964). Centric diatoms (Centricae) are radially symmetrical in valve view with the structural features of the diatom radiating from or being concentric around a central point. The Pennate (Pennatae) diatoms have a feather like structure on either side of a median line and are laterally symmetrical in valve view. Wolff (1987) stated that in muddy habitats, the Pennate diatoms are the dominant forms although certain Euglanoid flagellates may occur and in polluted areas, the Cyanobacteria (blue greens) may be abundant. Diatoms are very sensitive to environmental conditions and pollution and therefore, community changes would be expected not only in different parts of an estuary but also with increasing distance from a pollution outfall.

Light, temperature and particularly salinity are all of significance in determining diatom abundance and distribution, with each individual species having particular requirements (Hendey, 1964; Admiraal & Peletier, 1980; Wolff, 1987). Nutrient availability, including nitrate, phosphate and silicate, also plays an important role in controlling diatom populations. Benthic diatoms are seasonally influenced (although to a lesser degree than phytoplankton (Cadee & Hegeman, 1974, in Underwood and Paterson, 1993a) and dense communities of diatoms may exist in the mud when planktonic communities have declined due to exhaustion of the nutrient supply in the water column (Hendey, 1964). This is probably because the sediment provides protection against extremes of temperature, sunlight and salinity and the supply of nutrients is relatively constant. In general though, epipelic diatom biomass increases in the summer months (Colijn & Dijkema, 1981; Admiraal *et al.*, 1982; Montegana *et al.*, 1983; Colijn & de Jonge, 1984) in response to increased light and temperature although

there may be peaks at other times of the year. Diatom densities are also found to be higher in upper shore areas than in lower shore areas (Underwood & Paterson, 1993a).

Epipelic diatoms are able to migrate vertically within the sediment), a process which occurs in response to tidal fluctuations (Hendey, 1964; Wolff, 1987; Paterson, 1989). This is dependent on environmental conditions (particularly light) and it is common for communities to remain within the sediments at low tide during the night or on particularly dull days (Hendey, 1964; Wolff, 1987). This characteristic makes them extremely well adapted to life in muddy habitats since they are able to make maximum use of the available light and cope with the high rate of sedimentation which generally occurs in estuaries.

Many explanations have been offered as to why diatom motility is possible (Hendey, 1964). These explanations include the use of cilia; propulsion by osmotic currents being injected into the cell; vibrations within the outer layers of the diatom cell; expulsion of gasses produced by the chromatophores on exposure to light and cytoplasmic or mucilaginous stream flows within the raphe (Drum & Hopkins, 1966, in Hoagland *et al.*, 1993; Edgar & Pickett-Heaps, 1984). Modes of movement also vary between species, for example, *Suriella* spp. move by rolling whilst *Bacillaria paxilifer* cells move by sliding over each other with the whole colony moving in concert (Hendey, 1964). Movement by diatoms results in (or may be caused by) the production of mucopolysaccharides (Paterson, 1989; Madsen *et al.*, 1993). This mucilage, which is also produced by bacteria and as a result of the locomotion and burrowing behaviour of macro and meiofauna (Meadows & Tufail, 1986; Reichelt, 1991; Grant & Daborn, 1994), has been found to increase adhesion between sediment particles and is therefore thought to have a stabilising effect. The potential stabilising effect of these carbohydrate secretions will be discussed in greater detail in Chapter 8. In addition, these mucliages may enhance microbial growth and increase the nutritional value of the sediment to deposit feeding organisms. This is known as gardening (Hylleberg, 1975; de Wilde, 1991) and also occurs as a result of animal burrowing activity, as described in section 2.1.3. Therefore, the measurement of microalgal populations and sediment carbohydrate concentrations are of relevance to both faunal community studies and sediment erosion studies.

2.1.3. Animal – sediment relationships

The relationship between sediment properties and benthic invertebrates has been described in brief in Chapter 1 and can be found in more detail in Rhoads (1974), Hall (1994) and Snelgrove & Butman (1994). It is evident that a combination of factors such as particle size, organic content, cohesive properties, hydrodynamic regime, presence of other species, sediment chemistry, mobility and porosity and microbial properties play a key role in

structuring benthic communities. Elliott *et al.* (1998) described the relationships between an environment and its biology, the relationships between the biological components and processes (biologically mediating relationships) and the biological processes which modify the environment which all interact to define habitats and the communities they contain.

In the past, it has been accepted that sediment particle size played an important role in the structuring of benthic communities. It has generally, but not always, been observed that muddy sediments are primarily dominated by deposit feeding organisms and / or those which are able to change between deposit and suspension feeding according to the conditions whereas sandy sediments tend to be dominated by suspension feeders (Rhoads, 1974). Species which are not capable of deposit feeding or whose respiratory structures are susceptible to clogging by fine particles and which are not tolerant of high sulphide concentrations and salinity fluctuations are not found in estuarine muds.

According to Snelgrove & Butman (1994), although correlations have been found between sediment type and community structure, these relationships are sometimes weak and individual species are rarely confined to the habitat with which they are generally associated. For example, Tenore *et al.* (1968, in Snelgrove & Butman, 1994) did find that muddy sediments reduced the growth rate of the filter feeding bivalve *Rangia cuneata* but also stated that reduced growth rate did not necessarily mean exclusion from a particular habitat. In contrast, Davis (1960, in Snelgrove & Butman, 1994) observed enhanced growth in juveniles of *Mercenaria mercenaria* exposed to various levels of silt although extremely high concentrations did result in death. A possible reason for these weak relationships could be that although correlations have been found, none of them provide any insight into the mechanisms responsible for such associations. It is likely that grain size is a correlate of other factors, such as hydrodynamic regime, organic content, chemistry and micro-organisms, which also play an important role in defining community structure. Therefore, although it is of significance, grain size alone cannot be expected to be an adequate descriptor of the sedimentary environment.

Larval settlement and subsequent survival will explain the presence of various organisms in particular habitats (Gray, 1974; Butman, 1987, in Snelgrove & Butman, 1994). Larval settlement is largely governed by tidal and wind driven currents and turbulence (Gray, 1974; Butman, 1987, in Snelgrove & Butman, 1994). Similarities between particle and larval specific gravity and settling velocity may therefore, in part, explain the relationship between particle size and benthic infauna. However, there are various biological cues for larval settlement including the presence of biofilms, food availability, the release of natural inducers

from conspecific individuals, the presence of predators and community structure (Qian, 1999). Some species actively select the sediment type on which they settle and Meadows (1964a; 1964b; 1964c), Rhoads & Stanley (1965), Fenchel *et al.* (1975), Taghon (1989, in Wheatcroft, 1994) and Wheatcroft (1992) noted the selection of specific particle sizes by various species whilst feeding. Fenchel *et al.* (1975) found that this active selection of particle sizes was species specific and could allow the co-existence of two potentially competing deposit feeding organisms (e.g. *Corophium volutator* and *Hydrobia ulvae*). Gray (1974) suggested that the size of the particles selected was related to their organic films and therefore their nutritional value. Rhoads (1974) and Nowell & Jumars (1981) also highlighted the importance of sediment stability and near bed flow regime, respectively, to particle size distribution and organism feeding and distribution and therefore animal distribution is likely to be dependant on a combination of factors.

Sediment water content or, porosity, is also an important factor influencing the distribution of infaunal organisms. For example, Chapman (1949) found that the time taken for lugworms to burrow into muddy sands decreased with increasing water content, suggesting that too little water in the sediment is unfavourable. In contrast, Longbottom (1970) suggested that fine, fluid, sediments could be unfavourable for organisms such as *Arenicola marina* due to their inability to maintain their burrows and maintain contact with the overlying oxygenated water. According to this author, *A. marina*, unlike many estuarine infaunal species, is unable to produce sufficient amounts of mucous to line and, thus, stabilise its burrow walls.

Organic matter is the dominant food source for most organisms in sediments and is strongly correlated with animal distribution and abundance. For example, Pearson & Rosenberg (1978) demonstrated an association between specific organisms (e.g., the Polychaete *Capitella capitata*) and increased abundance of these organisms with increasing levels of organic carbon. Longbottom (1970) also found the abundance and biomass of *A. marina* to be highly correlated with organic matter. Microbial populations are also an important food source and have been shown to influence the distribution of deposit feeders in mud (Snelgrove & Butman, 1994). Therefore, animal distribution depends on the habitat meeting the chemical, physical and nutritional requirements of each particular species.

In addition to the physical and chemical conditions within the sediment, biological activity is also thought to have some influence over the community structure (Rhoads, 1974). The burrowing and feeding activity of deposit feeders causes the production of faeces and the destabilisation and resuspension of sediment. This results in an increase in near bottom turbidity which suspension feeders cannot tolerate (Rhoads & Young, 1970; de Wilde, 1991).

According to these authors, the larvae of suspension feeders are also prevented from establishing themselves due to the continual disturbance. This exclusion of suspension feeders through the activity of deposit feeders is an example of trophic group amensalism which was defined by Sanders (1958) as an interaction in which one organism or species adversely affects a second but where the second species has no effect on the first. In sandier sediments, suspension feeders are thought to inhibit deposit feeders by destroying the larvae.

In contrast, the production of faecal pellets, together with the secretion of mucous, increased depth of oxygen penetration and the increase in the surface area of the sediment-water interface (through the construction of burrows) by burrowing macrofauna encourages the growth of bacteria, benthic microalgae and meiofauna. These beneficial effects of bioturbation are known as 'gardening' (Hylleberg, 1975) and burrowing communities are thought to be a key factor in the success of small zoobenthos and microorganisms (de Wilde, 1991). Reise (1985) found intertidal meiofaunal densities to be positively correlated with the number of polychaete tubes and also found the burrows of *Arenicola marina* to be responsible for the presence of 93% of the total meiofaunal abundance for the entire subsurface sediment. However, de Wilde (1991) stated that extremely high densities of burrowing macrofauna may have a negative effect on microalgal populations due to the subduction and smothering of diatom cells. For example, this author found that intertidal mudflats, from which burrowing animals had been removed, became overgrown with microalgae whereas those with dense populations of *A. marina* remained comparatively bare.

The mechanisms determining organism distribution are clearly poorly understood and it is evident that the occurrence of a species in a particular habitat is not due to one factor alone. Rather, it is due to the complex interaction between particle size, organic and microbial content, hydrodynamic and chemical conditions, and biological interactions (including competition and predation) which makes a habitat suitable for a particular species. Conditions such as these are variable on a spatial and temporal scale and it is therefore not surprising that correlations between fauna and grain size have been weak. The trophic group amensalism theory was heavily criticised by Snelgrove & Butman (1994) due to a lack of direct evidence that the activity of deposit feeders in mud could destroy the larvae of suspension feeders or that deposit feeders were limited by food availability in sandy environments. However, it is unlikely that the activities of the dominant groups within a community do not affect the activity and distribution of other species, at least to some degree.

2.2. AIMS

As indicated above, factors such as particle size, water content, cohesive and adhesive properties, level of sediment contamination and diatom abundance all affect animal distribution but also influence the erosion characteristics of sediments. It is therefore important that the sediment properties are examined in order to aid the interpretation of data regarding the faunal community and erosion characteristics of sediments. These properties are expected to change on a seasonal basis as a result of temperature changes (and other climatic factors such as precipitation) and the resultant changes in biological activity and the frequency of storms and their effects on the hydrodynamic regime. The aim of the present chapter is to examine the properties of the sediment at the four key sites at Paull and Saltend over a period of ten months. Specifically, the following physical, chemical and biological properties of the sediment are to be examined:

- Particle size
- Water content
- Organic content
- Interstitial pH and salinity
- Sediment contamination in terms of metal concentration and redox potential (Eh)
- Microalgal abundance and carbohydrate production (both as total carbohydrate and that secreted by the diatoms (extracellular polymeric substances or EPS)).
- Sedimentation rate

These data will then be used, in part, to interpret differences between the faunal communities (Chapter 4), differences in bioturbation (Chapter 5) and differences in the erosion characteristics (Chapters 7 and 8) between sites.

2.3. METHODS

The locations of the sampling sites are given in Figure 1.3 and Table 1.1. Four key sites (Saltend 25 m, 75 m, 200 m and Paull 150 m) were sampled approximately every six weeks between June 1999 and April 2000. The remaining sites (S0 m, S50 m, S100 m, S150 m, P200 m, P100 m, P50 m, P25 m) were sampled only quarterly since, due to time constraints, it was not considered possible to sample the mudflats so intensively throughout the whole year. At the key sites, five replicate samples were taken on each sampling occasion whilst samples were taken in triplicate at the other sites.

2.3.1. Particle size analysis

Sediment samples were taken to a depth of 10 cm, using a small plastic core and prepared according to the method described by Buchanan (1984). Samples of oven-dried sediment were weighed, placed in a solution of 6% hydrogen peroxide and left overnight. They were then heated in a water bath (at 40°C), adding further amounts of hydrogen peroxide until the effervescence stopped. The solutions were diluted to 250 ml and 10 ml sodium hexametaphosphate (6.2 g l^{-1}) was added. This was stirred, allowed to soak overnight, restirred and passed through a 63 μm sieve to separate the silt/clay fraction from the sand fraction. The sand fraction was dried (in the sieve) for 24 hours at 105°C and weighed.

The silt/clay fraction was analysed using a Z1 Coulter Counter with a 100 μm aperture suitable for counting particles within 2-60% of its size. Samples were diluted to 1L, placed on a magnetic stirring plate to keep the particles in suspension and 10 ml 4% formalin was added to prevent microbial growth (FRPB, 1988). 1 drop (approximately 0.1 ml) was added to 100 ml electrolyte solution (Isoton II diluent, Coulter Electronics). Particles were counted between 9 and <4.5 φ, at 0.5φ intervals, to give an analysis of the particles within the range of 2-60 μm. Prior to analysis, a 50 ml sub-sample was removed from a depth of 10 cm, transferred to an evaporating dish and dried. The dry weight was multiplied by 50 to give the total weight of the silt/clay fraction in 1 L, which can be expressed as a percentage of the total weight of the original sample. This figure can then be used to convert the number of particles in each size class to a percentage of the total weight. These values can then be combined with the weight of the sand fraction (also expressed as a percentage) so that values such as mean and median phi and the sorting coefficient can be calculated.

The instrument was calibrated to allow for coincidence (error caused by more than one particle passing through the aperture at any one time). In order to keep coincidence to a minimum (within 10%), the count / 0.5 ml should not exceed 40,000. Calibration was carried

out before analysis using 1-2 drops of a solution containing latex beads of a standard diameter (median diameter of 9.7 μm).

2.3.2. Water content

Sediment samples (five replicates) were collected to a depth of approximately 10-15 cm, using a small plastic corer (3 cm i.d.), and homogenised. Fresh, wet sediment samples of approximately 20 g were placed in pre-weighed evaporating dishes and the weight recorded. Samples were then dried to constant dry weight (48 hours) at 105°C and the weight loss calculated. This was expressed as a percentage and was assumed to be equivalent to the percentage water content of the sediment. In May 2000, core sampling (three replicates) was carried out to a depth of 15 cm, using a plastic core (68 mm i.d.) with holes drilled at 1 cm depth intervals, protected by a stainless steel sheath. Upon return to the laboratory, the sheath was removed and sub-samples were taken at 1 cm intervals to a depth of 10 cm, using a curved metal spatula. Depth profiles of water content for the four key sites and for an upper shore site at Paull (P25 m) were then produced.

Wet bulk density was calculated by dividing the mass of the sample by its volume (Flemming & Delafontaine, 2000). Interstitial salinity and pH of the top 5 cm of the sediment were determined by centrifuging a small amount (approximately 1-2 g) fresh sediment at 3000 rpm for 15 minutes. pH of the supernatant liquid was measured and salinity was determined using an ATAGO salinity refractometer.

2.3.3. Organic content

Following calculation of water content, the dry sediment samples were ground using a mortar and pestle, reweighed in their respective evaporating dishes and placed in a muffle furnace for 4 hours at 475°C (Buchanan, 1984). Samples were allowed to remain in the furnace until cool, weighed and organic content was expressed as percentage weight loss on ignition (%LOI). In May 2000, depth profiles of organic content were produced with sampling being carried out as described in section 2.2.2.

2.3.4. Sediment contamination

Redox potential (Eh) profiles were plotted as a means of assessing the degree of contamination in the sediment. Eh was recorded at each cm to a depth of 10 cm using a Russell CMPtR11/150 silver/silver chloride electrode (4M KCl/AgCl) and a Russell RL100 meter. Readings were given as absolute millivolts and therefore, no correction for the electrode potential was necessary. Calibration of the electrode was carried out prior to each

use using the solutions potassium ferric cyanide, potassium ferrocyanide and potassium fluoride as follows:

Solution A	**234 mV**	
0.1 M $K_4Fe(CN)_6$	4.22 g	
0.05 M $K_3Fe(CN)_6$	1.65 g	in 100 ml distilled water
Solution B	**300 mV**	
0.01 M $K_4Fe(CN)_6$	0.42 g	
0.05 M $K_3Fe(CN)_6$	1.65 g	
0.36 M KF	3.39 g	in 100 ml distilled water.

Due to the difficulties associated with working in soft, deep and unconsolidated sediments, undisturbed core samples (5 cm i.d.) were taken to a depth of 20 cm, capped at both ends using plastic lids and parafilm and redox measurements made immediately upon return to the laboratory (within 5 hours of collection). Due to the short distance between the field site and the laboratory and the number and volume of the samples, no attempt was made to keep the samples cool. In addition, redox reactions are temperature dependent so it was considered appropriate to keep the samples at ambient temperature so that the Eh of the sample remained as close to that of the field conditions as possible.

Sediment metal concentration data, provided by Nikitik (in prep), are also used as an indication of the level of sediment contamination. Data are available for May, August and November 1997 and February and May 1998. For the purpose of this study, these data were averaged to give an annual mean although a full analysis is given in Nikitik (in prep).

Nikitik (in prep) collected sediment samples from eight sites at Saltend (seven of which correspond to this study) and one site at Paull. He Digested the sediment samples by boiling 1 g oven-dried sediment in 10 ml aqua regia (3:1 HCl:HNO_3). The cool digest was then diluted and filtered to a final volume of 50 ml and metal concentrations determined using a Perkin Elmer Plasma 40 Emission Inductively Coupled Plasma instrument (ICP-OES). It should be noted that the use of aqua regia allows an extremely high degree of metal extraction from the sediment sample and does not necessarily relate to the concentration of metals which is biologically available to sediment dwelling organisms (Nedwell, 1997; Al-Suhaimi, 1999)

2.3.5. Microalgae

Sediment chlorophyll-*a* content is usually expressed as mg m^{-2} and involves the collection of surface cores (e.g. the tube of a 10 ml plastic syringe) to the desired depth (Wolff, 1987). However, due to the unconsolidated nature of the sediment, this proved to be impossible. Therefore, surface scrapes (from the top 5 mm) of the sediment were taken using a metal spatula and stored in small (20 ml) plastic vials. The samples were then frozen over night, covered with parafilm (with several perforations) and freeze dried. Analysis of chlorophyll-*a* and pheopigment (degradation product of chlorophylls, including pheophytin and pheophorbide) was carried out immediately according to the method of Lorenzen (1967). Since both chlorophylls and pheopigments absorb within the same range of wavelengths (although their absorption maxima differ slightly (Eaton & Moss, 1966)) the presence of pheopigments can cause interference and their determination is therefore necessary. Freeze dried sediment samples of approximately 1 g (0.5 g for samples taken from the S25 m site) were ground using a mortar and pestle with 5 ml 90% acetone (AR grade) buffered with magnesium carbonate, washed into centrifuge tubes with a further 5 ml acetone and capped. Following overnight extraction at 4°C, samples were centrifuged at 3000 rpm for ten minutes and the absorbance measured at 665 nm (the wavelength of maximum absorbance of chlorophyll-*a* in acetone). Two drops of 1 mol HCl were added and the absorbance measured again. The absorbance of a turbidity blank (90% acetone) was also measured at 750 nm, before and after acidification and chlorophyll-*a* and pheopigment concentration calculated as follows (Tett, 1987):

$$\text{Chlorophyll-}a = K_e(e_o - e_a) * E/V \quad (\mu g \text{ chl-}a \text{ equivalent}) \, l^{-1}$$

$$\text{Pheopigment} = K_e(H_e * e_o - e_a) * E/V \quad (\mu g \text{ chl-}a \text{ equivalent}) \, l^{-1}$$

Where K_e and H_e are constants with the values 27.7 and 1.7 (maximum ratio of $665_o : 665_a$ in the absence of pheopigments), repectively; e_o and e_a are the optical densities before and after acidification, minus that of the blank; E is the extract volume (i.e. 10 ml) and V is the sample volume in litres. Sample weight in grams was substituted for V in order to give pigment concentrations as $\mu g \, g^{-1}$ rather than $\mu g \, l^{-1}$.

2.3.6. Sediment carbohydrate analysis

Total sediment carbohydrate concentration (expressed as $\mu g \, g^{-1}$ glucose equivalents) was determined using the phenol-sulphuric acid assay (Dubois *et al.*, 1956) with the extracellular polymeric substance (EPS) component being measured according to Underwood *et al.* (1995). Surface sediment samples (top 5 mm) were taken using a metal spatula, placed in plastic vials (20 ml) and freeze dried. Total carbohydrate was determined by adding 2 ml of distilled

water to a pre-determined weight of freeze dried sediment (30-50 mg samples), vortex mixing and adding 1 ml 5% aqueous phenol (wt/vol). 5 ml concentrated sulphuric acid was then rapidly added and the tubes allowed to cool and stand for 30 minutes to allow colour development. Analysis was carried out using 100 ml centrifuge tubes which were sufficiently large to prevent any overflow or spillage during addition of the sulphuric acid. Following this initial 30 minute period, the colour remains stable for several hours so repeat readings are possible (Dubois *et al.*, 1956). Samples were centrifuged at 4000 rpm for 15 minutes (at 4°C) and absorbance was measured against a reagent blank at 485 nm. Carbohydrate concentration (as glucose equivalents) was determined using the following regression equations:

$$y = 9.3778x + 0.0496 \qquad (r^2 = 0.996, p<0.01)\ (5\%\ \text{phenol})$$

$$y = 10.16x + 0.0763 \qquad (r^2 = 0.994, p<0.01)\ (10\%\ \text{phenol})$$

Concentrations of extracellular (colloidal) carbohydrate were determined following extraction (15 minutes at 20°C) in either 5 ml of 25 psu saline water (colloidal S) or 100 mM Na_2EDTA (colloidal EDTA) from approximately 100 mg freeze dried sediment (Underwood *et al.*, 1995). Following 15 minute centrifuging at 4000 rpm, a 2 ml sample of the supernatant was taken and analysed as described above. In addition, a 2 ml sample was taken and ethanol added (to give a final ethanol concentration of 70%) and allowed to precipitate over night at 4°C as described by Underwood & Smith (1998). Following centrifugation, the pellet was washed in 70% ethanol and analysed for carbohydrate using the phenol-sulphuric acid assay. This allowed determination of the concentration of EPS in the colloidal (S and EDTA) extracts.

Calibration curves were plotted using a series of glucose standards and the above regression equations derived. Initially, concentrations ranging from 0-1 mg ml^{-1} glucose were used but, as shown in Figure 2.2, the relationship between glucose concentration and absorbance became non-linear at concentrations higher than approximately 0.3 mg ml^{-1} and an absorbance of greater than about 2 - 2.5. Therefore, the calibration curve in Figure 2.3 was plotted using concentrations within the linear range. Regression equations were derived using phenol concentrations of 5 and 10% with the 10% phenol solution being used for the analysis of samples with low carbohydrate concentrations.

Since it is the extracellular carbohydrate which is of potential importance to sediment stabilisation rather than the intracelluar carbohydrate, the measurement of total carbohydrate is of limited value and is not given in most recent studies (e.g. Underwood & Smith, 1998; Blanchard *et al.*, 2000; de Dekere, 2003). However, the extraction of extracellular material

using EDTA can cause leakage from within the cell and Underwood *et al.* (1995) showed that repeated extractions using saline water were required to obtain the maximum yield of EPS. It was therefore considered appropriate to use both these extraction techniques. Correlations between total carbohydrate and EPS extracted by either EDTA or saline water can then be used as in indication of the efficiency of each extraction technique or as an indication of leakage of carbohydrates from within the cells (Underwood *et al.*, 1995). Therefore, total carbohydrate was also measured.

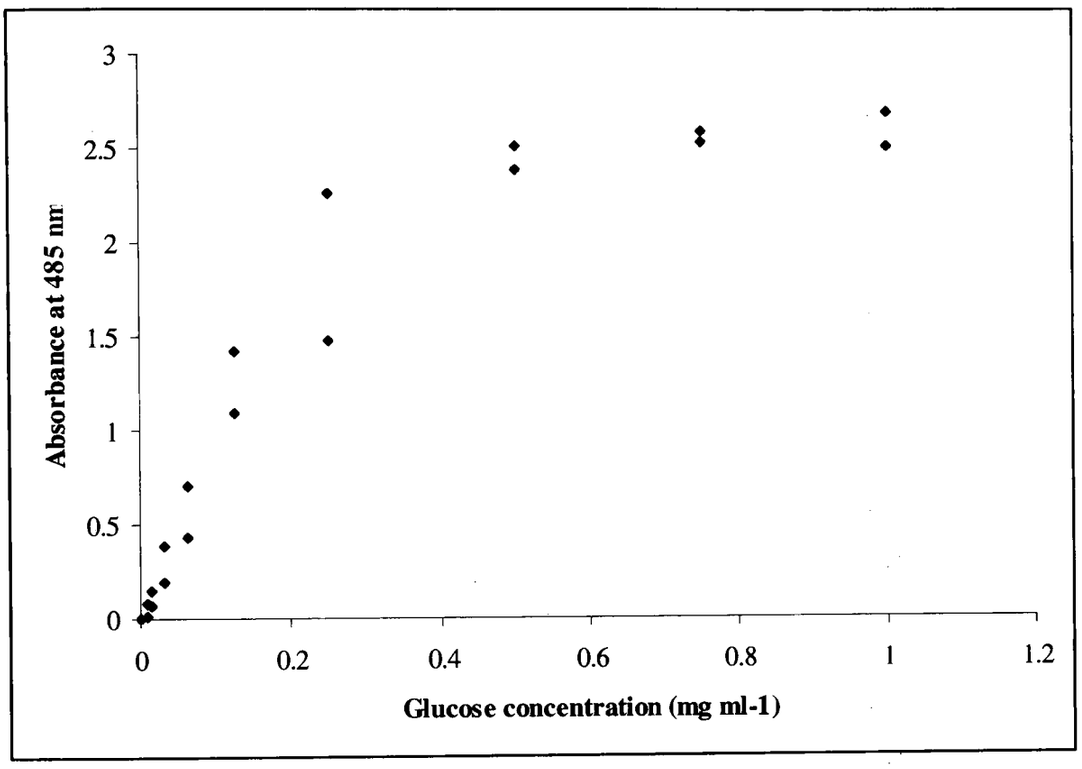


Figure 2.2. Initial calibration curve plotted to examine the relationship between glucose concentration and absorbance.

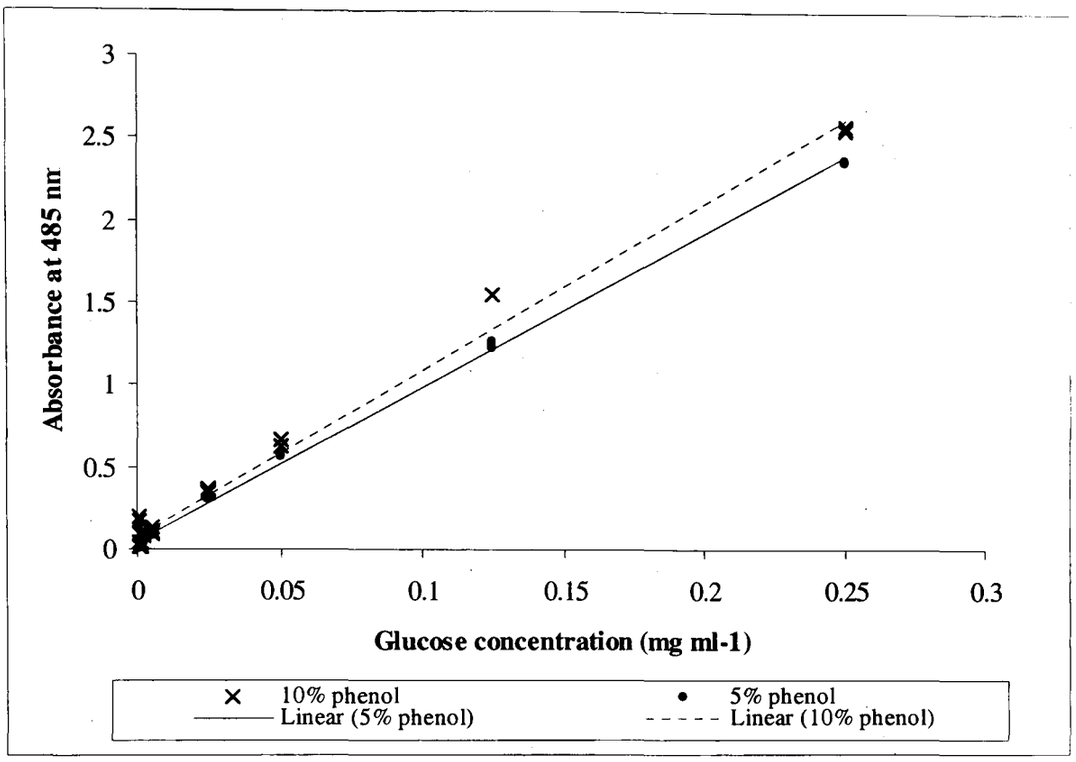


Figure 2.3. Calibration curves used to calculate sediment carbohydrate content.

2.3.7. Sedimentation

Two sedimentation studies were carried out, one short term and one long term. The methods used in the short term study were an adaptation of those used by Brown (1998) to study sedimentation in the saltmarshes of the Skeffling mudflat. Pairs of pre-weighed glass fibre filters (GF/C) were placed, one on top of the other, inside 10 cm diameter plastic lids with a 6 cm diameter hole cut out of the middle and secured using autoclave tape. The diameters of the top and bottom filters were 7 and 10 cm, respectively. The purpose of the bottom filter was to protect the top filter from collecting sediment directly from the sediment surface itself. This set up left a 28.3 cm^2 area (i.e. 6 cm diameter) of filter paper free for sediment to settle.

Three sets of lids (five replicates per set) were placed at the Saltend 25, 75 and 200 m sites and the Paull 150 and 25 m sites. The lids were placed face up and pushed into the mud so that the surface was flush with that of the mud. The lids were left for 24, 48 and 36 hours, i.e. 2, 4 and 6 tidal inundations. After each time period, one set of lids was removed from the mud. The bottom filter was removed in the field, taking care to keep the lid face up, and separated from the top filter. The top filters were then placed in plastic bags and the bottom filters discarded. The filters were dried at 105°C for 72 hours (taking care to wash out any sediment which had stuck inside the bag) and weighed. Sedimentation was expressed as g m^{-2} (dry weight) and was calculated as both cumulative sedimentation after 6 tidal inundations and as sedimentation between tidal inundations.

Longer term studies were carried out between April and September 2000. Pairs of canes (1.5 m long) were placed, vertically, 1.5 m apart with a third cane being placed across the two tops of the upright canes. The canes were pushed into the sediment to a depth of at least 0.5 m in order to prevent displacement by the currents. The depths of the two canes were adjusted, with the aid of a spirit level, so that the cane placed across the top was level. 25 cm divisions were marked along the top cane and the distance from that cane to the sediment surface measured at each division. Measurements began at 25 cm from the beginning and ended 25 cm before the end of the cane since erosion was expected to occur around the base of the canes. Two sets of canes were placed at each of the five sites, corresponding to those used in the short term study. Measurements were made monthly.

2.3.8. Statistical analysis.

Statistical testing between site parameters was carried out by one way analysis of variance (ANOVA) followed by *a posteriori* comparison of means (Zar, 1999). All data were tested for homogeneity of variance and in cases where this assumption was severely violated, attempts were made to transform the data or the appropriate non-parametric test (Kruskal-Wallis) test was carried out (Fowler *et al.*, 1998; Zar, 1999; Dytham, 2003). *A posteriori* testing of such data was carried out using the Games Howell test (Games & Howell, 1976). Two-way ANOVA tests were carried out in order to determine the interaction effects between site and month (Zar, 1999; Dytham, 2003).

Relationships between chlorophyll-*a* and sediment carbohydrate concentration were determined using regression analysis (Fowler *et al.*, 1998; Zar, 1999; Dytham, 2003) and cluster analysis (Euclidean distance) was used to determine similarities between sites in terms of all site parameters combined (Ludwig & Reynolds, 1988).

The statistical techniques used in the present chapter were also used in subsequent chapters. Further reference to statistical analysis is not made unless different, previously unmentioned techniques are used. All statistical analysis was carried out using SPSS version 11, with the multivariate (cluster) analysis being carried out using PRIMER (version 5.2).

2.4. RESULTS

2.4.1. Particle size analysis

The sediments at Saltend and Paull may be classified as soft muds with a silt/clay content greater than 97% in all cases. No statistically significant spatial or temporal differences were found for any of the parameters determined. Median particle diameter ranged from 6.8 φ (8.8 μm) at the S200m site in April to 7.9 φ (4.13 μm) at S75 m site in April although there were no statistical differences between sites or months (Figure 2.4). Whilst the particle size was not uniform between sites or over time, the difference between the maximum and minimum recorded particle size (4.67 μm) is not considered to be large enough to be of any significance in terms of its influence over the macrofauna present. The small sorting coefficient, or standard deviation, (0.29-0.41) indicated well sorted sediments consisting of a small range of particle sizes.

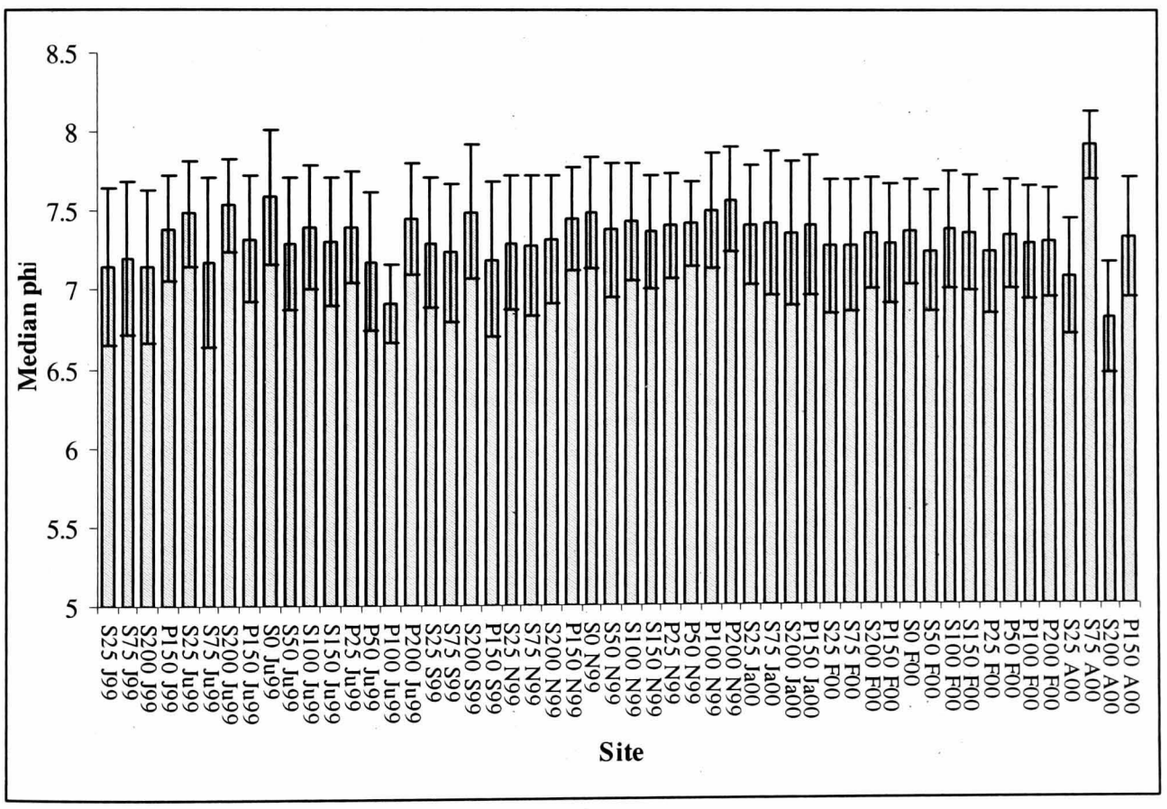


Figure 2.4. Spatial and temporal trends in median particle diameter (mean ± SE).

2.4.2. Water content

Sediment samples were analysed for water content at the four key sites between June 1999 and April 2000 (Figure 2.5). In general, water content at the S200 m and S75 m sites showed a slight increase during this period. Maximum and minimum water content values were 70.1% (April) and 57% (July and September) for the S200 m site and 65.5% (April) and

57.6% (June) for the S75 m site. Patterns at the S25 m and Paull sites were more variable and contrasting. That is, an increase in water content between months for the S25 m site was coupled with a decrease in water content at the Paull site. Maximum and minimum water content values were 61.1% (January) and 40.81% (April) at the Paull site and 65-66% (September and June) and 53.4% (January) at the S25 m site. Maximum water content for all of the Saltend sites occurred in April whilst water content at Paull was lowest for this month. Water content did not show a trend of decrease with increasing shore height.

One-way ANOVA tests, together with Tukeys' test, showed that differences in water content between sites were statistically significant for all months ($p<0.01$ and $p<0.05$ for April) except for July. In June and January, water content at the S25 m site was significantly lower than at all other sites whereas in September and November, water content was highest at this site with sites being in the order of P150 m < S200 m < S75 m < S25 m. In contrast, water content at the Paull and S200 m sites was significantly higher than at the other sites in January. In both February and April, water content at Paull was lower than that at all other sites.

Prior to statistical testing, all data were tested for homogeneity of variance and, with the exception of the June and April data, were found to meet the assumptions of the ANOVA test. Data for June and April could not be transformed and therefore a Kruskall-Wallis test was used to confirm the ANOVA output.

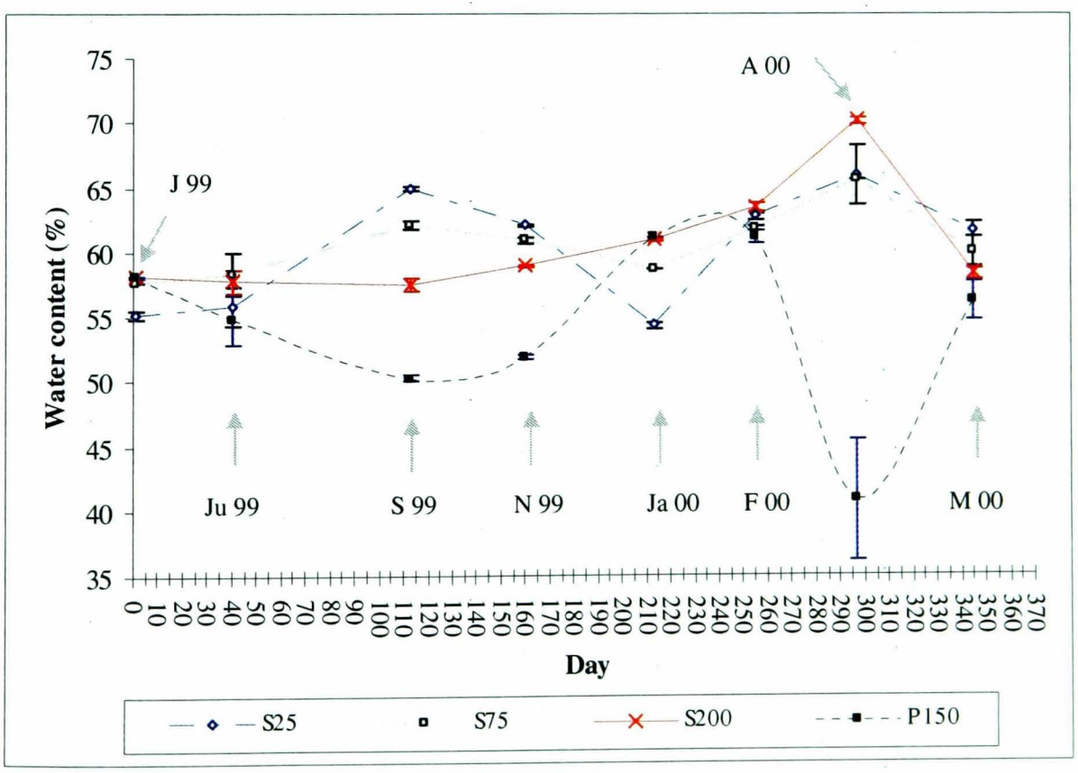


Figure 2.5. Trends in mean (± SE) water content at the key sites, between June 1999 and May 2000.

Water content profile data for May 2000 are presented in Figure 2.6. Whilst surface water content was found to be lowest at the upper shore Paull 25 m site (56.11%) and highest at the lower shore Paull 150 m (62.2%) and S 200 m sites in May 2000, this pattern was not consistent throughout the year. This suggests that position on the shore is not necessarily a key factor determining water content on these mudflats. As would be expected, water content decreased with increasing depth into the sediment with water content at the Paull 150 m site remaining lower than at all other sites at all depths. Two-way ANOVA tests revealed significant differences ($p<0.01$) between both sites and depths but did not indicate any significant interactive effect between site and depth. That is, sediment water content decreased with depth at all sites but site had no influence on the degree of this reduction.

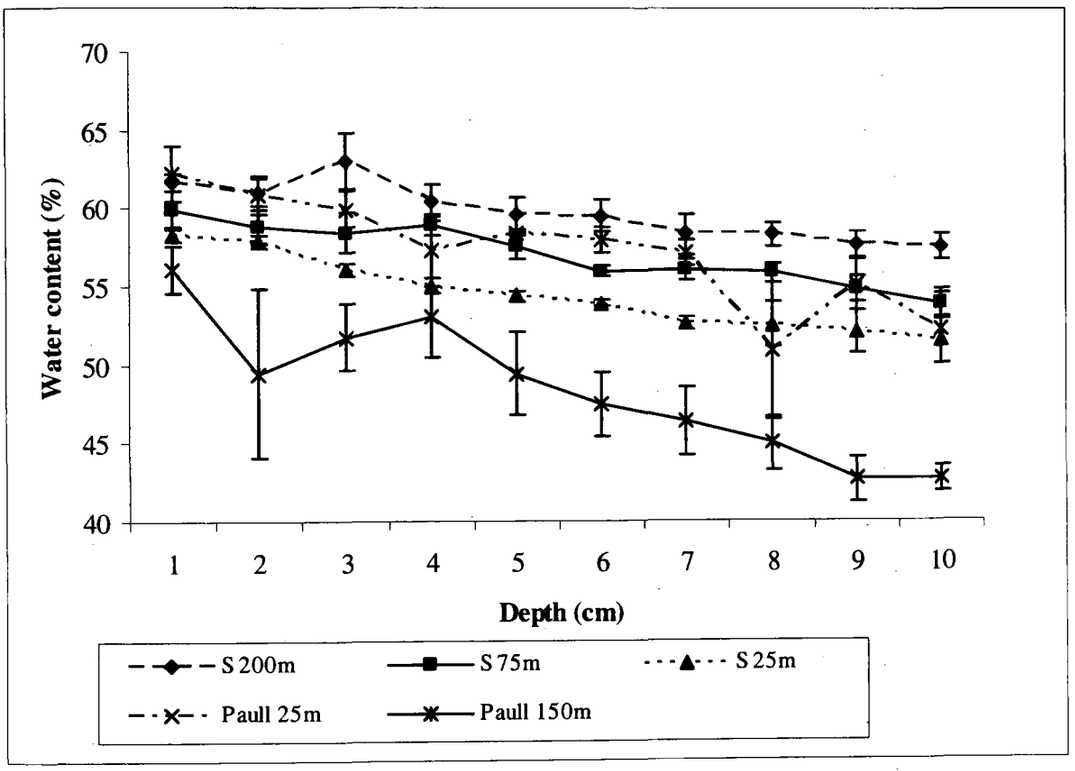


Figure 2.6. Mean (± SE) water content profiles for the four key sites and the Paull 25 m site for May 2000.

Wet bulk density ranged from 1.56 g cm^{-3} at the S200 m site in April to 1.73 g cm^{-3} at the P150 m site in April (Table 2.2). Whilst statistical differences between sites and months were found using both one and two-way ANOVA tests, no obvious trends could be seen.

Table 2.2. Maximum and minimum values (mean ±SE) of wet bulk density (g cm^{-3}) and the month in which they were recorded for the four key sites.

Site	Minimum bulk density	Month (min)	Maximum bulk density	Month (max)
P150 m	1.62 ±0.003	January	1.73 ±0.07	April
S200 m	1.56 ±0.001	April	1.63 ±0.003	September
S75 m	1.59 ±0.0001	April	1.64 ±0.0004	May
S25 m	1.59 ±0.013	April	1.66 ±0.0013	January

The salinity and pH of the interstitial water was measured in June 1999. Salinity ranged from 16.6-17.5 and pH ranged from 7.72-7.89. Since no statistically significant differences were found between sites for either parameter, salinity and pH were assumed to be the same at all sites. Therefore, measurements were not repeated.

2.4.3. Organic content

Organic content of the sediment was recorded between June 1999 and April 2000 for the four key monitoring sites (Paull 150 m and Saltend 25, 75 and 200 m) (Figure 2.7). In May 2000, data were collected for all sites and a depth profile of organic content produced for the key sites together with the Paull 25 m (upper shore) site (Figures 2.8 and 2.9). For the S25 m and S75 m sites, there appears to be some pattern of seasonality with organic content declining between June 1999 and January 2000 then increasing to a maximum in May. At the 25 m site, significant differences were found between January and April, May and June values ($p<0.01$) and between January and July, November and February ($P<0.05$). Homogeneity of variance could not be achieved through transformation of the data so a non-parametric Kruskal-Wallis test in combination with the Games Howell (equal variances not assumed) post-hoc test was carried out. At the S25 m site, organic content ranged from 7.89% in January to 13.75% in May. At the Saltend 75 m site, Figure 2.7 suggests some seasonal trend but significant differences were only found between June and May ($p<0.01$) and February and May ($p<0.05$) indicating that the organic content of the sediment at this site did not change according to season. Organic content values for this site ranged from 8.6% in January to 12.8% in May. Patterns for the Saltend 200 m and Paull 150 m sites were less clear, showing no obvious trend. No significant differences were found between months for the Paull site whilst differences between July and April ($p<0.05$) and November and January ($p<0.05$) were found for the S200 m site. Again, there was no indication of a seasonal trend. Maximum and minimum values of organic content were 11.58% (May) and 6.01% (September) for the S200 m site and 11.91% (January) and 8.44% (June) at the Paull 150 m site.

Figure 2.7 shows that, with the exception of January where sediment organic content at Paull is high and in September when sediment organic content at the S200 m site is uncharacteristically low, organic content at the Saltend sites is higher than that at Paull. Statistical analysis showed little difference between sites and significant differences could only be found for certain months. Organic content at Paull was significantly lower than that at the Saltend sites in November, April and May ($P<0.05$) and higher in January ($P<0.01$). Values were significantly higher at the S25 m site in July ($p<0.05$) and May ($P<0.01$).

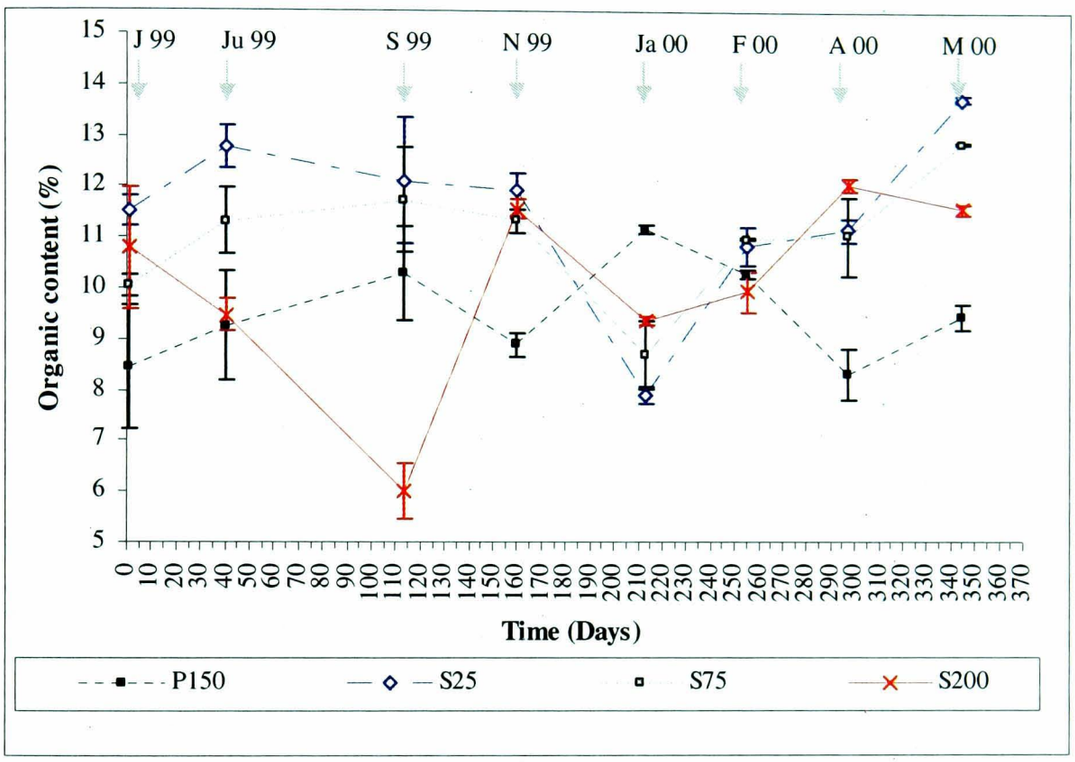


Figure 2.7. Changes in mean (± SE) sediment organic content (expressed as percent LOI) between June 1999 and May 2000 for the four key monitoring sites.

In May 2000, a clear trend of increasing organic content towards the point of discharge was found (Figure 2.8). Organic content at the S0 m, S25 m and S150 m sites was significantly higher ($p<0.05$) than that at all the Paull sites and at the S75 m, S100 m and S200 m sites. Organic content at the Paull 150 m and Paull 200 m sites was significantly lower than at all other sites. However, the data covering 10 months give no suggestion that sediment organic content within the vicinity of the outfall is consistently higher than that in other parts of the mudflat.

There was a slight decrease in organic content with increasing depth at all sites and, in general, organic content at the S25 and S75 m sites was higher than that at the S200 m or Paull sites (Figure 2.9). Organic content decreased from a maximum of 13.5% at the S25 m site to a minimum of 8.5 % at the P150 m site. One-way ANOVA tests revealed that this reduction in

organic content with increasing depth was statistically significant ($p<0.01$) at all sites except the P150 m site. A two-way ANOVA test showed significant effects of site and depth ($p<0.01$) but no significant interactive effects between the two variables ($p>0.05$).

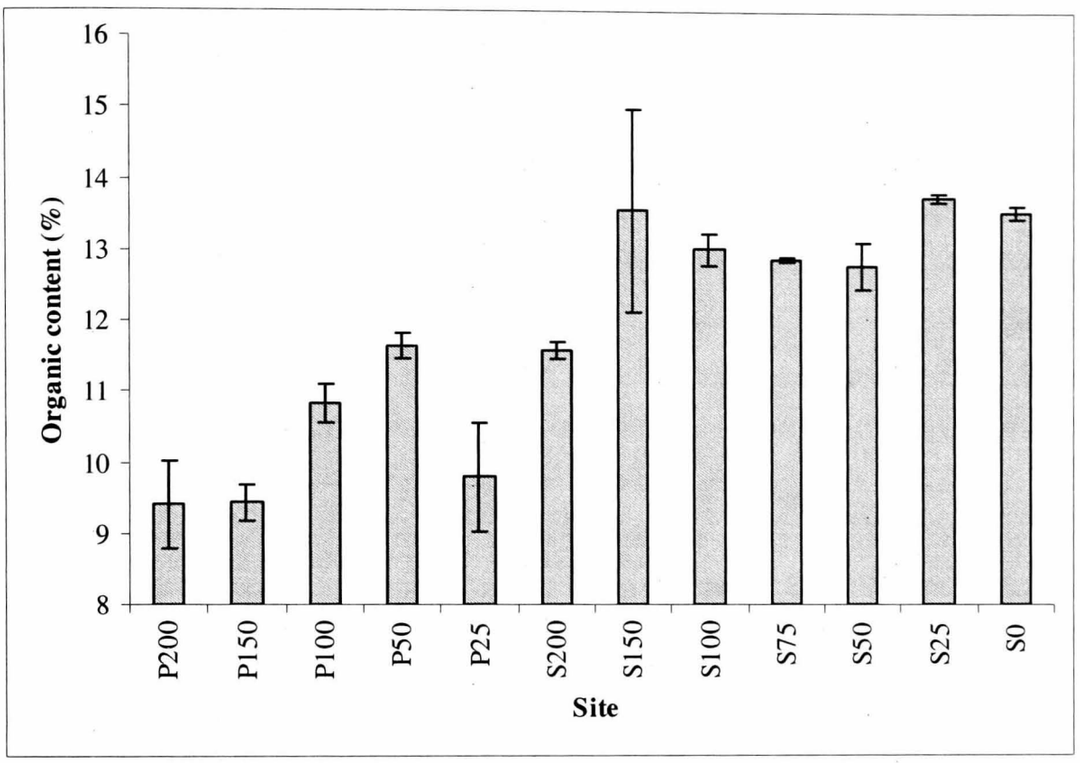


Figure 2.8. Changes in mean (± SE) sediment organic content (expressed as percent LOI) with increasing proximity to the discharge for all sites, May 2000.

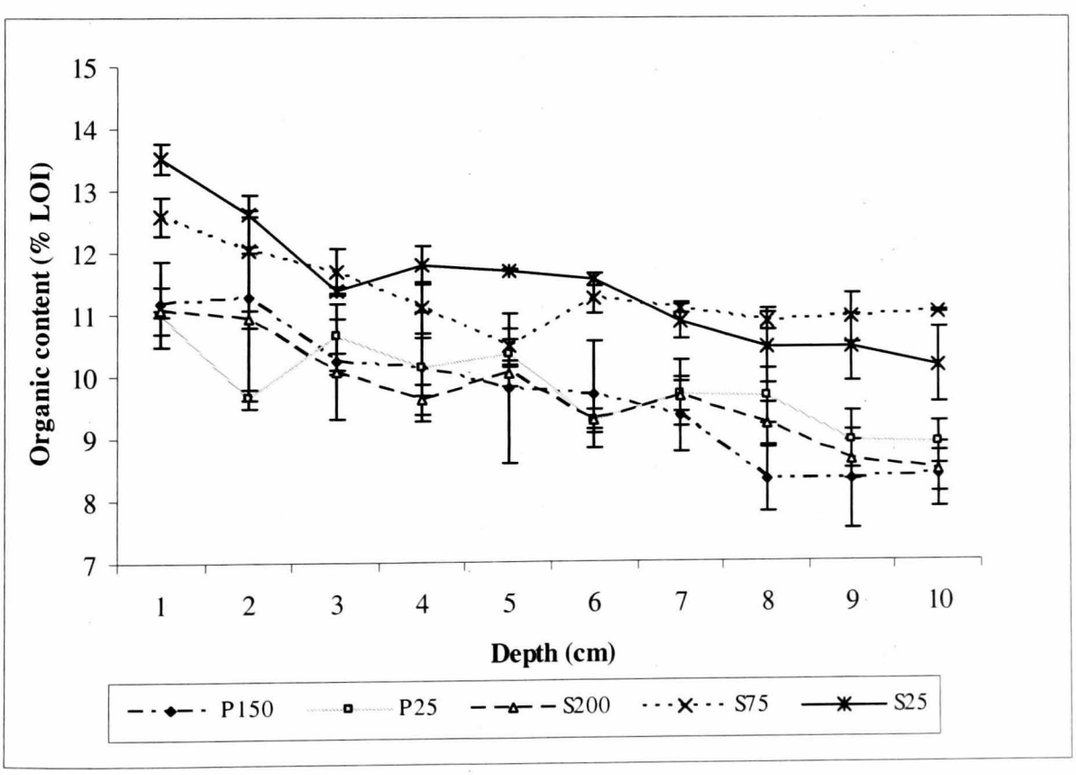


Figure 2.9. Organic content profiles (mean ± SE) for the four key monitoring sites, May 2000.

2.4.4. Sediment contamination

Redox potential was recorded at the four key monitoring sites as an indication of the degree of sediment contamination (organic and oxygen status) (Figure 2.10). In addition, sediment metal concentration data (C. Nikitik, University of Hull. Pers. comm.) were examined (as an annual mean) for the period May 1997 to May 1998. As was expected, Eh became more negative with increasing depth. In general, the S25 and S75 m sites were considerably more anoxic than the S200 m and Paull sites with Eh at the S25 m site being consistently more negative than that of the S75 m site (with the exception of the July 1999 profiles). Surface Eh values at the S25 m site were as low as -249 mV in June / July with values at the 10 cm depth being around -384 mV. Eh became less negative throughout the autumn and winter, at all depths (except during September) with surface values being close to or approaching zero in November and January and reaching 34 mV in February. Surface Eh values then became negative again in April. Eh values at the S75 m site were generally similar (although slightly higher in some cases) to those at the S25 m site with surface values being negative or close to zero for much of the year. In contrast, surface Eh at the Paull 150 m site remained positive throughout the year with maximum values being in February when Eh reached 214 mV. With the exception of June and July, surface Eh at the S200 m site also remained positive throughout the year.

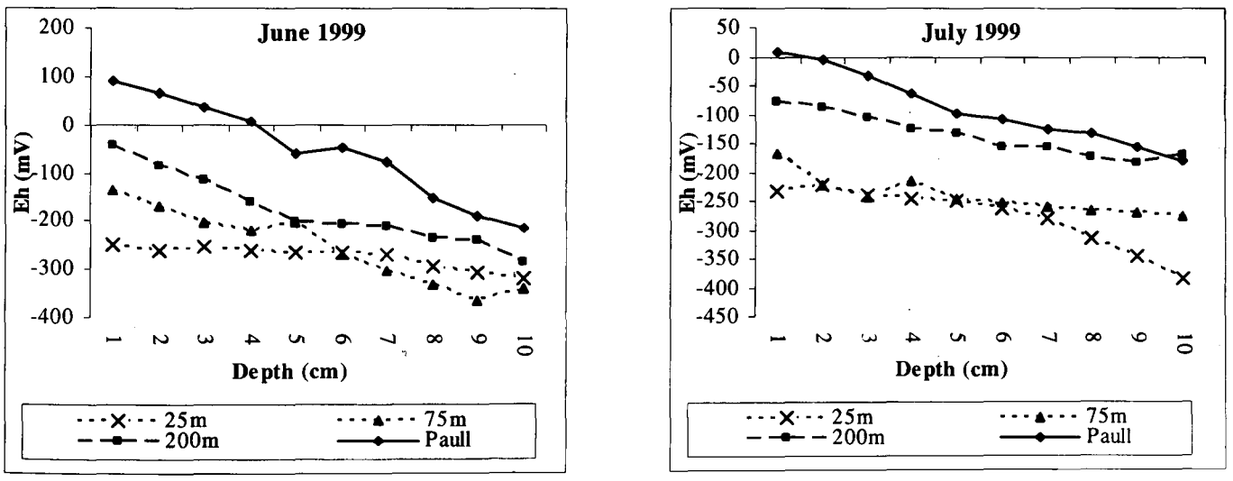


Figure 2.10. Redox potential (mean) profiles for the key monitoring sites for June 1999 - April 2000 (SE omitted for clarity).

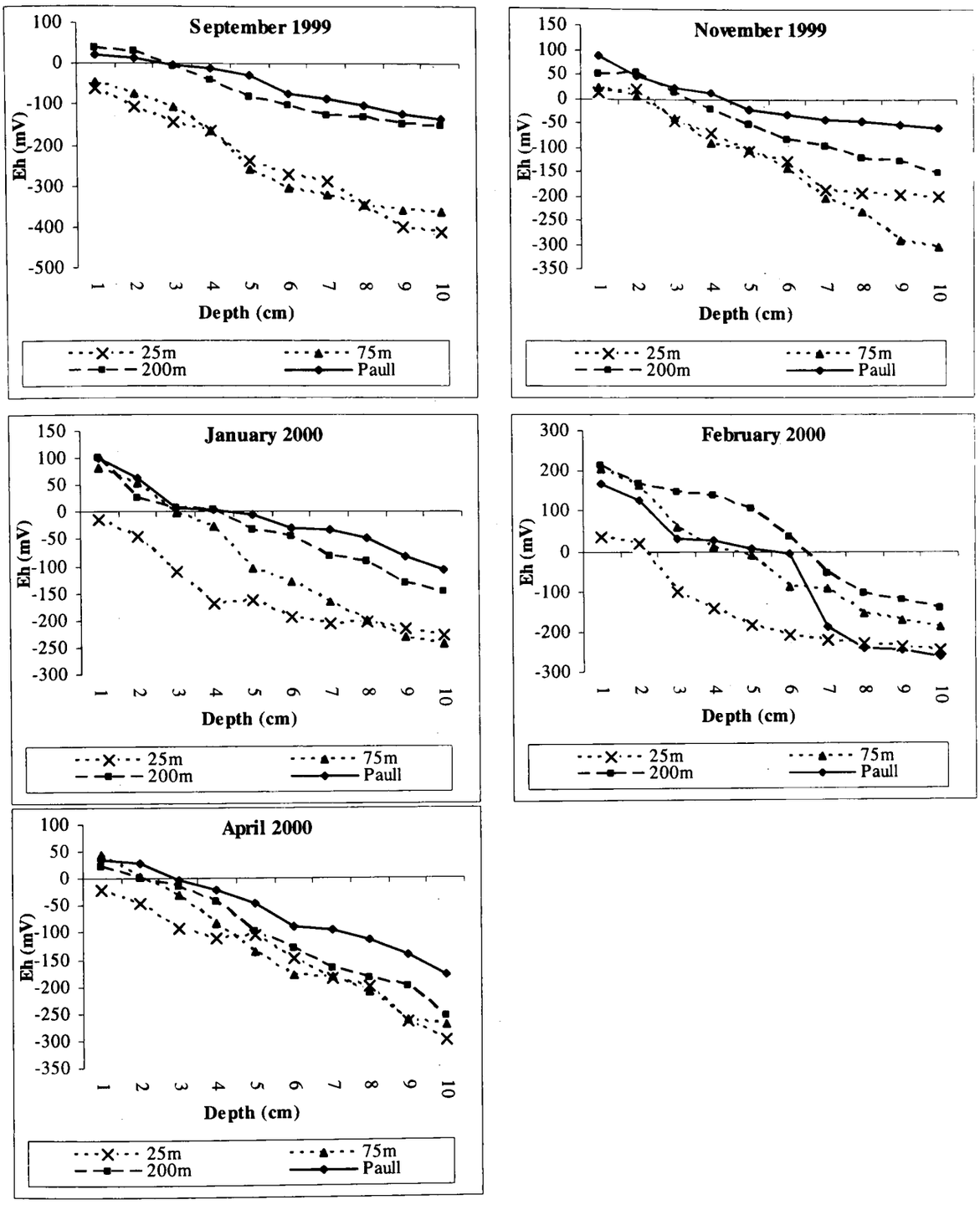


Figure 2.10 (cont).

Due to unequal variances and the fact that the data could not be transformed, it was not considered worthwhile to examine the interactive effects of depth, site and month (i.e. 3-way ANOVA). Therefore, the Eh at a depth of 4 cm has been examined with respect to site and month and the interactive effect of the two variables, using 2-way ANOVA, determined. Again, homogeneity of variance could not be achieved and therefore the output of the analysis was reinforced using a non-parametric Friedman test.

The 2-way ANOVA (Table 2.3) shows that, at a depth of 4 cm, Eh differs significantly between sites ($p<0.01$) and months ($p<0.01$) and that there is a significant interaction between the two factors ($p<0.01$). This was also demonstrated by the result of the Friedman test ($\chi^2 = 673.49$, $p<0.01$) but is most apparent in Figure 2.11. As would be expected, given the inverse relationship between Eh and temperature, the most negative values were recorded during the summer months although sediments at the S25 m site were also highly negative during the winter. Eh was consistently higher at the Paull site and became positive in June, November, January and February. Whilst the 2-way ANOVA indicates differences in Eh between sites and months, it does not indicate which data were responsible for this difference. Therefore, a Kruskal-Wallis test was carried out together with Games-Howell post hoc analysis in order to detect differences between sites.

For the majority of the year (July, September, November and April), the S25 and S75 m sites were significantly different ($p<0.01$) to the S200 m and Paull sites. In January and February, the S200 m and Paull sites were similar but the S75 m and S25 m sites were significantly different to each other suggesting that the characteristics of the S75 m site became closer to those of the S200 m and Paull sites. This can be seen in Figure 2.11 which shows that in January, the Eh at the S75 m site was close to zero, in comparison with the highly negative value at the S25 m site, and that in February, it became positive. In contrast, during the summer months (June, July and September), the S200 m site was more similar to the S25 and S75 m sites and, in June, there was no statistical difference between these three sites. This implies that during the winter, reducing conditions at the S75 m site are ameliorated by the low temperatures and the characteristics of the site become more similar to those at Paull and the S200 m site. During the summer, the redox conditions at the S200 m site became more negative due to higher temperatures so that the sediment characteristics became more similar to those at the more contaminated S75 and S25 m sites.

Table 2.3. Two-way ANOVA model output examining the effect of site and month on Eh at 4 cm.

Eh at 4 cm					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Model	1137754.37	28	40634.08	128.00	0.00
MONTH	240259.33	6	40043.22	126.14	0.00
SITE	280603.56	3	93534.52	294.63	0.00
MONTH * SITE	132192.24	18	7344.01	23.13	0.00
Error	17777.73	56	317.46		
Total	1155532.10	84			

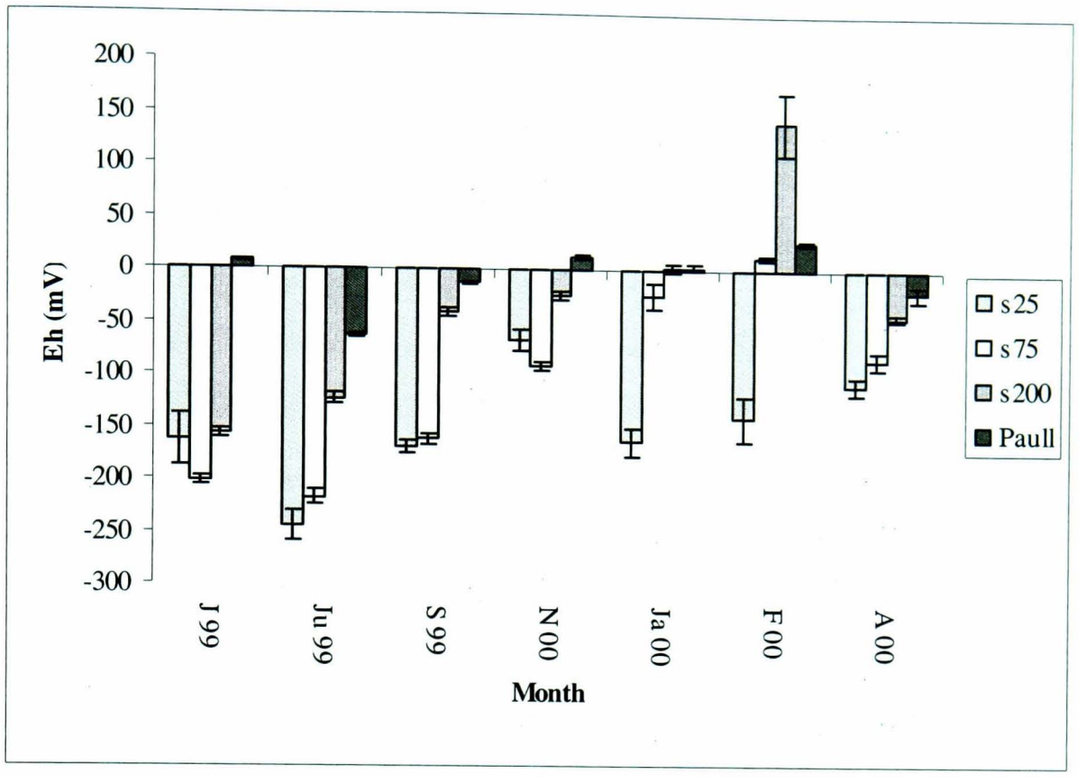


Figure 2.11 Difference between redox potential (mean ± 95% CL) at a depth of 4 cm for the four key sites between June 1999 and April 2000.

Sediment metal concentrations were analysed quarterly by C. Nikitik (University of Hull. Pers. comm..). Concentrations of all metals decreased progressively with increasing distance from the outfall although these differences were only statistically significant for copper, zinc and iron ($p<0.01$). Copper concentrations decreased from 65 mg kg^{-1} at the S0 m site to 34 mg kg^{-1} at the Paull site whilst zinc concentrations were 238 mg kg^{-1} and 169 mg kg^{-1} for these sites, respectively. Iron concentrations decreased from 41 g kg^{-1} to 31 g kg^{-1} between these sites.

2.4.5. Microalgae

Sediment microalgal biomass (expressed as chlorophyll-a concentration) was consistently higher at the S25 m and S75 m sites, a difference which was greatest during the spring and summer months (Figure 2.12). At the S25 m site, concentrations ranged from 41 μg g^{-1} in November to 96 μg g^{-1} in April. Chlorophyll-a concentrations were consistently lower at the Paull site (with the exception of November) with minimum concentrations of 6 and 5 μg g^{-1} in January and September and a maximum concentration of 53 μg g^{-1} being recorded in June. One-way ANOVA tests showed statistically significant differences ($p<0.01$) in chlorophyll-a concentration between sites for all months. Tukey tests showed concentrations at the Paull and S200 m sites to be significantly lower than at the S75 m and S25 m sites during June, July and April. Whilst chlorophyll-a concentrations remained highest at the S25 m site throughout the year, concentrations at the S200 m and S75 m sites did not differ statistically from each

other during the winter months. This trend of the S200 m and S75 m sites being similar during the cooler months but becoming more similar to the Paull and S25 m sites, respectively, during the summer was also observed for sediment redox conditions.

One-way ANOVA tests also showed significant differences in sediment microalgal density between months. Tukey tests showed that within each site, the summer (April, June and July) months tended to differ significantly to the autumn and winter months in terms of sediment chlorophyll-a concentration. Two-way ANOVA showed significant ($p<0.01$) effects of both site and month and a significant ($P<0.01$) interaction between the two variables. These results suggest that microalgal density is influenced both by season and distance from the BP outfall.

Pheopigment concentrations (Table 2.4) followed the same trends as chlorophyll-a concentration with the highest values being recorded at the S25 m site in April, June and July. One-Way ANOVA tests, together with Tukeys' test revealed significant differences between sites and months as described above.

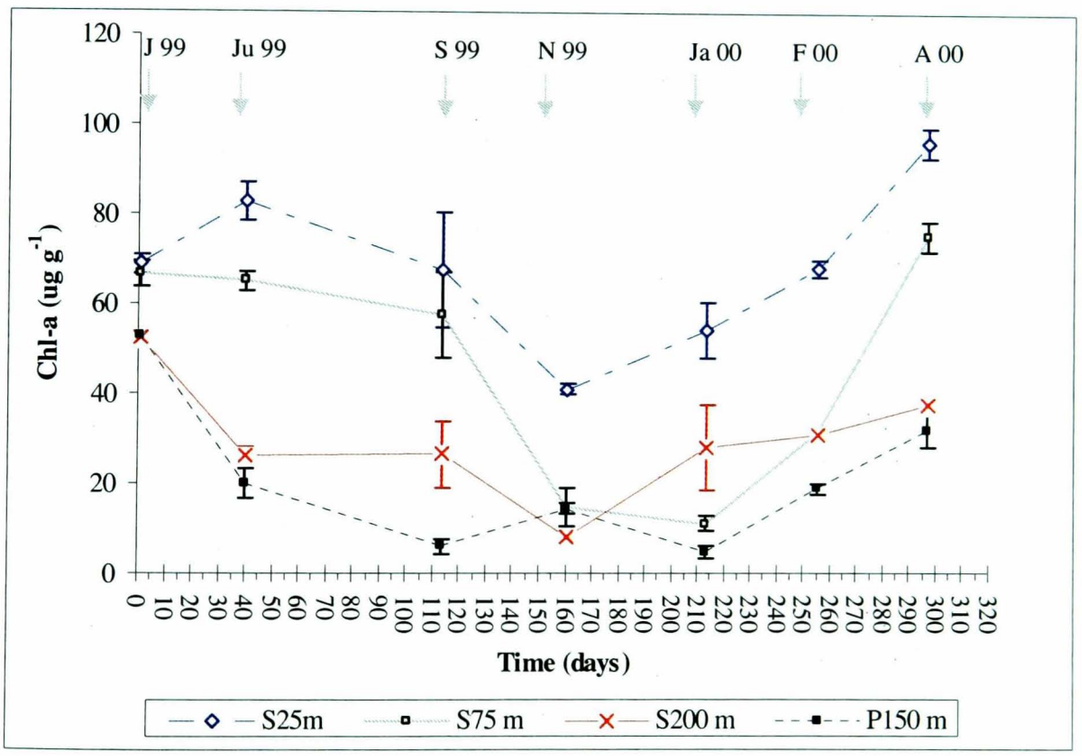


Figure 2.12. Changes (mean ± SE) in chlorophyll-a content of the sediment at the four key monitoring sites between June 1999 and April 2000.

Table 2.4. Pheopigment concentrations (mean ± SE) for the four key sites for each month.

	June-99	July-99	Sept-99	Nov-99	Jan-00	Feb-00	April-00
S25 m	117.2 ±3.6	140.9 ±7.5	114.9 ±21.8	69.9 ±2.2	92.3 ±10.1	115.6 ±3.4	163.6 ±5.9
S75 m	113.4 ±4.7	110.6 ±3.5	98.1 ±16.1	25.1 ±7.1	18.9 ±2.8	53.4 ±2	128 ±5.3
S200 m	88.9 ±2.1	44.5 ±3.1	45.1 ±12.4	13.9 ±2.3	47.9 ±16.4	52.8 ±1.2	64 ±1.4
P150 m	89.5 ±1.7	33.8 ±5.7	10.4 ±2.9	24.5 ±1.9	8.1 ±2.5	32.1 ±2.2	54.5 ±6.6

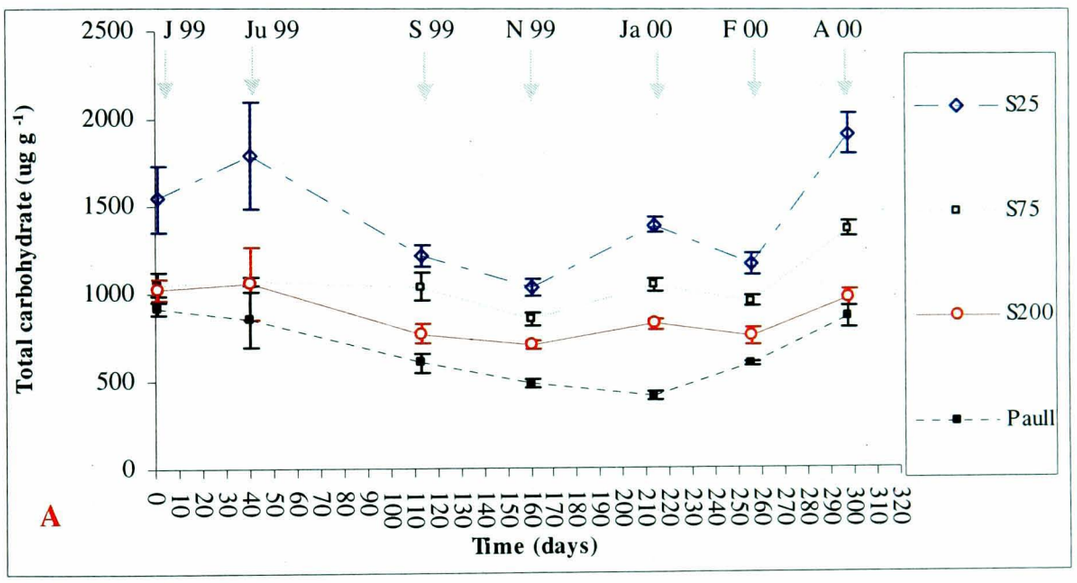
2.4.6. Sediment carbohydrate analysis

Figure 2.13 shows concentrations of total and colloidal (EDTA extractable and saline extractable) carbohydrate, expressed as glucose equivalents, at the key sites. Attempts to determine the EPS content of the colloidal extracts were largely unsuccessful and the data highly variable. These data have therefore been omitted. It can be seen that, in all cases, concentrations of carbohydrate were highest at the S25 m site throughout the year, showing a trend of decreasing concentration with increasing distance from the outfall. Maximum total carbohydrate concentrations ranged from 0.9 mg g^{-1} at Paull in June to 1.9 mg g^{-1} at the S 25 m site in April. Minimum concentrations occurred in November and January (Paull) and ranged from 0.4 mg g^{-1} at Paull to 1 mg g^{-1} at the S 25 m site. Colloidal (EDTA) carbohydrate concentrations reached a maximum in April at all sites with values being in the range of 0.9 mg g^{-1} at Paull to 1.4 mg g^{-1} at the S 25 m site. Minimum values were, again, recoded in November and January (Paull) and ranged from 0.2 mg g^{-1} at Paull to 0.7 mg g^{-1} at the S 25 m site. Colloidal (S) carbohydrate concentrations followed the same trend of maximum and minimum values being at the S25 m site and Paull, respectively. Maximum values ranged from 0.9-1.4 mg g^{-1} with minimum values being in the range of 0.2-0.7 mg g^{-1}.

For total (bulk) and colloidal (EDTA) carbohydrate, concentrations at the S25 m site were significantly higher than those at the S200 m and Paull sites for the whole year. Statstically significant ($p<0.01$, $p<0.05$ for June and July) differences were found between sites for all months with the S200 m and Paull sites differing to the S25 m site throughout the year. Significant differences were found between the S200 m site and the S75 m site between September and April for total carbohydrate and between September and January for colloidal carbohydrate (EDTA). For saline extractable carbohydrate (colloidal S) concentrations, the Paull and S200 m sites remained the same throughout the year (with the exception of January and April) and remained significantly lower than concentrations at the S75 and S25 m sites. In January, concentrations at the S200 m and S75 m sites did not differ and in April, all sites were different from each other. The S25 and S75 m site did not differ significantly from each

other during any month except January and April. There were clear seasonal differences in carbohydrate concentration at all sites and two-way ANOVA tests revealed a significant interaction between site and month ($p<0.01$) for all carbohydrate measurements. One-way ANOVA together with Tukey tests showed that the summer months generally differed from the winter months in terms of all carbohydrate measurements.

At all sites, sediment carbohydrate concentrations followed the same seasonal trend as chlorophyll-a with maximum concentrations being recorded during the warmer months (Figure 2.13). Significant ($p<0.01$) positive correlations existed between chlorophyll-a concentration and all carbohydrate measurements with r^2 values being 0.54, 0.66 and 0.64 for total, colloidal (EDTA) and colloidal (S) carbohydrate, respectively (Figure 2.14). Colloidal (S), colloidal (EDTA) and total carbohydrate were all also significantly correlated (Table 2.5).



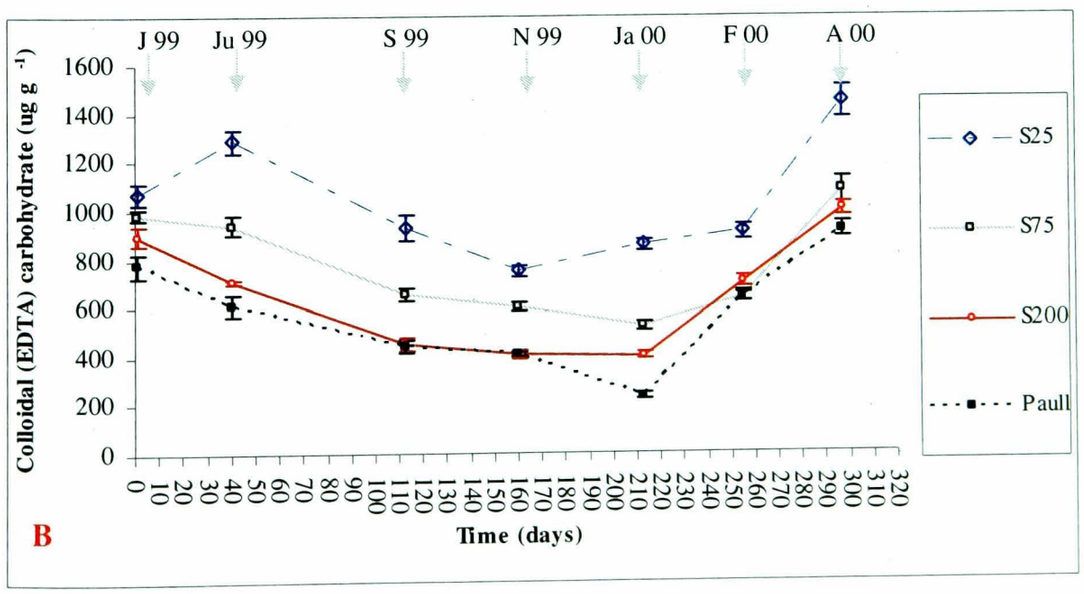


Figure 2.13. Trends in total (A) and colloidal (B,C) sediment carbohydrate content between June 1999 and April 2000 (mean ± SE).

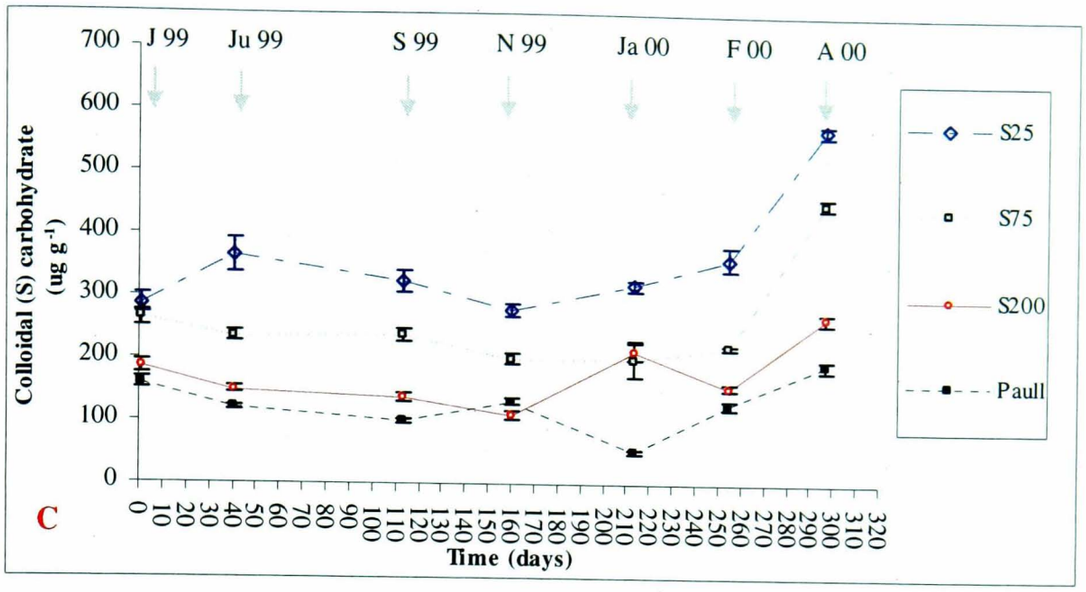


Figure 2.13 (cont.).

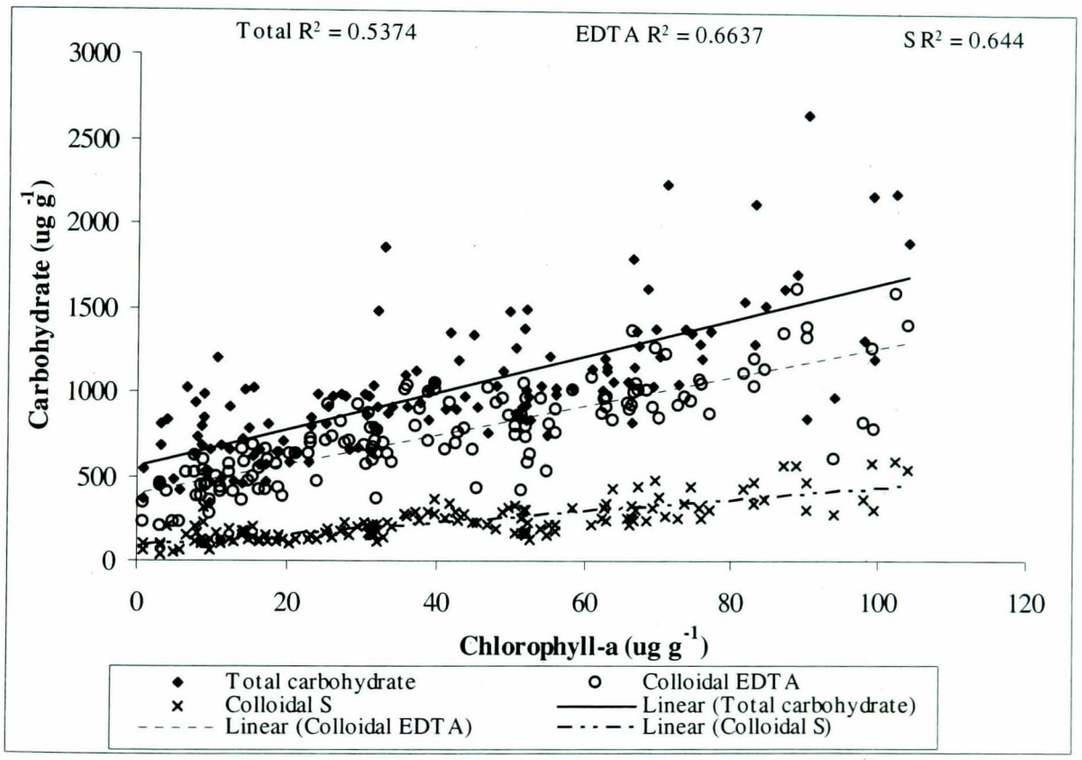


Figure 2.14. Correlations between sediment chlorophyll-a and carbohydrate (total and colloidal) content.

Table 2.5. Relationships (expressed as the coefficient of determination, r^2) between each fraction of the carbohydrate measured and the sediment chlorophyll-a concentration.

	Total carbohydrate	Colloidal (S)	Colloidal (EDTA)
Colloidal (S)	0.59 ($p<0.01$)		
Colloidal (EDTA)	0.52 ($p<0.01$)	0.65 ($p<0.01$)	
Chl-a	0.53 ($p<0.01$)	0.64 ($p<0.01$)	0.66 ($p<0.01$)

2.4.7. Sedimentation studies.

The amount of sediment deposited after 2, 4 and 6 tidal inundations showed a general pattern of increase between 2 and 6 tidal inundations at the S200 m site (Figure 2.15 A). In contrast, following the initial deposition after 2 tidal inundations, the amount of sediment deposited appeared to decline at the S25 m, S75 m, P25 m and P150 m sites. This indicates that erosion took place between 2 and 4 and 4 and 6 tidal inundations at these sites. Cumulative sedimentation ranged from 2505 g m^{-2} at the P25 m site to 16 831 g m^{-2} at the S200 m site and showed a trend of decreasing deposition with increasing shore height (Figure 2.15 B). One-way ANOVA tests showed that deposition was consistently higher at the S200 m site ($p<0.01$), both in terms of sedimentation between tidal inundations and cumulative sedimentation. However, there were no statistical differences between any of the other sites.

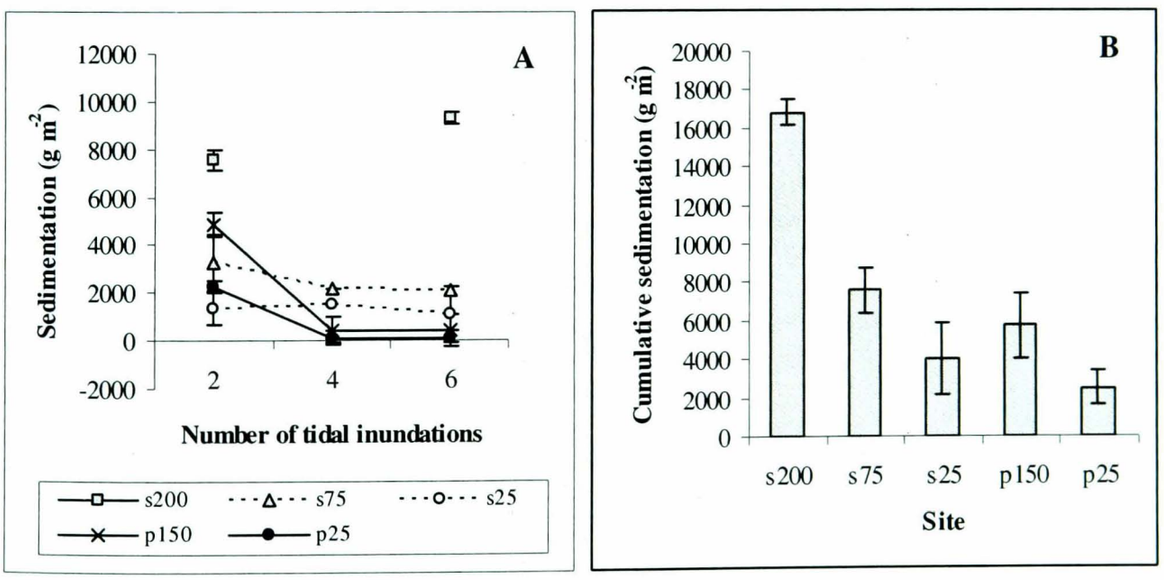


Figure 2.15. Short term differences in sedimentation (mean ± SE) between sites over six tidal innundations (A) and cumulative sedimentation after six tidal innundations (B). Data for S200 m after four tidal inundations are missing.

During the longer term study, sediment deposition varied considerably at each site over time and showed no consistent spatial trend (Figure 2.16). In general though, sedimentation was

greater at the Saltend sites (particularly at S200 m) than at the Paull sites. As was demonstrated by the short term study, cumulative sedimentation was greatest at the S200 m site and also showed a trend of decreasing deposition with increasing shore height (Figure 2.17). After May 2nd, sedimentation increased sharply at the S200 m and S75 m sites making cumulative deposition at these sites significantly ($p<0.01$) higher than at the other sites. Following this, no significant erosion or deposition took place although deposition remained higher at these sites with deposition at the S200 m (total of 7.4 cm) site remaining higher ($p<0.01$) than that at the S75 m site (total of 5.2 cm). Cumulative sedimentation was low at the S25 m site during the first part of the study but increased after June 21st and by the end of the study, total sedimentation (2.7 cm) at this site was significantly higher ($p<0.01$) than at either of the Paull sites. Sediment deposition at the p150 m site occurred between April 7th and May 2nd whilst no change was recorded from the P25 m site. Slight (but not statistically significant) erosion occurred at both sites between May 22nd and August 22nd. By the end of the study, significant ($p<0.05$) accretion (1.5 cm) had taken place at the P150 m site whilst no overall statistically significant change in sediment height at the P25 m site was recorded.

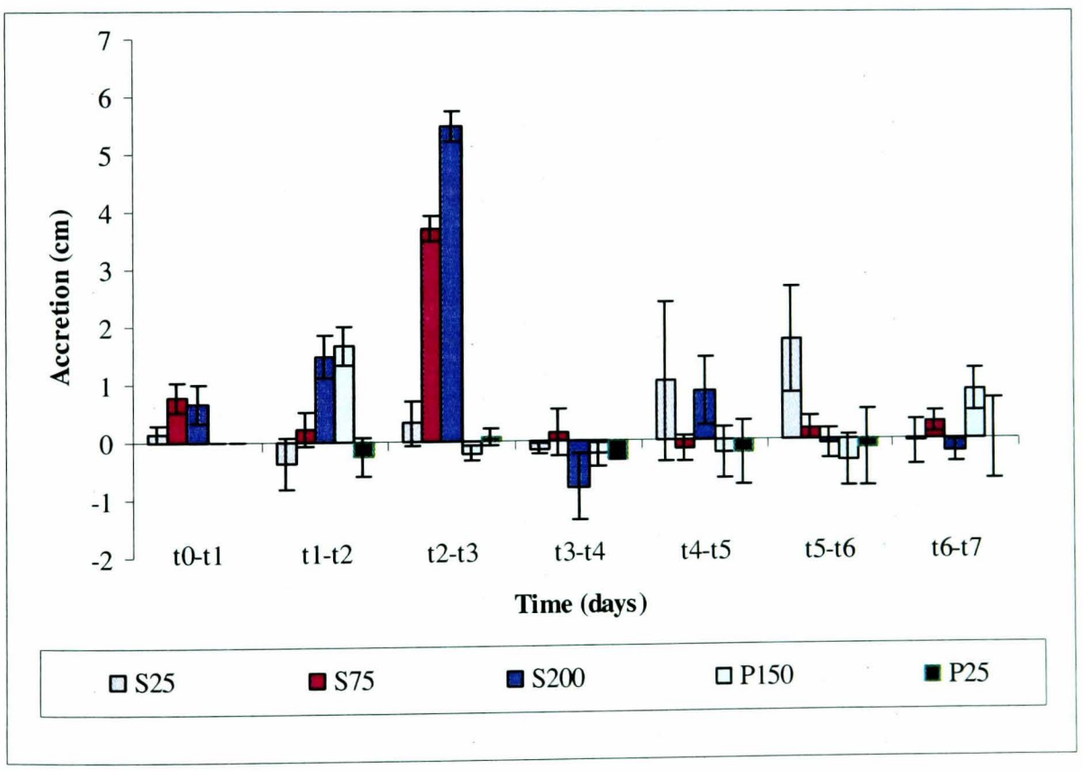


Figure 2.16. Differences in sedimentation (mean ± SE) between sites between April 2000 and September 2000 (sticks).

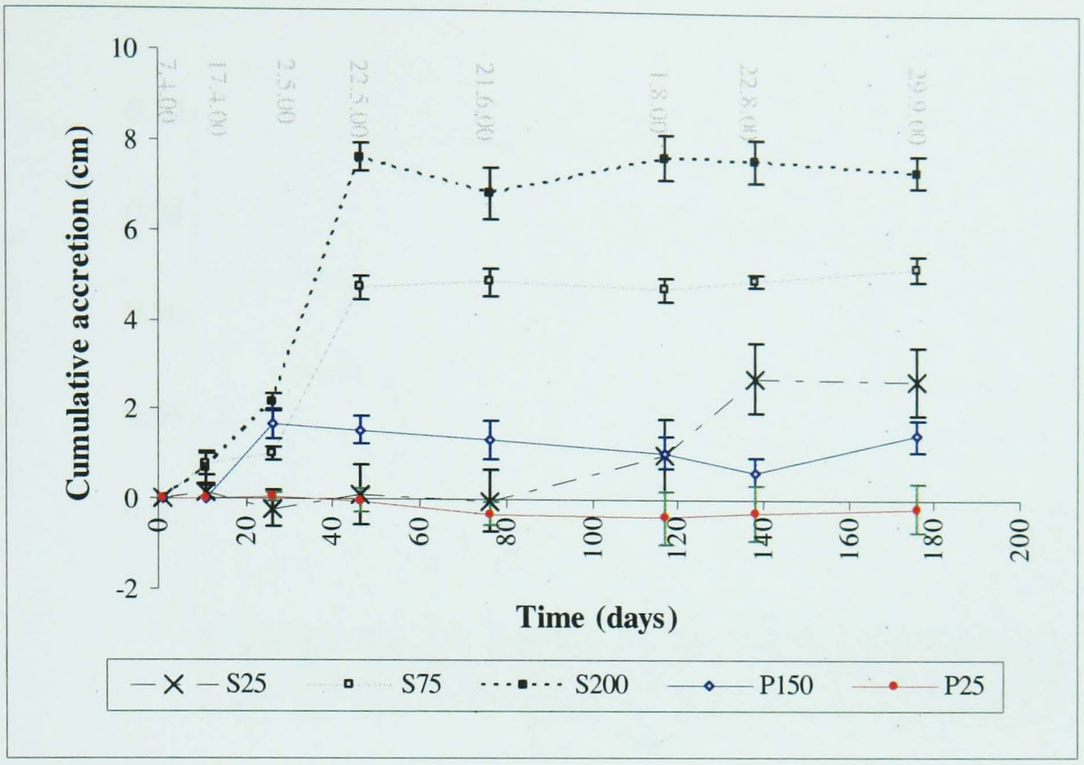


Figure 2.17. Differences in cumulative sedimentation (mean ± SE) between sites between April 2000 and September 2000 (sticks) (grey numbers indicate sampling dates).

2.4.8. Overall differences between sites.

When all the sedimentary parameters measured are considered collectively (using standardised units) for the four key sites for each month, the sites can be divided into two major groups (Figure 2.18). The first contains the majority of the S25 m and S75 m sites with sites generally being clustered according to season (e.g. S25 m and S75 m in April (1A); S25 m in June and July (1B); S75 m in June and July (1C) and S25 m in September, January and February (1E)). Cluster 1D contains the S200 m site for June and the S75 m site for September which reflects the fact that, in terms of Eh and chlorophyll-a concentration, during the autumn and winter, the conditions at the S75 m site become more similar to those at the S200 m site during summer (see section 2.4.4 and 2.4.5). This is again demonstrated in cluster 2A where the S75 m site is grouped with the P150 m site for June and April and the S200 m site for January. Cluster 2B contains the Paull sites for the cooler months and the S200 m site for November. Clusters 2C and 2D again show the way in which the sediment characteristics at the S25 and S75 m sites during the cooler months become more similar to those at the S200 m and Paull sites during the warmer months.

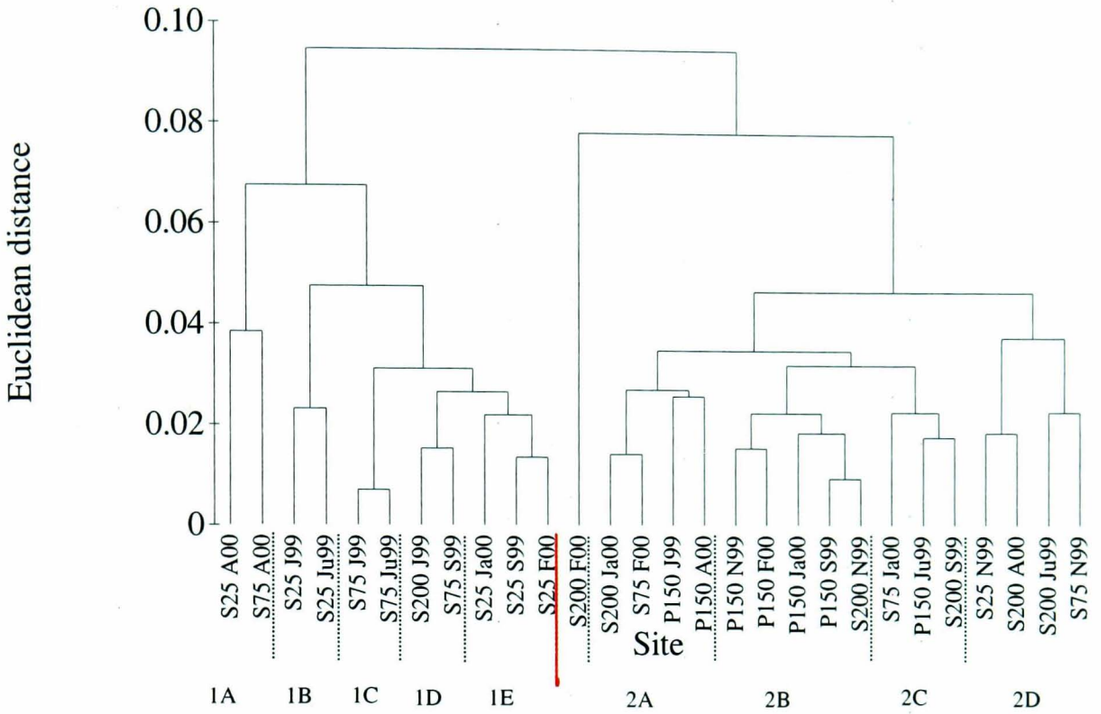


Figure 2.18. Cluster analysis showing similarities between sites taking all parameters into consideration.

2.5. DISCUSSION.

2.5.1. Physical properties of the sediment.

The sediments at Saltend and Paull comprised soft muds with a high silt/clay content (>97%) and a median particle diameter ranging from 6.8 - 7.9 φ. Whilst small variations in median particle diameter were recorded, there were no obvious spatial or temporal patterns and the differences were not considered to be large enough to be of any significance in terms of their influence over the macrobenthic community structure. As was concluded by Allen (2000a), the sediment characteristics are similar at Paull and Saltend and do not vary significantly in terms of time or site.

Allen (2000a) provides a detailed description of the sediment properties of the Paull and Saltend mudflats between 1998 and 2000. The findings were consistent with those of the present study, showing the sediments to be composed of soft muds containing 97-99% silt and clay (at sites which broadly correspond to those sampled during the present study). Median particle diameter ranged from 6.24 to 7.07 φ (in May 2000), indicating slightly coarser sediment than that recorded in the present study. Small differences such as this may be due to sampling error and method of analysis. Coulter counter analysis was used in the present study and sediment samples were prepared according to the method of Buchanan (1984) with organic matter removal and disaggregation using sodium hexametaphosphate. In contrast, Allen (2000a) did not dissagregate the samples and analysis was carried out using a Malvern Mastersizer. Furthermore, Allen (2000a) demonstrated a general trend of decreasing particle size and increasing silt content between 1998 and 2000, indicating that the sediments in this region are becoming increasingly fine. C. Nikitik (University of Hull, pers. comm.) recorded median φ values of 3.62 - 5.87 (81 and 17 μm, respectively) with the silt / clay content being in the range of 75-95% in 1997 which, compared with the data in the present study and that presented by Allen (2000a), also suggests that the sediments in this area are becoming finer. Allen (2000a) attributed this change in the sediment characteristics to construction works at the adjacent ABP (Associated British Ports) dock and their effect on the hydrodynamic regime.

Water content was high at all sites (generally greater than 55% but greater than 70% at some sites) with the highest and lowest values being recorded during the spring and summer months, respectively, for the S200 and S75 m sites. Well defined temporal patterns were not observed at the S25 m or Paull sites and contrary to expectation, although spatial differences in water content were observed, there was no trend of decreasing water content with increasing shore height. The exception to this was in May when the water content of the high shore sites was slightly lower than that of the lower shore sites, a difference which increased with increasing

depth. Wet bulk density of the sediment ranged from 1.56 - 1.73 g cm^{-3} and whilst statistical differences were found between sites and months, there were no consistent patterns. Widdows *et al.* (1998a) monitored the physical properties of the sediments at Skeffling and, as in the present study, found no apparent high to low shore trends.

Levels of organic carbon were moderate to high, ranging from 6.01% to 13.8% with the highest values generally being recorded from the Saltend sites. High organic content is thought to be related to inputs from the coal store in the adjacent ABP dock area. The May 2000 data indicated a reduction in organic content with increasing distance from the discharge. However, whilst statistical differences between sites were found throughout the year, there was no consistent pattern of decreasing organic content with increasing distance from the discharge. Organic matter was found to decrease with increasing depth at all sites with higher levels of organic matter being found at greater depths at the S25 ms and S75 m sites. Possible explanations for this include differences in the rate of primary production (e.g. microalgae or bacteria), differences in the rate of organic matter input and degradation and differences in bioturbation by the infaunal communities at each site (Dauwe et *al.*, 1998).

Allen (2000a) reported low to moderate organic carbon levels of 5.47 - 6.01% at sites which broadly correspond to those sampled during the present study. Organic content was found to be relatively uniform over the entire area. C. Nikitik (University of Hull. Pers. comm.) found organic content to be in the range of 5-6% and also found no differences between sites. It should be noted that the organic carbon levels recorded in the present study were considerably higher than those recorded by Allen (2000a) or C. Nikitik (University of Hull. Pers. comm.).

With the exception of the P25 m site, a general trend of sediment accretion was observed at Saltend and Paull between May and September 2000. Sediment deposition was highest at the S200 m site (7.4 cm) and generally decreased with increasing shore height, a trend which was also demonstrated during the short term study. Read *et al.* (2000) monitored topographic changes at Saltend and Paull between July 1998 and July 2000, in order to determine the effects of the construction of a waste water treatment plant on the sediment properties and mudflat topography. The mudflat profiles were described as 'typical' for a middle estuarine area with a gently sloping upper shore and steeply sloping lower shore. The profile was said to reflect the position of the mudflat, on the outside of a bend, subject to long term deposition but undergoing cycles of erosion and deposition according to changes in current velocity. The findings of the present study are consistent with those of Read *et al.* (2000) who reported a mean increase in shore height (averaged over the entire mudflat) of 14.4 cm at Saltend (in the region of the present study) in comparison to 8.3 cm at Paull between 1999 and 2000.

Sediment deposition at Paull ranged from -1 cm to 15 cm with the lowest values (indicating slight erosion) being associated with the upper shore sites. This is, again, consistent with the findings of the present study. Accretion at the Saltend mudflat was attributed to land claim by ABP (Associated British Ports) and extension of the adjacent ABP dock and the amount of sediment deposited was found to decrease with increasing distance (i.e. towards the Paull mudflat) from the construction site. Any effect of the construction of the waste water treatment works was masked by the effects of construction works on the ABP site (Read *et al.*, 2000).

2.5.2. Sediment contamination.

The most obvious, constant, difference between sites in terms of the sediment properties was redox potential with the sediments in the vicinity of the discharge being consistently more anoxic than sediments further away. Reducing conditions are generally associated with high organic content and the associated high oxygen demand (Perkins, 1957; Rhoads, 1974; Pearson & Stanley, 1979) and patterns of Eh would therefore be expected to follow similar patterns to organic carbon distribution. This was not observed in the present study or in that of C. Nikitik (University of Hull. Pers. comm.). However, the effluent discharged from BP Chemicals (Saltend) Ltd. contains several volatile organic compounds which may increase the oxygen demand of the water (at high tide) or come into contact with the sediment, before becoming volatilised, and influence the redox conditions without leaving a residue. It is also possible that volatilisation of these compounds could occur during oven drying of the samples. Seasonal variation in Eh was also noted with more negative values being recorded during the summer although it should be noted that it is often difficult to detect anthropogenic influences against high natural background levels.

Sediment metal concentrations were analysed quarterly by C. Nikitik (University of Hull. Pers. comm.) between May 1997 and May 1998 and are summarised in Figure 2.19. Concentrations of all metals decreased progressively with increasing distance from the outfall although these differences were only statistically significant for copper, zinc and iron. However, Scott (1996) found a high degree of variability (both between sites and between replicates) in sediment metal concentrations at Saltend and Paull and concluded that metal concentrations were primarily controlled by bioturbation and diagenetic processes, leading to adsorption or desorption and subsequent remobilisation, following their deposition. No direct link could be made between metal concentrations found in the sediments with those in the effluent. This was thought to be due to the frequent cycle of erosion and deposition of the surface layers and the fact that metals deposited at Saltend and Paull may be from other sources within the estuary.

Scott (1996) summarised sediment metal concentration data for the Humber estuary, from various sources, and noted an overall pattern of decline between 1974 and 1995. Nedwell (1997) reported copper concentrations of 18 - 103 μg g^{-1} and zinc concentrations of 180 - 384 μg g^{-1} in sediments from Paull in 1994. These concentrations are considerably lower than those recorded by C. Nikitik (University of Hull. Pers. comm.), despite the different digestion techniques used (nitric acid vs aqua regia), suggesting that metal concentrations are continuing to decline. Concentrations of lead and chromium reported by Nedwell (1997) were also higher than those recorded by C. Nikitik (University of Hull. Pers. comm.). However, given the variability in the metal concentration data, as highlighted by Scott (1996), assumptions about the long term temporal trends in the level of metal contamination in the area should not be made from such a limited data set.

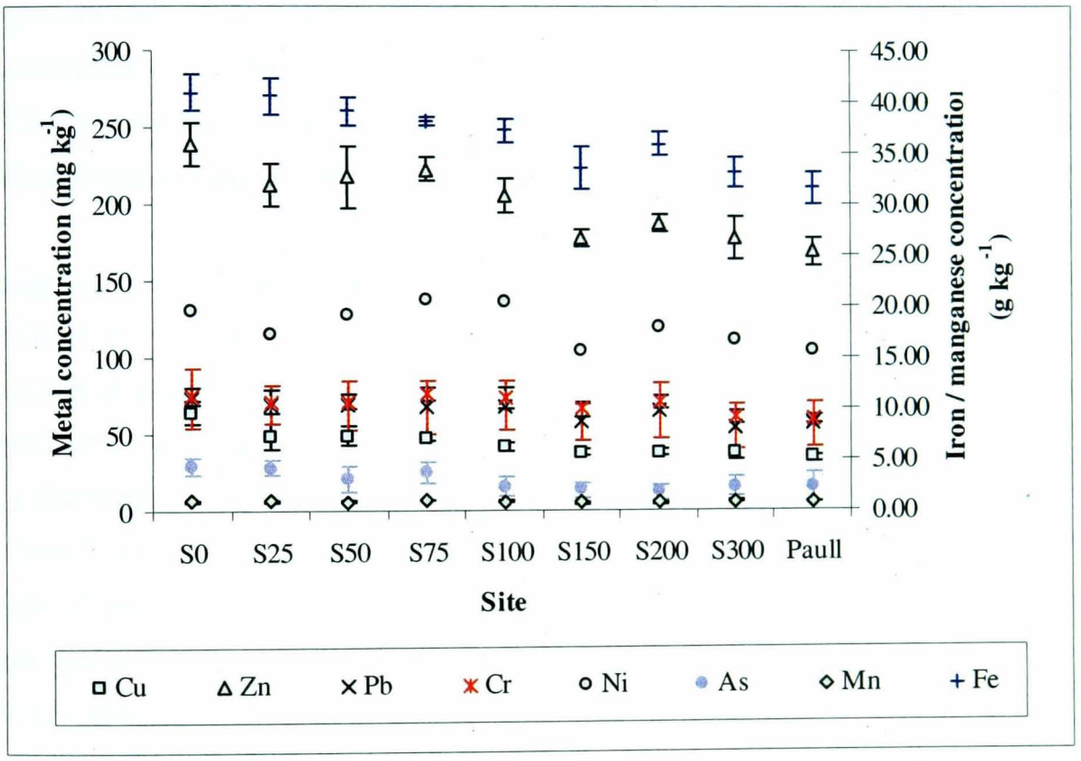


Figure 2.19. Trends in mean sediment metal concentration for samples analysed between May 1997 and May 1998 (C. Nikitik. University of Hull. Pers. comm.). (SE omitted from Ni data for clarity).

2.5.3. Microalgal communities.

2.5.3.1. Microalgal biomass.

Microalgal biomass (expressed as Chlorophyll-a concentration) was higher at the S25 m and S75 m than at the S200 m and P150 m sites throughout the year. These results suggest that the microalgal communities are enhanced as a result of exposure to the effluent and / or the change in the sediment conditions caused by the discharge of the effluent. Chlorophyll-a concentration increased during the summer months at all sites although this increase was most

pronounced at the S25 m and S75 m sites. Chlorophyll-a concentrations ranged from 5 $\mu g\ g^{-1}$ (January, P150 m) to 96 $\mu g\ g^{-1}$ (April, S25 m). These concentrations are similar to those recorded by a number of authors working with estuarine sediments (e.g., Underwood & Paterson, 1993a; 1993b; Underwood *et al.*, 1995; Underwood & Smith, 1998; Kornman & de Dekere, 1998; de Winder *et al.*, 1999; de Brouwer *et al.*, 2000; de Dekere, 2003).

Diatom populations are controlled by salinity, light, temperature (McIntire, 1978; Asmus & Bauerfeind, 1994), sediment characteristics, hydrodynamic conditions and the availability of inorganic nutrients (Asmus & Bauerfeind, 1994). Various studies on epipelic (sediment dwelling) diatoms have shown an increase in diatom biomass during the warmer months (Colijn & Dijkema, 1981; Admiraal & Peletier, 1980; Admiraal *et. al.*, 1982; Underwood & Paterson, 1993a; 1993b; Asmus & Bauerfiend, 1994) with biomass being positively correlated with temperature (Underwood & Paterson, 1993a). de Brouwer *et al.* (2000) and de Dekere (2003) also reported increased algal biomass during the summer months, resulting from higher temperatures and greater light availability.

Admiraal & Peletier (1980) examined the spatial and temporal distribution of diatom species in relation to environmental stress including salinity, sulphide and ammonia. A clear succession of species was observed throughout the year with species dominance being related to temperature and irradiance with species tolerant of high temperatures (e.g. *Nitzschia* spp.) being dominant during the summer months. However, successional changes in species were also found to be highly related to changes in pollution levels and freshwater flow. Clear patterns of species distribution in relation to pollution were demonstrated with the biomass of certain species increasing as a result of exposure to organic pollution and the associated high levels of ammonia and sulphide. Admiraal (1984, in Asmus & Bauerfeind, 1994) stated that many benthic diatom species are highly tolerant of hydrogen sulphide and Admiraal & Peletier (1980) found ammonia to enhance the growth of *Navicula salinarum*. This was consistent with the findings of van Raalte *et al.* (1979, in Admiraal & Peletier, 1980) who reported the growth of this species to be enhanced on artificially fertilised plots of saltmarsh. Since the effluent discharged from BP Chemicals (Saltend) Ltd. contains ammonia and appreciable concentrations of nitrate and phosphate, it is not unexpected that the microalgal communities appear to be enhanced within the immediate vicinity of the outfall.

Organic matter on polluted mudflats may promote the growth of *Navicula salinarum, N. cryptocephala* and *Nitzschia thermalis* which are all capable of heterotrophic growth in dark conditions (Admiraal & Peletier, 1980). Hellebust & Lewin (1977, in Admiraal & Peletier, 1980) proposed that the ability to utilise organic matter may be widespread among the

pennate diatoms. Peletier (1996) studied changes in diatom communities (species composition and total biomass) following a reduction in the discharge of organic waste from the potato flour and cardboard industries (Ems-Dollard estuary). Under conditions of high discharge, the microalgal community was dominated by the sulphide and ammonium tolerant species *Navicula salinarum* and *N. pygmaea* and seasonal changes in abundance (i.e. spring and autumn blooms) were not recorded. Following a reduction in organic input, a decline in microalgal biomass was coupled with changes in species composition with the community being dominated by species less tolerant of sulphide and ammonium (e.g. *Navicula phyllepta* and *Navicula flanatica*). In addition, a seasonal cycle of high spring and autumn biomass and low winter biomass was also observed. This was, in part, attributed to the reduction in the organic content of the sediment but also to the increase in the density of *Corophium volutator* and *Hediste diversicolor* which both feed on diatoms.

Admiraal & Peletier (1980) found the presence of sulphides to promote the growth of diatoms although details of the species were not given. van den Hock *et al.* (1979, in Admiraal & Peletier, 1980) found diatom populations on an organically polluted mudflat to be among the highest in the Ems-Dollard estuary.

2.5.3.2. Sediment carbohydrate concentration.

Sediment carbohydrate concentrations (in terms of total and colloidal carbohydrate) followed a similar trend to that of chlorophyll with concentration decreasing with increasing distance from the outfall and the highest concentrations being recorded during the summer. High concentrations of carbohydrate and high microalgal biomass at the S25 m and S75 m sites could, in part, explain why the organic content of the sediment at these sites was generally higher than at the other sites during the warmer months. Sediment carbohydrate concentrations were lowest at the P150 m site in January and highest at the S25 m site in April, ranging from 416- 1919 $\mu g\ g^{-1}$ (total), 234 - 1447 $\mu g\ g^{-1}$ (colloidal EDTA) and 53-571 $\mu g\ g^{-1}$ (colloidal S). These concentrations are within the same rage as those reported by several other authors (e.g., Underwood *et al.*, 1995; Underwood & Smith, 1998; Kornman & de Dekere, 1998; de Winder *et al.*, 1999; de Brouwer *et al.*, 2000; de Dekere, 2003).

The phenol-sulphuric acid assay (Dubois *et al.*, 1956) can be used to detect a wide range of carbohydrates including sugars, methylated sugars and neutral and acidic polysaccharides which comprise most of the components of microbial EPS (extracelluar polymeric substances) (Decho, 1990). EPS comprises the high molecular weight mucous secretions (thought to be of relevance in terms of sediment stabilisation) surrounding cells and is largely composed of polysaccharides (Hoagland *et al.*, 1993; Decho, 1990; Decho, 2000). The phenol-sulphuric

acid assay is a widely accepted technique and has been used by a number of authors (e.g. Underwood & Paterson, 1993a; 1993b; Underwood & Smith, 1998; de Brouwer *et al.*, 2000; Galois *et al.*, 2000; Paterson *et al.*, 2000; deDekere, 2003). However there are a number of factors which can cause variation in the amount of carbohydrate and EPS detected. These sources of variation were reviewed by Underwood *et al.* (1995) who made a number of recommendations regarding sample size, sample preservation techniques and storage conditions, extraction media (for the determination of EPS) and extraction times.

In the present study, high concentrations of total carbohydrate extracted from the sediments often resulted in densely coloured solutions, despite the small sample size (10-30 mg, as recommended by Underwood *et al.*, 1995), which required dilution to obtain absorbance readings within the linear range of the calibration curve. Underwood *et al.* (1995) also recommended that samples to be used for total carbohydrate analysis should be frozen and stored at -70°C. In contrast, freeze drying was recommended for samples to be used for EPS extraction. Since the extracelluar material was considered to be of greater importance, samples were freeze dried during the present study. This together with the fact that intense colour development (due to the high concentration of total carbohydrate) meant that dilution of the reagents following colour development was necessary, could explain why the variability in the total carbohydrate data was greater than that for the concentration of colloidal carbohydrate (S and EDTA). Other factors responsible for the variability included the reagents and equipment used, the heterogeneous nature of the mud and that associated with sub-sampling. The data presented in Figure 2.14 were derived from different sites at different times of the year and this could also have increased the variability. Furthermore, total carbohydrate analysis not only detects the intra and extracelluar, material of microalgae and bacteria, it would also detect the intracellular carbohydrate present in meiofauna in the sample. Underwood *et al.* (1995) also found the carbohydrate and chlorophyll-a content of sediments to vary according to the organisms present (e.g. diatoms, cyanobacteria, filamentous bacteria). The microbial community was not characterised but it is possible that spatial and temporal variations in the community could have contributed to the variability within the data set.

Despite this, there were statistically significant correlations between total carbohydrate and chlorophyll-a. Since temporal variation in chlorophyll-a concentration was found, it is not surprising that carbohydrate concentration also varied according to season. This relationship between chlorophyll-a and sediment carbohydrate concentration, together with seasonal variation in carbohydrate concentration, has also been reported in various studies (e.g.

Underwood & Paterson, 1993a; Underwood *et al.*, 1995; de Brouwer *et al.*, 2000; de Dekere,2003).

Extraction of colloidal carbohydrate using EDTA can increase the carbohydrate yield over that extracted using saline since it can release carbohydrate bound to metal ions. It can, however, also cause leakage of intracellular material (Underwood *et al.*, 1995). If intracelluar contamination were a problem, colloidal EDTA concentrations would show a greater correlation with total carbohydrate than with the colloidal (S) concentrations (Underwood *et al.*, 1995). These authors found correlation coefficients of 0.4 ($p<0.01$) between total carbohydrate concentration and colloidal (EDTA) concentration and of 0.67 ($p<0.01$) between chlorophyll-a / colloidal (S) carbohydrate and colloidal (EDTA) concentration. They therefore concluded that the leakage of intracellular material had not occurred. In the case of the present study, the coefficient of variation (r^2) for total carbohydrate concentration and colloidal (EDTA) was 0.52 ($p<0.01$) which is lower than that between colloidal (EDTA) carbohydrate and colloidal (S) carbohydrate (0.65, $p<0.01$). It is therefore assumed that intracelluar contamination of colloidal material was not a problem in the present study. However, it should be noted that Underwood *et al.* (1995) also found high percentages of uronic acids in EDTA extracts and, since uronic acids are only present in extracelluar material, this provided further indication that the carbohydrate present in the EDTA extracts was of extracellular origin. No such measurements were made during the present study.

The reasons for the unsuccessful measurement of the EPS fraction of the colloidal extracts are not clear. Whilst the EPS fraction is considered to be of importance in terms of its effect on sediment stability (Hoagland *et al.*, 1993; Decho, 1990; Decho, 2000), its composition, and therefore its physical and chemical properties, can be variable according to species composition and physiological state (Hoagland *et al*, 1993; Decho, 2000). EPS may be present in various states ranging from viscous gels to a fully dissolved state and its cohesive properties are thought to be related to the ratio of sugar monomers (rhamnose and fucose) present (Zhou *et al.*, 1998, in Decho, 2000). Since the cohesive properties of EPS are dependent on so many factors, the presence of diatoms and EPS does not always lead to increased sediment stability (Decho, 2000). Therefore, had EPS been successfully measured in the present study, inferences about its relationship with the sediment properties could not have been confidently made without further characterisation of the EPS. Therefore, the measurement of the extracellular, colloidal, material was considered to be sufficient. Underwood *et al* (1995) found the percentage of EPS to range from 22-25 in the colloidal (S) fraction and from 19-38% in the colloidal (EDTA) fraction and van Duyl *et al.* (2000) found

the percentage of EPS to range from 38-53% (EDTA fraction) in samples taken from diatom mats.

2.6. SUMMARY AND CONCLUSIONS

- The sediments at all sites can be classed as fine-very fine silts with median φ values being in the range of 6.8-7.9 and the silt/clay fraction exceeding 97% in all cases. No statistically significant spatial or temporal differences were found.

- Water and organic content and bulk density were variable at all sites but showed no consistent spatial or temporal trends. No high-low shore increase in water content was found and whilst bulk density was found to be higher at the Paull site than at any of the other sites on some occasions, the differences were only slight. During the summer months, organic content was highest at the S25 m and S75 m site and was possibly related to increased microalgal biomass and microbial populations caused by high summer temperatures, together with greater exposure to nutrients (nitrate and phosphate) in the effluent.

- Eh values decreased with depth with the greatest degree of anoxia being associated with the S25 m and S75 m sites. Eh showed a seasonal pattern at all sites with the lowest values occurring during the summer. Sediment metal concentration data (measured by C. Nikitik. University of Hull. Pers. comm.) suggest that levels of copper, zinc and iron may be elevated at sites close to the discharge.

- Microalgal biomass and carbohydrate concentrations (in terms of total and colloidal carbohydrate) were consistently higher at the S25 m and S75 m sites with the highest concentrations being recorded during summer.

- Sedimentation rates were highest at the S200 m sites and showed a trend of decreasing accretion with increasing shore height. This is consistent with the findings of Read *et al.* (2000).

- Eh, microalgal biomass and sediment carbohydrate concentration were considered to be the most important factors in determining consistent differences between sites in relation to the discharge. For the majority of the year, the S25 m and S75 m sites were broadly similar in terms of their sediment characteristics, as were the S200 m and P150 m sites. However, in January and February, the sediment characteristics of the S75 m site become more similar to those at the S200 m and P150 m sites and during the summer (June, July and September), the sediment characteristics of the

S200 m site become more similar to those at the S75 m site. This suggests that season (principally temperature) has some influence over the effect of the discharge on the sediment properties.

CHAPTER 3

EFFLUENT AND SEDIMENT TOXICITY ASSESSMENT

3.1. INTRODUCTION

In the past, the effects of industrial discharges on the quality of the receiving body of water have been monitored and controlled by means of discharge consents based on the chemical and physical constituents of the discharge, perhaps with some knowledge of the biological impact (Williams *et al.*, 1993). However, according to Cairns & Pratt (1989), the ability to detect a compound does not ensure that biological effects can be predicted and failure to detect a compound does not preclude its effects. Both biological and chemical information are therefore required in order to effectively assess the level risk to biological systems. The use of bioassays is considered to be superior to chemical analysis since they have the ability to measure directly the levels of stress experienced by test organisms exposed to various contaminants and may indicate the presence of a substance which is undetectable by chemical analysis (Crane & Maltby, 1991).

Whilst toxicity testing in the past has focused on testing of the individual components of an effluent, it is now widely accepted that whole effluent or 'Direct Toxicity Assessment' (DTA) is a more appropriate way of assessing the potential effects of the discharge of industrial effluents (Doi, 1980; Williams *et al,* 1993). Pollutants in the environment do not occur in isolation and under natural conditions, and/or where complex effluents are discharged, organisms are usually exposed to a wide variety of substances which may have interactive properties. It is generally assumed that the combined toxicities of the various components of a solution will be approximately additive (Walker *et al.,* 1996). However, the presence of a particular substance may also reduce the overall toxicity of a combination of chemicals. This, antagonistic effect, described by Walker *et al,* (1996), may be the result of substance A causing the induction of detoxifying enzymes which act on substance B. Detoxification of lipophilic (fat soluble) compounds occurs in two stages. In the first stage, monooxygenase enzymes are involved in the oxidation, hydrolysis and hydration of the xenobiotic, producing hydroxyl containing metabolites (biotransformation). In stage 2, these metabolites form conjugates with endogenous molecules which are water soluble and readily excretable. One substance may also neutralise the acidic or alkaline toxic effects of another. However, the induction of these enzymes may lead to the production of active metabolites and the presence of a particular compound may increase the toxicity of a combination of substances. This 'synergistic' effect may be the result of compound A inducing an enzyme which increases the rate of activation of

compound B, thus causing a potentiation in toxicity. It may also be the result of one compound interrupting the detoxification mechanism of a second compound. An example of this synergistic effect is given by Jones (1939, in Hellawell, 1986) where the occurrence of nickel and chromium in combination was found to cause a ten fold increase in the toxicity of nickel.

Other factors influencing the toxicity of a substance, or combination of substances, include pH, salinity, temperature and dissolved oxygen and, during a laboratory test, these factors should be controlled and monitored in order to remove their influence over the toxicity of the substance. However, it should be noted that these controlled test conditions will differ considerably to those prevailing in the environment where the nature of a chemical can be mediated by the physical and chemical conditions. This might lead to variations in the bioavailability and toxicity of pollutants. For example, an increase in pH may favour the adsorption of toxins such as metals, onto particulate matter, therefore reducing concentrations in the water column (Knezovich, 1994). In addition, for marine organisms, there is generally an inverse relationship between salinity and toxicity although this is thought to be due to the physiological effects of low salinity rather than changes in toxin availability (Knezovich, 1994). The characteristics of the test solution will also be subject to change over time with degradation of the components of the solution, evaporation, photo-oxidation, excretion (e.g. ammonia) and absorption of various components of the solution by the test organism (Abel, 1989). It is therefore important that the test solution is frequently changed. This can be achieved by setting up a flow through test with constant renewal of the test solution or by means of a static test with renewal at set time intervals. For these reasons, the test conditions should reflect those prevailing in the environment as far as possible without jeopardising the accuracy of the test (Cairns & Pratt, 1989). This is particularly important for an effluent such as that being discharged from BP, which degrades and loses toxicity rapidly as a result of photo-oxidation and volatilisation of organic compounds (Fitton, 1995), and which is continually discharged (i.e. constant renewal).

Life stage, sex, ecology, nutritional and reproductive status and the general health of an organism also affects toxicity. For example, Naylor *et al.* (1990) found brooding females of the freshwater amphipod, *Gammarus pulex,* to be markedly more tolerant to zinc than males of the same age group, an observation which was attributed to the fact that females do not moult. The juveniles were identified as the most sensitive group. Finally, there are inter and intra-specific variations in toxicity where a substance which is highly toxic to one species may be relatively harmless to another. It is also common for an organism in a polluted environment to develop increased tolerance to the contaminants to which it is exposed thus making it less susceptible than members of the same species in a cleaner environment (Naylor *et al.*, 1990; Nedwell, 1997).

Toxicity testing can generally be divided into two categories. Acute tests are of short duration, typically 24-96 hours whereas chronic tests are generally conducted over longer time periods (Abel, 1989). Both may be used to assess the lethal and sub-lethal effects of a toxicant. Lethal toxicity is defined as the median lethal concentration or time at which 50% of the test population die (LC_{50} or LT_{50}) and sub-lethal toxicity defined as the median effective concentration at which 50% of the test population show a defined response. Traditionally, the majority of toxicity testing has been laboratory based, involving single species, acute lethal tests. Whilst these tests are of value, the concentrations of pollutants in the environment are often much lower than those which would cause mortality but which may have far reaching ecological consequences. For this reason, there is a growing interest in the use of sub-lethal toxicity tests which provide an early warning of effects so that action can be taken to prevent more serious, lethal effects. Examples of sub-lethal responses to stress include changes in an organisms biochemistry, genetics, physiology, morphology, and behaviour which may jeopardise the chances of survival, growth and reproduction of an individual and its population and in turn, impact upon the status of predatory populations.

Behavioural changes in organisms are regarded as the final integrated result of a diversity of biochemical and physiological changes and a single behavioural parameter is generally more comprehensive than a biochemical or physiological parameter (Walker *et al.*, 1996). Kittredge (1980, in Forbes & Forbes, 1994) described four classes of behavioural response to pollution. *Appetitive* and *operant* responses were considered to be the most damaging, including changes in feeding and mating behaviour and damage to the sensory organs, respectively. *Defensive* responses include adaptation to tolerate certain levels of stress and finally, organisms may display *detective* behaviour, for example, leading to avoidance of polluted conditions.

3.2. AIMS

The lethal and sublethal toxicity of the BP effluent has been studied by various authors using various invertebrate (Fitton, 1995; Henry, 1996; O'Brien, 1997; Baynes, 2000; Nikitik, in prep) and algal (Osman, 1999) species and it was therefore not considered necessary to carry out a detailed study of the effluent's toxicity. The primary aim of the present chapter was to provide a broad indication of the lethal effects the effluent, based on timescale and concentration ranges used in previous studies, and of the changes in the lethal toxicity of the sediment taken from sites at various distances from the outfall. In addition, in March 2003 BP Chemicals (Saltend) Ltd. proposed to change the nature of the effluent currently discharged to Old Fleet Drain by diverting effluents from plants DF2, DF3 and A5 (acetic acid production) to the Yorkshire Water Treatment Works. These process effluents were then be discharged via a subtidal outfall, following treatment, with the Yorkshire Water effluent. In addition to effluents from the DF2, DF3 and A5 plants, a component of the diverted effluent would also include shutdown effluents from the ethyl acetate (EtAc), vinyl acetate monomer (VAM) and A4 (acetic acid) plants. Contaminants in this effluent predominantly include acetic acid, formic and propionic acid together with some esters, ketones and alcohols. The removal of these effluents would result in changes to the composition (in terms of the concentrations of its components) of the effluent and the volume that is currently discharged into Old Fleet Drain (A. Kirton, BP Saltend pers. com.). This would result in a substantial decrease in the concentration of zinc, chromium, nickel, manganese, copper and organic compounds.

Whilst it was anticipated that the overall toxicity of the effluent would be reduced, the conservation designations imposed upon the Humber estuary as a whole and on the Saltend mudflat in particular dictate that work must be undertaken to ensure that any change to the effluent characteristics will not adversely affect the interest features of the area. BP Chemicals (Saltend) Ltd. Therefore requested that the Institute of Estuarine and Coastal Studies (University of Hull) provide a comparison of the toxicities of the current and proposed effluents.

The present study therefore has the following primary objectives:

- Determination of the lethal (LC_{50} and LT_{50}) of the effluent to provide an appropriate range of concentrations with which further, sub-lethal, tests can be carried out (Chapter 5). *Hediste diversicolor, Corophium volutator* and *Macoma balthica* were used due to their local abundance, ecological importance, the ease with which they could be collected and maintained in the laboratory and to give an indication of the inter-specific effects of the effluent.

- Determination of the lethal effect (LT_{50}) of sediments from different distances from the outfall, using the above species.

- This information will then be used to explain both the changes in benthic community structure with increasing distance from the discharge (Chapter 4) and the changes in bioturbation potential by various individual species and community types (Chapter5).

The secondary objectives were as follows:

- Comparison of the acute lethal (LC_{50} / LT_{50}) and sub-lethal (EC_{50} / ET_{50}) toxicities of the current and future effluents in the form in which they are discharged into Old Fleet Drain.

- To use local, ecologically important species (*H. diversicolor* and *C. volutator*) in toxicity testing using an initial 'range finding' test followed by a more refined 'definitive' test. The range finding test will involve the use of a wide range of effluent concentrations (0-100%) and will allow the identification of the broad range of concentrations which produce a lethal effect over a given time period.

- Definitive toxicity testing where the concentrations used will be narrowed down to those which are in the region of LC_{50} / LT_{50} values calculated during the range finding test in order to give a more precise value of toxicity. This will be carried out both with and without salinity correction in order to determine the effects of the freshwater component of the effluent on its toxicity.

3.3. METHODS

3.3.1. Animal collection.

H. diversicolor and *C. volutator* were collected from the upper shore area of the Paull mudflat. *H. diversicolor* were removed from the mud by simply digging by hand, to a depth of approximately 15 cm, and the worms were individually picked out. *C. volutator* is most commonly found in high numbers small areas of standing water, where the animals can usually be seen swimming (Watkin, 1941). The surface layers (top 5-10 cm) of mud were swept into a bucket in order to capture both swimming organisms and those in their burrows within the mud. *M. balthica* were collected from the Skeffling mudflat due to their relatively low abundance at Paull. Animals were sieved through a 1 mm sieve, using diluted sea water and maintained at 10°C in aerated sea water at salinity of 20 (a salinity characteristic of the area) until testing.

3.3.2. Effluent toxicity testing

Effluent toxicity testing was carried out in May 1998. Raw effluent was diluted using a stock solution of diluted sea water (20) to concentrations of 0, 4, 8, 16, 32 and 64% and solutions of 200 ml were used in 500 ml glass beakers containing 2 cm depth of clean, defaunated sediment. Test solutions were changed every three days over a period of 32 days. This was to allow for any chemical changes to the effluent which may have occurred as a result of degradation or the presence of the test organisms (e.g. absorption of certain components of the effluent, ammonia excretion etc). No correction was made for changes in salinity or pH as a result of the effluent concentration since dilution upon discharge to seawater would automatically change the pH and salinity of the effluent. Furthermore, the low salinity and pH of the effluent were considered to be important factors which may influence the toxicity of the effluent.

Three replicate solutions for each concentration, for each species were set up with each replicate containing 10 animals. The animals chosen were all of a similar size and in the case of *H. diversicolor*, individuals in spawning condition (i.e. those which had turned green and become uncharacteristically delicate) were rejected. In the case of *C. volutator*, starvation induces moulting which would have increased the likelihood of all animals being of equal sensitivity. When added to the test solutions, any animals which did not immediately become active (swimming) were removed and replaced. The number of mortalities was recorded and the dead animals removed after 2, 4, 8, 16 and 32 days. In the case of *H. diversicolor*, dead animals were classed as those which had lost their colour and showed no reaction to stimulation. *C. volutator* was classed as dead if there was no reaction to stimulation and if, when turned on their backs, beating of the pleopods did not commence upon disturbance. *M. balthica* were classed as dead if the shells were slightly open, revealing the decaying animal, or, if when

placed in clean seawater (20), there was no movement or reaction from the animal (e.g. shell opening or protrusion of the siphons). The results of the test were considered to be valid if the mortality in the control solutions was less that 20% (Unpublished protocol, Clyde River Purification Board). pH and salinity were recorded every three days, before and after changing the test solutions.

3.3.3. Sediment toxicity testing

Eighteen replicate sediment cores were collected from the S0 m, S25 m, S75 m, S200 m and P 150 m sites to give a total of 90 cores. Sediment cores were collected to a depth of 12 cm using plastic cores 15 cm deep with an internal diameter of 10 cm. The bottoms were capped with plastic discs and parafilm and the cores transported back to the laboratory in plastic trays. In order to maintain the structure of the sediment, defaunation was carried out by pouring boiling water over the sediment surface. This process was repeated ten times over the period of one day, was found be effective in killing the organisms present and did not alter the properties of the sediment. Initially, freezing of the cores at -20°C (for 48 hours) was carried out but several of the organisms were able to survive this and, due to its cohesive nature, the freezing process caused the sediment to crack and form plate-like structures due to expansion of the interstitial water.

The defaunated sediment cores were placed in plastic tanks according to the site from which they were taken, covered with clean seawater (20) and maintained at 10°C for 32 days. The water in each tank was aerated with one air stone, recirculated using a small aquarium pump and changed every 36 hours. Ten individuals of *H. diversicolor, C. volutator* and *M. balthica* were added to each core and the cores covered with 500 µm mesh lids to prevent the animals from escaping. Any animals which had not burrowed into the sediment after 1 hour were removed and replaced with healthy animals. Three cores were removed from each tank after 2, 4, 8, 16 and 32 days, passed through a 500 µm sieve and the number of remaining live animals from each species recorded.

75 of the 90 cores were used for toxicity testing with the remaining 15 cores being used for redox measurements (at a depth of 4 cm) at the beginning of the experiment. Redox measurements were taken from the cores containing the animals immediately before sieving.

3.3.4. Comparison of the toxicity of the current and proposed effluents (BP Chemicals (Saltend) Ltd.).

For each species, a range finding test was used to identify the broad range of concentrations which produced an effect over a given time period (in terms of mortality). Following this, a definitive test was carried out in order to give a refined value of toxicity. Both tests were carried out over a 96 hour period with the number of mortalities being recorded every 24 hours. This allowed the calculation of LC_{50} values (median lethal concentration where 50% of the test population are killed) for each time (24, 48, 72 and 96 hours) and LT_{50} (Median lethal time) for each concentration. pH and salinity were monitored throughout all experiments in order to ensure that differences in lethal and sub-lethal effects between replicate test solutions were not due to differences in these two parameters. In addition, the pH of the raw effluent was recorded daily as a means of monitoring degradation of the effluent.

3.3.4.1. Range finding test

5L of each effluent (current and modified) were delivered on 4th April 2003. Concentrations of 0, 20, 40, 60, 80 and 100% were used. Effluent was diluted using a stock solution of diluted sea water (20) and solutions of 100 ml were used in 500 ml glass beakers.

Test solutions were changed twice (i.e. after 36 and 72 hours) during the 96 hour test period. This was to allow for any chemical changes to the effluent which may have occurred as a result of degradation or the presence of the test organisms (e.g. absorption of certain components of the effluent, ammonia excretion etc).

3.3.4.2. Definitive test (with and without salinity correction)

The results of the initial range finding test were used to determine the range of concentrations to be used in the definitive test and the above experiment was repeated. Concentrations (%) used were as follows:

	H. diversicolor	*C. volutator*
Current effluent	0, 75, 80, 85, 90, 95	0, 40, 50, 55, 60, 65
Modified effluent	0, 80, 85, 90, 95, 100	0, 55, 60, 65, 70, 75

20L of each effluent were delivered on May 6th 2003 and tests using *H. diversicolor* were carried out between 6.5.03 and 10.5.03. 100ml solutions (three replicates) of both the current and modified effluents were made up and the salinity corrected to 20 using Instant Ocean artificial sea salt. A further set of solutions were made up without salinity correction.

The above experiment was repeated between 13.5.03 and 17.5.03, using *C. volutator*. However, the results of these tests are not directly comparable to those using *H. diversicolor* since different effluents were used on each occasion. In addition, a problem with the Etac/VAM plant meant that this component of the effluent was missing during testing using *C. volutator*.

3.3.5. Data analysis

Data were analysed using probit regression analysis, using the package SPSS for Windows, v11. This analysis gives median (or any other percentile) time and concentration values, together with 95% confidence limits on those values as an indication of the accuracy of the output. In addition to deriving regression equations from which endpoints (e.g., LC_{50} and LT_{50}, values) can be calculated, probit analysis also gives a chi-square (χ^2) statistic as an indication of goodness of fit. Essentially, this gives an indication of the validity of the regression equations used in the prediction of endpoint values. In general, a low χ^2 statistic, with a high (>0.05) associated p value indicates that the observed response does not deviate significantly from the expected response. Therefore, it can be assumed that accurate calculations of end points can be made using the regression equations. A low (<0.05) value of p would indicate significant deviation from the expected response (e.g. very few responses) and therefore, the calculated endpoints should be treated with caution. In cases where endpoints would occur at concentrations greater than the maximum of those used in the test, the regression equations will be used to predict what the value of a given end point may be. These values should be treated with caution since they are derived through extrapolation of the data. In general, the confidence limits are extremely wide in such cases.

One way ANOVA tests were used to determine statistical differences between LC_{50} and LT_{50} values between species, sites, times and concentrations. Homogeneity of variance was found in all data sets and post hoc testing was therefore carried out using Tukeys' test.

3.4. RESULTS

3.4.1. Effluent toxicity (in the presence of sediment)

In general, animals exposed to clean sea water were more active than those exposed to high effluent concentrations with *Hediste diversicolor* and *Corophium volutator* actively swimming, investigating the substratum and burrowing. At higher concentrations, the animals appeared lethargic and response to stimulation was slow with animals displaying spasmodic behaviour rather than swimming as was noted in the controls. *Macoma balthica* exposed to clean sea water were found to extend their siphons both into the water column and out along the sediment surface, actively feeding or investigating the bottom of the beaker in search of a suitable substratum in which to burrow. In contrast, animals in high effluent concentrations kept their shells closed.

Figure 3.1 shows the way in which toxicity increases over time suggesting increased sensitivity of all three species with increasing exposure time. The 32 day LC_{50} values were 27%, 40% and 50% for *C. volutator, M. balthica* and *H. diversicolor*, respectively, suggesting that *H. diversicolor* is the more tolerant of the three species. One-way ANOVA tests showed significant ($p<0.05$) differences in LC_{50} values between times for all species with low toxicity being detected after 2 and 4 days and higher toxicity being detected after 32 days of exposure. However, whilst the data suggest that *C. volutator* is consistently the most sensitive of the three species and *H. diversicolor* the least sensitive, no statistical differences between species in terms of toxicity were found at any of the exposure times. It should be noted that below 16 days and, in the case of *C. volutator*, below 8 days, the LC_{50} values were greater than the maximum concentration (i.e. 64%) and have been calculated based on extrapolation of the data. Whilst these values should therefore be treated with caution, they are useful in indicating the low toxicity of the effluent to all species tested.

LT_{50} values for 16 and 64% effluent concentration were 29 and 16 days for *C. volutator*; 30 and 25 days for *M. balthica* and 39 and 28 days for *H. diversicolor* (Figure 3.2), again suggesting that *C. volutator* is the most sensitive of the three species. One-way ANOVA tests showed toxicity to increase significantly ($p<0.01$) with increasing effluent concentration for all species and at a concentration of 64%, showed *C. volutator* to be significantly ($p<0.05$) more sensitive than the other two species. However, no statistically significant inter-specific differences were found at any other effluent concentration. Again, particularly in the case of *H. diversicolor*, LT_{50} values exceed the maximum exposure time of 32 days and calculations were based on extrapolation of the data. It is possible that longer exposure times would have revealed greater differences in toxicity between the species.

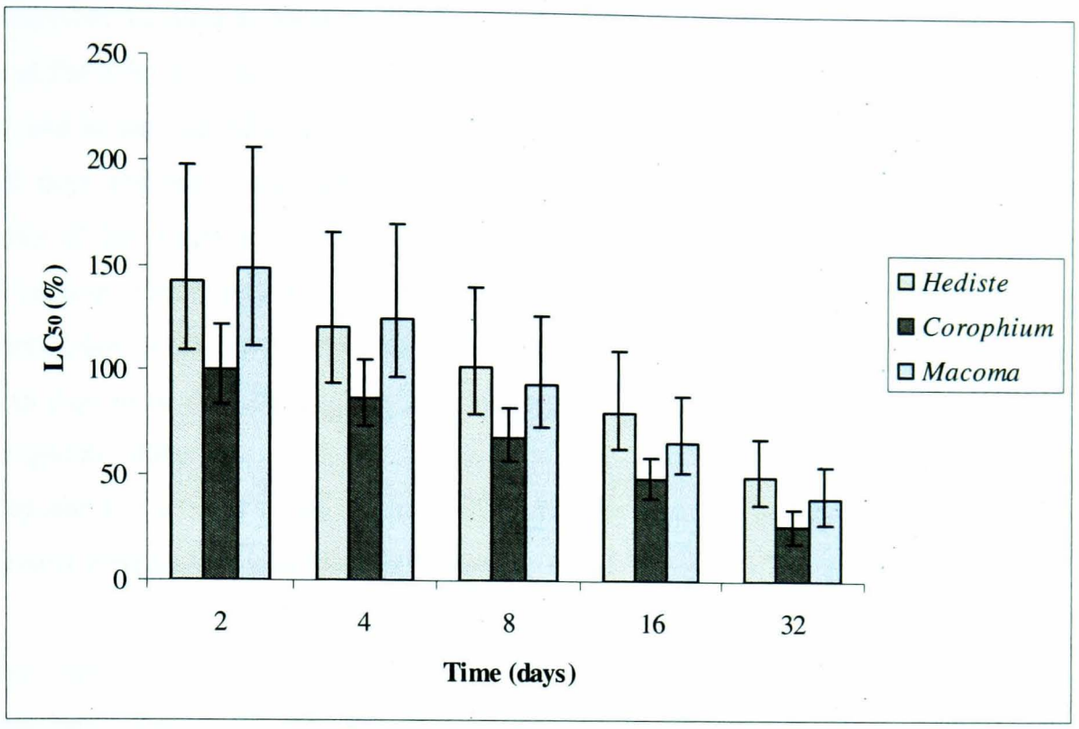


Figure 3.1. LC_{50} (± 95% CL) values of the effluent for *H. diversicolor, C. volutator* and *M. balthica* at different time intervals.

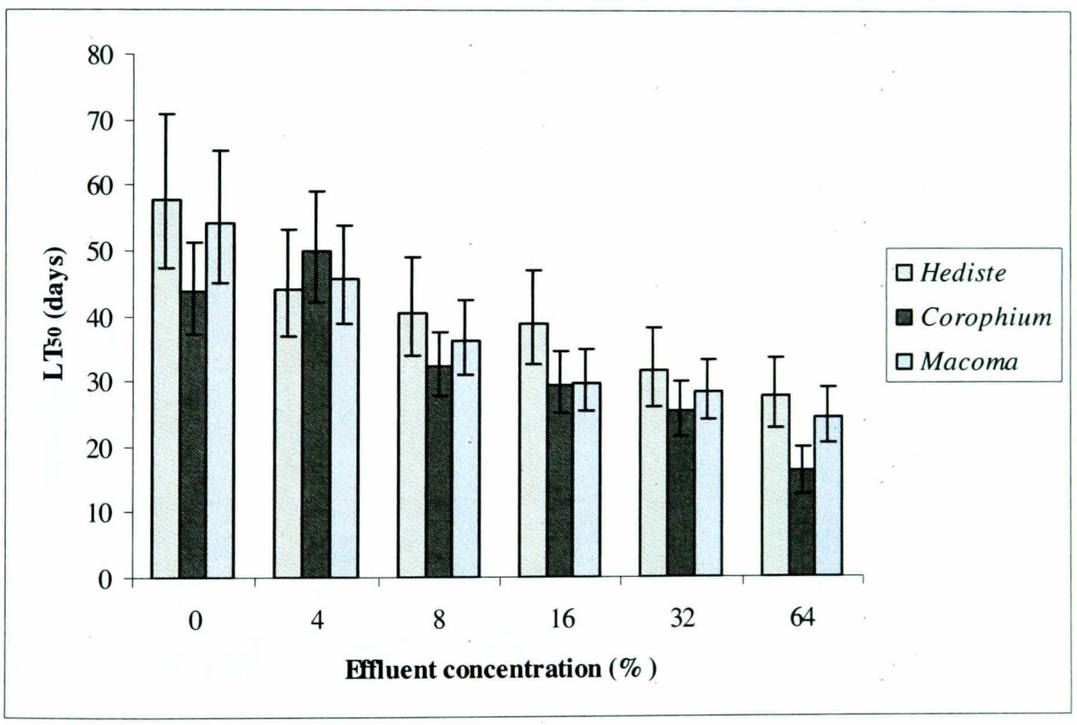


Figure 3.2. LT_{50} (± 95% CL) values of the effluent for *H. diversicolor, C. volutator* and *M. balthica* at each concentration.

3.4.2. Sediment toxicity

The sediment LT_{50} increased with increasing distance from the discharge (Figure 3.3) indicating a reduction in the toxicity of the sediment between the S0 m and Paull sites. LT_{50} values ranged from 27 days at the S0 m site to 79 days at the Paull and S200 m sites for *H.*

diversicolor; 12 days at S0 m to 61 days at Paull for *M. balthica* and 6 days at S0 m to 46 days at Paull for *C. volutator*. However, the LT_{50} values calculated for the Paull and S200 m sites, and in the case of *H. diversicolor*, the S75 m site, exceed the maximum exposure time of 32 days and were calculated based on extrapolated data. Whilst they indicate the low toxicity of the sediment (in terms of lethality) at these sites, these values should be treated with caution. One-way ANOVA tests revealed significant differences ($p<0.05$) in the number of mortalities at each site for all species for all times (2-32 days) and showed the S0 m and S25 m sites to be consistently different to the other sites (in that they had a greater toxicity). Inter-specific differences ($p<0.01$) in lethal response to the sediment were found for the S0 m, S25 m and S75 m sites with *C. volutator* consistently being the most sensitive species and *H. diversicolor* being the most tolerant species.

Redox potential at a depth of 4 cm was found to increase over time (although not significantly) and was consistently higher (i.e. less anoxic) at the Paull and S200 m sites than at the S0 m, S25 m and S75 m sites (Figure 3.4). Two-way ANOVA tests showed significant differences between sediments ($p<0.01$) but no significant change in Eh over time and no interaction between site and time.

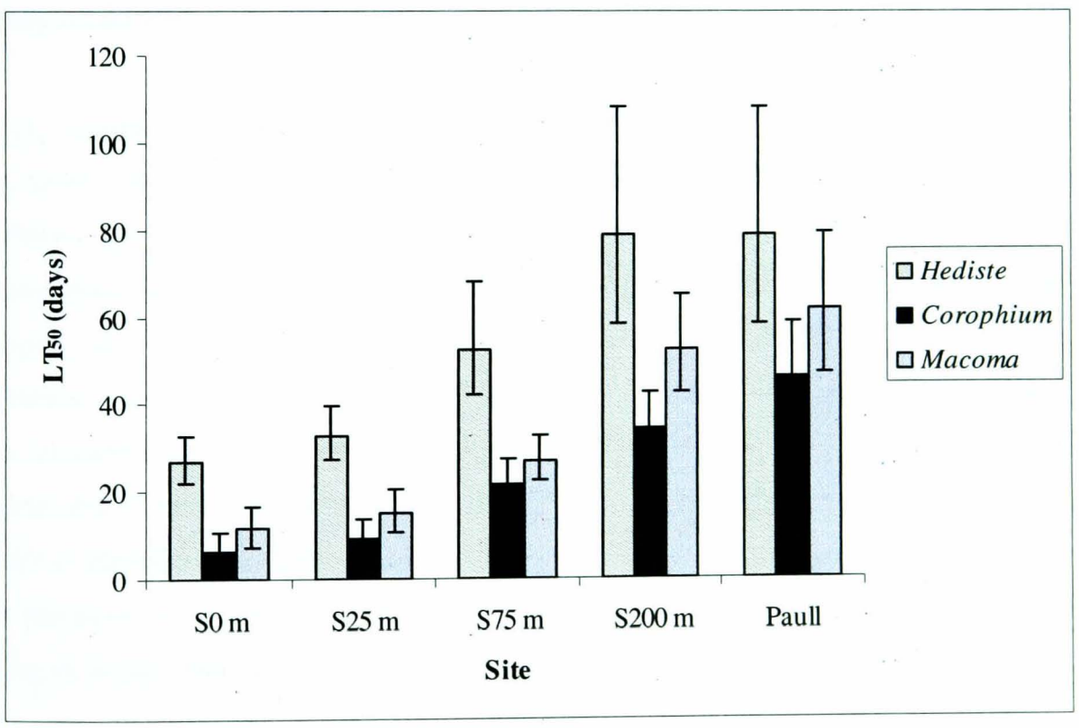


Figure 3.3. LT_{50} (± 95% CL) values for *H. diversicolor*, *C. volutator* and *M. balthica* exposed to sediments taken from various distances from the discharge.

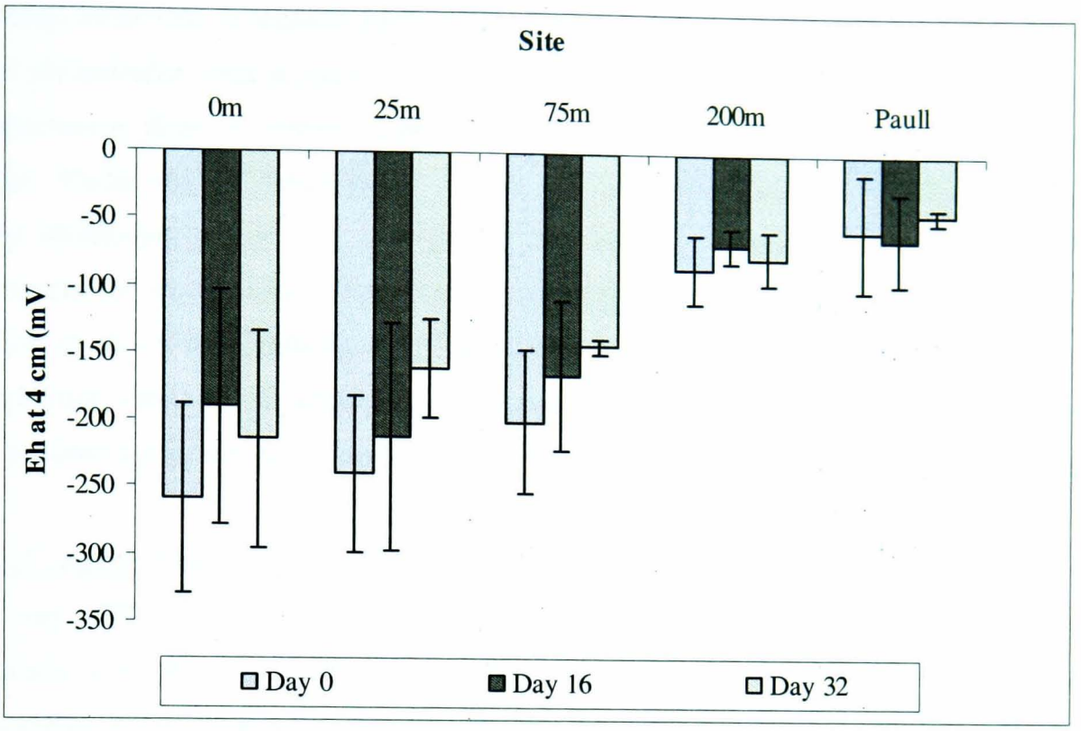


Figure 3.4. Changes in redox potential (mean ± SE) at a depth of 4 cm over time for each site.

3.4.3. Comparison of the toxicity of the current and proposed effluents (BP Chemicals (Saltend) Ltd.)

3.4.3.1. General observations (exposure to effluent)

In general, animals (*Hediste diversicolor* and *Corophium volutator*) kept under control conditions (i.e. clean sea water with no effluent) were more active than those kept in high concentrations of effluent. Whilst motionless behaviour is not abnormal in either species, the frequency of motionless animals increased with increasing concentration. The number of swimming animals declined over time, at all concentrations, as the animals settled. However, most animals remained active (crawling) but those in higher effluent conditions showed considerably slower movement than those in the control conditions. In the case of *C. volutator*, motionless animals in control conditions would lie on their dorsal surface beating their pleopods (rear swimming legs). Animals in high effluent concentrations only began beating their pleopods upon stimulation.

In both species, the time taken to respond to stimulation increased with increasing effluent concentration. In the case of *H. diversicolor*, animals in the control conditions would either crawl quite rapidly or display rapid S-shaped swimming behaviour within 1-3 seconds of being disturbed. In contrast, animals in higher concentrations took much longer to respond and either displayed spasmodic swimming or crawling (curling / twitching of the body or

writhing) behaviour or showed short pulses of swimming or crawling behaviour. Individuals of *H. diversicolor* were found to lose their colour and become a yellowish white colour in comparison to those in control conditions which were the characteristic red / reddish brown colour. Under control conditions, *C. volutator* either crawl or rapidly flick their bodies and begin swimming within 1-3 seconds of stimulation. Animals in the higher effluent concentrations would either not respond to stimulation or would show short pulses of slow swimming or crawling behaviour. In many cases, animals would bend backwards, curling their bodies, and then roll before attempting to swim. In these cases, swimming lasted only a few seconds before the animal sank to the bottom of the container and remained motionless.

3.4.3.2. Range finding test

One-way ANOVA tests revealed that salinity and pH did not differ significantly between replicates ($p>0.05$) but did differ between treatments due to the effect of dilution. LC_{50} and LT_{50} values for *H. diversicolor* and *C. volutator* are presented for each effluent in Table 3.1. For both the current and the proposed effluents, *C. volutator* was the more sensitive of the two species as it had 96 hour LC_{50} values of 59.53 and 64.26% for the current and proposed effluents, respectively in comparison with 88.94 and 204.89% for *H. diversicolor*. However, given the overlap of the 95% confidence limits, there is no significant difference (in statistical terms) between the overall patterns.

It should be noted that the LC_{50} for the proposed effluent for *H. diversicolor* is an extrapolation based on the calculated regression equation, since 50% of the animals did not die at the maximum (100%) effluent concentration. In addition, given the nature of the data, the 95% confidence limits are extremely wide. Since very few animals died (a maximum of two after 96 hours), it can be said that the 96 hour LC_{50} for this effluent is >100%. It can also be seen that the proposed effluent was marginally less toxic than the current effluent. This was more apparent in *H. diversicolor* than in *C. volutator* although this finding was based on extrapolated results rather than on an actual calculated LC_{50}. The LC_{50} values were consistently higher at each time interval (24, 48, 72 and 96 hours) for *H. diversicolor* than for *C. volutator* for both the current and proposed effluents with the LC_{50} values being consistently higher for the proposed effluent for both species.

As would be expected, the LT_{50} values for both species were lowest at the highest effluent concentrations (i.e., survival time reduces with increasing effluent concentration). These values, again, indicate the lower toxicity of the proposed effluent with 100% effluent concentration LT_{50} values of 67.53 hours and 154.8 hours for the current and proposed

effluents, respectively, for *H. diversicolor* and values of 4.88 and 15.64 hours for *C. volutator*. This also demonstrates the greater sensitivity of *C. volutator*.

Table 3.1. LC/LT_{50} values for *H. diversicolor* and *C. volutator* exposed to both the current and proposed effluents for each concentration and time.

	Hediste diversicolor		*Corophium volutator*	
	Current	**Proposed**	**Current**	**Proposed**
Concentration effects				
24 h LC_{50}	160.06	305.89	101.52	112.37
95% CL lower	97.01	205.39	85.33	94.55
95% CL lower	305.72	686.26	121.03	135.22
48 h LC_{50}	143.07	299.63	75.08	89.60
95% CL lower	100.61	200.88	61.49	74.37
95% CL lower	250.12	676.27	90.33	108.13
72 h LC_{50}	115.64	229.26	67.83	76.86
95% CL lower	87.95	161.33	54.78	62.75
95% CL lower	185.1	495.77	82.19	93.35
96 h LC_{50}	88.94	204.89	59.53	64.26
95% CL lower	67.23	146.33	46.65	50.78
95% CL lower	135.34	434.32	73.18	79.11
Time effects				
20% LT_{50}	130.2	242.5	121.3	125.2
95% CL lower	114.41	157.75	103.66	106.33
95% CL lower	152.05	400.51	143.57	149.57
40% LT_{50}	146.12	179.3	107.36	117.59
95% CL lower	117.75	140.31	92.22	100.16
95% CL lower	182.85	266.44	126.26	139.85
60% LT_{50}	146.06	174.7	101.57	101.57
95% CL lower	117.76	137.75	87.32	86.62
95% CL lower	182.73	257.26	119.12	120.26
80% LT_{50}	125.98	161.47	80.51	100.86
95% CL lower	111.93	128.34	68.28	86.06
95% CL lower	145.50	236.32	94.59	119.44
100% LT_{50}	67.53	154.8	4.88	15.64
95% CL lower	6097	124.04	-11.31	0.07
95% CL lower	74.66	224.11	18.61	29.16

3.4.3.3. Definitive test (with and without salinity correction)

No lethal toxic effects could be detected for either species exposed to the current and proposed effluents when salinity correction was carried out. Therefore, calculation of LC_{50} and LT_{50} values was not possible and they were assumed to be >100% and >96 hours, respectively. This suggests that increasing the salinity of the test solutions to that of the control solutions reduces the toxicity of the effluent. That is, the freshwater component of the effluent was contributing to the toxicity of the effluent to these estuarine animals. Figures 3.5 and 3.6 give a summary of the LC_{50} and LT_{50} values for the current and proposed effluents without salinity correction. Again, it can be seen that the proposed effluent is less toxic than the current effluent and that *C. volutator* is the more sensitive of the two species. The 96 hour LC_{50} values for the current and proposed effluents for *H. diversicolor* were 102.48% and 155.827%, respectively. The 95% concentration LT_{50} for the current effluent for this species was 89.12 hours with a 100% concentration LT_{50} for the proposed effluent of 139.6 hours. In

comparison, the 96 hour LC_{50} for *C. volutator* exposed to the current effluent was 61.98% which is considerably lower than that of *H. diversicolor*. In terms of time effects, the 65% concentration LT_{50} for this species exposed to the current effluent was 119.06 hours. This, again indicates the greater sensitivity of *C. volutator* since the 75 - 90% concentration LT_{50} for *H. diversicolor* was 223.79 hours. No lethal effects could be detected for *C. volutator* exposed to the proposed effluent. However, neither the current of proposed effluents to which *C. volutator* was exposed in this experiment contained material from the Etac/VAM plant and therefore, a reduction in toxicity would be expected.

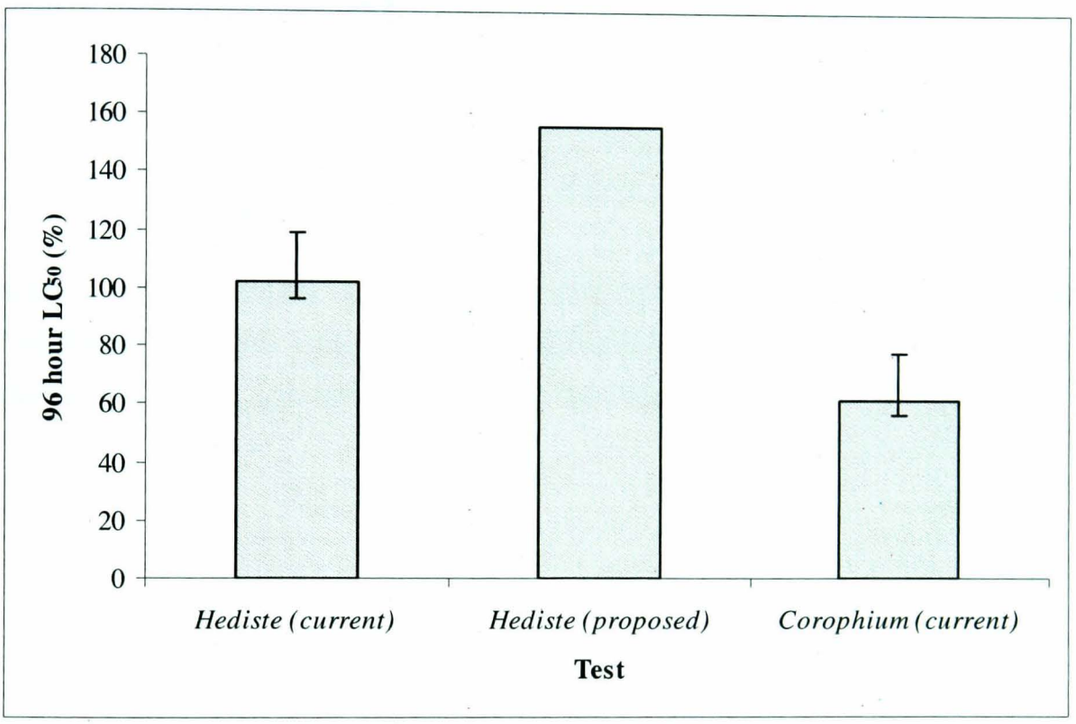


Figure 3.5. 96 hour LC_{50} values (± 95% CL) for *Hediste diversicolor* (current and proposed effluent) and *Corophium volutator* (current effluent) without salinity correction. 95% CL could not be calculated for *H. diversicolor* (proposed effluent) due to the low response.

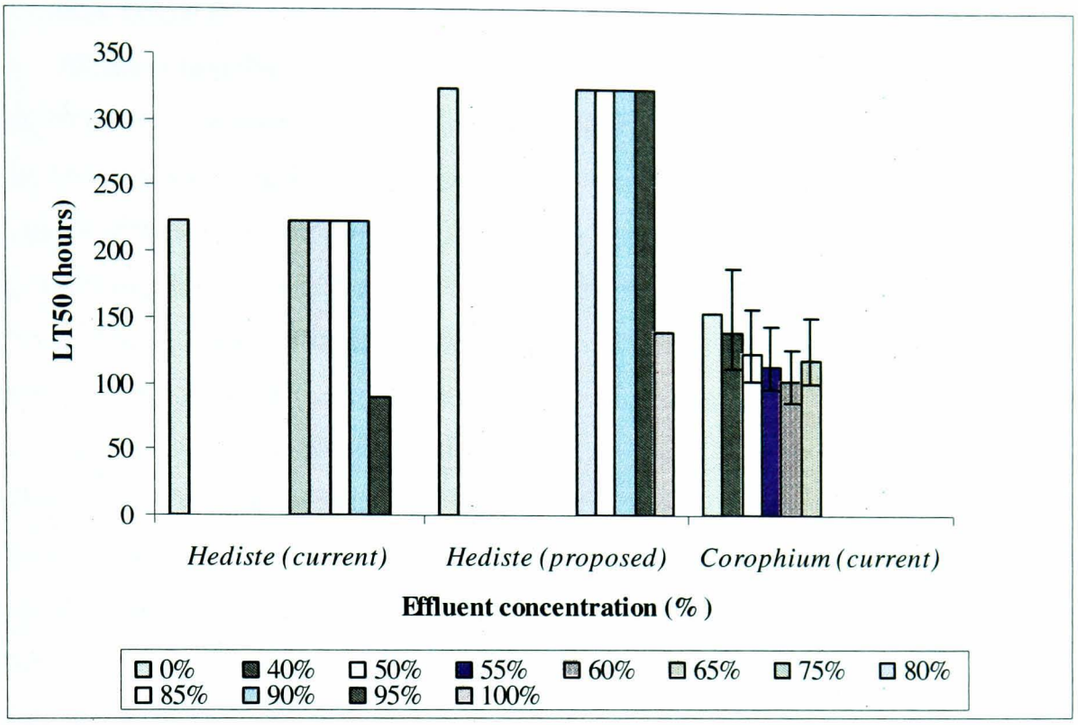


Figure 3.6. LT_{50} values for each concentration for *H. diversicolor* exposed to the current and proposed effluents and *C. volutator* exposed to the current effluent (no salinity correction). 95% CL omitted from *H. diversicolor* results for clarity.

3.5. DISCUSSION

3.5.1. Effluent toxicity

In the presence of sediment, toxicity tests showed the effluent to be of low toxicity with the 32 day LC_{50} values being 27%, 40% and 50% for *Corophium volutator*, *Macoma balthica* and *Hediste diversicolor*, respectively. The 64% LT_{50} values for these species were 16, 25 and 28 days, indicating that *C. volutator* was the most sensitive species and *H. diversicolor* the least sensitive. These values, although slightly higher, are similar to those in O'Brien (1997) who reported 21 day LC_{50} values of 11.8, 28.9 and 36% for *C. volutator, M. balthica* and *H. diversicolor,* respectively and also found *C. volutator* to be the most sensitive species. The difference in toxicity found between these studies not only reflects the variable nature of the effluent's composition (Appendix 1) but also the fact that the toxicity of the effluent is significantly reduced on exposure to light and air (Fitton, 1995). This would add to the variability due to differences in the experimental set up between studies and different values of toxicity are therefore to be expected.

Nikitik (in prep.) exposed *H. diversicolor* to a range of effluent concentrations (0-40%) for 50 days and found a strong inverse relationship between survival and effluent concentration with the greatest number of mortalities (60%) occurring at the highest concentration. However, percent survival remained higher than 80% for all concentrations between 0 and 20%. A similar relationship was found between the survival of *M. balthica* and effluent concentration with 100% mortality being observed in concentrations of 20 and 40% after 148 days.

The fact that *C. volutator* was found to be more sensitive of the two species is consistent with the findings of several other authors who state that crustaceans are known to be the most sensitive of all marine taxa (e.g., Rand & Petrocelli, 1985, in Warwick, 2001; McLusky *et al.,* 1986; Nedwell, 1997). The effects of xenobiotics on crustaceans are also often complicated by moulting and cannibalism (McLusky *et al.,* 1986) which can periodically increase susceptibility.

In a comparison of the toxicity of the effluent currently being discharged with that of a proposed, cleaner, effluent, tests where a lethal toxic response could be detected showed the proposed effluent to be less toxic than the current effluent (in the absence of sediment). This was demonstrated both by concentration and time effects and by both the range finding and definitive tests. For *H. diversicolor* exposed to the current effluent, the 96 hour LC_{50} values ranged from 88.95% in the range finding test to 102.48% in the definitive test. In comparison, exposure of this species to the proposed effluent gave LC_{50} values of greater than 100% (155.83% to 204.89%) suggesting that the proposed modifications to the effluent will

result in a reduction in toxicity. For *C. volutator* exposed to the current effluent, the 96 hour LC_{50} values ranged from 59.55% to 61.98%. The LC_{50} value for this species exposed to the proposed effluent was 64.26%. No lethal toxic effects could be detected during the definitive test. This maybe due to the fact that the Etac/VAM component of the effluent was omitted due to a short term plant shut down. These results again demonstrate that the proposed effluent was less toxic than the effluent currently being discharged but also that *C. volutator* is more sensitive than *H. diversicolor.* Thomson (1995, in Nikitik, in prep.) reported a 48 hour LC_{50} of 7% for *H. diversicolor* whilst Sykes (1996, in Nikitik, in prep.) reported a 48 hour LC_{50} of 22% for the same species (in the absence of sediment). Baynes (2001) reported a 96 hour LC_{50} of 68% which, together with the above results, suggests that the toxicity of the effluent has been reduced over time. The results of the present study, together with those mentioned above, highlight the way in which the toxicity of this particular effluent can change.

Differences in the composition of the two effluents included significant reductions in organics, BOD, COD and metals. Whilst no data regarding the concentration of organic chemicals in either effluent were available, the proposed effluent contains considerably lower concentrations of metals (Table 3.2) and it is therefore not surprising that its' toxicity was found to be lower. Whilst concentrations of all metals still exceed the EQS, information from ENTEC (provided by A. Kirton, BP Chemicals (Saltend) Ltd) suggests that concentrations will be well below the EQSs upon dilution following discharge. It is assumed that the reduction in organics also contributed to the reduction in toxicity.

Table 3.2. Comparison of metal concentrations (μg l^{-1}) in the 95th percentile between the current and future effluents (A. Kirton, BP Chemicals (Saltend) Ltd. Pers. comm).

	Zn	Cr	Ni	Cu	Mn
Dirty stream (current)	709	197	411	65	22,687
Clean stream (proposed)	270	37	42	28	-
% difference	69%	81%	90%	54%	100%

When compared to the LC_{50} values derived from experiments where animals were exposed to the effluent in the presence of sediment for 32 days, the above 96 hour values (comparison of the two BP effluents) suggest that the effluent is more toxic in the absence of sediment. The presence of sediment can ameliorate toxicity by acting as a sink for the various components of an effluent, thus reducing bioavailability and reducing the toxicity (Calmano *et al.*, 1996,

Linnik & Zubenko, 2000) and by buffering external environmental fluctuations. Furthermore, whilst both *H. diversicolor* and *C. volutator* may spend time swimming or crawling on the surface of the mud, they are essentially sediment dwelling, burrowing organisms. Prolonged periods where the animals are maintained in water without sediment are likely to induce abnormal behaviour to some degree since the absence of sediment is stressful in itself (Bat *et al.*, 1998). Bat *et al.* (1998) reported that even in control conditions, *C. volutator* mortality was increased as a result of the absence of sediment.

In terms of lethal time, the results, again, indicate the lower toxicity of the proposed effluent and the greater sensitivity of *C. volutator*. As would be expected, there was a general trend of decreasing lethal time (LT_{50}) with increasing effluent concentration for both species exposed to both effluents. The 100% effluent concentration LT_{50} values (range finding test) were 67.53 hours and 154.8 hours for the current and proposed effluents, respectively, for *H. diversicolor* and 4.88 and 15.64 hours for *C. volutator*. This was also demonstrated by the results of the definitive test where the maximum concentration LT_{50} for *H. diversicolor* was 89.12 hours (95% concentration) for the current effluent and 139.6 hours (100% concentration) for the proposed effluent. For the current effluent, the 65% concentration LT_{50} for *C. volutator* was 119.06 hours. This is considerably lower than the minimum concentration (75%) LT_{50} of 223.79 hours for *H. diversicolor*.

In addition to endpoints (e.g. LC_{50} values), probit analysis also allows the calculation of 95% confidence limits. These indicate the limits between which 95% of the statistical data lie, thus giving an indication of the spread of the data. In cases where the calculated LC_{50} values are within the concentration range of the test (i.e., they are less than 100%), these 95% confidence intervals are reasonably small indicating that the variation in the data is not great and the calculated result is reliable. However, in many cases (particularly for *H. diversicolor*) the calculated LC_{50} value was greater than 100%, that is, the calculated LC_{50} value was beyond the concentration range of the test. LC_{50} values were therefore calculated based on extrapolation of the data and are subject to statistical error. This is not considered a problem since an effluent concentration of greater than 100% is not possible and therefore the toxicity of the effluent discharged will be less than the predicted 96 hour LC_{50}. Whilst most of the results indicated a general trend of reduced toxicity of the proposed effluent, the overlap of the 95% confidence limits shows that there is no statistical difference between the toxicities of the two effluents. This implies that any reduction in toxicity as a result of the proposed modifications to the effluent will be marginal. There is, however, no evidence to suggest that removing certain components of the current effluent will result in increased toxicity.

With the exception of one case, correction of salinity in the test solutions to that of the control solutions had the effect of reducing toxicity. No lethal effects were observed in any of the salinity corrected solutions, for either species. The toxicity of a variety of metals to marine organisms has been found to be related to salinity with a decrease in toxicity being observed with an increase in salinity (McLusky *et al.*, 1986). There are several explanations for this. Firstly, as the chloride ion concentration (i.e. salinity) increases, the free metal ion concentration decreases (relative to the total metal concentration) due to complexation with the chloride ions, thus reducing its bioavailability. Metals such as cadmium are also known to compete with major cations (e.g. calcium and magnesium) for uptake sites such that as salinity reduces, metal uptake increases. At the sub-lethal level, increased toxicity at reduced salinities may be linked to osmoregulatory impairment and according to Thurberg (1973, in McLusky *et al.*, 1986), a characteristic feature of copper is the depression of the blood osmotic pressure in decapod crustaceans (that is, the ability of the organism to maintain its blood sodium concentration is reduced). According to Dorgelo (1973) and Mckenny *et al.* (1981), marine and estuarine organisms show metabolic compensation to salinity stress. The oxygen consumption rate of the amphipod *Chaetogammarus marinus* was found to decrease with increasing salinity, possibly reflecting the increased energy expenditure associated with osmoregulation at low salinities (Dorgelo, 1973). Given that the gills are a major site for the intake of toxins, increased oxygen consumption could also lead to the increased uptake of pollutants at low salinities, thus resulting in increased toxicity at low salinity. Finally, the test animals used in the present study are tolerant of brackish and marine conditions and any exposure to freshwater will induce stress regardless of the added effect of any toxins which may be present.

3.5.2. Sediment toxicity

The sediment LT_{50} increased in sediment samples taken from increasing distances from the point of discharge. The decrease in toxicity with increasing distance from the outfall correlates ($r^2 > 0.9$, $p<0.01$ for all times and all species) with the decrease in the level of anoxia (expressed as Eh at a depth of 4 cm). LT_{50} values ranged from 27 days at the S0 m site to 79 days at the Paull and S200 m sites for *H. diversicolor*, 12 days at S0 m to 61 days at Paull for *M. balthica* and 6 days at S0 m to 46 days at Paull for *C. volutator*. As with the effluent toxicity data, these results indicate the greater sensitivity of *C. volutator*. The predicted sediment LT_{50} values were greater than the exposure time (32 days) for *H. diversicolor* exposed to sediments taken from all sites except the S0 m site with the LT_{50} value for S25 m sediments being 33 days. In contrast, *C. volutator* and *M. balthica* were considerably more sensitive with LT_{50} values only exceeding the exposure time at the S200 m and Paull sites. However, no information about the sub-lethal effects of the sediment is given

(e.g. effects on reproduction, growth, feeding) and therefore, no inference about the long term survival of the three species in the different sediments can be made from these data.

Following 90 day exposure (*in-situ*), Nikitik (in prep.) reported 100% mortality in *H. diversicolor* at sites between 0 and 50 m from the discharge and noted decreased survival between 50 and 150 m in comparison to that further away from the discharge. Nikitik (in prep.) also demonstrated a general (but not statistically significant) trend of increasing growth rate (expressed as increase in mean weight) with increasing distance from the discharge with maximum growth being recorded from the S200 m and S150 m sites. Growth was also found to increase with decreasing effluent concentration with minimum growth being recorded at 40% concentration. During a 263 day study (*in-situ*), the survival of *M. balthica* was also found to increase with distance from the outfall with 100% mortality occurring within 122 days at the S0 m site in comparison to 214 days at the S50 m site and 263 days at the S100 and S150 m sites. As with *H. diversicolor*, the growth of *M. balthica* was reduced as a result of exposure to the effluent and contaminated sediment. Nikitik (in prep.) also recorded reduced burrowing rates of *C. volutator* and *M. balthica* in sediments taken from sites close to the discharge (S0 m and S25 m) in comparison with those further away (e.g. Paull).

3.5.3. Possible causes of toxicity.

Examination of the effluent characteristics for the period September 1999 to February 2000 shows that the majority of the compounds present in the effluent do not exceed either the EQS, as defined by Cole *et al.*(1999), or the Canadian water quality guidelines for the protection of aquatic life (CCME, 2001a) (Table 3.3). Many of the Canadian guidelines relate only to freshwaters since sufficient data are not available to derive guidelines for the marine environment. Therefore, these have only been referred to in the absence of any marine guidelines or EQS values. The only organic compounds found to exceed these guidelines were pentachlorophenol (2.4 times greater than the Canadian guideline), trichloromethane (chloroform, up to 5 times greater than the Canadian guideline), endosulphan (up to 3.75 times greater than the Canadian guideline) and hexachlorocyclohexane γ (lindane, up to 5 times greater than the Canadian guideline). Standards or guidelines for PCBs, some of the organochlorine pesticides, and a number of the chlorinated ethanes, ethenes, benzenes and halogenated methanes present in the effluent do not exist and so it was not possible to determine whether or not these compounds were present in higher than recommended concentrations.

The concentrations of organic compounds in the raw effluent exceed the guidelines by a maximum factor of 5. It is expected that, following initial dilution upon discharge,

concentrations of these compounds would be reduced sufficiently so as not to breach the guidelines. In addition, organic compounds are subject to rapid degradation by photo-oxidation, as described by Lee (in press). Many of the products of photo-oxidation are water soluble and more rapidly degraded by bacteria than are the compounds from which they originated. Whilst photo-oxidation has been shown to increase the toxicity of various polycyclic aromatic hydrocarbons (PAH) (which are absent from the effluent), this toxicity is thought to be short lived and photo-oxidation does not increase the toxicity of all substances (Lee, in press). Fitton (1995) also found that exposure to light and aeration considerably reduced the toxicity of the BP effluent. Schratzberger *et al.* (2003) suggested that the addition of fertiliser (in the form of nitrogen and phosphorus) can also aid the biodegradation of hydrocarbons through the stimulation of microbial and microalgal growth. Whilst it is not known whether the nitrate and phosphate concentrations in the effluent are high enough to impact on biodegradation, there is certainly evidence of some response of the microalgal community as demonstrated by the high chlorophyll-a content of the sediment in the immediate vicinity of the outfall (Chapter 2). Direct lethal toxic effects on the organisms in the receiving environment as a result of exposure to these organic compounds is therefore unlikely.

In contrast, with the exception of triphenyltin (TPT) arsenic, cadmium and for the majority of the time, lead, all metals present in the effluent exceed their respective EQS values. The greatest exceedance for metals is copper which is present in concentrations of up to 165 times greater than the EQS. Nickel, zinc, chromium and tributyltin (TBT) are present in concentrations of up to 41, 31, 29 and 8.5 times higher, respectively, than their EQSs. In the immediate vicinity of the outfall, dilution may not necessarily be sufficient to reduce some of these metals (particularly copper) to concentrations below the EQS. It is therefore likely, as indicated by Nikitik (in prep), that any direct toxic effects as a result of exposure to the effluent are due to the high concentrations of the metals present, particularly copper, rather than the organics.

Table 3.3. Effluent components and degree of exceedance of the Canadian Water Quality Guidelines.

Compound	Effluent concentration (μg l^{-1})	Guideline	Exceedance
Pentachlorophenol	1.2	0.5	2.4
Trichloromethane	0.2-9	1.8	0.1 - 5
Endosulphan	0.045-0.075	0.02	2.3 - 3.8
Hexachlorocyclohexane γ (Lindane)	0.03-0.05	0.01	3 - 5
Copper	104-826	5	21 - 165
Zinc	121-313	10	12 - 31
Nickel	56.1-619	15	3.7 - 41.3
Chromium	40.1-146	5	8 - 29
Tributyltin	0.004-0.017	0.002	2 - 8.5

Matthiessen *et al* (1999) stated that prolonged exposure to copper concentrations of 50 - μg l^{-1} was likely to result in mortality in most marine organisms and that 20 μg l^{-1} could result in reduced growth and reproductive success. Conradi & Depledge (1998) found concentrations of 1 mg l^{-1} to cause juvenile mortality in *C. volutator*. The raw effluent concentration was less than this (maximum of 0.826 mg l^{-1}) and would have been considerably reduced following dilution upon discharge. However, concentrations of 0.2-0.4 mg l^{-1} were found to be sufficient to reduce specific growth rate. Conradi & Depledge (1998) stated that *C. volutator* must reach a length of 4 mm before it can reproduce and found that the time taken to reach this length was significantly increased at copper concentration 0.6-0.8 mg l^{-1}. Animals exposed to 1 mg l^{-1} were unable to reach maturity. An increase in the time taken to reach maturity can have a major negative impact on fitness. In addition, copper reduced fertility (number of new born individuals / female) suggesting that exposure to copper could have negative effects on the survival and growth of young animals and on the reproductive success of mature animals.

Nedwell (1997) reported 96 hour LC_{50} values of 1.34 and 0.3 mg l^{-1} for Humber (collected from Paull) populations of *C. volutator* and *H. diversicolor* (respectively) and found that tolerance to copper in different populations was largely related to the concentrations in the sediments from which they came. In addition, pre-exposure of *C. volutator* to sub-lethal copper concentrations resulted in the development of copper tolerance. Conradi & Depledge (1999) also found that zinc concentrations of 0.2 to 0.8 mg l^{-1} were sufficient to cause a reduction in specific growth rate, reduced survival of ovigerous females and reduced length with the effect increasing with increasing concentration. Following initial dilution upon discharge, concentrations of metals in the effluent may be reduced to concentrations below

their lethal level but the possibility of sub-lethal effects should be considered. It should also be considered that the toxic effects of several of the organic compounds are not known.

Based on the fact that Thompson (1995) and Sykes (1996, in Nikitik, in prep.) found high toxicity values for *H. diversicolor* exposed to effluent in the absence of sediment, compared to the low toxicity values derived when sediment was incorporated into the experiment, Nikitik (in prep.) concluded that any toxic response was related to the effluent rather than the sediments. However, in the present study, the 32 day LC_{50} values ranged from 27-50% with the 96 hour LC_{50} values being greater than 50% in all cases. This suggests that, following discharge, an initial dilution factor of 2 would be sufficient to effectively remove any toxicity (according to 96 hour LC_{50} values). The toxicity of the effluent has to be interpreted against the behaviour of the effluent once it has been discharged. At low water, the effluent enters Old Fleet Drain and mixes with the water in the drain whereas at high water, it mixes immediately with the water in the estuary. During the flood tide, the effluent may be pushed up the drain and then be diluted whereas on the ebb tide, the effluent will be diluted as the water moves off the mudflats. Therefore, following discharge, it is unlikely that the mudflat organisms would be exposed to the concentrations required to produce a lethal effect, particularly at high water when the level of dilution is greatest. The fact that the 32 day and 96 hour LT_{50} values were so high (with the exception of those for *C. volutator* exposed to 100% effluent concentrations) suggests that, in order to observe a lethal effect, the animals would have to be bathed in the effluent for long periods of time - several days in some cases. Given that the effluent is discharged to the upper shore of an intertidal area, and that dilution occurs immediately upon discharge to the drain, it is highly unlikely, if not impossible, that the mudflat organisms would ever be exposed to the effluent for the length of time required to produce a lethal effect.

This, together with the fact that a toxic response to the sediment in the absence of effluent was detected in all three species during the present study, suggests that it is unlikely that exposure to the effluent alone is responsible for the toxicity. In terms of sediment contamination, no data are available with regard to the concentration of organic compounds and their degradation products. Concentrations of metals determined by Nikitik (in prep) are summarised in Chapter 2. Table 3.4 gives details of sediment quality guidelines and Predicted Effects Levels (PEL) for various metals together with the degree of exceedance at each site. It can be seen that all metals measured breached their respective Canadian sediment quality guideline (CCME, 2001b) values at all sites although the degree of exceedance decreased with increasing distance from the outfall. Arsenic and copper showed the greatest degree of exceedance with concentrations of arsenic being 2.6 - 4.7 times higher than the

Canadian sediment quality guideline for the protection of aquatic life and copper concentrations being 1.8 - 2.6 times higher. Chromium, zinc and lead exceeded the guidelines by factors of 1.59 -2.05 (Cr); 1.38 - 1.81 (Zn) and 1.61 - 1.97 (Pb). Sediment quality guideline values were not available for nickel. Of these metals, only arsenic (factor of 1.47 - 1.64) exceeded the predicted effects level (PEL) as defined in CCME (2001b).

Table 3.4. Canadian sediment quality guidelines and Predicted Effects Level (PEL) for metals and degree of exceedance at the key monitoring sites. (* indicates value from Cole *et al.*, 1999)

Site	Sediment concentration (mg kg^{-1})	Metal	Guideline (mg kg^{-1})	Guideline exceedance	PEL (mg kg^{-1})	PEL exceedance
S25 m	48.41	Copper	18.7*	2.6	197	0.25
S75 m	47.45			2.5		0.24
S200 m	37.9			2.0		0.19
Paull	34.38			1.8		0.17
S25 m	71.39	Chromium	37.3	1.91	90	0.79
S75 m	76.6			2.05		0.85
S200 m	70.27			1.88		0.78
Paull	59.58			1.59		0.66
S25 m	69.26	Lead	35	1.97	91.3	0.75
S75 m	67.27			1.93		0.74
S200 m	65.68			1.87		0.71
Paull	56.49			1.61		0.62
S25 m	212.15	Zinc	123	1.72	315	0.67
S75 m	222.35			1.81		0.71
S200 m	187.43			1.52		0.6
Paull	169.16			1.38		0.54
S25 m	27.95	Arsenic	5.9	4.7	17	1.64
S75 m	24.94			4.23		1.47
S200 m	12.17			2.2		0.72
Paull	15.21			2.58		0.89

Nikitik (in prep.) found a correlation between the distribution of sediment-bound metals (particularly copper) and proximity to the outfall and suggested that this, in combination with elevated copper concentrations in the effluent, may be related to community structure in the vicinity of the outfall and the observed toxic responses. Evidence from a variety of studies suggests that there are sufficient levels of certain metals in the sediments at Saltend and Paull to cause sub-lethal effects. For example, Roper *et al.* (1995) found concentrations of 25 mg kg^{-1} to be sufficient to reduce the burrowing of *Macomona liliana* and 10 mg kg^{-1} caused avoidance behaviour. Marked mortality occurred at 50 mg kg^{-1} and according to these authors, copper is particularly toxic to molluscs. McGreer (1979, in Roper *et al.*, 1995) found that 30 mg kg^{-1} copper was sufficient to reduce burrowing in *Macoma balthica* and 95 mg kg^{-1} induced avoidance behaviour. Bat *et al.* (1998) stated that copper was less toxic to *C.*

volutator than zinc or cadmium and Bryan (1976a,b, in Bat *et al.*, 1998) found *C. volutator* to be one of the most copper tolerant species either through regulation of uptake, impermeability or by direct excretion in an insoluble form. This is consistent with the result of Nedwell (1997) who found *C. volutator* to be considerably more tolerant of copper than *H. diversicolor*, despite the fact that crustaceans are generally regarded as being one of the more sensitive groups (Rand & Petrocelli, 1985, in Warwick, 2001). *C. volutator* was shown to actively avoid copper contaminated sediments and no animals were found in sediments containing 127 mg kg^{-1} (Bat *et al.*, 1998).

With regard to zinc, Roper *et al.* (1995) found that 80 mg kg^{-1} reduced burrowing and 40 mg kg^{-1} induced an escape response in *M. liliana*. Mortality occurred at 160 mg kg^{-1}. A concentration of 73 mg kg^{-1} reduced burrowing in *M. balthica* and 134 mg kg^{-1} initiated avoidance behaviour (McGreer, 1979, in Roper *et al.*, 1995). *C. volutator* was found to avoid sediment with 152 mg kg^{-1} or more of zinc (Bat *et al.*, 1998). Watzin & Roscigno (1997) treated sediments with zinc concentrations of 0.25 - 5 mg kg^{-1}. Zinc contaminated sediments always contained fewer spionids (principally *Streblospio benedicti*) than did uncontaminated sediments and it was concluded that zinc contamination in sediments had potential to alter the abundance and composition of benthic recruits. Most macrobenthic species were found in lower abundances in contaminated sediments, regardless of the concentration. Austin *et al.* (1994, in Watzin & Roscigno, 1994) found reduced survival of nematodes exposed to sediment zinc concentrations of 1100 mg kg^{-1}. There is also a large amount of evidence that zinc concentrations above 250 mg kg^{-1} can cause lethal and sub-lethal effects to a variety of macroinvertebrates and that by altering recruitment dynamics, macrobenthic communities can become significantly altered (Watzin & Roscigno, 1997).

However, it should be noted that whilst differences in toxicity were detected between sediments taken from different distances from the outfall, the sediments at Paull (the lowest response) also contain concentrations of metals which exceed the EQSs. Metal concentrations generally decline between the Saltend 25 m site and the control site at Paull but, although statistically significant, these differences are small. Considering this, together with the spatial and temporal variability of metal concentrations (Scott, 1996) and the fact that EQSs are breached at the Paull site, it is questionable whether the copper and zinc concentrations in either the effluent or the sediment play such a significant role in the structuring of the benthic community as that suggested by Nikitik (in prep).

Furthermore, the presence of high concentrations of sediment bound metals bears little relevance to the actual bioavailable concentration and is not necessarily an indication of the

likely biological effect. Bioavailability of certain metals is reduced in fine grained, organic rich sediments (Bat *et al.*, 1998; Stark, *et al.*, 2003) and is further reduced in the presence of sulphides (Di Torro *et al.* in Stark *et al.*, 2003; Power & Chapman, 1992, in Roper *et al.*, 1995). In sulphidic environments (e.g. anoxic environments such as those close to the discharge), organisms may be considered to be relatively safe from the direct toxic effects of metals (although the sulphide itself is toxic) due to strong fixation of metal ions by S^{2-} or HS^- (Calmano *et al.*, 1996). However, disturbance of contaminated, sulphidic sediments can result in an increase in redox potential, leading to the oxidation of sulphides and the subsequent breakdown of sulphide-metal complexes. This can cause the release of metals into the water column or the interstitial water in their bioavailable form (Calmano *et al.*, 1996; Petersen *et al.*, 1996). Anoxic conditions are generally associated with the uptake of copper, zinc and cadmium from the water column. In contrast, the release of arsenic, cobalt and chromium from the sediment has been associated with anoxia. Jørgensen (1980) and Petersen *et al.* (1996) explained this by the fact that whilst copper, zinc and cadmium form complexes with sulphides under anoxic conditions, arsenic, cobalt and chromium are precipitated by hydrous ferrous oxides (under aerobic conditions). These precipitates dissolve in the absence of oxygen, thus leading to the release of these metals.

Given the degree of anoxia at the S25 m and S75 m sites recorded in the present study (Chapter 2), together with the fact that arsenic concentrations in the sediment exceed both the EQS and PEL, it might be reasonable to assume that arsenic toxicity could contribute significantly to the overall toxicity of the sediment. However, changes in the aerobic-anaerobic balance, caused by bioturbation and temperature changes, mean that the bioavailability of all metals present will be variable on a spatial and temporal scale. It should also be noted that whilst sediment bound contaminants may not be available to organisms which feed on plankton and detritus in the overlying water they may be available to deposit feeding organisms which actively ingest the sediment (Meador *et al.*, 1995). There are also a number of persistent organic compounds (e.g. PCBs) present in the effluent which could potentially accumulate in the sediment but the concentration of such compounds has not been determined. Craft (1999) examined levels of phthalate esters in the sediments at Saltend and found concentrations to decrease with increasing distance from the discharge. However, studies regarding the toxicity of sediment bound phtalates are lacking and no conclusions about their potential biological effects (with regard to the organisms present at Saltend) could be drawn (Craft, 1999). It is therefore not considered appropriate to associate any toxic response detected with the presence of a single contaminant.

Nedwell (1997) studied the sediment metal concentrations at Paull in 1995 and found copper, lead, zinc and chromium to be present in higher concentrations than were recorded at Saltend by Nikitik (in prep.). All concentrations exceeded their respective EQS and PEL values yet abundant populations of *C. volutator* and *H. diversicolor* were still present. The fact that these two species both showed a toxic response to the sediments at Saltend, but are clearly tolerant of high metal concentrations, suggests that metals are not necessarily the primary mechanism of toxicity at these sites. Nedwell (1997) showed that toxic response was related to the environment from which the organisms were taken with organisms from cleaner environments being more sensitive. It was also demonstrated (as mentioned above) that short term exposure to sub-lethal copper concentrations could result in the development of increased copper tolerance in *C. volutator.*

The low level of toxicity detected in the present study, together with the fact that most components of the effluent comply with their respective EQSs suggests that whilst the effluent may have some direct impact on the organisms present, secondary effects through its effect on the sediment quality may be more important. This has also been suggested as a mechanism of toxicity in contaminated Antarctic sediments (Stark *et al.,* 2003). Stark *et al.* (2003) stated that oil in sediments represents a chemical and physical disturbance that could result in primary toxic effects and secondary changes in sediment properties such as organic enrichment. The physical properties of the sediment are summarised in Chapter 2 and differences in the physical properties (e.g. particle size, water content etc.) were not considered to be sufficiently large to cause the observed differences in toxic response between the sites within the immediate vicinity of the outfall and those at Paull. However, the redox potential profiles for each site show that the sediments at sites close to the outfall are severely reduced in comparison to those at sites further away. Low concentrations or the complete absence of oxygen are highly correlated with the formation of hydrogen sulphides (Theede *et al.,* 1969) and iron plays an important role in the retention of sulphides in the sediment (Jørgensen, 1980). It was also stated by de Zwann & Babarro (2001) that bacterial outbreaks (i.e., bacteria associated with the sediment particles) are part of every anoxic event. Given the high organic content of the effluent together with the high iron concentrations and the extremely low redox potential at the surface of the sediment at sites within the vicinity of the outfall, it would not be unreasonable to assume that hydrogen sulphide concentrations could be high. Survival of benthic macrofauna is limited by low dissolved oxygen and further limited by the presence of high levels of hydrogen sulphide (Theede *et al.,* 1969; Llansó, 1991; Laine *et al.*, 1997 in Modig & Ólafsson, 2000). Communities in sulphide contaminated areas are characterised by low numbers of species and the sudden occurrence of low oxygen

and high sulphide concentration can result in complete defaunation (Theede *et al.,* 1969; Llansó, 1991; Rosenberg, 1980; Rainer & Fitzhardinge, 1981, in Llansó, 1991.

Sulphide was not measured successfully during the present study but there was a strong odour of it at sites very close to the discharge. Sulphide has a neutral charge and therefore diffuses easily through biological membranes (Julian & Arp, 1992, in Jahn & Theede, 1997). Sulphide toxicity results from the inhibition of the electron transport chain in aerobic respiration (Torrans & Clemens, 1982, in Miron & Kristensen, 1993a). Hydrogen sulphide binds to the haem (oxygen carrying group in proteins) of cytochrome-C-oxidase and other enzymes containing porphyrin bound metals, inhibiting their function (Evans, 1967, in Llansó, 1991). This inhibits the last enzymatic reactions of the respiratory chain and leading to a breakdown of oxidative metabolism (Nicholls, 1975; Nicholls & Kim, 1981, 1982, in Jahn & Theede, 1997) and therefore interrupting the oxygen supply to the cells (Theede *et al.*, 1969). Jahn & Theede, (1997) also suggested that the presence of sulphide may inhibit the synthesis of the cytochrome-C-oxidase enzyme. According to Theede *et al.,* (1969), the toxic action of hydrogen sulphide on cellular metabolism can be attributed to the fact that it forms insoluble sulphides with heavy metals. It has a high affinity for iron, causing a similar reaction to an elimination of the cytochrome oxidases by hydrogen cyanide. According to these authors, the toxic action of hydrogen sulphide is pH dependent with low pH being associated with the dissociation of hydrogen sulphide, that is at lower pHs, sulphide is less likely to form toxic, insoluble sulphides with metals.

3.6. SUMMARY AND CONCLUSIONS.

- The toxicity of the effluent can be considered to be low. The 32 day LC_{50} values (in the presence of sediment) ranged from 27% (*C. volutator*) to 50% (*H. diversicolor*) with the 64% (maximum concentration) LT_{50} values ranging from 16 - 28 days for these species.
- Comparison of the current and proposed effluents showed both effluents to be of low toxicity (96 hour LC_{50} of more than 50%) but indicated reduced toxicity of the proposed effluent. This suggests that a dilution factor of 2 upon discharge would be sufficient to remove any direct toxic impact of the effluent (in terms of lethal effects).
- Sediment toxicity studies showed reduced survival of all three species at the S0 m and S25 m and, in the case of *C. volutator* and *M. balthica*, the S75 m site. LT_{50} values for the S0 m and S25 m sites were 27 and 33 days for *H. diversicolor*; 6 and 9 days for *C. volutator* and 12 and 15 days for *M. balthica*.
- *C. volutator* was found to be the most sensitive species in all studies and confirms the generally accepted sequence of crustaceans being the most sensitive group to pollution, followed by molluscs then polychaetes.
- The concentrations of certain compounds present in the effluent and the sediment exceeded their respective EQS and PEL values suggesting that direct toxicity as a result of exposure in the environment may be possible. However sediment metal concentrations were high at all sites and variable on a spatial scale and LT_{50} values suggested that the organisms would have to be continually exposed for long periods of time for any lethal effects to occur. It is therefore suggested that secondary toxic effects in terms of changes to the sediment properties (e.g. changes in Eh) as a result of exposure to the effluent may, in part, be responsible for the difference in toxicity between sites.

CHAPTER 4

FAUNAL COMMUNITY ANALYSIS

4.1. INTRODUCTION

The general characteristics of estuarine communities and the natural processes influencing their structure and function are briefly described in Chapter 1 with a detailed description of the biology of the dominant species occurring in this part of the estuary. This chapter will specifically examine the communities at Saltend and Paull in relation to the physical characteristics (particle size, water and organic content, salinity, pH, redox potential, chlorophyll-a and carbohydrate content) of the environment described in Chapter 2. In addition, it gives a closer examination of the community structure at these sites in relation to seasonality and proximity to the BP Chemicals discharge at Saltend. Parameters such as species diversity, abundance, biomass (total community biomass and individual biomass) and body size are expected to change spatially with changing levels of pollution but also temporally in relation to seasonal changes in predation pressure, growth, mortality and fecundity. It is hypothesised that these changes will have an impact on bioturbation potential and ultimately sediment erosion potential which will be addressed in Chapters 5, 7 and 8.

Seasonal patterns in abundance and biomass of primary producers are well documented (Watling, 1975), with population dynamics being determined by temperature, light intensity, nutrient availability, abundance of grazing species and weather conditions (e.g. storms versus calm conditions). Various studies on epipelic (sediment dwelling) diatoms have shown an increase in diatom biomass during the warmer months (Colijn & Dijkema, 1981; Admiraal *et. al.*, 1982; Underwood & Paterson, 1993a; 1993b) with biomass being positively correlated with temperature (Underwood & Paterson, 1993a). However, Cadeé & Hegeman (1974, in Underwood & Paterson, 1993a) state that benthic diatoms are less seasonally influenced than phytoplankton communities and are known to peak at other times of year due to their ability to respond rapidly to favourable environmental conditions (Underwood & Paterson, 1993b).

Estuarine intertidal mudflats are naturally stressed areas in that the conditions are harsh and variable and therefore tolerated by only a few species. Therefore significant year to year fluctuations in the abundance and biomass of benthic species are to be expected (Beukema, 1976). Furthermore, as most biological reactions are temperature driven and that seasonally influenced primary producers provide a source of food, either by direct consumption or indirectly through the consumption of grazing species, for all organisms, then seasonal patterns

in abundance, biomass, diversity and possibly species composition may be observed in benthic macrofaunal communities. These seasonal patterns could be expected to occur as a result of species immigration / emigration, recruitment, death, predation or body weight changes resulting from changes in food availability, temperature driven metabolic reactions and reproductive state. In addition, seasonal changes in sediment characteristics (e.g., changes in sediment stability during winter storms, hypoxia or anoxia in bottom waters and surface sediments induced by high summer temperatures) may impact upon the community structure. Despite this, studies on seasonal patterns in benthic, and particularly intertidal communities, are not common (Watling, 1975; Beukema, 1974; 1976) and since the work of these authors, very few studies appear to have dealt with seasonality in intertidal benthic communities as a primary focus.

In the context of this study, seasonal changes in community structure are of relevance not only with reference to bioturbation but also in determining the effects of pollution from the BP outfall. Maurer *et al.*(1979) stated that without some knowledge of the degree of seasonal variation in benthic populations, estimates of change, with respect to disturbance, are flawed. Hence, seasonal and annual fluctuations and spatial heterogeneity of populations must be studied before valid conclusions concerning the effects of a polluting discharge can be drawn.

4.1.1. Effects of organic pollution

The effects of pollution are evident at all levels of biological organisation from the cellular and biochemical level through to the community and ecosystem level (Stebbing *et al.*, 1992). Capuzzo (1981) and Stebbing & Dethlefsen (1992) described how effects at the biochemical and cellular level could lead to physiological, genetic, morphological and behavioural changes at the organism level. These effects can be lethal, affecting the survival of an organism or species, or sublethal, leading to changes in the reproductive and recruitment success of a population. Bayne *et al.* (1988) provide an evaluation of some of the biochemical, cellular and physiological techniques used in the determination of the biological effects of pollutants. At the community level, changes in species composition, size class structure, abundance and biomass of the organisms occur and communities in this situation generally do not progress beyond the pioneering stages of development (Pearson & Rosenberg, 1978). The ultimate effect of this can be ecosystem change, as described by Walker *et al.* (1996).

Along a pollution gradient it is generally accepted that there will be a changing pattern of species abundance as each species will have a different level of response to the pollutant. Species living in contaminated sediments must move, tolerate it or die (Gray, 1982; Gray *et al.*, 1988). Polluted communities are characterised by a change in their species abundances

(Magurran, 1988, McLusky & Elliott, 2004) and in organically polluted areas, it is frequently observed that some species increase in abundance, many decrease in abundance and some remain unaffected (Gray *et al.*, 1988). The effects of organic pollution on marine and estuarine benthic communities are well documented and were reviewed, predominantly, in relation to sewage and pulp mills, by Pearson & Rosenberg (1978). Undisturbed communities were found to be relatively diverse, containing high numbers of species, high biomass but relatively low numbers of individuals. In contrast, communities influenced by organic pollution tend to be composed of very few species at very high abundances with comparatively low community biomass. The species present in organically polluted environments are generally opportunistic with growth and reproduction characteristics which allow them to take immediate advantage of a sudden change providing them with a favourable environment (Pearson & Rosenberg, 1978).

Pearson & Rosenberg (1978) used these basic, quantitative, parameters to derive the species, abundance, biomass (SAB) curve (Figure 4.1) which have been widely used as an indication of organic pollution impacts on marine benthic communities. Sediments at the point of maximum pollution are generally devoid of life but as the organic input decreases slightly, the sediments become colonised by large numbers of small opportunistic organisms. Biomass and the number of species remain low but at the peak of opportunists (**PO**), there is a temporary peak in biomass which is due to the shear number of organisms. As the organic input further decreases, the number of species increases whilst abundance and biomass both decrease. This area of low biomass, caused by the dramatic decrease in animal abundance, is known as the ecotone (**E**) and marks the point where two distinct community types merge. Throughout the transition zone (**TR**), as organic input further declines, there is a progressive change from a community characteristic of polluted sediments, through a community which benefits from slight organic enrichment, to one which is characteristic of undisturbed conditions. It is worth noting that this generalised model has been tested and validated in subtidal, marine areas where species diversity is considerably higher than that in an estuary. Given that estuarine communities are characterised by low species diversity and high abundance, it is possible that such clear trends, as shown in Figure 4.1, might not necessarily be observed.

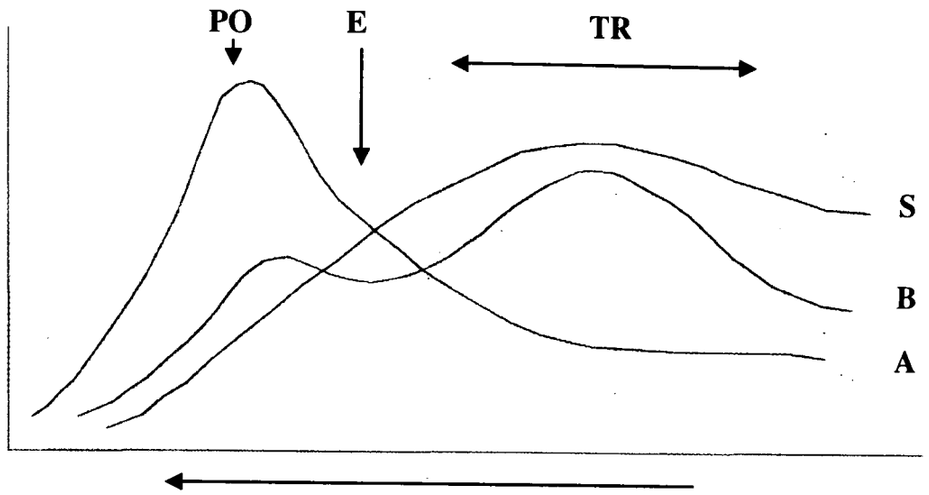


Figure 4.1. Generalised SAB diagram (re-drawn from Pearson & Rosenberg, 1978) showing changes in species (S), abundance (A) and biomass (B) along an organic gradient. PO = Peak of opportunists; E = ecotone point; TR = transition zone.

4.1.2. Detecting community change

Most approaches to determining the impact of a pollutant involve the analysis of community composition (i.e., has it changed from normal) (McManus & Pauly, 1990; Elliott, 1994; Little, 2000). Statistical methods for analysing changes in community structure may be univariate, graphical / distributional or multivariate (Gray *et al.*, 1988; Warwick & Clarke, 1991). Elliott (1994) gives a review of the techniques commonly used in the interpretation of benthic community data. Univariate measures are useful in that they can be used to determine whether or not community differences can be attributed to conditions causing stress (Gray *et al.*, 1988) since they generally give an absolute or derived value. This can be used to make an assessment of the severity of the change, on a temporal or spatial scale (Warwick & Clarke, 1991). Univariate techniques for the comparison of the responses of macrofaunal communities to pollution range from the use of simple statistics such as species number (S), abundance (A) and biomass (B), A/S and B/A ratios (where a high value of A/S together with a low value of B/A would indicate a disturbed community) (Gray *et al.*, 1988). More complex methods involve the analysis of size / biomass spectra (Schwinghamer, 1988) or the examination of the trophic structure of the community (e.g. the UK Infaunal Trophic Index (UKITI), Codling & Ashley, 1992; Allen, 2000b).

Environmental variability in estuaries presents problems when trying to find control sites for comparison. Several attempts have therefore been made to devise a method whereby no control site is necessary (Dauer *et al.*, 1993). These involve the use of indicator species (Pearson &

Rosenberg, 1978) and the nematode:copepod ratio (Raffaelli & Mason, 1981). However, all have their associated problems and none has been widely accepted. For the purpose of this study, the use of indicator species was not considered appropriate since there are very few species present in this part of the estuary. Many of these are classed as indicator species which would give no more information than changes in abundance between sites. The nematode:copepod ratio does not always give a good indication of pollution induced stress and it was considered beyond the scope of this study to use a meiofaunal technique such as this.

Graphical techniques include the use of the log-normal distribution (Gray, 1979; 1981) and the use of *k*-dominance curves (Lambshead *et al.*, 1983; Platt *et al.*, 1984, in Magurran, 1988), where cumulative percent dominance (in terms of abundance or biomass) is plotted against the species rank, on a logarithmic scale (Warwick, 1986). Warwick (1986) proposed a variation on the use of these curves whereby the abundance and biomass curves were overlaid on the same graph. This method is known as the ABC (abundance-biomass comparison) method and is based on the assumption that unstressed or stable environments are characterised by one, or few large species, each represented by few individuals. Whilst these species are rarely the numerical dominants in marine or estuarine communities, they are dominant in terms of the biomass. In contrast, stressed communities are characterised by high numbers of short lived *r*-strategists with small body size, a high reproductive capacity and a variable population size. This method, together with the SAB method (Pearson & Rosenberg, 1978), indicates the value of assessing the biomass of whole communities or for individual taxa (Elliott, 1993; Elliott, 1994).

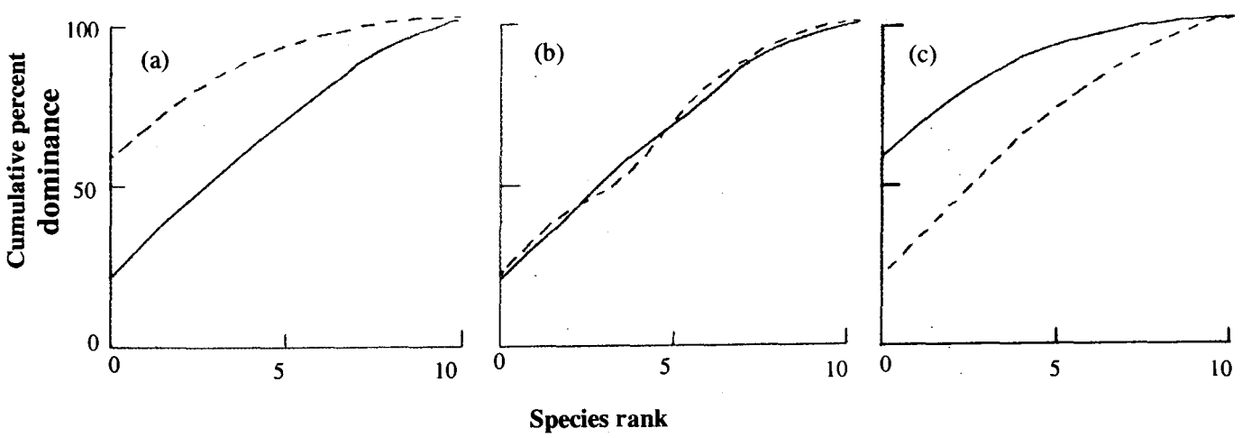


Figure 4.2. Hypothetical *K*-dominance curves for species abundance (—) and biomass (-----) showing unstressed (a), moderately stressed (b) and stressed (c) conditions (re-drawn from Warwick (1986)).

In an unstressed community, the biomass curve would lie above the abundance curve, implying that the total biomass of species 1 (the highest ranking species) was greater than the abundance of the highest ranking (in terms of abundance) species in terms of percent contribution to the community. In contrast, the curves for a polluted, or otherwise disturbed community, would be reversed with the abundance curve being above the biomass curve. This would then imply that the large number of organisms associated with species 1 did not make a proportional contribution to the biomass. In moderately disturbed communities, the curves would lie close to each other, perhaps crossing. The distance between these curves can therefore be used as an indication of the degree of stress to which the community is subjected. Clarkes *W* statistic (Clarke, 1990; Warwick & Clarke, 1994) is a single statistic describing the degree and direction of the separation of these curves. It is appropriately scaled so that the difference between the two curves gives a value of -1 in the case of abundance domination (i.e. stressed), and a value of +1 in the case of biomass domination (i.e. unstressed). In this method, one curve acts as an internal control against which the other can be compared, therefore eliminating the need for reference to control samples (Warwick, 1986; Clarke & Warwick, 1994).

Another useful, but widely debated, measure of community health is the diversity index (Southwood, 1978; Krebs, 1980; Magurran, 1988). Simple measures of diversity include recording the number of species present (Fowler *et al.*, 1998) but according to Krebs (1980) and Magurran (1988), diversity is a measure of both species richness and the relative abundance of each species (evenness or equitability). A community with high evenness, which is not dominated by one or very few species, is considered to be more diverse than one with low evenness (Clarke & Warwick, 1994). According to Clarke & Warwick (1994), the Shannon-Weiner diversity index (H') is the most commonly used diversity index, incorporating both species richness and evenness. Margalef's index (D_{Mg}) is an expression of species richness which incorporates the number of individuals to give a measure of the total number of species for a given number of individuals. In terms of evenness or equitability, Pielou's index, J' (Pielou, 1975) is the most commonly used (Clarke & Warwick, 1994).

As already stated, the use of diversity indices has been the subject of considerable debate. Washington (1984) provides a review of the relative merits and drawbacks of the various indices which have been derived. This review does not provide any insight into which indices provide the most effective measure of diversity. Dauer *et al.*(1993) state that all measures of the biological effects of pollution are subject to error, particularly because of the immense variability inherent in real population dynamics and 'spot' sampling strategies. Therefore, no single method of analysis is likely to give reliable pollution stress classifications and it was recommended that several complimentary methods be used to ensure robustness (Elliott, 1994).

It is not the aim here to further evaluate the use of the various measures of pollution induced stress but to highlight the methods which have been developed in the past. In terms of univariate statistics, the SAB method (Pearson & Rosenberg, 1978) together with the ABC method (Warwick, 1986) will be used in order to determine the impact of the BP effluent on the mudflat communities at Paull and Saltend. In addition, the diversity indices H', J' and D_{Mg} will be used in combination to provide a range of diversity calculations, each with a slightly different emphasis. The justification for choosing these indices over the others which are available is the fact that they have been so widely used by other authors (Clarke & Warwick, 1994). However, there is an inherent circular argument here: it is recognised that the more frequently a technique is used, the more easily it becomes established as a standard method and therefore, the more frequently it is chosen above all other techniques. This does not, however, mean that it is the best technique.

Univariate techniques have the advantage that a value can be attached to the observed changes (e.g. Clarkes *W* statistic or a diversity index) which can be used to determine whether or not these community differences can be attributed to conditions causing disturbance or stress (Warwick & Clarke, 1991). However, these techniques are not species specific and two communities with entirely different taxonomic compositions could appear to have the same structure using these techniques (Warwick & Clarke, 1991). Multivariate techniques compare communities on the basis of their component species and their relative importance in terms of abundance and biomass. Species dependent, multivariate, methods have repeatedly been found to be more sensitive than univariate or graphical methods in discriminating between sites of times (Warwick & Clarke, 1991). In addition, the multivariate methods gave consistent results when used on different components of the fauna (e.g. macrofauna and meiofauna) whereas univariate and graphical techniques did not. A commonly used technique is the calculation of Bray-Curtis similarity coefficient (also know as the Czekanowski coefficient) which represents the overall similarity between each pair of samples, taking the abundance or biomass of all species into consideration (Clarke & Warwick, 1994). The Bray-Curtis similarity coefficient does not give equal weighting to rare and common species and therefore, no information about the prevalence of a species is lost (Clarke & Warwick, 1994).

Similarity matrices, showing the Bray-Curtis similarity coefficient for each pair of sites, can be represented graphically in the form of a dendogram using hierarchical agglomerative clustering. This allows the identification of groups of samples or sites with a distinct community structure, implying that the different patterns of the species present and their abundance or biomass occur consistently within the different groups (Clarke & Warwick, 1994). Alternatively, the Bray-Curtis similarity matrix can be used to create an ordination of the samples using

multi-dimensional scaling (MDS) (Bray & Curtis, 1957) where the distances between pairs or groups of samples reflect their dissimilarity (Clarke & Warwick, 1994). Bayne *et al.* (1988) indicated that the choice between using hierarchical clustering or MDS was largely one of personal preference although it is emphasised that at least two complimentary techniques should be used to indicate robust trends.

Whilst being more sensitive than univariate techniques, multivariate techniques give no indication of the underlying causes of species differences between sites and should therefore be used to complement, rather than replace the use of univariate techniques (Gray *et al.*, 1988; Warwick & Clarke, 1991; Elliott, 1994).

4.2. AIMS

It is hypothesised that the number of species and individual biomass will be reduced within the vicinity of the BP discharge whereas organism abundance will be increased. In addition, changes in abundance and biomass are expected to occur on a seasonal basis as a result of changes to the sediment characteristics, food availability, weather conditions, recruitment and predation pressure. Structural changes to the community, brought about by pollution or seasonality will have an impact on bioturbation potential, as described in Chapter 5.

The aims of the present chapter are therefore as follows:

- Determine spatial and temporal changes in community structure;
- assess the impact of the BP discharge on the benthic communities in the vicinity of the outfall in terms of species present, abundance, biomass and size and biomass spectra;
- identify a number of different communities with which bioturbation experiments can be carried out.

The above will be carried out using a variety of univariate and multivariate statistical techniques and the sediment quality data presented in Chapter 2 will be used to explain differences in community structure.

4.3. METHODS

4.3.1 Sample collection and laboratory analysis

Five 15 cm cores (68 mm i.d.) were taken from the four key sites (Figure 1.3) with three replicate cores being taken from all other sites. The cores were sieved through a 300 μm sieve in order to retain the macrofauna and the larger meiofauna and the sieve residue preserved in a solution of 4% formaldehyde with Rose Bengal vital stain. Samples were left for at least 24 hours to allow staining to take place and rinsed over a 212 μm sieve before sorting. Animals were identified to species level using a binocular microscope and their abundance recorded as numbers / m^2. It was assumed that the impact of nematodes and oligochaetes on the sediment would be the same regardless of the species and it was therefore not considered necessary to identify these organisms to species level. However, to complete the data set for wider interest, for the June 1999 samples, subsamples of oligochaetes (50 worms / sample) were mounted on slides in lactophenol, cleared by warming at 35°C for approximately 35 minutes (or for a sufficient length of time to remove enough stain to allow identification) and examined under a high power microscope.

Abundance was expressed as the number of animals m^{-2} and biomass was recorded for individual species as wet (tissue dry) and shell free weight. Both of these parameters were calculated as total abundance and biomass for individual species and for the whole community. Size frequency measurements were carried out on samples from the four key sites for June 1999 and January 2000 in order to cover periods of high and low abundance and biomass. Length measurements were carried out under a binocular microscope, using a graticule with 0.1 mm divisions, as follows:

Hediste diversicolor	peristomium width
Corophium volutator	telson to rostrum
Macoma balthica	Shell length across widest point
oligochaetes, nematodes, spionids, *Manayunkia*	body length (whole animals only)

The length of individuals of *Hediste diversicolor* was calculated from peristomium width using the regression equation $y = 19.115x + 0.5248$ ($r^2 = 0.871$, $p<0.01$), as shown in Figure 4.3.

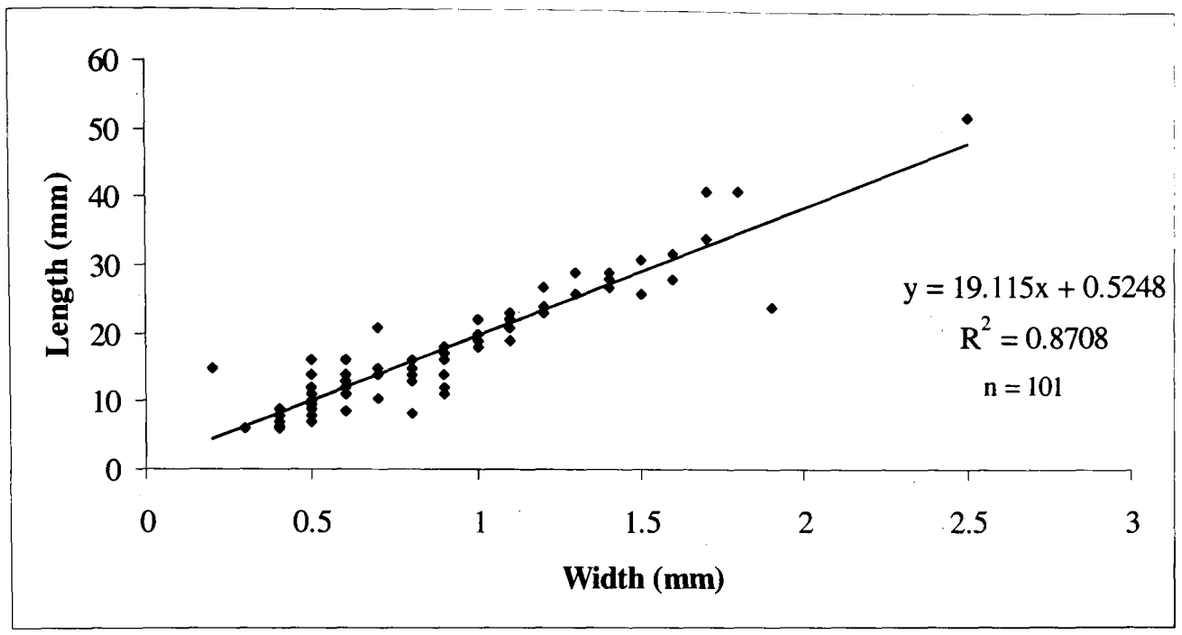


Figure 4.3. Relationship between body length and peristomium width for *Hediste diversicolor*.

Biomass frequency analysis was also carried out for June 1999 and January 2000 samples for the four core sites. For incomplete worms, the regression equation $\log_{10}y = 2.9208 \times \log_{10}x + 1.0398$ was used ($r^2 = 0.9404$, $p<0.01$) to calculate wet biomass from peristomium width, as shown in Figure 4.4.

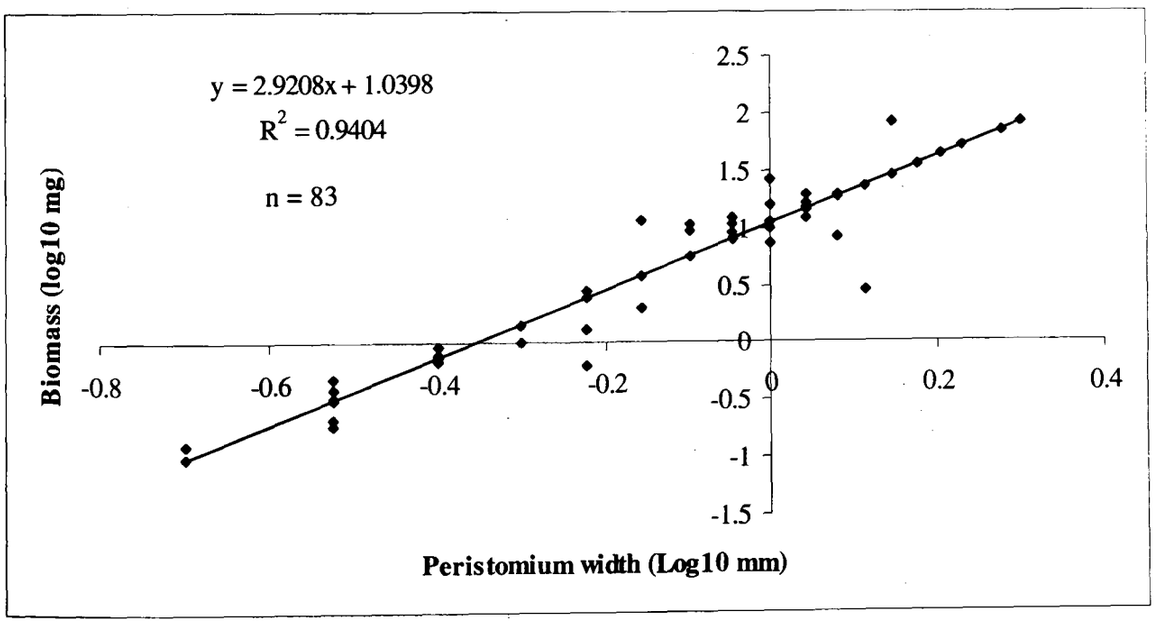


Figure 4.4. Relationship between peristomium width and wet weight biomass for *Hediste diversicolor*.

With the exception of oligochaetes, all animals in the sample were measured and weighed to give sufficient representation of the different size/biomass classes. Due to the abundance of oligochaetes, subsamples of 30 worms / replicate (150 worms in total) were taken and corrections made, as shown below, to give the true abundance of worms in each size class.

Total number of worms in sample / number or worms measured

This gave a correction factor by which the number of worms in each class could be multiplied to give an estimation of the number of worms in each class for the whole sample.

4.3.2. Univariate statistics

The relative composition of the species, in terms of abundance and biomass, was expressed as a percentage of the total and calculated using mean data for each site in each season. Descriptive statistics for species, abundance and biomass (mean, standard error, standard deviation, range, maximum and minimum values and 95% confidence intervals) were calculated for each data set and these variables were plotted against sites as SAB curves (Pearson & Rosenberg, 1978). Mean biomass ratio (B/A) and abundance ratio (A/S) values were also calculated. Diversity indices calculated included the Shannon Weiner diversity index, H' (Krebs, 1980; Magurran, 1988; Clarke & Warwick, 1994), species richness, D_{Mg} (Margalef's diversity index, Clifford & Stephenson, 1975, in Magurran, 1988; Krebs, 1980) and Pielous index of evenness, J' (Pielou, 1975), using the following formulae:

$$D_{Mg} = (S-1) / \ln N$$

$$H' = - \Sigma p_i \ln p_i$$

$$J' = H' / \log S$$

Where: S = number of species

N = total number of individuals

p_i = proportion of individuals in the ith species

Clarke's W statistic was calculated as a measure of community stress or disturbance using the following formula:

$$W = \sum_{i=1}^{S} (Bi - Ai) / [50(S-1)]$$

where species abundance and biomass are ranked and the proportion of each species' contribution calculated. The cumulative percent abundance of species i (A_i) is then subtracted from the respective cumulative percent biomass (B_i) value. The sum of these values is multiplied by 50 and divided by $S-1$ where S is the number of species.

The data were tested for homogeneity of variance (Levene's test) and statistical testing of site parameters was by one way analysis of variance (ANOVA) followed by *a posteriori* comparison of means (using Tukey's HSD or Scheffe's test where appropriate) (Zar, 1996). In cases where homogeneity of variance could not be achieved through transformation of the data, a Kruskal-Wallis test was used, followed by the Games-Howell test which assumes unequal variance (Games & Howell, 1976). A two way ANOVA test could have been used to simultaneously test for between site and between season differences. However, the size of the data set combined with the inequality of the variences, in most cases, prevented the generation of any easily interpretable or meaningful results.

4.3.3. Multivariate statistics

The Bray-Curtis coefficient was calculated for each pair of samples to create a similarity matrix with which cluster analysis (using group average linking) was performed, as described by Gray *et al.* (1988). Dendograms were plotted using both replicate data and mean data in order to ensure that replicate samples were sufficiently similar. All multivariate analyses were carried out using PRIMER (Plymouth Routines In Multivariate Ecological Research). Clarke & Warwick (1994) stated that similarities calculated using original abundance or biomass data can often be dominated by a small number of highly abundant species so that they fail to reflect similarities between the overall composition of communities. Data are therefore commonly transformed in order to define a balance between contributions from common and rarer species. Clarke & Warwick (1994) recommended the use of the 4^{th} root transformation which retains quantitative information but down-weights the importance of highly abundant species. However, the dominance of one species may be an important characteristic of a community (e.g. the dominance of opportunistic species in organically polluted sediments), in which case, down-weighting their importance would be inappropriate. Analysis was therefore carried out using both original data and data which had been subjected to a number of transformations of increasing severity to ensure that actual patterns in the data were not masked by the method of analysis.

4.3.4. Statistical analysis of size / biomass frequency data.

Frequency histograms of mean length / biomass were plotted on a $\log_{10}$ scale to account for the wide range in size classes, for each of the key sites in June and January. Differences between mean length/biomass were calculated from grouped frequency data and plotted as means ± 95% CL. 95% confidence limits were calculated as follows:

$n<30$ $\quad 95\%\ CL = \bar{x} \pm (t \times SE)$

$n>30$ $\quad 95\%\ CL = \bar{x} \pm (1.96 \times SE)$

where t = the tabulated value of t for a given number of degrees of freedom and 1.96 represents the number of standard deviations to either side of the mean equal to 95% of the total area under a normal curve (Elliott, 1977; Fowler *et al.*, 1998)

Prior to any statistical analysis, data were standardised to percent to account for the different sample sizes. There appears to be no single, objective, means of statistically examining differences between frequency distributions and therefore several methods were attempted. Friedmans test (a non parametric, multiple paired t-test) and a two sample Kolmogorov-Smirnov test (Cryer *et al.*, 1986; Zar, 1999; Dytham, 2003) were used to determine differences between the frequency distributions for each site and season. The Kolmogorov-Smirnov test examines the overall difference between the observed distribution of a data set against the expected distribution (e.g. a normal distribution). The two sample test allows comparison of the distributions of two data sets. Although Dytham (2003) states that the Kolmogorov-Smirnov test was originally considered to be appropriate for continuous data, Zar (1999) states that it is also applicable to discrete data sets. Both the Friedmans and the Kolmogorov-Smirnov tests give a statistic indicating whether or not there is an overall difference between the two distributions but neither test gives any indication as to where the differences lie. Cluster analysis was therefore used to aid the interpretation of the data, in addition to a linear index of selection, originally used as an index of food selection (Strauss, 1979, Cowx *et al.*, 2001):

$$\mathbf{L = r_i - p_i}$$

where r_i is the proportion of measurements in class *i* for site *a* and p_i is the proportion of measurements in class *i* for site *b*. A positive value would indicate a greater proportion at site *a* for a particular size class. Values of L were then plotted against size or biomass class, for each pair of sites, to highlight which classes differed from each other in terms of the proportion of measurements in each.

Polymodal decomposition of length/biomass frequency distributions was carried out according to Battacharyas (1967) method, using FiSAT (version 0.1. FAO-ICLARM Fish Stock Assessment Tools). This is a goodness of fit test and is generally used to demonstrate the growth of fish, calculating a separation index between two modes calculated from a normal curve fitted by eye. Unfortunately data sets could not be superimposed on one graph and separation indices between modes at different sites could not be calculated. Therefore, modes from each site were plotted and differences determined from the 95% confidence limits. Values of less than 0.25 cannot be input into FiSAT and therefore values less than this had to be summed and the class widths adjusted from 0.1 to 0.25. The modes calculated using FiSAT therefore differ slightly to those shown by the histograms.

The above analyses were carried out on data for all species combined, to give a total size / biomass frequency distribution for each site, and on frequency data for oligochaetes and *Hediste diversicolor*. There were too few data for all other species to carry out the latter analysis.

4.4. RESULTS

4.4.1. Seasonality

4.4.1.1. Species, abundance and biomass

Figures 4.5-4.6 show seasonal trends in abundance, biomass and species data for the four key monitoring sites between June 1999 and April 2000. Whilst there is variation in the data between months for all sites and not all sites follow exactly the same patterns, Figures 4.5 and 4.6 do show a general trend of lower abundance and biomass in the colder months (November and January). In terms of abundance, at the Saltend 25 m site mean values range from a minimum of 2147 individuals m^{-2} in January to a maximum of 128093 individuals m^{-2} in June with high abundances also occurring in September and April. Differences between abundances recorded in January and June are significant at the 1% level with differences between September and June and February and June being significant at the 5% level. Maximum abundances occurred in February (72252), April, June and July at the Saltend 75 m site with a minimum of 16834 in November. Statistical analysis was carried out on $\log_{10}$ transformed data in order to achieve homogeneity of variances within the data set. Significant differences were found between February, April, June and November ($p<0.01$) and July and November ($p<0.05$). Abundance at the Saltend 200 m site remained relatively stable between months with no particular seasonal trend. In contrast to the other sites, maximum abundance occurred in November (32212) with abundance recorded in January (13657) being significantly lower ($p<0.05$) than that recorded in April, July, September and November. Abundance also remained stable throughout the year at Paull (150 m) with no significant variation between months.

Biomass also shows a clear seasonal trend with minimum values generally being recorded in November or January and maximum values being recorded in April or June. The highest biomass at the Saltend 25 m site was 117 g m^{-2}, recorded in April. This is significantly greater ($p<0.01$) than the biomass recorded in January (33 g m^{-2}), June, July and September. This site had a higher winter biomass than that recorded at any of the other sites. Biomass at the 75 m site ranged from 17 g m^{-2} in November to 112 g m^{-2} in April. The November biomass was significantly lower than that recorded in most of the summer months ($p<0.01$) and the January value was also relatively low. Summer biomass was considerably higher at the Saltend 200 m and Paull sites that at the other two sites with values ranging from 19 g m^{-2} in November to 147 g m^{-2} in April at the 200 m site. The November biomass was found to be significantly ($P<0.01$) lower than that recorded in all months except January and February. At Paull, biomass ranged from 10. g m^{-2} in November, the lowest recorded biomass for all sites, to 148 g m^{-2} in June which was the highest recorded biomass between all sites. Again,

November values were found to be significantly lower ($p<0.01$) than summer values (June and July).

Averaging the data over the entire mudflat (Figure 4.7) for Saltend showed a more obvious pattern of seasonality with peak abundance occurring during the warmer months in April, June and July and minimum abundance in January. This was carried out by collating the abundance and biomass data from all sites on each mudflat and calculating the mean for each month. This should therefore not be treated as an estimate of abundance and biomass for the whole mudflat, rather as an average of the collective data from each site.

A one-way ANOVA test (with Scheffe's test) on log transformed data showed the difference between the January (25442 individuals m^{-2}) minimum and July maximum (79515 individuals m^{-2}) to be significant ($p<0.05$). Significant differences were also found between summer and winter values of biomass where April and June figures were considerably higher than the other months. Biomass ranged from 16 g m^{-2} in November to 148 g m^{-2} in April. However, the data for the S25 m and S50 m sites were missing for November and the total mudflat biomass for this month was therefore an underestimate and should be treated with caution. Despite this, biomass is still clearly higher during the warmer months at the Saltend site. No seasonal trends were apparent at the Paull site and abundance was considerably lower ($p<0.05$) than at the Saltend site for all months except January and November. Abundance at this site ranged from 8329 individuals m^{-2} in September to 22789 individuals m^{-2} in February. In contrast, biomass showed a clear seasonal pattern, reaching a maximum in April and June (148 g m^{-2}). The minimum biomass value, recorded in November, was 11 g m^{-2}. Biomass at Paull did not differ significantly from that at Saltend except in June when it was considerably higher and in September when it was much lower.

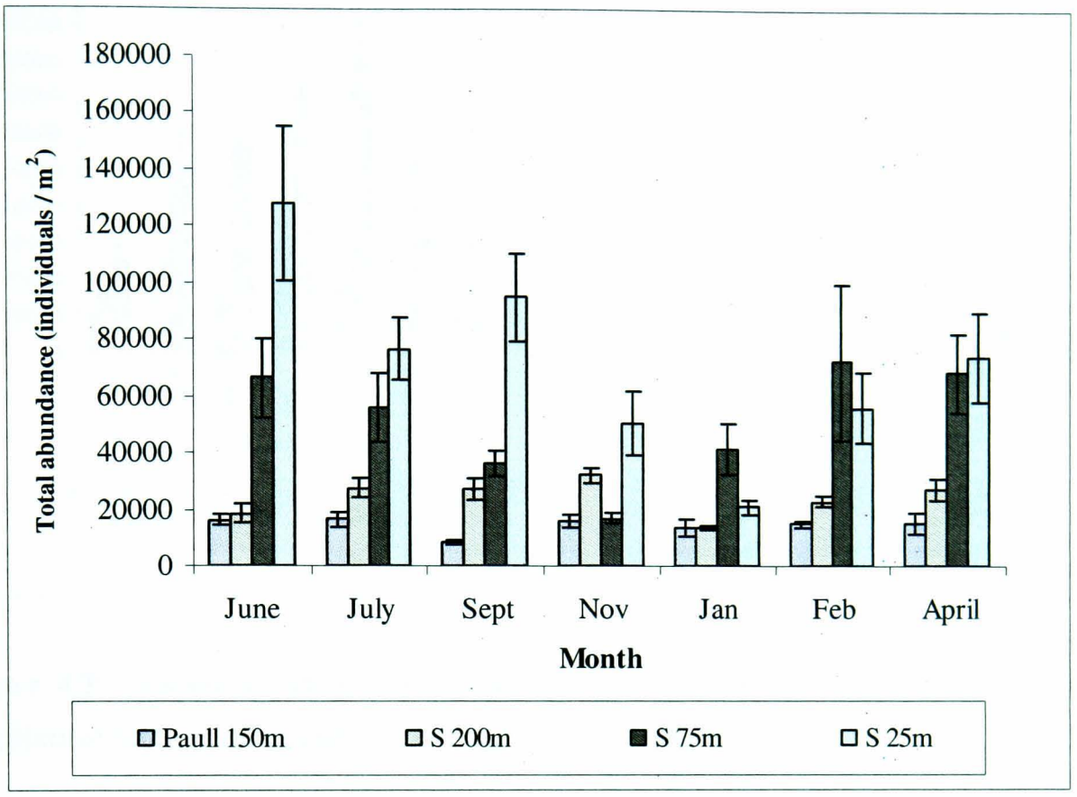


Figure 4.5. Trends in abundance (mean ± SE) at the key monitoring sites between June 1999 and April 2000.

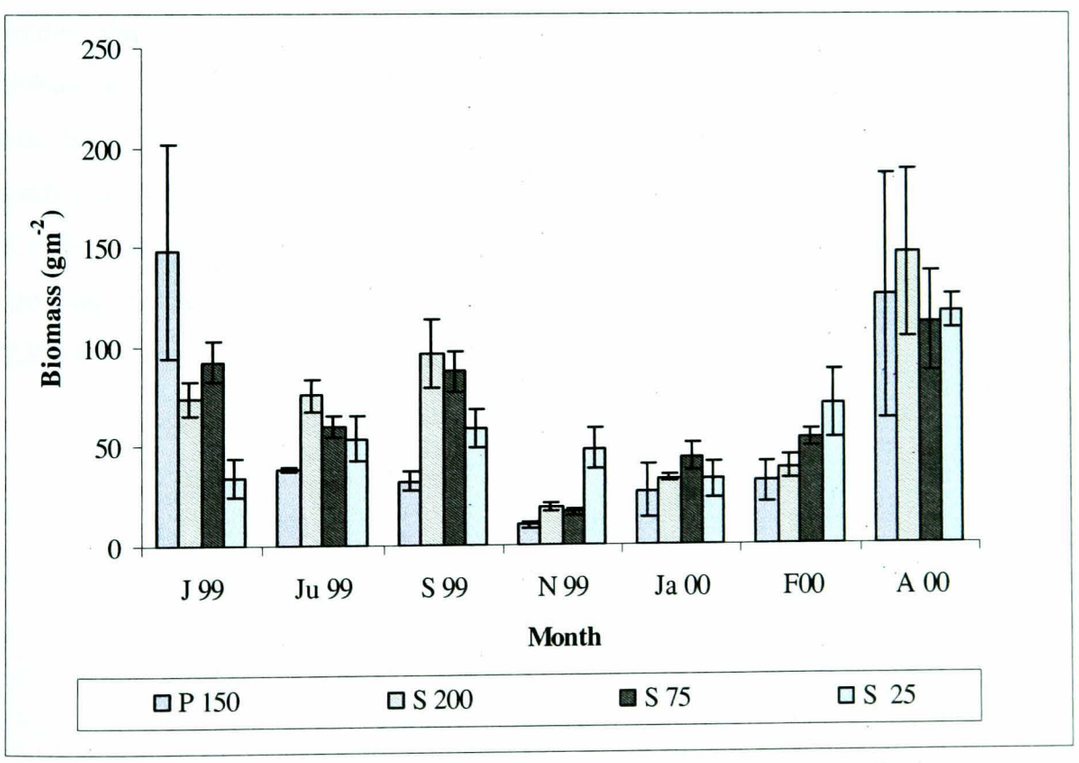


Figure 4.6. Trends in biomass (mean ± SE) at the key monitoring sites between June 1999 and April 2000.

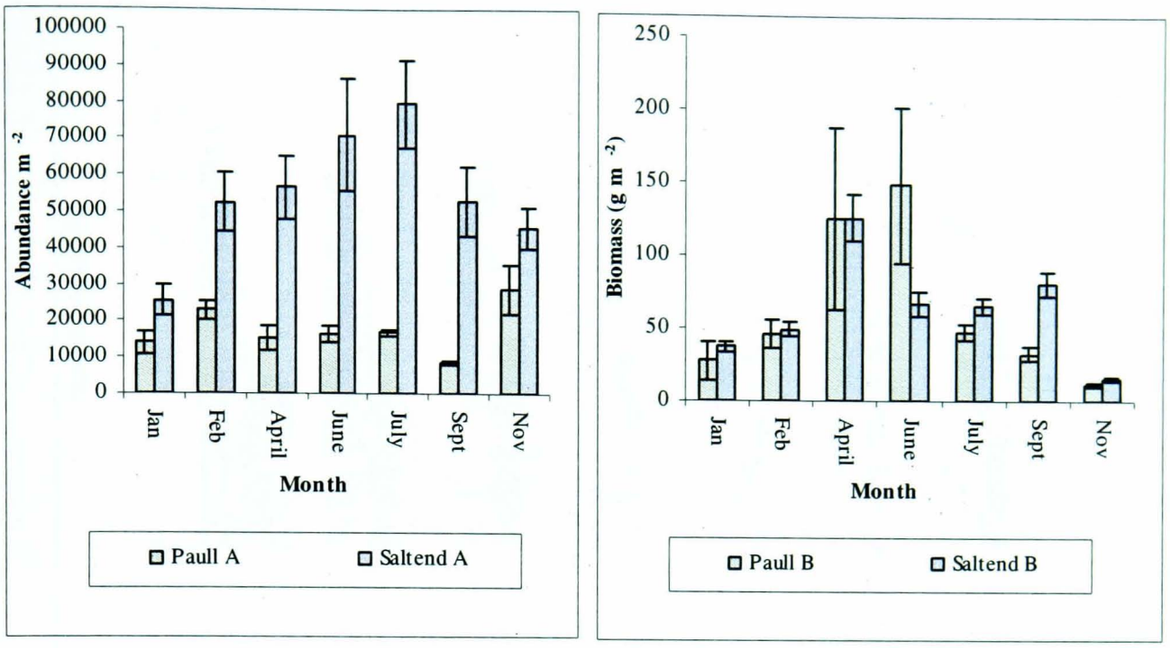


Figure 4.7. Seasonal patterns in abundance and biomass averaged over the entire mudflats at Saltend and Paull (A = Abundance; B = Biomass).

The number of species present (Figure 4.8) was low for all sites and did not change significantly over time or show any clear seasonal pattern at any of the sites. The exception to this was at Saltend 75 m where the number of species present was higher ($p<0.05$) in September than in January, February, June or July. This is due to the presence of both *Corophium volutator* and *Manayunkia aestuarina*, as shown in Figure 4.11 and Appendix 2A. Despite this, the highest number of species was generally recorded between April and September with minimum numbers of species generally being recorded in January, February, April or September. The number of species present increased between the Saltend 25 m site and the control site at Paull with mean numbers of species being in the range of 2.2-2.6 (25 m); 2.8-4.6 (75 m); 4.8-6 (200 m) and 4.75-6.5 (Paull).

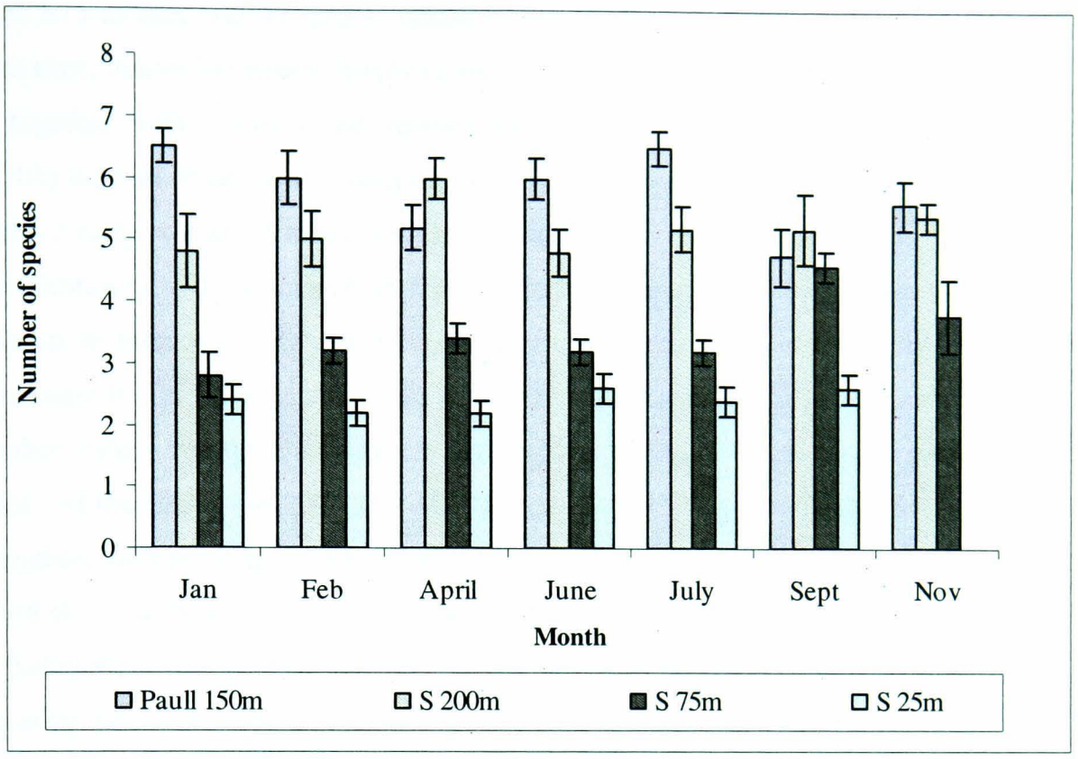


Figure 4.8. Trend in number of species (mean ± SE) present over time (November data missing for S25 m).

4.4.1.2. Diversity

Diversity indices and the abundance (A/S) and biomass (B/A) ratios for the four main sites are presented in Table 4.1. For all three diversity indices, values for Paull and S200 were consistently higher than those at the S75 and S25 m sites, with diversity at the S75 m site also being higher than that at the S25 m site ($p<0.01$).

At the Paull site, all three indices followed the same trend with peaks in January, July and November although there was no apparent seasonal pattern. Minimum values occurred in September with the maximum being in January, ranging from 1.09 – 1.65; 0.7-0.88 and 0.42-0.58 for H' (diversity), J' (evenness) and D_{Mg} (species richness), respectively. A one-way ANOVA, followed by Scheffes test revealed that for H', January values were significantly higher than April, June or September values ($p<0.05$). At the 200 m site, D_{Mg} and H' both increased between January and April / June and decreased to November, showing slightly elevated diversity during the summer. However, these differences were not statistically significant, indicating that no seasonal patterns exist at this site. Maximum values of H' and D_{Mg} occurred in April and June (1.3 and 0.5, respectively) with the minimum being in November (0.98 and 0.42, respectively). J' showed a pattern of slight decline between January (0.75) and November (0.59) but was relatively stable throughout the year.

At the S75 m site, both H' and J' remained relatively stable between January and July after which time, values increased sharply to reach a maximum in November. A one-way ANOVA test together with Tukey's test showed that November values were significantly higher ($p<0.01$) than all other months with the exception of September, indicating higher diversity in autumn / early winter. Values were in the range of 0.39 (January) – 0.91 (November) and 0.33 (February) – 0.7 (November) for H' and J', respectively. The value of D_{Mg} was at a minimum in January (0.17), remaining relatively stable throughout the summer until reaching a maximum (0.35) in September. The September value was significantly ($p<0.05$) higher than all other values except November, again indicating higher diversity in autumn and early winter. At the S25 m site, H' and J' showed a general pattern of decline between January and September, both reaching a minimum in July. Values ranged from 0.8 to 0.38 for H' and 0.1 to 0.48 for J' with the January value of J' being significantly greater ($p<0.05$) than the July and September values. This indicates higher diversity during the colder months. Values of D_{Mg} were relatively stable throughout the year, being only slightly elevated ($p>0.05$) in January and September. The maximum (0.14) value occurred in September with the minimum being in February (0.11).

In terms of the abundance ratio (A/S), values at all sites were high indicating dominance of the community by few species occurring in high abundances. With the exception of September and November (at the S75 m site), S25 m and S75 m values were consistently higher than Paull and S200 m values ($p<0.01$). Values at the Paull and S200 m site did not differ but S25 m values were notably higher than those at the S75 m site in April, June, July and September ($p<0.01$). In contrast, the Paull and S200 m values of B/A were higher than those at the S25 and S75 sites for the majority of the year, particularly in April and June ($p<0.01$). This indicates a higher mean individual biomass at Paull and S200 m. Paull values were also considerably higher than S200 values during these months ($p<0.01$). As with diversity, the differences between sites were most pronounced during summer and in September, November and January, the S75 m site did not differ to the Paull and S200 m sites.

At the Paull site, A/S values increased steadily from January to reach a maximum of 3016 in April and then declined to a minimum of 1773 in September. The biomass ratio reached a maximum in spring and early summer with lower values occurring in July and during the winter months. Values range from 0.0007 in November to 0.01 in April and statistical testing revealed significant differences ($p<0.05$) between maximum and minimum values for B/A. A/S values for the 200 m site showed a pattern of steady decline throughout the year, ranging from 3115 in January to 5991 in November. Statistical analysis revealed no difference between months and therefore no pattern of seasonality. As at the Paull site, the biomass ratio

reached a maximum in spring and early summer with lower (but not significantly) values during the colder months. Values ranged from 0.00062 in November to 0.006 in April. At the 75 m site, A/S values ranged from 4707 in November to 23349 in February and, again with values generally being slightly higher between February and July than between September and January. In terms of B/A, values tend to increase throughout the summer to reach a maximum of 0.003 in September. Minimum values occurred in November and February (0.001) although differences between maximum and minimum values were not statistically significant. Seasonal trends were clearer at the S25 m site where A/S values increased sharply from the January minimum (8793) to the June maximum (53638), a difference which was significant at the 5% level. No such pattern of seasonality was evident for the B/A values where maximum values were in April (0.002) and minimum values were in June (0.0003) ($p<0.01$). In general, summer and early autumn values were lower than those in winter and late spring.

Table 4.1. Diversity indices, A/S and B/A ratios (mean ± SE) for June 1999 to April 2000 for the main monitoring sites.

		June	July	Sept	Nov	Jan	Feb	April
H'	P150	1.17 ±0.097	1.545 ±0.05	1.085 ±0.12	1.31 ±0.104	1.65 ±0.06	1.41 ±0.11	1.17 ±0.097
	S200	1.296 ±0.09	1.128 ±0.137	1.154 ±0.074	0.98 ±0.053	1.136 ±0.071	1.05 ±0.066	1.296 ±0.092
	S75	0.448 ±0.059	0.416 ±0.066	0.824 ±0.071	0.91 ±0.084	0.388 ±0.035	0.386 ±0.044	0.448 ±0.059
	S25	0.21 ±0.128	0.08 ±0.016	0.11 ±0.03	_	0.384 ±0.022	0.162 ±0.037	0.21 ±0.128
J'	P150	0.71 ±0.04	0.825 ±0.031	0.695 ±0.04	0.768 ±0.06	0.88 ±0.02	0.796 ±0.055	0.71 ±0.04
	S200	0.726 ±0.049	0.692 ±0.075	0.714 ±0.027	0.586 ±0.042	0.752 ±0.042	0.662 ±0.02	0.726 ±0.049
	S75	0.368 ±0.039	0.368 ±0.065	0.54 ±0.033	0.742 ±0.069	0.432 ±0.084	0.334 ±0.0395	0.368 ±0.039
	S25	0.224 ±0.108	0.098 ±0.027	0.134 ±0.044	_	0.48 ±0.069	0.21 ±0.047	0.224 ±0.108
d	P150	0.44 ±0.04	0.57 ±0.039	0.42 ±0.05	0.48 ±0.039	0.58 ±0.025	0.518 ±0.045	0.44 ±0.042
	S200	0.496 ±0.037	0.41 ±0.036	0.414 ±0.055	0.424 ±0.023	0.402 ±0.062	0.398 ±0.045	0.496 ±0.037
	S75	0.216 ±0.02	0.204 ±0.017	0.346 ±0.025	0.288 ±0.058	0.17 ±0.033	0.202 ±0.0198	0.216 ±0.021
	S25	0.114 ±0.024	0.124 ±0.02.	0.14 ±0.018.	_	0.138 ±0.023	0.112 ±0.017	0.114 ±0.024
A/S	P150	2732.5 ±362.6	2584.7 ±489.04	1772.6 ±90.52	2780.9 ±410.14	2106.1 ±413.6	2517.7 ±194.7	3016 ±750.3
	S200	4030.2 ±892.4	5361.1 ±667.9	5390.6 ±724.6	5990.8 ±534.1	3115.13 ±589.4	4726.8 ±559.6	4659.4 ±906.7
	S75	20499.8 ±3966	17255.3 ±3471.7	8081.52 ±1229.1	4707.1 ±530.3	14859.7 ±2483.3	23349.7 ±9323.2	20421.8 ±4631.3
	S25	53638.2 ±16087.3	31913.1 ±2817.8	36080 ±5097.4	_	8792.84 ±596.7	25543.3 ±5695.4	36520.6 ±8492.5
B/A	P150	0.0086 ±0.003	0.0025 ±0.0004	0.0039 ±0.0006	0.0007 ±0.00004	0.0017 ±0.0005	0.002 ±0.0006	0.01 ±0.005
	S200	0.004 ±0.0009	0.003 ±0.0001	0.0038 ±0.0009	0.00062 ±0.00009	0.0025 ±0.0002	0.0018 ±0.0003	0.0059 ±0.002
	S75	0.0017 ±0.0004	0.0015 ±0.0005	0.0026 ±0.0004	0.0011 ±0.0001	0.0013±0.0 004	0.00104 ±0.0002	0.0017 ±0.0003
	S25	0.0003 ±0.00002	0.0008 ±0.0002	0.0007 ±0.00008	-	0.0016 ±0.0005	0.0016 ±0.0004	0.0023 ±0.0009

The data for the remaining, non key, monitoring sites are presented in Table 4.2. Again, there was great variation within the data set and in some cases, significant differences between months could not be detected. Despite this, the same trend of high abundance and biomass during the summer months, with minimum values occurring in November was found at the majority of the Saltend sites. The exception to this is the S0 m site where maximum and minimum biomass was recorded in February and November, respectively and minimum abundance occurred in July. The number of species present varied only slightly throughout the year with no particular pattern of maximum or minimum values occurring in any particular month. In terms of diversity, minimum values were generally recorded in July although both diversity and evenness were both highest in July at the S100 m site. In terms of A/S and B/A, there were no obvious seasonal patterns at any of the Saltend sites although B/A tends to decline between February and November at S0 m, S100 m and S150 m.

At the Paull sites, with the exception of the P200 m site, these trends were not observed. Abundance showed a general pattern of decline between February and November with intermediate values being recorded in July. In terms of biomass, no particular seasonal trends were observed with maximum and minimum values occurring in different months between sites. There was no significant difference between months in terms of diversity (all three indices) at any of the sites although there was a general pattern of decline between February and November in species richness (D_{Mg}), diversity (H') and evenness (J') at the 25 m site. In contrast, evenness and diversity increased slightly at the 100 m site. B/A ratios were all at a maximum in July whereas A/S ratios showed no particular pattern. Minimum values were calculated in July for sites P100 m and P50 m with values declining between February and November at the P25 m site but increasing at the P200 m site.

In terms of diversity (all three indices) and B/A, values at the Paull sites were consistently higher ($p<0.01$) than those at the Saltend sites. In contrast, A/S Values were consistently lower ($p<0.01$) at the Paull sites indicating a higher level of disturbance or stress at Saltend.

Table 4.2. Species, abundance and biomass values and diversity indices for the non-key sites.

Site	S	A	B	A/S	B/A	DMg	H'	J
P25 February	6.33 ±0.33	26892.53 ±3433	28.43 ±3.16	4318.19 ±724.4	0.0011 ±0.0003	0.52 ±0.04	1.68 ±0.003	0.91 ±0.025
P25 July	5.67 ±0.33	17805.93 ±2250.11	57.59 ±6.57	3138.98 ±319.14	0.0032 ±0.000008	0.48 ±0.03	1.44 ±0.11	0.83 ±0.04
P25 November	5 ±1.53	10187.95 ±3179.47	11.03 ±1.81	2093.53 ±199.8	0.0013 ±0.0005	0.43 ±0.16	1.13 ±0.47	0.67 ±0.18
P50 February	7.67 ±0.33	28269.27 ±1883.23	67.35 ±27.71	3679.53 ±89.88	0.0023 ±0.0009	0.65 ±0.03	1.56 ±0.06	0.77 ±0.02
P50 July	5.67 ±0.33	13583.93 ±1171.81	32.44 ±2.03	2389.43 ±76.85	0.0024 ±0.0003	0.49 ±0.03	1.33 ±0.1	0.77 ±0.03
P50 November	6 ±0.58	12207.18 ±183.57	8.84 ±0.89	2067.75 ±172.6	0.0007 ±0.000008	0.53 ±0.06	1.34 ±0.05	0.75 ±0.02
P100 February	6.33 ±0.33	37539.42 ±2623.49	73.17 ±43.8	5928.77 ±305.03	0.0018 ±0.001	0.5 ±0.03	1.19 ±0.2	0.64 ±0.09
P100 July	6 ±0	16337.43 ±1923.07	46.29 ±22.31	2722.91 ±320.51	0.0031 ±0.002	0.51 ±0.01	1.4 ±0.08	0.78 ±0.04
P100 November	6.67 ±0.33	21018.38 ±875.56	11.86 ±4.33	3175327 ±252.11	0.0006 ±0.0002	0.57 ±0.04	1.53 ±0.02	0.81 ±0.03
P200 February	6 ±0.58	11564.7 ±2617.03	38.81 ±26.5	1892.05 ±263.61	0.0028 ±0.001	.054 ±0.05	1.49 ±0.09	0.83 ±0.01
P200 July	6.25 ±0.25	18260.78 ±1951.04	59.56 ±11.22	2915.62 ±276.39	0.0036 ±0.001	0.54 ±0.02	1.41 ±0.04	0.77 ±0.02
P200 November	6 ±0.58	19842.1 ±3175.64	12.8 ±3.41	3382.23 ±688.44	0.0007 ±0.0002	0.51 ±0.06	1.49 ±0.1	0.83 ±0.02
S0 February	1.67 ±.033	40109.32 ±3924.21	24.28 ±4.06	27791.12 ±4520.5	0.0006 ±0.00004	0.06 ±0.03	0.03 ±0.02	0.05 ±0.03
S0 July	2 ±0.58	29829.58 ±12559.23	11.28 ±9.2	17056.4 ±7511.68	0.0003 ±0.0002	0.097 ±0.05	0.08 ±0.04	0.1 ±0.06
S0 November	2.33 ±0.33	70673.17 ±14777.97	5.1 ±2.27	32246.54 ±9348.89	0.00007 ±0.000002	0.12 ±0.03	0.09 ±0.02	0.11 ±0.01
S50 February	4 ±0	52683.63 ±13764.13	37.92 ±5.62	13170.91 ±0.0009	0.0008 ±0.000008	0.28 ±0.01	0.85 ±0.15	0.61 ±0.11
S50 July	4.33 ±0.67	106009.8 ±52557.5	70.77 ±15.55	21507.89 ±3441.03	0.0024 ±0.0019	0.3 ±0.04	0.81 ±0.13	0.57 ±0.04
S100 February	3.33 ±0.33	115380.8 ±29180.96	75.01 ±10.4	35783.26 ±10896.9	0.0007 ±0.0002	0.17 ±0.05	0.17 ±0.01	0.17 ±0.05
S100 July	4 ±0	132810.5 ±25446.35	103.03 ±13.04	33202.62 ±6361.6	0.0008 ±0.000005	0.25 ±0.003	0.66 ±0.11	0.48 ±0.08
S100 November	4.33 ±0.88	65992.22 ±9068.92	10.84 ±1.8	15779.08 ±1956.39	0.0002 ±0.000003	0.3 ±0.08	0.35 ±0.05	0.24 ±0.021
S150 February	4.67 ±0.88	11748.27 ±3034.41	28.74 ±3.05	2432.26 ±255.51	0.0028 ±0.0007	0.39 ±0.09	1.03 ±0.23	0.67 ±0.08
S150 July	4.67 ±0.33	180537.8 ±38123.9	86.66 ±18.06	38553.59 ±6899.04	0.0005 ±0.000001	0.3 ±0.03	0.42 ±0.05	0.28 ±0.04
S150 November	4.33 ±0.33	27810.35 ±5412.1	22.38 ±4.61	6479.9 ±1410.33	0.0009 ±0.0003	0.33 ±0.03	0.77 ±0.04	0.53 ±0.04

4.4.1.3. Community structure

Figures 4.9 - 4.12 show changes in species composition, at the four key monitoring sites, throughout the year in terms of abundance and biomass. The percent contribution to the community of each species and cumulative percent in terms of abundance and biomass are presented in Appendices 2A and 2B. In terms of abundance, the dominant organisms at the Paull 150 m site were *Hediste diversicolor* and oligochaetes throughout the year, contributing from 53% to 80% to the community, in combination. Of these two, *H. diversicolor* dominated the community for all months with the exception of February with a maximum contribution of 60% in September and a minimum of 33% in January. Abundance was generally highest in summer and early autumn, ranging from 7709 individuals m^{-2} in June to 3745 individuals m^{-2} in February. Oligochaetes were most abundant in spring and early summer with numbers declining throughout the summer to reach a minimum in early autumn. Abundances ranged from 551 individuals m^{-2} in September (6.6 and 6.8% contribution in September and November, respectively) to 5672 individuals m^{-2} in April (37.27 and 37.32% contribution in February and April, respectively).

Corophium volutator, Spionidae and *Manayunkia aestuarina* were fairly evenly distributed within the community with the contribution of each being up to about 30 %, depending on the time of year. *C. volutator* appears to be most abundant in late summer and throughout the autumn. In terms of the contribution of the species to the community, a decline occurred between February and June, after which time, numbers increased to reach the November maximum. Minimum abundance occurred in April with only 275 individuals m^{-2} (1.82% of the community) with the maximum being in November (4290 individuals m^{-2}, 28% of the community). Spionidae (*Pygospio elegans* and *Streblospio shrubsollii*) were also most abundant during autumn and winter with relatively few individuals of this family being present in the summer. Maximum abundances were in November (2974 individuals m^{-2}, 19% of the community) with the minimum being in June (165 individuals m^{-2}, 1% of the community). *M. aestuarina* generally appeared in its greatest abundance during the summer, reaching maximum density in June (2478 individuals m^{-2}, 16% of the community) with relatively high numbers also being present in January and February. Minimum abundances were in September and November (0 and 110 individuals m^{-2}, respectively, contributing 0 and 0.71% to the community). However, *M. aestuarina* is a small organism, generally classed as meiofauna, and it is likely that many individuals were not retained during sieving. Therefore, seasonal patterns in the abundance of this species may not have been detected. Similarly, the newly settled larvae of all macroinvertabrate species may not be retained during sieving which may mask some of the seasonal patterns in abundance.

Macoma balthica, Nematodes and *Hydrobia ulvae* were all present in low numbers. No seasonal patterns were observed for *M. balthica* and abundance remained low but relatively stable throughout the year. Maximum abundance was in June (385 individuals m^{-2}) with maximum contribution to the community being in June and January (2.4.and 2.5%). Minimum abundance was in July (138 individuals m^{-2}) when only 0.84% of the community could be accounted for by this species. Numbers of nematodes generally increased between January and June / July, after which time they declined to reach the autumn minimum. Maximum abundances were in June (1487 individuals m^{-2}, 9% of the community) with the minimum being in September (69 individuals m^{-2}, 0.82% of the community). As with *M. aestuarina,* nematodes are also often classed as meiofauna and any pattern of seasonality seen in their abundance should be treated with caution. *H. ulvae* was absent from the community except for the appearance of a small number of individuals, contributing only 0.82% to the community, in September. Therefore, the presence of this species is considered to be of only minor importance.

The biomass at this site was dominated throughout the year by *Hediste diversicolor* and *Macoma balthica* with the other species being of minor importance. *H. diversicolor* dominated the biomass from late summer, throughout the autumn, to winter (July – January) with biomass ranging from 8.2 g m^{-2} in November to 70 g m^{-2} in June. In terms of contribution to the community, this species was most dominant in September, when it accounted for 69.5% of the biomass, and least dominant in June when it only accounted for 46.93%. The remaining species collectively made up less than 10% of the community in terms of biomass. Of these, *Corophium volutator*, spionidae and oligochaetes are of most significance. It should be noted that core sampling was only carried out to a depth of 15 cm and that larger, rarer organisms which may have been present would have been missed.

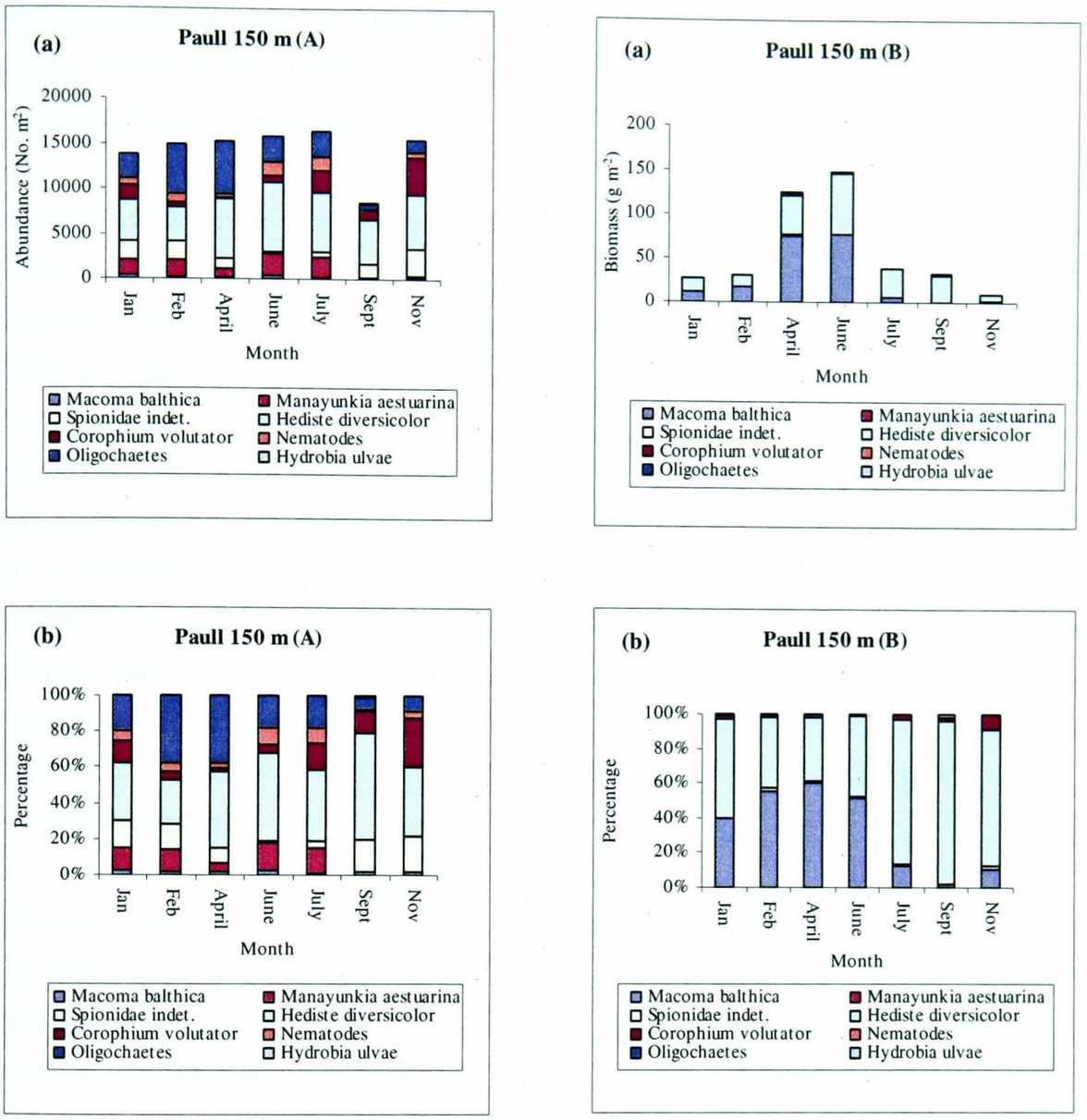


Figure 4.9. Community structure, showing actual (a) and percent (b) contribution of each species, in terms of abundance (A) and biomass (B) at Paull 150 m.

As at Paull, the S200 m site (Figure 4.10) was dominated by *Hediste diversicolor* and oligochaetes throughout the year in terms of abundance. *H. diversicolor* was the dominant organism during the winter months (January and February) with oligochates being dominant during the summer (April – July). There was little difference between the abundances of these two organisms in September and November suggesting that this marks the transition between summer dominance of oligochaetes and winter dominance of *H. diversicolor*. Maximum densities of *H. diversicolor* occurred in July (12299 individuals m^{-2}) although this is the month when this species made its minimum contribution to the community (27%). Minimum densities were in January (6058 individuals m^{-2}) with maximum dominance being in February (45%). Oligochaete density increased from January, throughout the summer to reach a maximum of 25791 individuals m^{-2} in July. A second peak occurred in November

which is when this group was most dominant (61% of the community). Minimum abundance was in January (5507 individuals m^{-2}) and September with minimum contribution to the community being in September (38%).

Numbers of *Manayunkia aestuarina* increased slightly between January and July and declined throughout September and November, indicating greatest abundance in summer. However, as previously mentioned, this should be treated with caution. Maximum abundance was in July (2570 individuals m^{-2}) with the minimum being in June (110 individuals m^{-2}). Maximum and minimum percent contribution to the community were in April (8.2%) and June (0.6%), respectively. As at the Paull site, *Corophium volutator* was most abundant in late summer and autumn with density ranging from 275 m^{-2} in February (1.2% of the community) to 3524 m^{-2} in September (13% of the community). Peak nematode densities occurred in spring and summer (with the exception of June), declining throughout autumn although there was no obvious pattern of seasonality. Densities were in the range of 330 individuals m^{-2} in September to 3121 individuals m^{-2} in July. In terms of percent dominance, nematodes made their maximum contribution to the community (9%) in February with the minimum (1.2%) being in September.

Spionid worms were present in relatively low abundances throughout the year, reaching a maximum in June (881 individuals m^{-2}, 5% of the community). Whilst numbers were relatively stable throughout the year, there was a slight tendency for abundances to be higher in winter and spring than in late summer and early autumn. Minimum densities occurred in September (165 individuals m^{-2}) with minimum percent dominance occurring in July and September (0.64%). *Macoma balthica* showed no pattern of seasonality, being present only in April, July and September. Densities ranged from 55 individuals m^{-2} in April to 110 individuals m^{-2} in September with the maximum % contribution to the community being only 0.4% (September).

The biomass at the S200 m site was dominated throughout the year by *H. diversicolor* (62% in April – 94% in November). Biomass of this species was generally highest during the summer with a maximum of 115 g m^{-2} in July and a minimum of 19 g m^{-2} in November. *M. balthica* made a significant contribution to the biomass for the months in which it was present in the community. Biomass ranged from 5 g m^{-2} (4 % of the community) in July to 42 g m^{-2} (29% of the community) in April. Again, this species did not show any trend of seasonality. Maximum oligochaete biomass was generally during summer, although the data do fluctuate, whereas percent contribution to the community was highest during the colder months between November and February (1.74% - 8.5%). Biomass was in the range of 1 g m^{-2} (November) to

9 g m^{-2} (April). Biomass of *C. volutator* was greatest during summer (April – June), declining after the June peak (7.3 g m^{-2}, 10% of the community) to a minimum of 0.1 g m^{-2} (0.3% of the community) in February. Spionid worms, nematodes and *M. aestuarina* contributed less than 0.1% to the community in terms of biomass for the majority of the year.

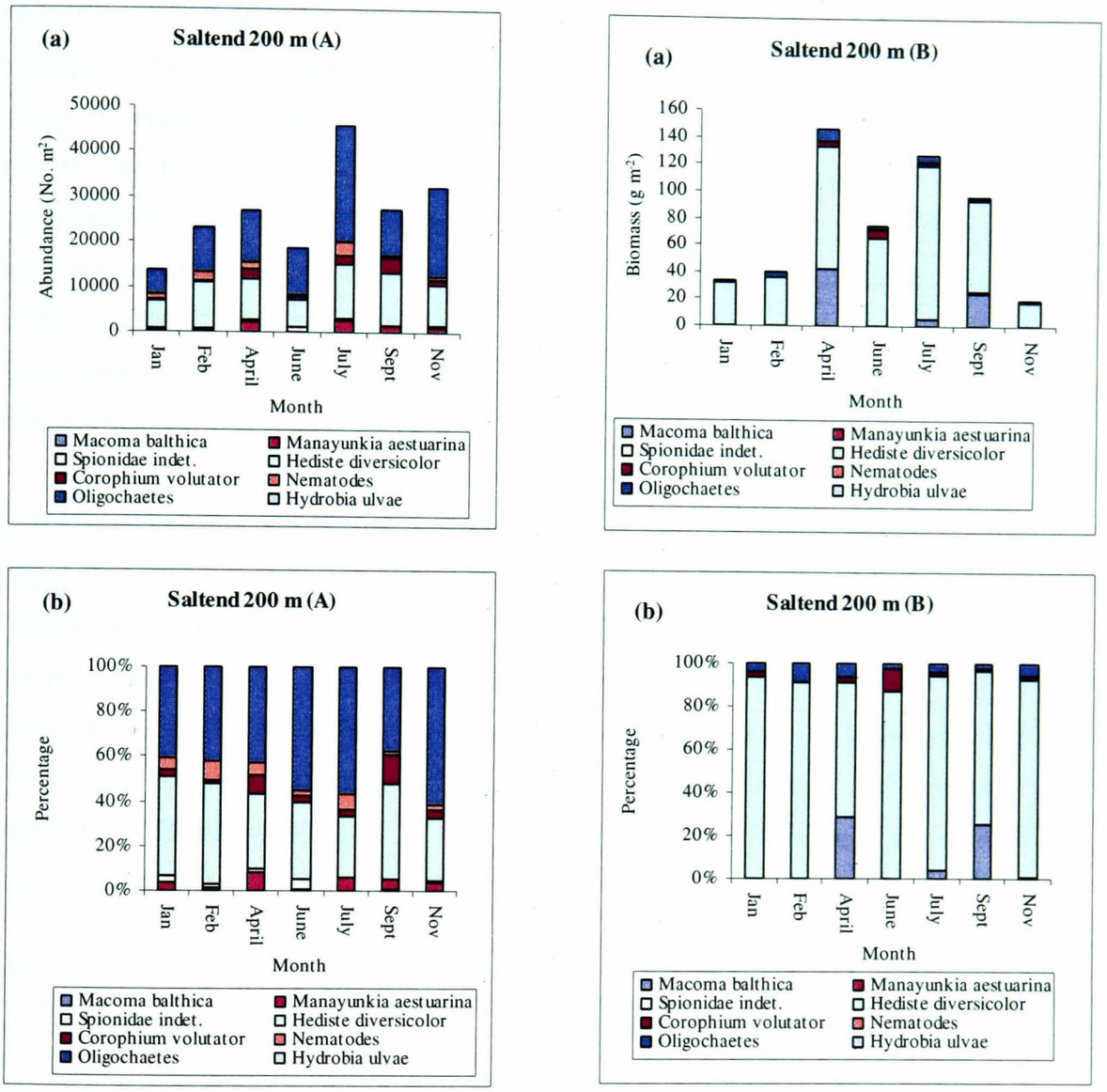


Figure 4.10. Community structure, showing actual (a) and percent (b) contribution of each species, in terms of abundance (A) and biomass (B) at Saltend 200

The S75 m site (Figure 4.11) was dominated by oligochates with maximum numbers being present between January and April. Abundance increased from January to reach a peak of 66414 individuals m^{-2} (92% of the community) in February. Numbers then declined to reach a minimum of 9014 individuals m^{-2} in November (54% of the community). There was no obvious pattern of seasonality. *H. diversicolor* was most abundant during the warmer months, showing a general pattern of increase from January to reach peaks in June and September, before declining in November. Maximum densities were in September (8426 individuals m^{-2}) although the maximum percent contribution of this species to the community was in

November (37%). Minimum abundance was in February (3910 individuals m^{-2}, 5% of the community).

Nematode numbers increased steadily between January and June then increased sharply to reach a summer peak in July (14814 individuals m^{-2}, 27% of the community) before declining throughout the autumn. Minimum abundance was in January (220 individuals m^{-2}) when this group of organisms contributed only 0.5% to the community. Although *M. aestuarina* was absent from the community in June, numbers of this organism varied little throughout the year. Maximum density occurred in September (991 individuals m^{-2}, 3% of the community) with the minimum being in January, February and July (55%) when the species accounted for only 0.008 - 0.13% of the community. *C. volutator* was present only in September and November when abundances were 275 and 110 individuals m^{-2}, respectively. This species contributed only 0.7% to the community.

In terms of biomass, the S75 m site was dominated throughout the year by *H. diversicolor* which contributed between 79% (April) and 97% (November) to the community biomass. In terms of actual biomass, this trend was reversed with values ranging from 16 g m^{-2} to 88 g m^{-2} for November and April, respectively. Oligochaete biomass was also at a maximum in April (24 g m^{-2}), contributing 21% to the community, which explains why the contribution of *H. diversicolor* was low for this month in comparison with its abundance. Minimum biomass occurred in November (0.5 g m^{-2}, 3% of the biomass). The other species present at this site contributed less than 1% to the biomass.

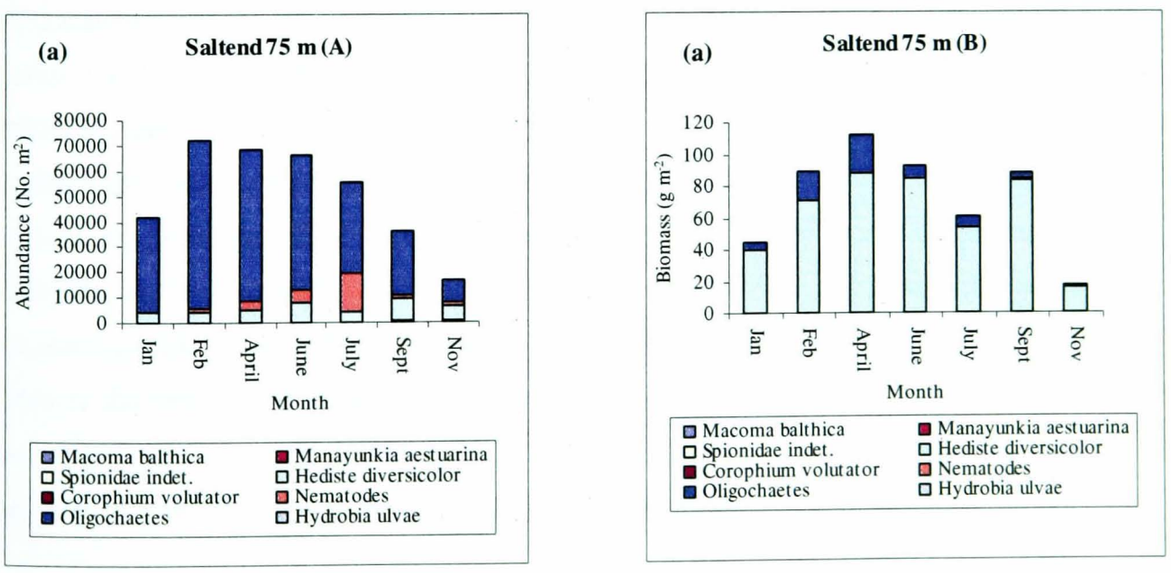


Figure 4.11. Community structure, showing actual (a) and percent (b) contribution of each species, in terms of abundance (A) and biomass (B) at Saltend 75 m

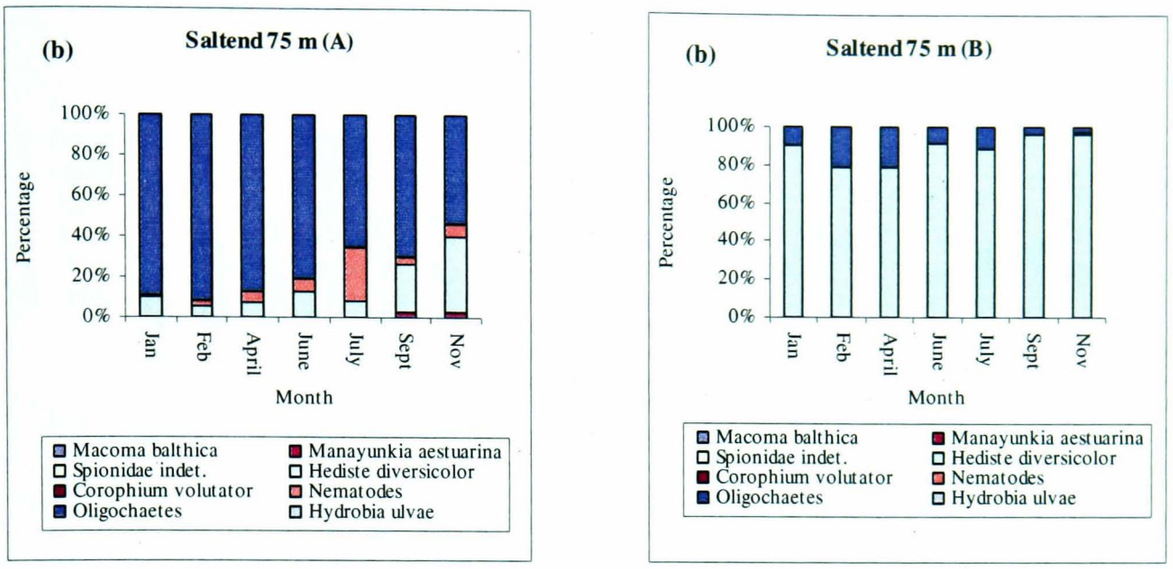


Figure 4.11. (cont.).

The community at the S25 m site (Figure 4.12) was dominated by oligochaete worms with very low numbers of any other species occurring. Maximum oligochaete densities were in June (123,797 individuals m^{-2}) with the maximum contribution to the community, 99%, being in July. Minimum abundance was in January (18,669 individuals m^{-2}, 88% of the community). In contrast to the other sites, *H. diversicolor* showed minimum abundance during the summer with peaks in January and September. Minimum abundance was in June with only 275 individuals m^{-2}, making up 0.2% of the community with maximum values being in January (2368 individuals m^{-2}, making up 11% of the community). The contribution of nematodes to the community was less than 0.1% throughout the year with all other species being absent.

Oligochaete biomass increased between January and June then decreased in July, remaining stable for the rest of the year, with the maximum contribution to the community biomass being in April, June and July. Maximum biomass was in April (72 g m^{-2}), contributing 92% to the total community biomass with the minimum being in January (6 g m^{-2}, 18% of the community biomass). *H. diversicolor* biomass increased slightly between January and April then declined to a minimum of 2.8 g m^{-2} in June (8% of the community biomass) before increasing slightly throughout July and September. Maximum biomass was in April (45 g m^{-2}, 54% of the community biomass). Nematode biomass reached a peak during summer (June and July) with low values in January, February and September. Maximum biomass was in June (0.02 g m^{-2}, 0.1%) with the minimum being in February (0.01 g m^{-2}, 0.002%).

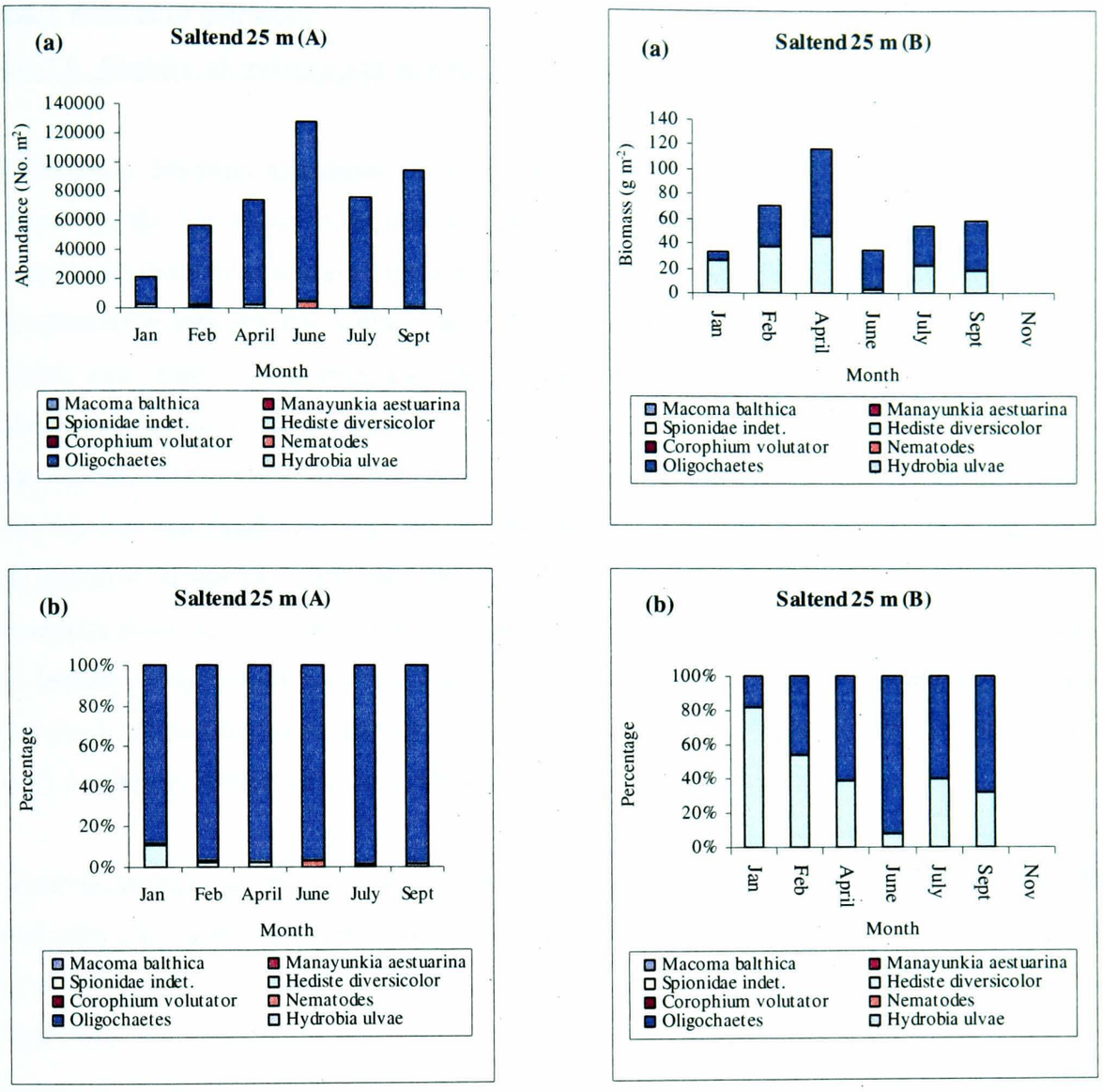


Figure 4.12. Community structure, showing actual (a) and percent (b) contribution of each species, in terms of abundance (A) and biomass (B) at Saltend 25 m.

Community structure at the non-key monitoring sites at Paull broadly follow the trends found at the P150 m site with an overall dominance of *H. diversicolor* and oligochaetes, in terms of abundance, and significant numbers of *M. balthica*, spionids and *M. aestuarina*. *H. diversicolor* and *M. balthica* dominated the biomass. The S150 m site was similar to the S200 m site in terms of the dominant species. All other Saltend sites followed the same patterns as that shown at the S75 m and S25 m sites with oligochaetes being the dominant organism throughout the year.

4.4.2. Effects of pollution

4.4.2.1. Species, abundance and biomass.

Differences between abundance, biomass and the number of species are shown for each month for the key monitoring sites in Figure 4.13 (A-G). In general, the data show trends resembling those of a typical Pearson & Rosenberg (1978) SAB curve with a distinct increase in abundance between the control site at Paull and the most polluted site at S25 m. These trends were most obvious between June 1999 and September 1999 (Figure 4.13 A-C) where abundances at the P150 m and S200 m sites were significantly ($p<0.01$) lower than those at the S75 and S25 m sites. In September (Figure 4.13C), the S75 m site became more similar to the S200 m and Paull sites than the S25 m site and in November (Figure 4.13D), the trend disappeared altogether with abundance being similar at the S75 m and Paull sites but distinctly lower at the S200 m site. No data were available for the S25 m site for this month. In January (Figure 4.13E), the trend reappeared with a notable increase in abundance between the Paull and S200 m sites and the S75 m site. However, abundance at the S25 m site was only marginally higher than that at the Paull and S200 m sites.

Seasonal changes in the difference in abundance between sites can be more clearly seen by examining the difference between the maximum and minimum recorded abundance (e.g., for June 1999, the maximum difference in abundance would be calculated by subtracting the abundance at the P150 m site from that at the S25 m site) (Figure 4.14). Maximum and minimum differences in abundance between sites were in June when abundance ranged from 16246 individuals m^{-2} at P150 m to 128093 individuals m^{-2} at S25 m (a difference of 111847) and in January when abundance ranged from 13657 individuals m^{-2} at S200 m to 41523 individuals m^{-2} at S75 m (a difference of 27865). This emphasises the fact that adherence to Pearson & Rosenberg's (1978) model was greatest during the summer months. This is shown more clearly for all three parameters in Figure 4.14.

Biomass was generally highest at the S200 m site, reducing at S75 m and S25 m with biomass at the S25 m site being slightly higher than that at Paull. This general trend is prevalent throughout July, September, November and January. In February, maximum biomass was at S75 m with biomass at both S75 m and S25 m being higher than that at S200 m or Paull, i.e. the pattern described by Pearson & Rosenberg (1978) is reversed. In April, patterns more typical of the Pearson & Rosenberg model emerged with biomass at S25 m and S75 m being lower than that at Paull or the S200 m site. By June, there was a clear reduction in biomass with increasing proximity to the discharge. There was no significant difference between sites in terms of the biomass, except in June where the P150 m biomass was significantly ($p<0.01$)

greater than that at the S25 m site. Again, this shows a greater degree of adherence to the Pearson and Rosenberg model in summer (Figure 4.14). The maximum difference between sites was in June where biomass ranged from 35 g m^{-2} at the S25 m site to 148 g m^{-2} at the P150 m site, a difference of 113. Minimum differences were in January with values ranging from 27 g m^{-2} at P150 m to 44 g m^{-2} at the S75 m site, a difference of 17.

In terms of the number of species, there was a clear pattern of decline between the P150 m site and the S25 m site with species richness at the S25 m site being consistently ($p<0.05$) lower than at all other sites for all months except November. It should be noted that the data for the S25 m site were missing for November. However, since the number of species at S25 m was low throughout the year, together with the fact that the number of species at the S0 m site was only 2.3, there is no reason to assume that the number of species should be any higher for the S25 m site. Maximum and minimum ranges for number of species were in January / July where S ranged from 2.4 at S25 m to 6.5 at P150 m (a difference of 4.1) and in November when S ranged from 3.8 at S75 m to 5.6 at P150 m, a difference of 1.8. Again, this difference between sites is less evident in November.

In January, April, June and July, the sites fell into two groups (statistically) with P150 m and S200 m being significantly higher in terms of the number of species than the S75 m and S25 m sites ($p<0.01$). In April the number of species at the S75 m was also higher than S25 m but still remained significantly lower than that at the P150 m and S200 m sites. For the rest of the year, the number of species at the S75 m site was statistically the same as that at the S200 m site whilst S200 maintained its similarity with the P150 m site and its difference to the S25 m site. The S75 m site remained similar to the S 25 m site and different to the Paull site.

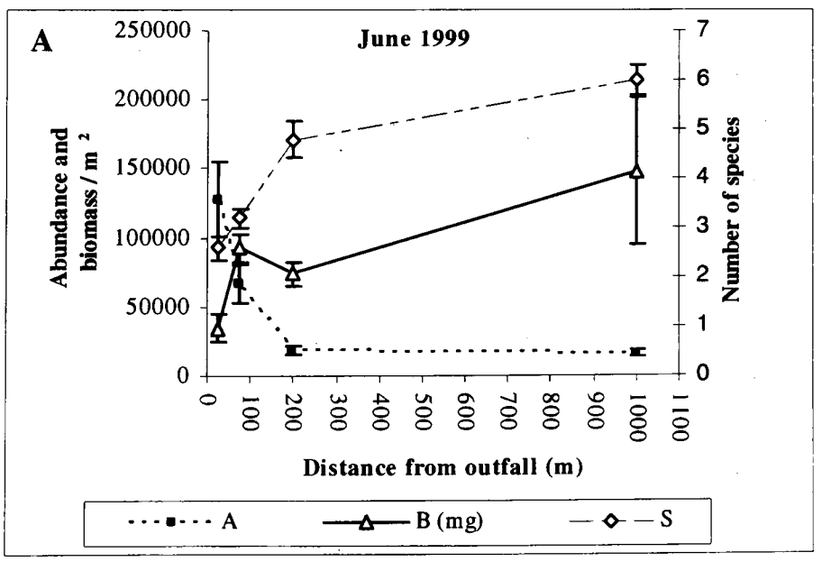
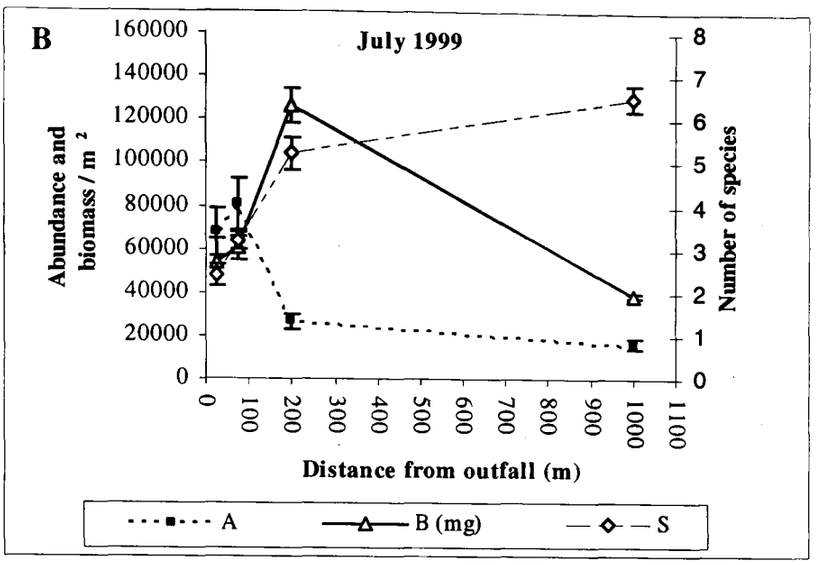
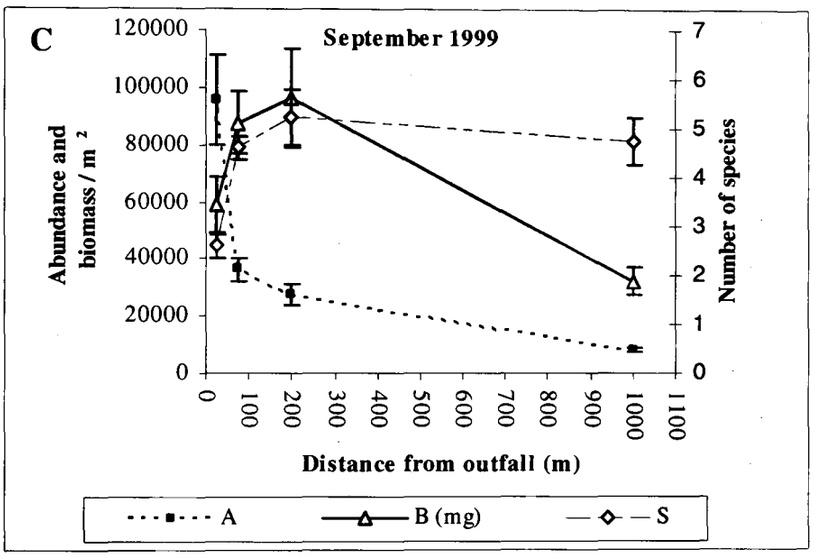


Figure 4.13. Species (S), abundance (A) and biomass (B) curves (mean +/- SE) for the key sites where 1000 m represents the P150 m site.





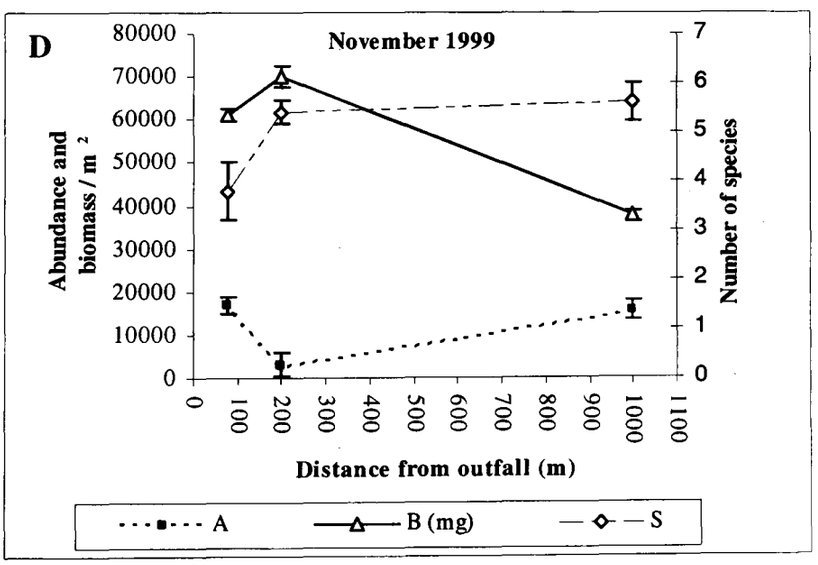
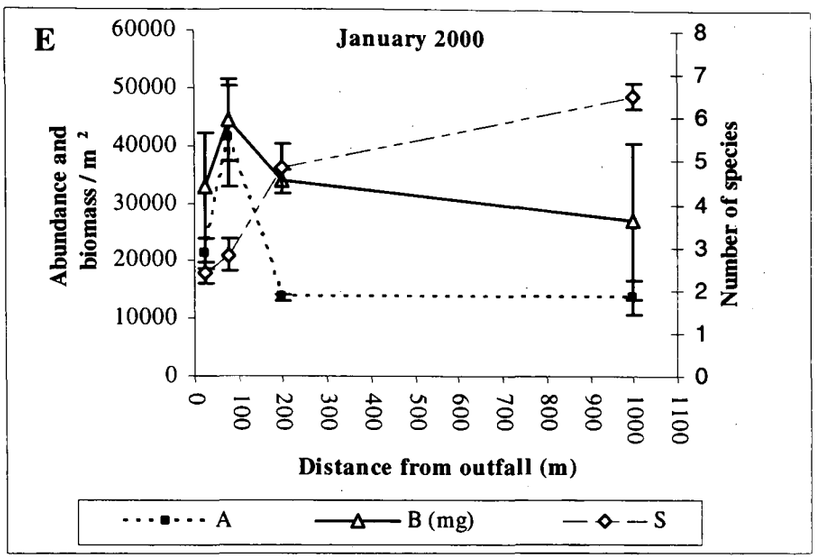
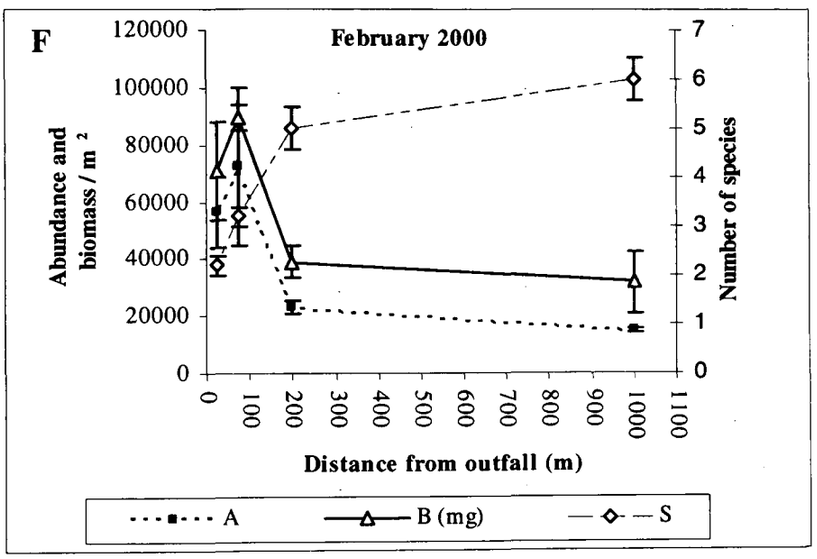


Figure 4.13 (cont)





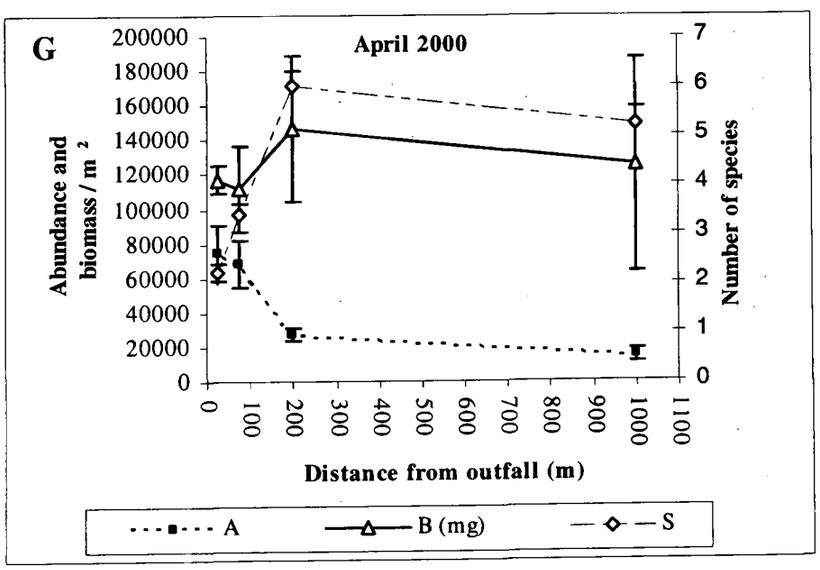


Figure 4.13 (cont)

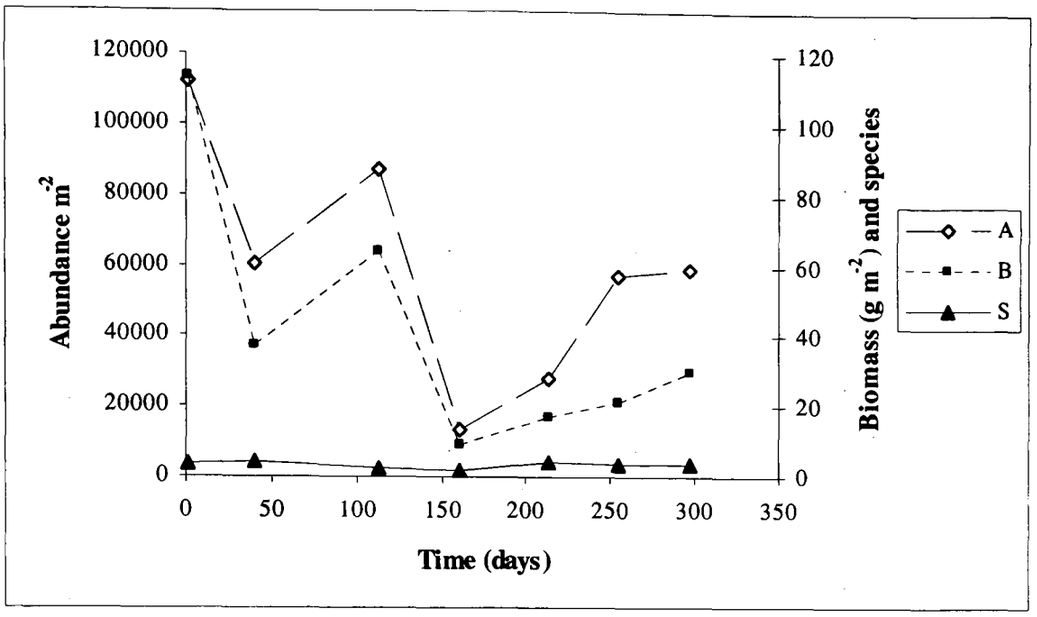


Figure 4.14. Differences between maximum and minimum recorded values of S, A and B for each month (all sites).

When SAB curves were plotted showing data from all sites, the patterns were less well defined. These curves are presented in Figure 4.15 A-C. Of the three variables, the number of species shows the most consistent pattern, declining from a maximum at the Paull sites to a minimum at the S0 m site. The maximum range in number of species between sites was in February 2000 with 1.67 at S0 m and 7.67 at Paull (Figure 4.15 C). There was little difference between the number of species at any of the Paull sites implying that low species numbers at some of the Saltend sites (namely S50 m, S25 m and S0 m) was attributable to pollution and not tidal height.

Abundance remained relatively stable between the Paull sites (P200 m to P25 m), increasing sharply between S200 m (July and November) (Figure 4.15 A, B) and S150 m (February 2000) (Figure 4.15 C) to reach a maximum at the S150 m and S100 m sites. In February and July, abundance declined to the S25 m and S0 m sites but still remained higher than that at the Paull sites. Maximum abundance was at the S150 m site (180538) in July with the minimum being at the Paull 200 m site in February.

Biomass patterns were quite erratic, only showing any clear reduction with increasing levels of pollution in July (Figure 4.15A). For this month, biomass was relatively stable between the Paull sites. The maximum was at the S200 m site (127 g m^{-2}), after which there was a steady, although not continuous, decline to the S0 m site (11 g m^{-2}). In November 1999, biomass was stable between the Paull sites but was low in comparison to the Saltend sites. The minimum was at the S200 m site with the maximum being at the S100 m and S0 m sites. Whilst there

was no clear pattern of a decline in biomass with increasing levels of pollution in February, the minimum value was at the most polluted site, S0 m (24 g m^{-2}). There was, however, no significant difference between this site and several of those further away from the outfall, including three of the Paull sites.

The coefficient of variation (%CV, a measure of the relationship between the mean and the standard deviation of a site) was calculated to indicate the within and between site variability (Elliott & O'Reilly, 1991). It has been suggested that variability within a community increases with increasing stress as communities characteristic of polluted areas merge with those characteristic of unpolluted areas. As stress further increases, the variability reduces as the community becomes more stable (Dr. M. Elliott, University of Hull. Pers. comm.). However, variability was generally high at all sites throughout the year and calculation of %CV provided no insight into the reasons behind any of the patterns observed. This information has therefore not been presented.

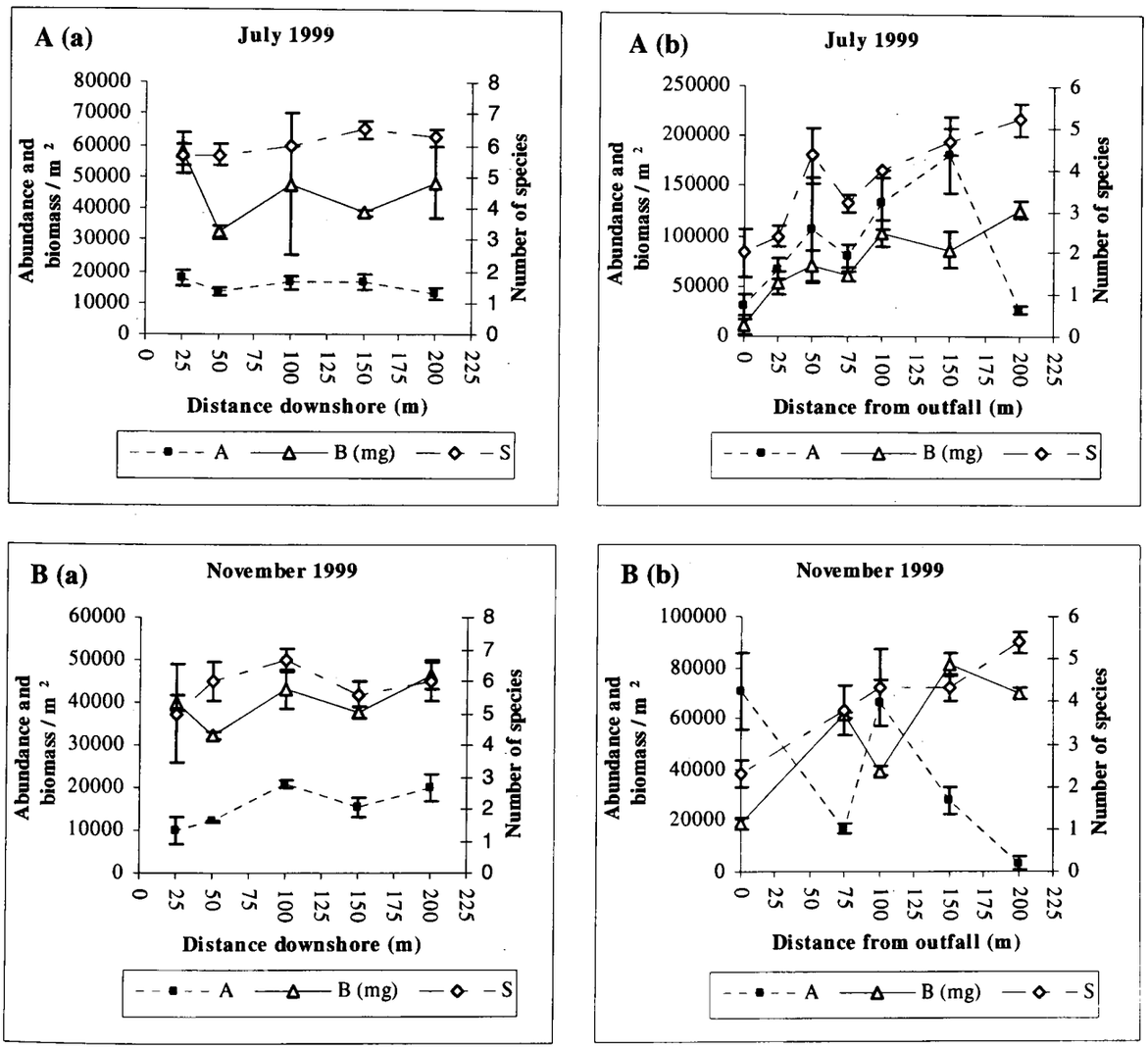


Figure 4.15. Species, abundance and biomass curves for the non key sites (mean +/- SE) at Paull (a) and Saltend (b) for July (A), November (B) and February (C).

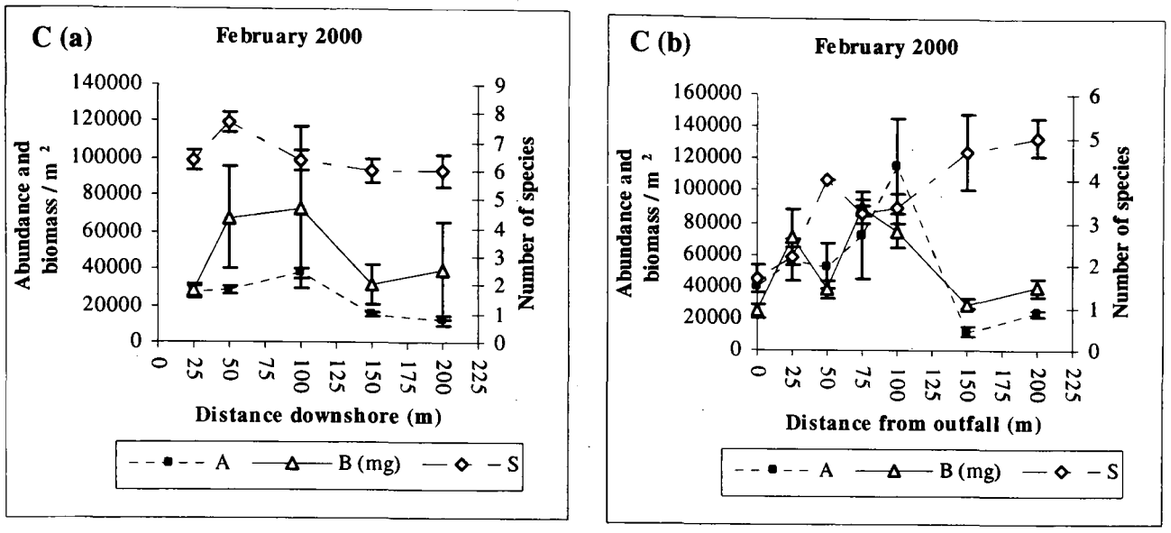


Figure 4.15. (cont.).

Diversity (H', J' and D_{Mg}) was consistently higher at the P150 m and S200 m sites than the S75 and S25 m sites and it is therefore unnecessary to repeat the description of trends in diversity here. It has also been noted that A/S was significantly higher at S25 m and S75 m than at the P150 m and S200 m sites ($P<0.01$) for most of the year and that the reverse was observed for the B/A ratio. These simple measurements, together with the SAB curves and diversity indices indicate a higher level of stress at the S25 m and S75 m sites, which are closest to the discharge.

Whilst the data are highly variable and do not strictly or consistently conform to the Pearson and Rosenberg (1978) model, there is a general pattern of declining numbers of species and community biomass and increasing abundance with increasing proximity to the discharge. In addition, this pattern is repeated over several months, being most evident in the summer. This suggests that differences in community structure between sites are attributable to the BP discharge. The impact of this discharge does, however, appear to have a rather localised impact on the benthic communities in the area with the community at a distance of 200 m being the same (statistically) as that at the control sites. The extent of this impact is dependent on season, being generally greater during summer.

4.4.2.2. Abundance - Biomass Comparison (ABC)

Figure 4.16 shows the abundance and biomass *k*-dominance curves (ABC plots) for contrasting summer and winter months (April and January) for the four key monitoring sites. The *W* statistic was calculated using mean biomass and abundance data and therefore differs slightly to the values presented in Figure 4.16 which are means calculated from replicate biomass and abundance data. All sites showed higher values of *W* in January than in April

indicating that the level of disturbance was greater during the warmer months. The value of *W* increased with increasing distance from the source of pollution in January and April with S25 showing negative (i.e.stressed) values in both months. These seasonal trends are considered in more detail in Figure 4.17.

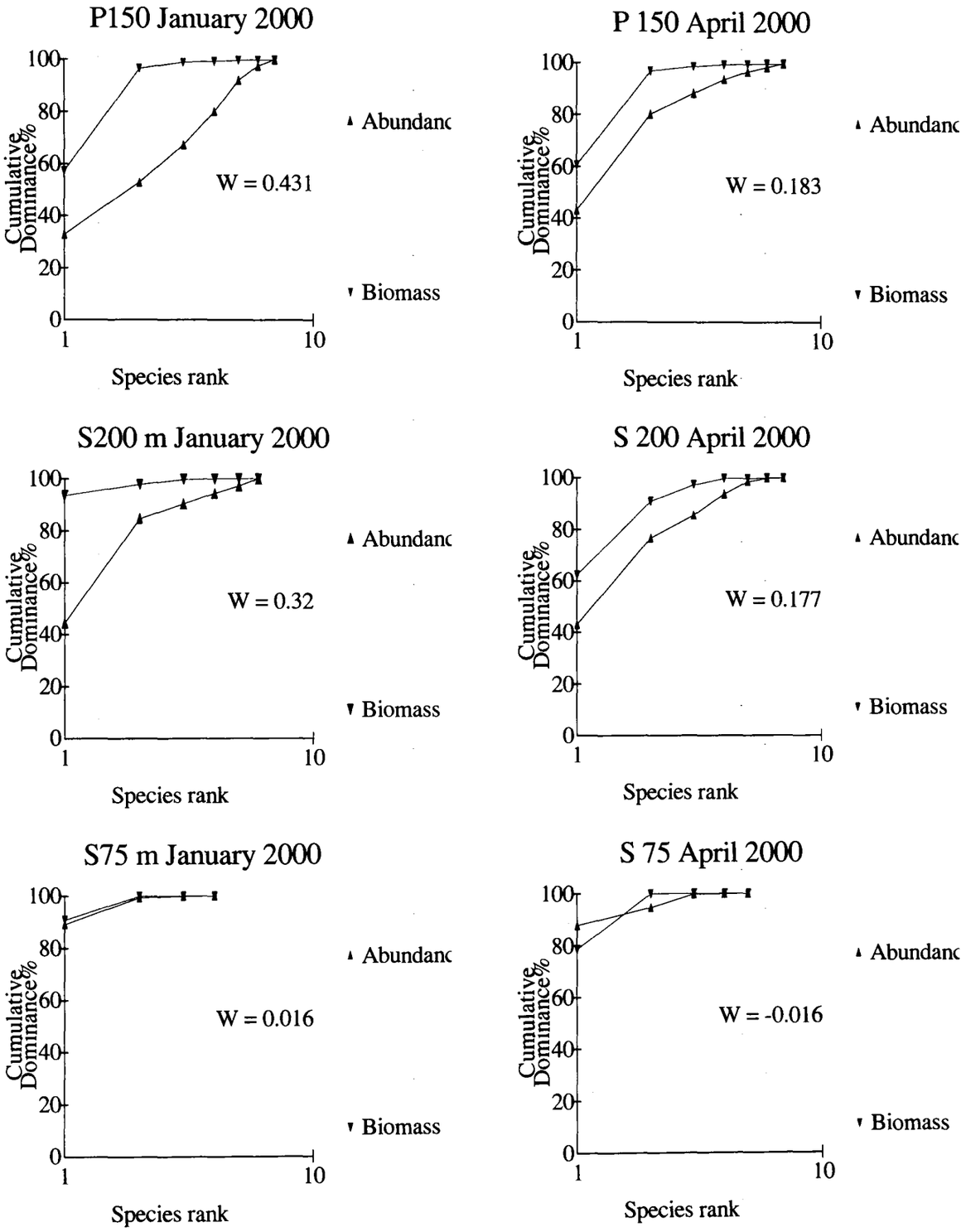


Figure 4.16. ABC plots for January 2000 and April 2000 for the four key monitoring sites

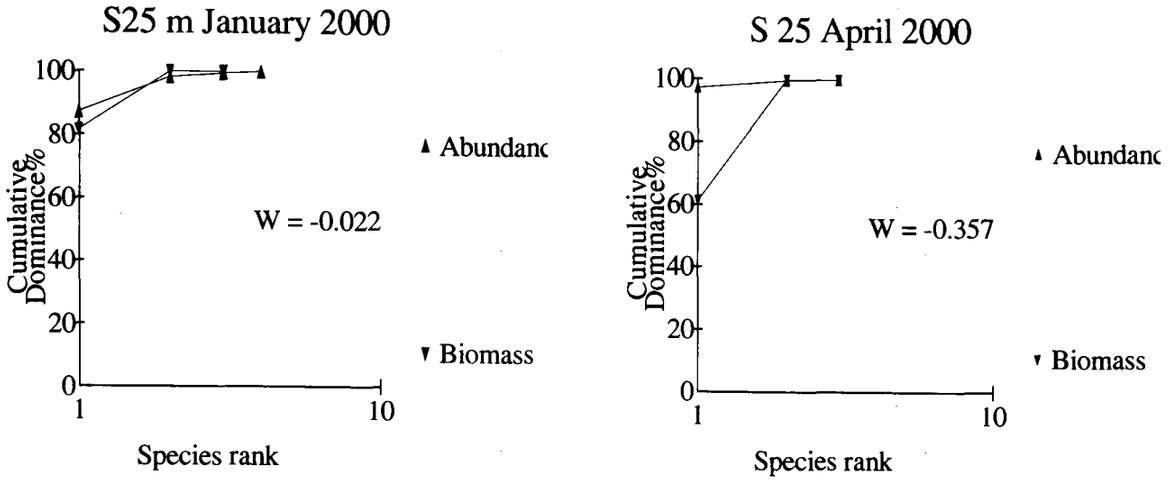


Figure 4.16 (cont)

Figure 4.17 shows changes in the value of Clarke's *W* statistic over time at the four key monitoring sites. The 25 m site showed a clear pattern of seasonality with negative values generally occurring in the warmer months between late spring and early autumn (April to September) and positive values in winter. January and February values were found to be significantly higher ($P<0.01$) than September values. Mean values of *W* at this site ranged from –0.45 in April to a maximum of 0.27 in February. Whilst statistical differences were detected between various months at the 75 m site, the pattern of seasonality was less well defined. Values of *W* ranged from –0.031 in April to 0.384 in November. Statistical testing revealed that, for several months of the year (January, June and April), the 75 m and 25 m sites were different to the Paull and S200 m sites ($p<0.01$) and, with the exception of the low January value, the S75 m site follows a similar trend to the S25 m site. No differences were found between months at either the Paull or Saltend 200 m site and whilst the data follow the same trend at both sites, no pattern of seasonality can be seen. Values ranged from 0.276 in February to 0.44 in January at the Paull site and 0.218 in November to 0.391 in January at the S200 m site with Paull values being consistently higher than Saltend values. Values of *W* calculated for these sites were significantly higher ($p<0.01$) than values at the 25 m site throughout the year.

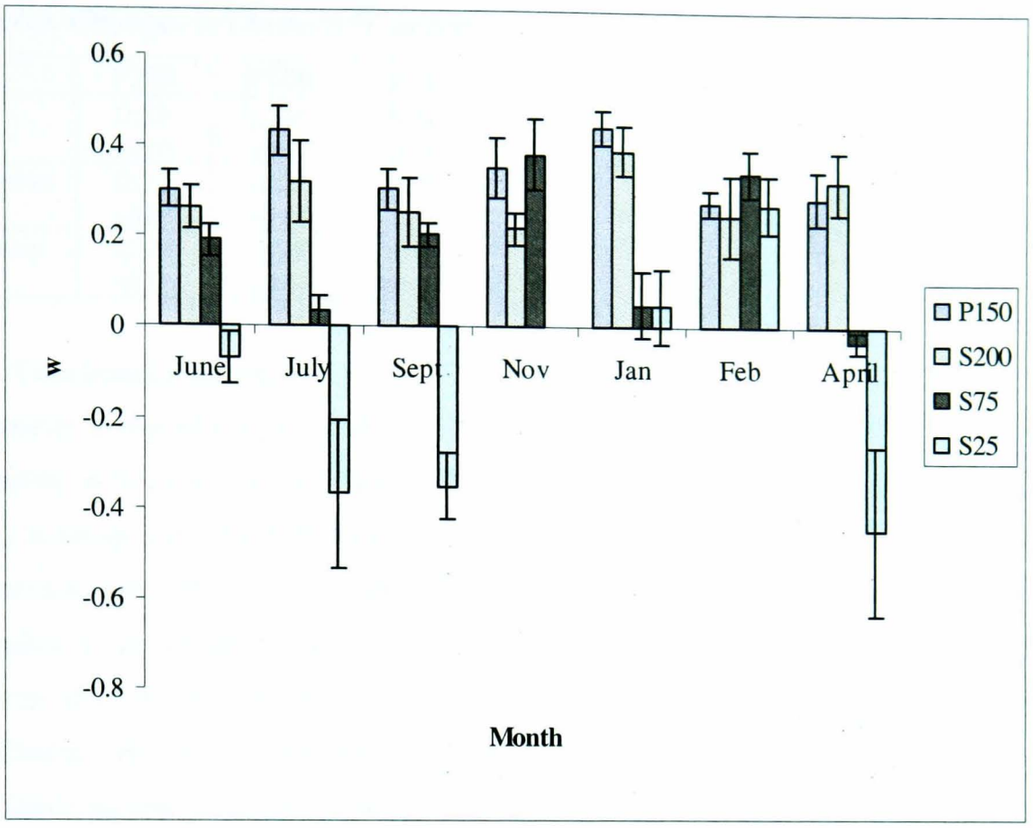


Figure 4.17 showing trends in Clarke's *W* statistic (mean ± SE) over time for the four key monitoring sites (November data for the S25 m site are missing).

Table 4.3 shows mean *W* statistic values for the non-key sites. Values at the Paull sites were generally lowest in November although there was no clear seasonal pattern and differences in values of *W* between months were not found to be significant for any of the sites. In general, values at the P25 m site were higher than at the other sites although there is only a significant difference ($p<0.01$) between P25 m and P50 m in February. The Saltend data do not appear to show any seasonal trend. Values of *W* at the S100 m site showed a pattern of decline between July and February, becoming strongly negative in February, whereas values at S150 m and S50 m increased from July to reach a maximum in February. Values calculated for S0 m reached a maximum, positive value, in November but were negative in July and February, most strongly so in February.

Paull values were generally higher than those calculated for Saltend, a difference which was most noticeable in July and February where values for S150 m and S0 m and S100 m and S0 m were negative, respectively.

Table 4.3. Changes in Clarke's *W* statistic (mean ± SE) for the non-key sites.

	P200	P100	P50	P25	S150	S100	S50	S0
July	0.38 ±0.03	0.397 ±0.09	0.42 ±0.03	0.49 ±0.06	-0.01 ±0.03	0.15 ±0.06	0.05 ±0.13	-0.15 ±0.16
November	0.31 ±0.03	0.38 ±0.03	0.29 ±0.05	0.31 ±0.06	0.127 ±0.03	-0.08 ±0.04	_	-0.31 ±0.14
February	0.35 ±0.07	0.25 ±0.09	0.3 ±0.03	0.6 ±0.03	0.35 ±0.05	-0.44 ±0.12	0.27 ±0.06	-0.28 ±0.25

4.4.3. Community structure

The species compositions for each site have been commented on in detail with reference to seasonality in Section 4.4.1.3. Figures 4.9-4.12 and Appendices 2A and 2B show a distinct change in community structure from the communities at Paull where oligochaetes and *Hediste diversicolor* may collectively dominate between 50 and 80% of the community but with a reasonably even spread of the less dominant species, to the communities close to the discharge (S75 m, S50 m, S25 m and S0m) which are almost completely dominated by oligochaetes. *Hediste diversicolor* is common at the S75 m site but its abundance becomes much lower and more random at the other sites. Rather than repeat the description of percent contribution of each species, differences between communities at each site have been expressed as dendograms in Figures 4.18 and 4.19 (A-G).

The results of the cluster analysis, carried out on replicate data sets, are presented in Appendix 3. These analyses show that, in general, clustering of replicate samples for each site was good with similarity being over 60% in the majority of cases and up to 80% in a large proportion of cases. As described in Section 4.3.3, the dominance of a few highly abundant species can prevent the detection of similarities between communities and data are therefore frequently transformed. Whilst the Bray-Curtis Similarity index does not down-weight the importance of the most abundant species, transformation can improve the balance between the contribution of each species to the community. The overall dominance of oligochaetes at the most polluted sites was considered to be an important characteristic of the communities in these areas suggesting that transformation to down-weight their importance would be inappropriate. However, the effects of transforming the data were examined to ensure that meaningful similarities between communities were detected.

The Bray-Curtis similarity index was calculated using the original data and a variety of transformations (only the results using the original data and the $\sqrt{\sqrt{}}$ transformation are presented). Whilst the $\sqrt{}$, $\sqrt{\sqrt{}}$ and log $(x + 1)$ transformations slightly increased the similarity between replicates, there were very few differences in the actual clustering of the sites and the overall effect of the transformation was minimal. It was therefore assumed that

transformation of the data was unnecessary and analysis was carried out using the original data. Figure 4.18 shows mean data for all sites for June 1999 - April 2000, with a more detailed analysis for each month being presented in Figure 4.19 (A-G).

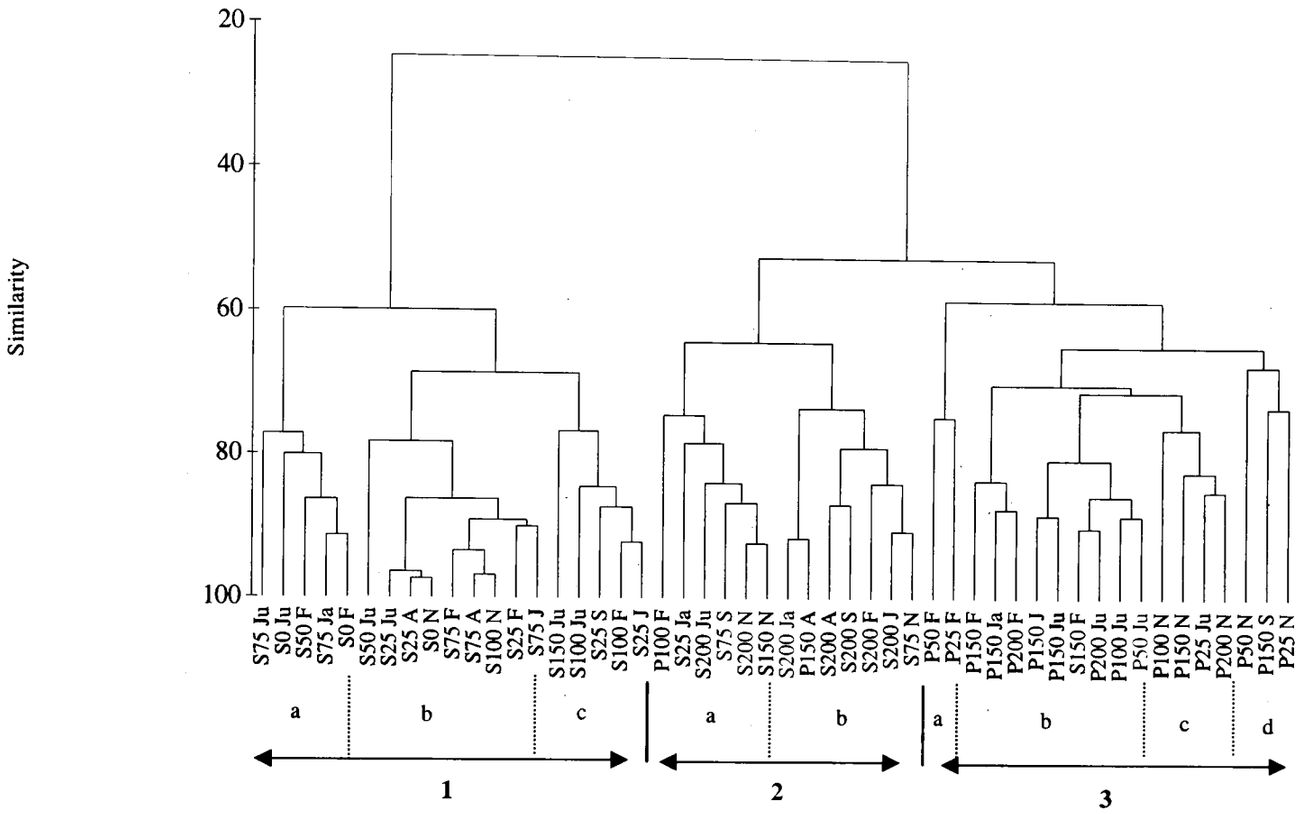


Figure 4.18. Cluster analysis of species abundance for all sites, June 1999 - April 2000 (Ja = January, J = June, Ju = July).

Figure 4.18 shows three main clusters, which generally reflect distance from the discharge, with a cluster of the most contaminated sites, a group of intermediate / uncontaminated sites and a group of unaffected sites (i.e. the Paull sites). The first includes sites S0 m, S25 m (except January), S50 m, S75 m (except the September and November samples), S100 m and S150 m (July). These communities are characterised by highly abundant oligochaetes with comparatively (in relation to other sites) low numbers of *H. diversicolor* and nematodes. Within this main group, there are three minor groups although there are generally no consistent patterns in the distribution of sites or months between these three sub-groups. There is, however, some degree of separation of the S100m and S150 m sites from those closer to the outfall.

The second cluster includes the S25 m (January sample), S75 m (September and November), S150 m (November), S200 m, P100 m (February) and P150 m (April). This cluster is only

20% similar to the first cluster and contained communities composed of much lower densities of oligochaetes with higher numbers of *H. diversicolor.* In addition, *C. volutator, M. aestuarina,* nematodes and spionid worms were present in low abundances. This main group can also be divided into two sub-groups although, again, there are no consistent patterns of the distribution of sites between the clusters. However, it is of note that the S25 m (January) and S75 m (September) sites occur within group 2a, together with the P100 m (February), S200 m (June and November) and S150 m (November). This indicates that the difference in community structure between the polluted and unpolluted sites is less well defined during the cooler months. Group 2b is largely composed of the S200 m sites with some degree of separation between the winter and summer months.

The third cluster includes the majority of the Paull sites and the S150 m (February sample) with overall similarity being 55% to samples in cluster 2 and 20% to samples in cluster 1. These sites differ from those in the second group in that the numbers of *C. volutator* and *M. aestuarina* were considerably higher. In addition, *M. balthica* was frequently recorded from these sites with *Hydrobia ulvae* also being present in low numbers at certain times of the year. Sites within this cluster are separated, to some degree, by season and, to a lesser extent, shore height. Group 3a contains the upper shore P25 m and P50 m sites for February and is separated from the lower shore P150 m and P200 m sites for January and February. Group 3c contains the majority of the summer samples (July), although some November samples are also included, whereas group 3d includes autumn samples.

In terms of seasonality (key sites only), the P150 m site shows almost 90% similarity between the June and July (i.e.summer) samples. These samples are approximately 72% similar to the winter / spring samples (January, February and April) with the January and February samples showing greater similarity to each other than to the April sample. The September and November samples (autumn) show 70% similarity to each other and 60% similarity to the other samples. Whilst similarity between all samples is quite high, there is a degree of clustering between samples in terms of seasonality at this site. This trend of similarity between months in each season is less evident at the other sites. The S200 m site shows over 70% similarity between all samples with three main clusters, showing over 80% similarity, consisting of the January and June; July and November and February, April and September samples. At the S75 m site, there is a high degree of similarity between the late winter / spring and summer samples (February, April and June) with these three samples also being 65-70% similar to those taken in January, July and September. The November sample shows just 40% similarity to the others. The S25 m site shows a high degree of similarity between the April, July and September sites and the June and February sites with these two groups

being over 70% similar to each other but less than 40% similar to the January sample, in terms of species abundance.

A more detailed analysis of Figure 4.18 is given in Figure 4.19, where data for individual months have been examined, which shows a definite split between the Paull and S200 m sites and the remaining Saltend sites in almost every month. This reflects the difference between the species compositions and abundances at the four sites which was demonstrated in sections 4.4.1.3 and 4.4.2.1. The June, January and April diagrams show a high degree of similarity between the P150 m and S200 m sites and the S75 m and S25 m sites (over 60%) but with only 20 - 30% similarity between the two clusters. In June, January and April, there are two main clusters with the S200 m and P150 m samples being around 60% similar and the S25 m and S75 m sites being between 60 and 85% similar, depending on the month. In all months, the similarity between these two groups of sites is 20-30%. In July, two major clusters are formed again, the first with all of the Paull sites showing over 80% similarity to each other and 60% similarity to the S200 m site. The second cluster, which is only 20% similar, contains the remaining Saltend sites (S0 m – S150 m). With the exception of the S75 m site, these sites appear to be grouped according to distance from the discharge with the S100 m and S150 m sites being more similar to each other than to the S25 m and S50 m sites and the S75 m and S0 m sites.

In the September and November diagrams, the degree of similarity between the S200 m and the S75 m sites is increased from approximately 20% throughout the rest of the year, to approximately 60%. In September, these two sites are less than 40% similar to the P150 m site and only 20% similar to the S25 m site. In November, the level of similarity between the P150 m and S200 m sites is also lower (approximately 50%) than at other times of the year. This diagram shows three main clusters, the first containing sites S0 m and S100 m and being 20% similar to the sites in the other two clusters. The second cluster includes the Paull sites (except P100 m) and is approximately 50% similar to the third cluster which contains the S200 m, S150 m, S75 m and P100 m sites. The September and November diagrams suggest that, during these months (i.e. autumn), the sites closest to the discharge (S0 m and S25 m) remain different but the degree of difference between the other sites reduces so that the distinction between them according to their position along the pollution gradient is less obvious.

The February diagram, again, shows the separation between those sites closest to the outfall and those which are far enough away for the impact to be minimal or undetectable. The first

cluster contains the sites S0 m – S100 m and is less than 30% similar to the second cluster which contains the Paull, S200 m and S150 m sites.

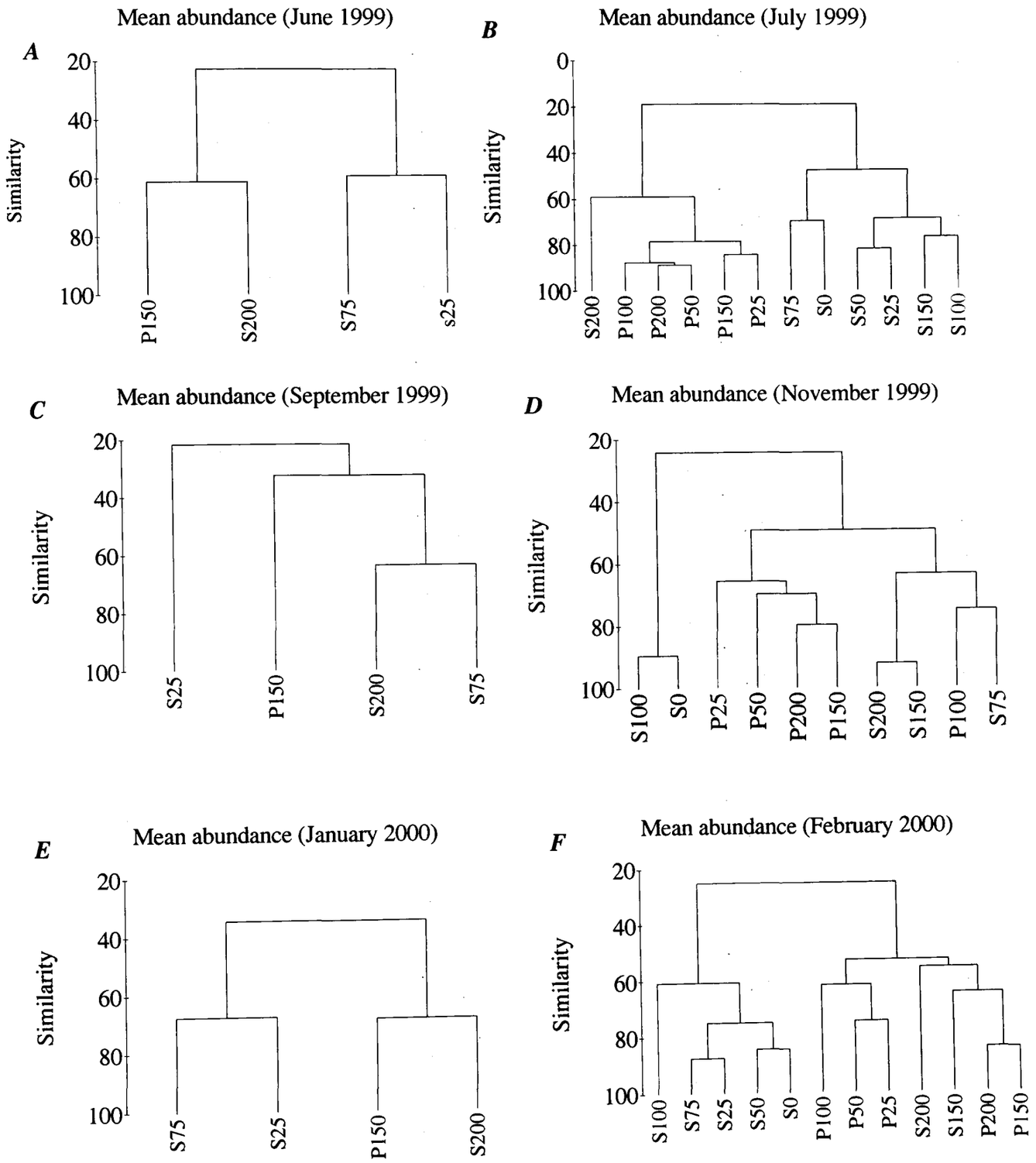


Figure 4.19. Cluster analysis of species abundance for June 1999 - April 2000.

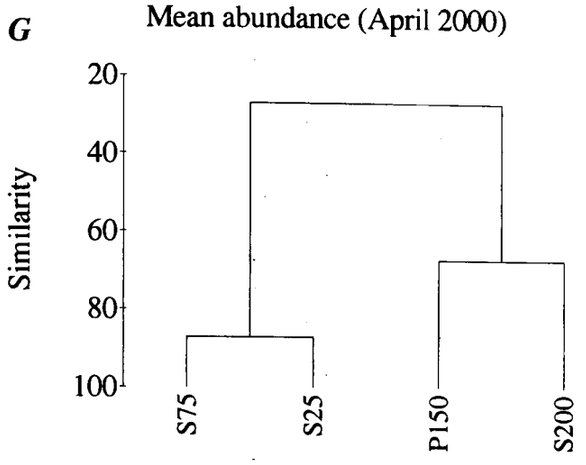


Figure 4.19. (cont.).

4.4.4. Size / biomass frequency analysis

4.4.4.1. Mean individual length and biomass

Figures 4.20 A and B show differences in mean individual length and biomass between sites for June and January for all species combined. Mean individual length ranges from 6.9 mm at the S25 m and S75 m sites in June to 12.84 mm at the S200 m site in June. It can be seen in Figure 4.20A that mean individual length is significantly higher (i.e., the 95% confidence limits do not overlap) at the Paull and S200 m sites than at the S75 m and S25 m sites. In addition, mean length appears to increase (although not significantly) between January and June at the Paull and S200 m sites. The opposite of this occurs at the S75 m and S25 m sites with June values being significantly lower than January values at the S25 m site.

The highest mean individual biomass is at the S200 m site in June (6.86 mg) where biomass is significantly higher than at any other site. Minimum biomass is at Paull in January and at the S25 m site in June where values are 0.4 mg and 0.45 mg, respectively. Biomass does not differ significantly between the other sites. Again, there is a pattern of increase in biomass between January and June at the Paull and S200 m sites whereas individual biomass decreases at the S75 m and S25 m sites. This is only of any significance at the S25 m site and could reflect large scale recruitment or the outward migration of larger species.

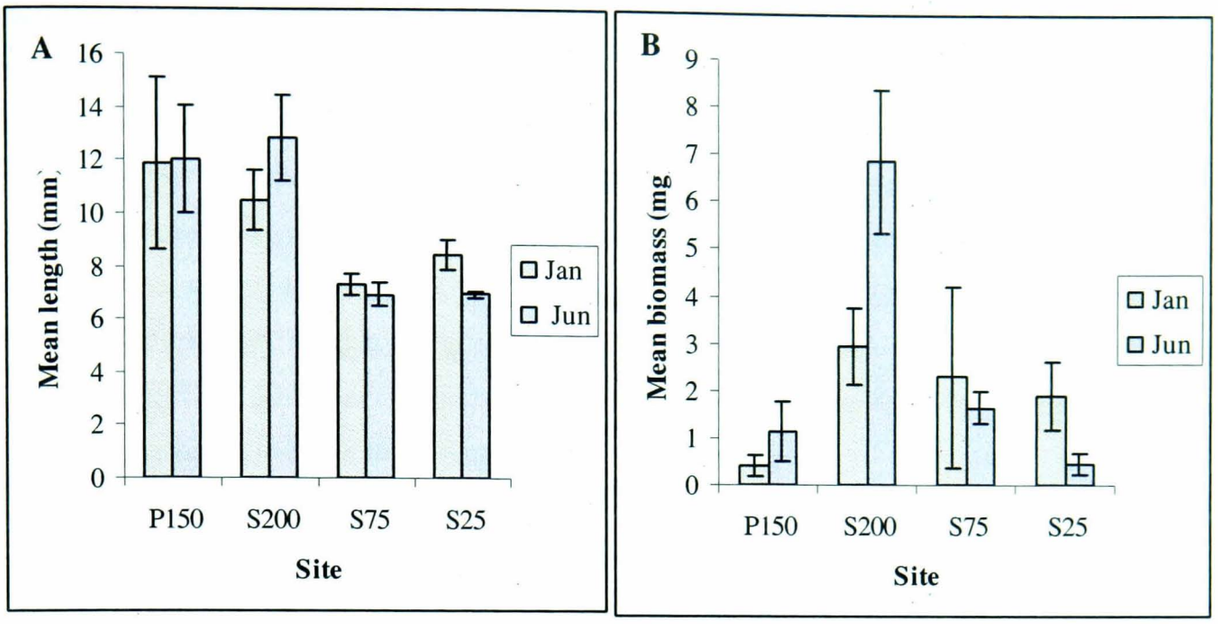


Figure 4.20 showing differences in mean (± 95% CL) individual length (A) and biomass (B) between sites (all species combined).

Differences between length and biomass of individuals within each species were tested using one-way ANOVA. Mean length/biomass data for individual species are presented in Figure 4.21 A-F. Data have only been presented for the dominant species for which significant differences between sites could be identified. Oligochaete length and biomass clearly increases with proximity to the discharge, the lowest value of length being at the Paull site ($p<0.01$) and the S200 m site (January). Length increases significantly to the S75 m site and again to the S25 m site ($p<0.01$). Oligochaete length decreases (although not significantly) between January and June at Paull, S75 m and S25 m, possibly due to recruitment. In terms of biomass, minimum values occur, again, at Paull (0.01 mg), increasing significantly to S200 m and S75 m and again to reach a maximum of 0.61 mg at the S25 m site in January ($p<0.01$). Mean individual biomass decreases between January and June at all sites although this is only significant at the S200 m and S25 m sites ($p<0.01$). In terms of length, nematodes show the same trend as the oligochaetes with low values at the Paull and S200 m sites and significantly higher values at the S75 m and S25 m sites. Mean individual length ranges from 1.95 mm at the S200 m site to 4.6 mm at the S25 m and S75 m sites in January and June, repectively. In addition, June values are significantly lower than January values at all sites except S200 m ($p<0.01$). No differences were found between sites of months in terms of Nematode biomass with values ranging from 0.01 mg at the S200 m site in June to 0.0 mg at the S75 m site in January.

Data for *Hediste diversicolor* are presented in Figure 4.21 (E and F). The greatest length value is at Paull in January (26 mm) and whilst there are significant differences between sites,

these do not correlate with distance from the source of pollution. Minimum individual length is at the S200 m site in January. Mean individual length increases significantly ($p<0.01$) between January and June at the S200 m and S75 m sites but decreases at the Paull and S25 m sites ($p>0.05$). Similarly, mean individual biomass shows no pattern in relation to the influence of the discharge with values at the S25 m site being greater than or equal to those at Paull. Biomass increases significantly ($P<0.05$) between January and June at Paull and the S200 m site and only marginally ($p>0.05$) at the S75 m site but decreases at the S25 m site (not significantly). Maximum individual biomass is 14.34 mg at the S200 m site in June with the minimum being 4.73 mg at Paull in January.

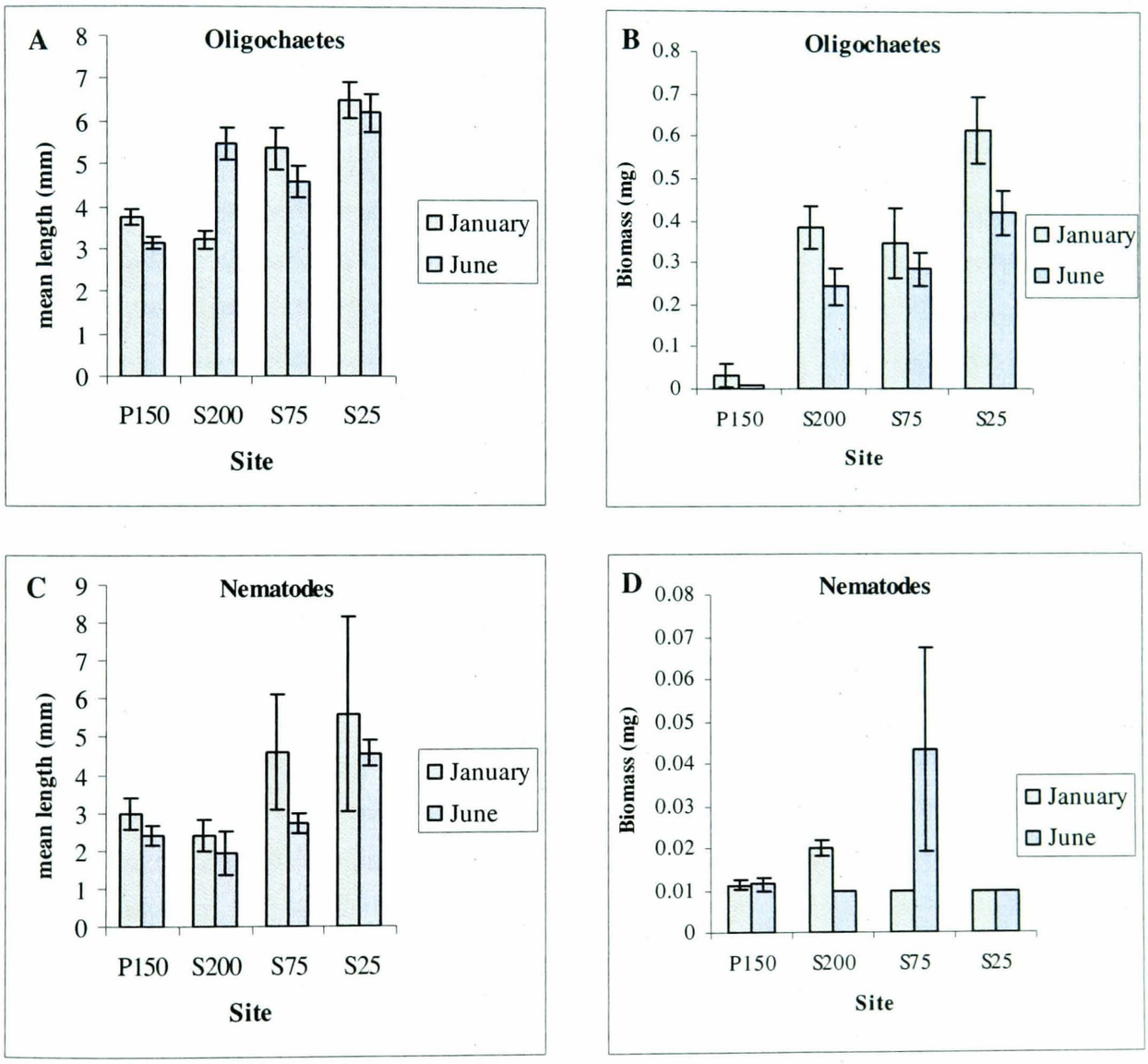
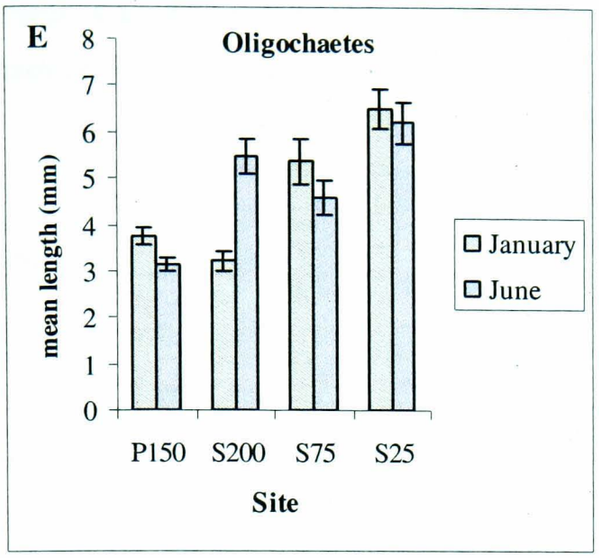


Figure 4.21 Mean length and biomass of Oligochaetes (A, B, ± 95% CL), nematodes (C,D, ± SE) and *Hediste diversicolor* (E,F, ± SE).



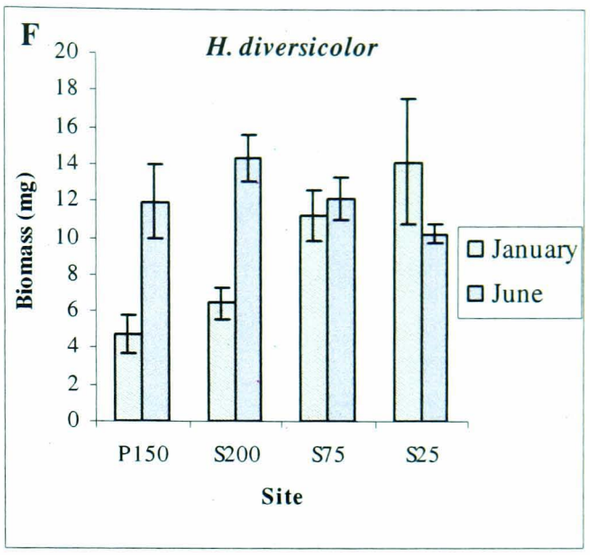


Figure 4.21 (cont).

Significant differences, or trends between sites and / or months were generally not found for the remaining species and the data have therefore not been plotted. Mean individual length and biomass are presented in Table 4.4. The S75m and S25m sites have been omitted from the table since the species listed were not found in either January or June. The mean length of *Corophium volutator* increased between January and June at both the Paull and S200 m site with the largest animals being recorded from the S200 m site in June (5.47 mm). Differences between mean lengths for *C. volutator* were not significant but in terms of biomass, individuals at the S200 m site were significantly larger ($p<0.01$) than at the Paull site in both months and showed a significant increase between January and June.

There were no significant differences in mean length or biomass between sites or months for *Manayunkia aestuarina* although values were marginally higher at Paull and there was a slight increase in size between January and June at both sites. Spionid worms showed a slight increase in length between January and June ($p>0.05$) with worms also being significantly ($p<0.01$) larger in terms of biomass at the Paull site. *Macoma balthica* showed a non-significant increase (largely due to the low numbers of animals and the high variability in size) in both length and biomass between January and June.

Table 4.4. Mean individual length / biomass for *Corophium volutator*, *Manayunkia aestuarina*, spionidae and *Macoma balthica*.

	Paull January	Paull June	S200 January	S200 June	Significance
Length (mm)					
Corophium volutator	2.78 ± 0.13	3.29 ± 0.5	2.58 ± 0.37	5.47 ± 1.01	ns
Manayunkia aestuarina	2.02 ± 0.11	2.2 ± 0.15	1.9 ± 0.18	2.1 ± 0.01	ns
Spionidae	4.43 ± 0.23	5.59 ± 0.46	3.22 ± 0.49	4.15 ± 0.05	ns
Macoma balthica	5.28 ± 1.38	10.4 ± 1.75	-	-	ns
Biomass (mg)					
Corophium volutator	0.53 ± 0.07	1.76 ± 0.58	0.82 ± 0.32	5.01 ± 1.3	P<0.01
Manayunkia aestuarina	0.048 ± 0.079	0.01 ± 0.00	0.015 ± 0.002	0.01 ± 0.00	ns
Spionidae	0.12 ± 0.09	0.36 ± 0.03	0.21 ± 0.04	0.2 ± 0.00	P<0.01
Macoma balthica	45.9 ± 25.01	172.97 ± 86.7	-	-	ns

Whilst calculating mean length and biomass clearly indicated differences between the sites, calculating the mean for data sets with two or more modes is not appropriate. Therefore, maximum length and biomass were calculated for the most dominant organisms (Figure 4.22 A and B) as an alternative means of showing the effect of pollution on animal size. Analysis of the modal size classes is presented in section 4.4.4.2. Figure 4.22 A shows an overall decrease in maximum length with increasing pollution for *H. diversicolor* and all species, in both January and June. One-way ANOVA (using $\log_{10}$ transformed data) together with Scheffe's test revealed that individuals of *H. diversicolor* were significantly ($P<0.05$) larger at the Paull site in January and June and significantly smaller at the S25 m site for both months. The same pattern was found when examining the maximum length of individuals from all species within the community ($P<0.05$). This is not surprising since, in terms of length, *H. diversicolor* was the largest organism. In contrast, maximum length of oligochaetes and nematodes increased between the Paull and S200 m sites and the S25 m and S75 m sites ($P<0.05$). No differences in maximum length between months were found for any of the sites with the exception of the S25 m site where maximum length was greater in January than in June ($p<0.05$).

A similar trend was observed for the biomass data with maximum biomass of *H. diversicolor* and all species combined decreasing between Paull and the S25 m site ($p<0.01$) and maximum biomass of oligochaetes increasing ($P<0.01$). There were no differences in nematode biomass between sites. For *H. diversicolor*, June biomass values were greater than

those in January with the reverse being true for the S25 m site ($p<0.01$). Seasonal differences in the maximum biomass of *H. diversicolor* were not observed at the S200 m or S75 m sites.

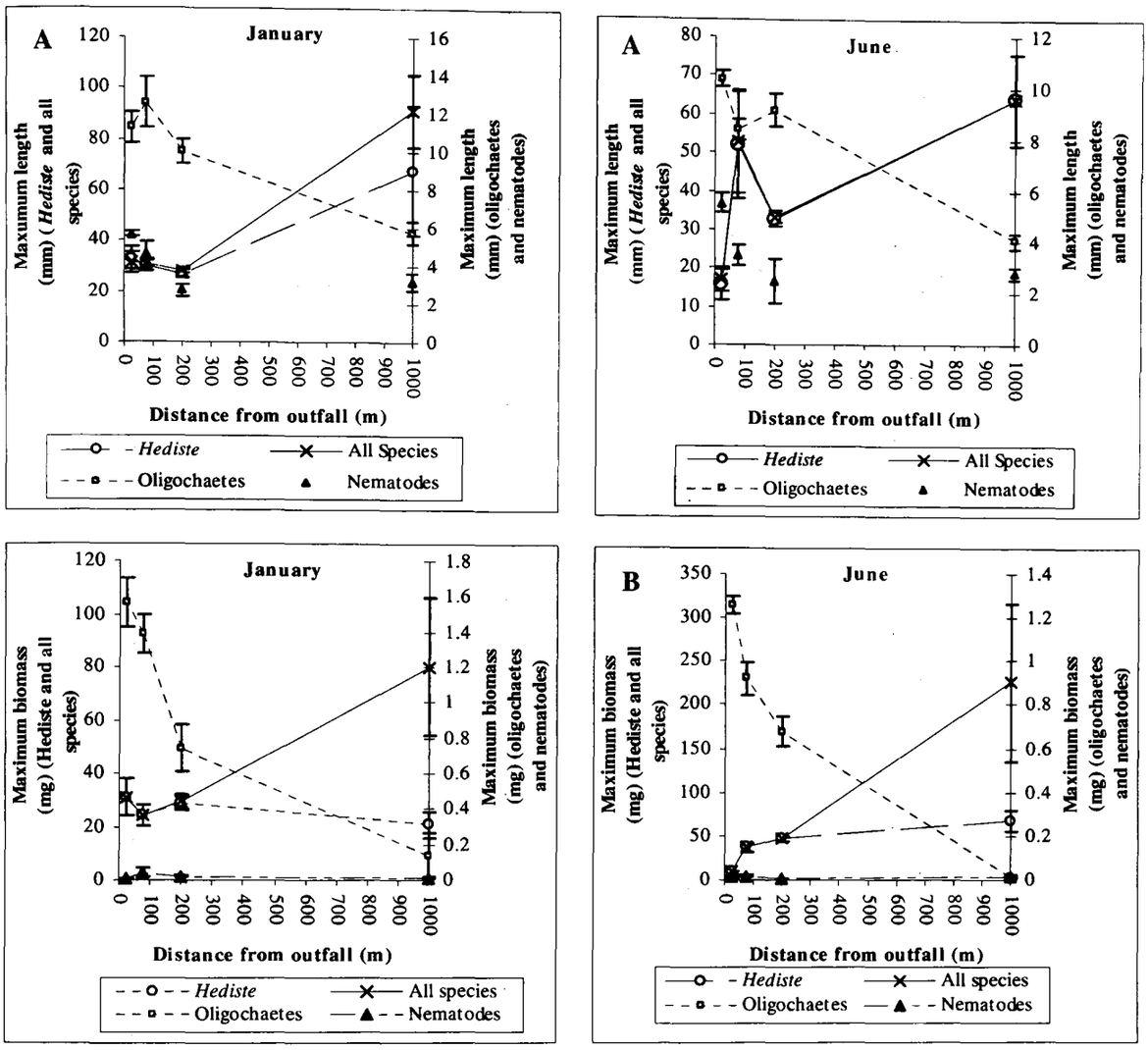


Figure 4.22. Maximum (mean ±SE) individual length (A) and biomass (B) for June and January.

4.4.4.2. Length / biomass frequency distributions

Figure 4.23 A-H shows length frequency distributions (on a $\log_{10}$ scale) for all species combined for the key sites in June 1999 and January 2000. In general, frequency distributions become more complex with increasing distance from the discharge. At the S25 m site, the distributions show only one mode with maximum frequencies being in the 0.9 and 0.8 classes for June and January respectively. Distributions at the S75 m site show maximum values of 0.6 and 1.3/1.4 for June and 0.7 and 1.4 for January. With increasing distance from the discharge, the frequency of individuals in the larger size classes increases so that at the Paull site, maximum frequencies are in the 0.7, 1.2 and 1.6 classes for June and the 0.6 and 1.5 classes for January. Modes, calculated using Bhattacharyas method, are presented in Figure

4.24, where the separation index was greater than 2 for all distributions for which more than one mode has been plotted. It can be seen that there is no significant difference between the first set of modes for each site, which range from 0.62 at Paull (January) and 0.88 (S25, January). However, the S75 m, S200 m and Paull sites all have a second, significantly higher mode (ranging from 1.28 at Paull in June to 1.55 at the S75 m site in June). This indicates a difference in the size frequency distributions which was found to be statistically significant ($p<0.01$) using Friedmans test. The Kolmogorov-Smirnov test did not show significant differences between the distributions although this is thought to be because the method is based on testing the difference between cumulative frequency distributions.

Plots of linear index of selection (section 4.3.4) against size class ($\log_{10}$ mm) are presented in Appendix 4A and provide a graphical means of interpreting the output of the Kolmogorov-Smirnov analysis which indicates a statistical difference between two distributions but gives no information as to where these differences lie. Appendix 4A generally shows that for the Paull and S200 m sites, the proportion of individuals in the larger size classes (0.9-1.3 and 1.2-1.6 for Paull and S200 m, respectively) is greater in January than in June. The opposite of this is observed for the S75 m and S25 m sites where the 1-1.2 and 1.1-1.5 size classes contain a higher proportion of individuals in January. This is in agreement with the calculations of mean length presented in Figure 4.20A, which shows a seasonal increase in length at the Paull and S200 m sites but a decrease at the S75 and S25 m sites. In terms of difference between sites the S200 m site shows a greater proportion of individuals in the larger size classes than Paull, S75 m and S25 m in both January and June. In contrast, the frequency of larger individuals is greater at Paull than at the S75 m and S25 m sites, a difference which is most noticeable in June. Comparison between the frequency distributions for the S75 m and S25 m sites shows a higher proportion of individuals in the smaller size classes (0.3 - 0.7) at the S75 m site with a higher proportion of individuals in the 0.8-1.2 size classes at the S25 m site. A greater proportion of individuals are in the 1.2-1.5 size class at the S75 m site although this difference is very small.

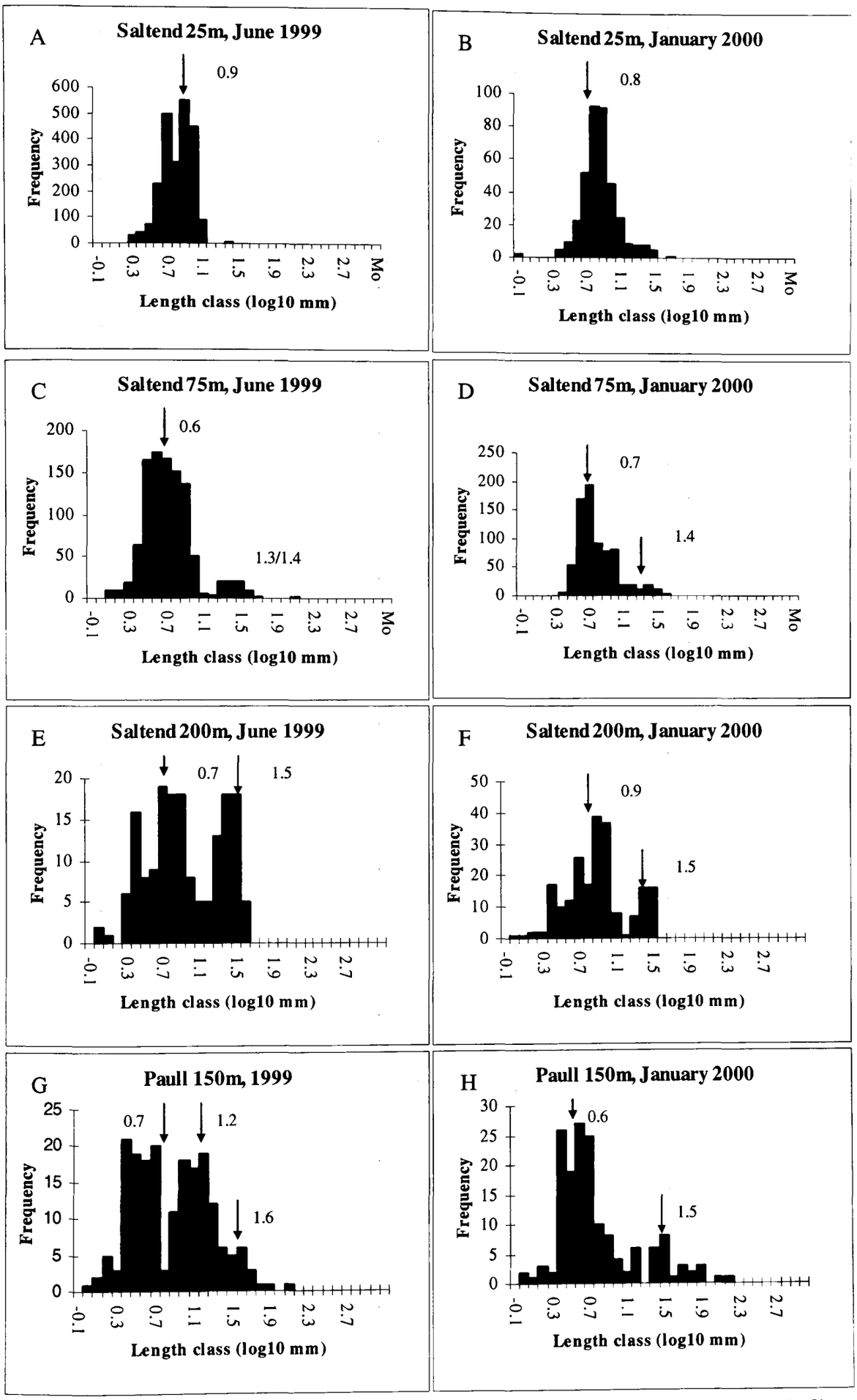


Figure 4.23. Length frequency distributions for all species combined (arrows indicate the position and size of the modes).

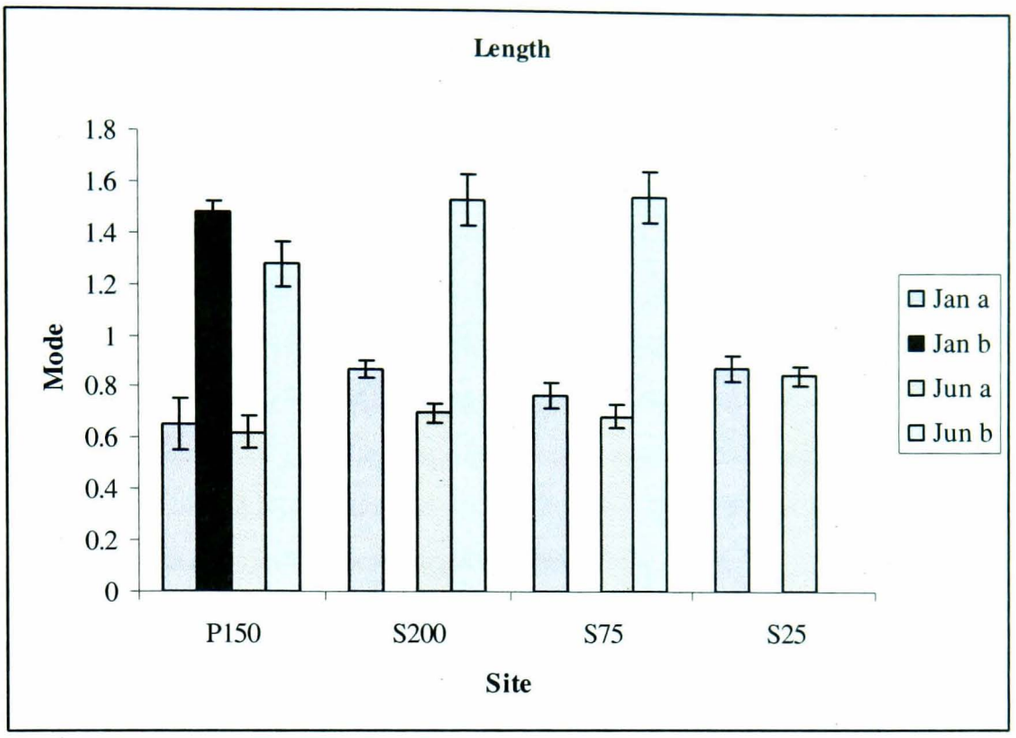


Figure 4.24. Modal length classes (± 95% CL), calculated using Bhattacharyas method.

Whilst there do not appear to be any patterns in differences in mean individual biomass between sites, the biomass frequency distributions shown in Figure 4.25 show a higher frequency of individuals in the larger size classes at the Paull and S200 m sites than at the S75 m and S25 m sites. Again this difference was found to be significant using Friedmans test ($p<0.01$) but not using the Kolmogorov-Smirnov test. As with the length frequency distributions, the biomass frequency distributions become more complex with distance from the source of pollution. All sites have high frequencies of individuals in the -2 and -1.2 classes. However, the S25 m site does not have a second mode in the 1.2 biomass class as the S75 m (January), S200 m and Paull (January) sites do. There appear to be higher frequencies of individuals in the larger size classes in January than in June at the S25 m and S75 m sites where as frequencies in the larger biomass classes either remain the same or increase between January and June for the two less polluted sites.

Modes, calculated using Bhattacharyas (1967) method are presented in Figure 4.26. It can be seen that, with the exception of the S25 m site in June, all sites have two modes and the S200 m site has three in January. The separation index for these modes is greater than 2 in all cases. The S200 m site has the largest modal class in each group ($p<0.05$) with the modal classes for the Paull, S200 m and S75 m sites generally being larger than those for the S25 m sites at the higher end of the scale. In contrast, the modal classes are larger for the S25 m and S75 m sites at the lower end of the scale.

Plots of linear index of selection against biomass class (log_{10} mg) are presented in Appendix 4B. Comparisons between January and June for each site show that the proportion of individuals in the 0-2 classes is greater in June for the Paull and S200 m site whereas the opposite can be seen for the S25 m site. There is no obvious pattern in the S75 m data. Comparison between sites generally shows the S200 m site to have a higher frequency of organisms in the larger size classes than all the other sites. The Paull site shows higher frequencies in the larger size classes than both the S25 m and S75 m sites, particularly in June. Comparison between the S75 m and S25 m sites shows a greater proportion of individuals in the larger size classes (-0.6 - 0.2) at the S25 m site although the frequency of individuals in the 0.2 - 0.4 and 0.8 - 1.2 size classes is marginally higher at the S75 m site in January. Differences between the sites are least pronounced in June.

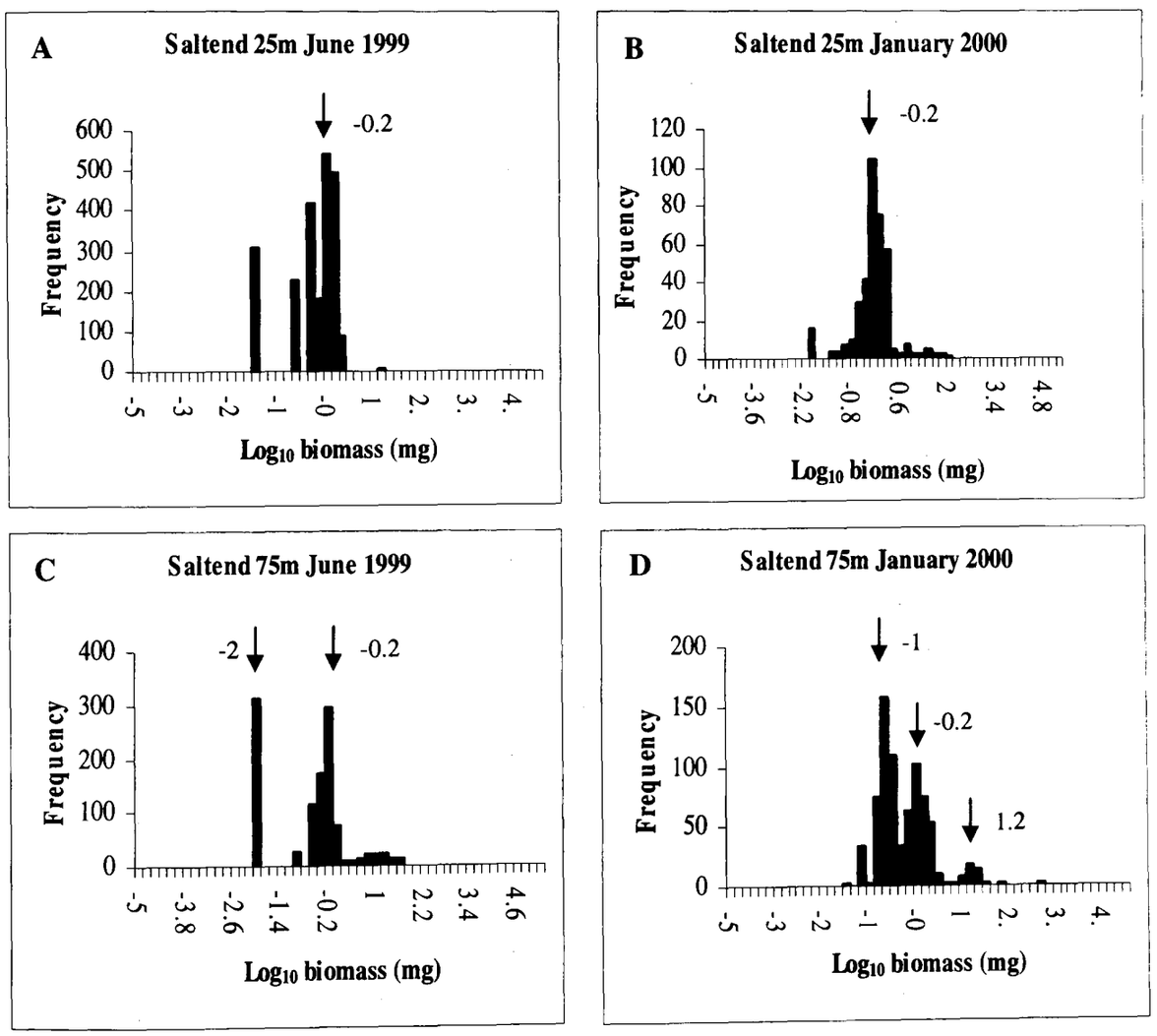


Figure 4.25. Biomass frequency distributions for all species combined (arrows indicate the position and size of the modes).

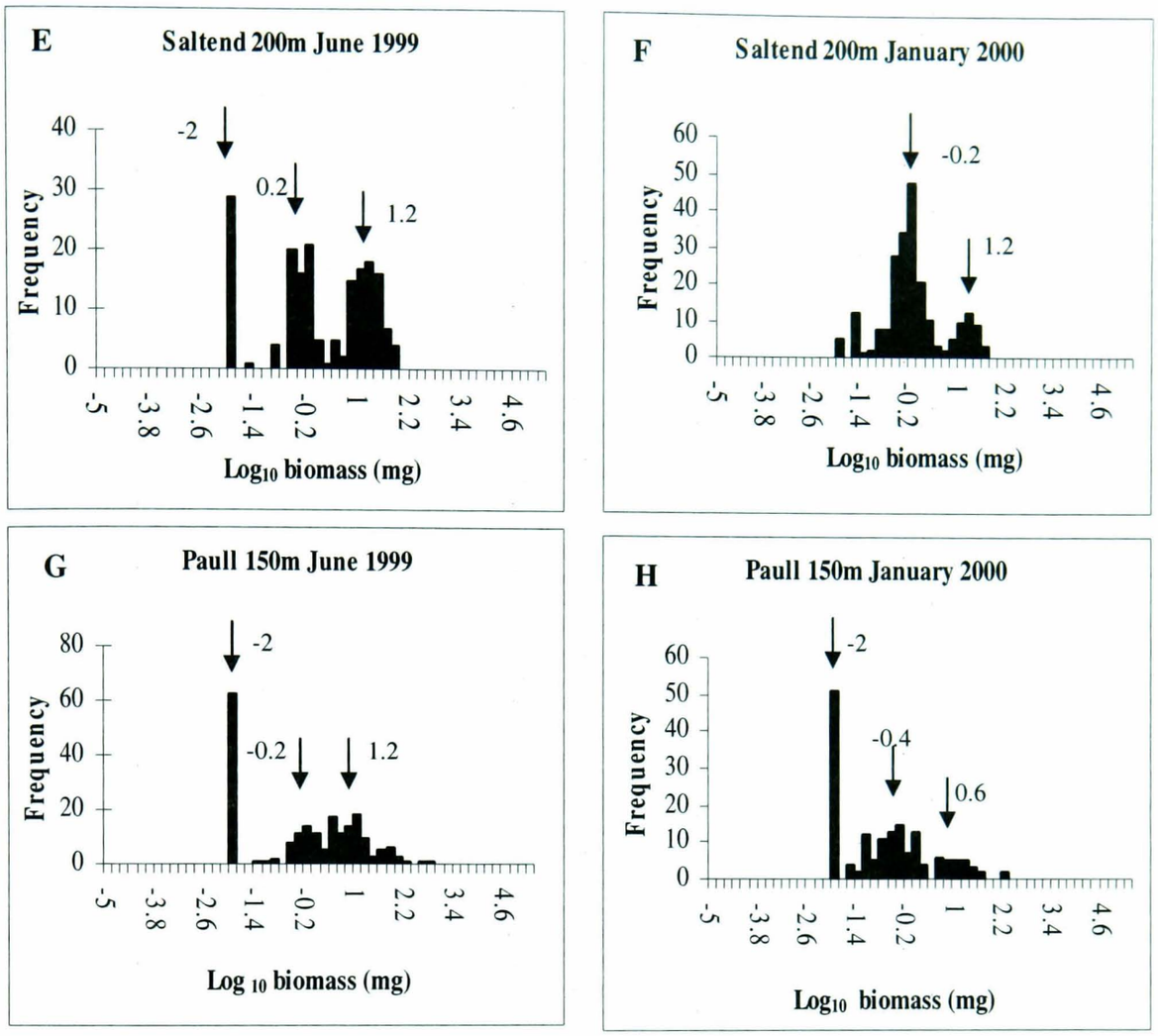


Figure 4.25. (cont). Biomass frequency distributions for all sites.

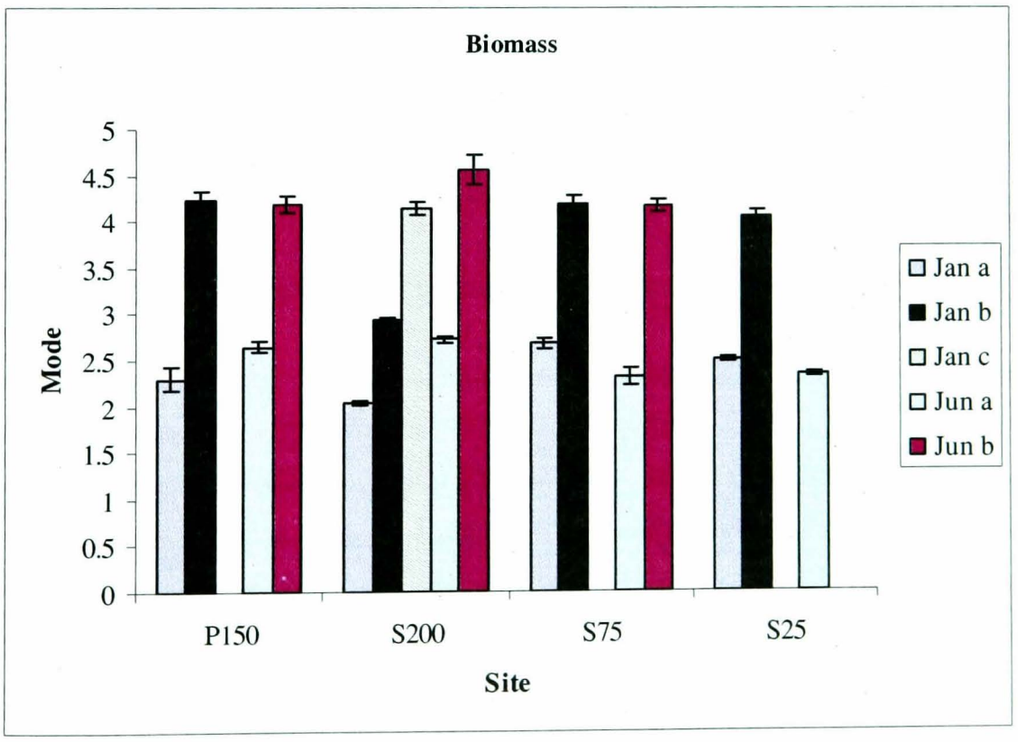


Figure 4.26. Modal biomass classes (± 95% CL), calculated using Bhattacharyas method.

These results, in combination with the length frequency data, suggest an increase in the frequency of larger (in terms of length and biomass) organisms with increasing distance from the discharge, particularly in summer. The frequency distributions also indicate that with increasing pollution, the community changes from one containing all size classes to one dominated by the smaller size classes. The difference between the Paull and S200m sites and the S75 m and S25 m sites can be seen in Figure 4.27 which shows cluster diagrams based on length (A) and biomass (B) frequency data. Diagram A shows approximately 85% similarity, in terms of length, between the S200 m (January and June) site and the Paull site (January and June), respectively. These two sites are 70% similar but are less than 60% similar to the S25 m and S75 m sites. Diagram B shows three clusters, in terms of biomass, with the Paull and S200 m site being separated from the S25 m and S75 m sites in June.

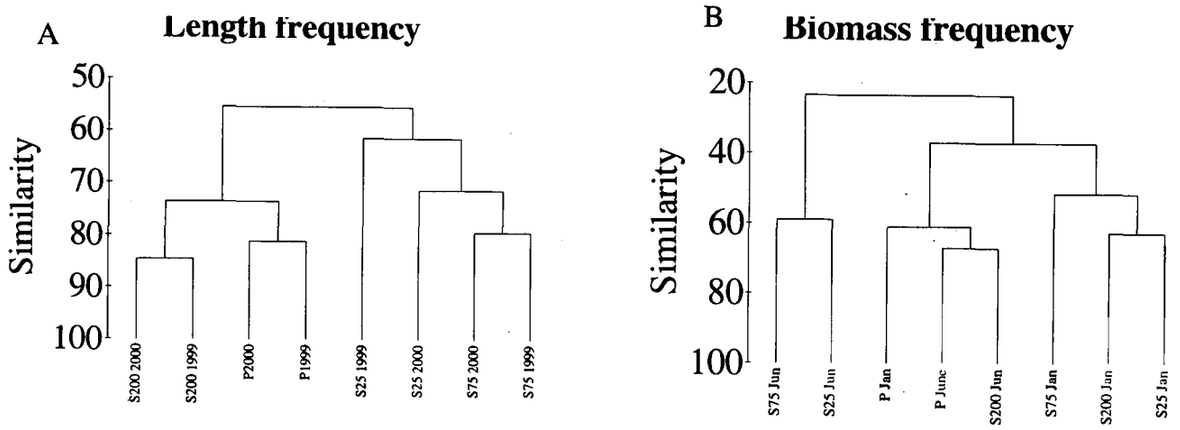


Figure 4.27. Cluster diagrams showing similarity between sites in January and June in terms of length (A) and biomass (B) frequency distributions.

4.4.5. Relationships between the faunal communities and the sediment properties.

Correlations between the faunal community characteristics and the environmental characteristics were carried out using BIOENV (PRIMER) which uses Spearman's rank correlation to determine the environmental variables which best explain the community patterns. The routine allows a correlation between the similarity matrices produced from the faunal analysis and the physico-chemical data. Weak correlations were found between the faunal community and all environmental variables (Table 4.5) although the strongest correlation was found by including the Eh at a depth of 4 cm, mean Eh, microalgal biomass (chl-a), sediment carbohydrate (as colloidal-S) and organic content (r_s = 0.38) in the analysis. However, the BIOENV analysis procedure does not allow the determination of statistical significance (Clarke & Warwick, 1994). Spearmans rank correlations were therefore carried

out for individual species abundances versus each individual environmental variable in order to give an indication of the strength of the relationship (Table 4.6.).

Table 4.5. Output of the BIOENV analysis showing the combinations of environmental variables which best explain the faunal community patterns.

Number of variables included	Variable combinations	Spearman rank correlation coefficient (r_s)
5	Eh 4 cm, mean Eh, Chl-a, colloidal-S, organics	0.382
4	Eh 4 cm, mean Eh, colloidal-S, organics	0.381
4	Eh 4 cm, mean Eh, bulk density, colloidal-S	0.378
3	Eh 4 cm, mean Eh, colloidal-S	0.378
4	Eh 4 cm, mean Eh, colloidal-S, water	0.378
6	Eh 4 cm, mean Eh, Chl-a, colloidal-S, organics, Md phi	0.376
6	Eh 4 cm, mean Eh, Chl-a, bulk density, colloidal-S, organics	0.376
6	Eh 4 cm, mean Eh, Chl-a, colloidal-S, organics, water	0.376
5	Eh 4 cm, mean Eh, chl-a, bulk density, colloidal-S	0.375
6	Eh 4 cm, mean Eh, Chl-a, colloidal-EDTA, colloidal-S, organics.	0.374

Statistically significant correlations were found between abundance and all environmental variables (with the exception of water content, bulk density and particle size) for all species except nematodes (Table 4.6). In general, the abundance of *M. balthica, H. diversicolor, M. aestuarina, C. volutator* and spionid worms was positively correlated with Eh (at 4 cm), showing an increase in these species with decreasing levels of anoxia, and negatively correlated with sediment organic content, carbohydrate concentration (colloidal-S, colloidal-EDTA and total carbohydrate) and microalgal biomass (chl-a). In contrast, the abundance of oligochaete worms was negatively correlated with Eh at 4 cm but positively correlated with all of the other parameters highlighted above.

The relationships between individual species abundance and sediment metal concentration were examined using metal concentration data provided by Nikitik (University of Hull, pers. comm.). It should, however, be noted that these data were collected between 1997 and 1998 (two years prior to the faunal data presented in this study). In addition, the sampling frequency (quarterly) did not match that of the present study (approximately every six weeks) and the data could therefore not be entered directly into the matrix used in the BIOENV analysis. In general, no statistically significant relationships were found and the correlation coefficients (r_s) were very low. The only exception to this was the positive correlation of oligochaete abundance with copper (r_s = 0.52, $p<0.05$) and with zinc (r_s = 0.57, $p<0.05$). However, these weak correlations appear to be the result of the spatial and temporal variability in sediment metal concentration as highlighted by Scott (1996).

Table 4.6. Spearman rank correlations between individual species abundance and environmental variables (r_s values in italics, significant p values given in bold). Water content, bulk density and particle size have been omitted since no correlation was found between these variables and individual species abundance.

	Macoma balthica	*Manayunkia aestuarina*	Spionidae	*Hediste diversicolor*	*Corophium volutator*	Nematodes	Oligochaetes	Eh 4 cm	CHL_A	Total carb	Colloidal EDTA	Colloidal S
Macoma balthica												
p												
M. aestuarina	*0.50*											
p	**0.01**											
Spionidae	*0.84*	*0.56*										
p	**0.00**	**0.00**										
H. diversicolor	*0.27*	*0.67*	*0.42*									
p	0.17	**0.00**	**0.03**									
C. volutator	*0.74*	*0.75*	*0.81*	*0.63*								
p	**0.00**	**0.00**	**0.00**	**0.00**								
Nematodes	*-0.07*	*0.29*	*0.01*	*0.30*	*-0.04*							
p	0.73	0.14	0.96	0.13	0.83							
Oligochaetes	*-0.74*	*-0.55*	*-0.85*	*-0.47*	*-0.79*	*0.08*						
p	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	0.69						
Eh 4 cm	*0.52*	*0.39*	*0.75*	*0.45*	*0.51*	*-0.01*	*-0.64*					
p	**0.01**	**0.05**	**0.00**	**0.02**	**0.01**	0.97	**0.00**					
CHL_A	*-0.44*	*-0.45*	*-0.56*	*-0.38*	*-0.57*	*0.11*	*0.68*	*-0.65*				
p	**0.02**	**0.02**	**0.00**	**0.05**	**0.00**	0.60	**0.00**	**0.00**				
Total carb	*-0.55*	*-0.53*	*-0.76*	*-0.45*	*-0.68*	*-0.03*	*0.78*	*-0.80*	*0.86*			
p	**0.00**	**0.00**	**0.00**	**0.02**	**0.00**	0.89	**0.00**	**0.00**	**0.00**			
Colloidal_EDTA	*-0.36*	*-0.41*	*-0.50*	*-0.31*	*-0.60*	*0.18*	*0.67*	*-0.63*	*0.90*	*0.83*		
p	**0.06**	**0.03**	**0.01**	0.11	**0.00**	0.37	**0.00**	**0.00**	**0.00**	**0.00**		
Colloidal_S	*-0.62*	*-0.56*	*-0.73*	*-0.45*	*-0.73*	*-0.02*	*0.77*	*-0.62*	*0.89*	*0.89*	*0.80*	
p	**0.00**	**0.00**	**0.00**	**0.02**	**0.00**	0.91	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	
ORGANICS	*-0.47*	*-0.21*	*-0.44*	*-0.33*	*-0.36*	*0.17*	*0.54*	*-0.40*	*0.33*	*0.30*	*0.36*	*0.37*
p	**0.01**	0.29	**0.02**	0.09	0.06	0.41	**0.00**	**0.04**	0.10	0.12	0.07	**0.05**

Canonical Correspondence Analysis (CCA) (using MVSP, version 3.1) was also used to determine the relative importance of each environmental parameter in influencing species distribution (Figure 4.28). As demonstrated by the BIOENV analysis, all the environmental parameters were found to be of importance although water content and bulk density were found to be the least important variables (as indicated by the short arrows (vectors) by which they are represented). The species found at the sites farthest away from the discharge (Spionidae, *C. volutator, M. aestuarina* and *H. diversicolor*) are distributed to the right hand side of axis 2 (vertical axis) (Figure 4.28). The abundance of these species is favoured by increasing Eh (i.e., decreasing anoxia), increasing Md phi and, to a lesser extent, increasing bulk density. In contrast, the oligochaetes and nematodes are placed close to or to the left hand side of axis 2 and favour conditions of increasing organic content, microalgal biomass (chl-a) sediment carbohydrate (colloidal-S, colloidal-EDTA and total carbohydrate) and, to a lesser extent, increasing water content.

Axis 1 (horizontal axis) represents the environmental gradient from the fine (i.e., increasing Md phi) , oxidised (increased Eh), drier sediments (right hand side of axis 2) to those with high organic content, low Eh values, high microalgal biomass and carbohydrate concentrations and increased water content. This axis was the dominant gradient and explained 44% of the variation in the data whilst axis 2 explained only 8% of the variation. The ordination has placed all the species very close together, and very close to the origin suggesting that there is little difference between their environmental preferences (with respect to the variables included in the present analysis) or little influence of these particular variables on the community structure. However, it is of note that the correlation matrix presented in Table 4.6 reinforces the patterns shown in Figure 4.28. For example, statistically significant positive correlations were found between Eh at 4cm and the species placed on the right hand side of axis 2 whilst negative correlations were found between these species and the other environmental variables which are placed on the left hand side of the axis.

The ordination of the sites shows that those classed as unpolluted (P150 m and S200 m) are largely placed on the right hand side of axis 2 reflecting the increase in Eh and reduction in organic matter, microalgal biomass and sediment carbohydrate content recorded from these sites. In contrast, the more polluted sites (S25 m and S75 m) are generally placed on the left hand side of axis 2. The exceptions to this general pattern are the presence of the S75 m (November and January) site on the right hand side and the presence of the S200 m site (June) on the left hand side. This supports the statement (Chapter 2 and section 4.4.3) that the characteristics of the S75 m site become similar to those of the less polluted sites during winter but that the characteristics of the S200 m site become more similar to those of the more

polluted sites during the summer. However, as with the species ordination, all sites are positioned very close together indicating homogeneity across the area.

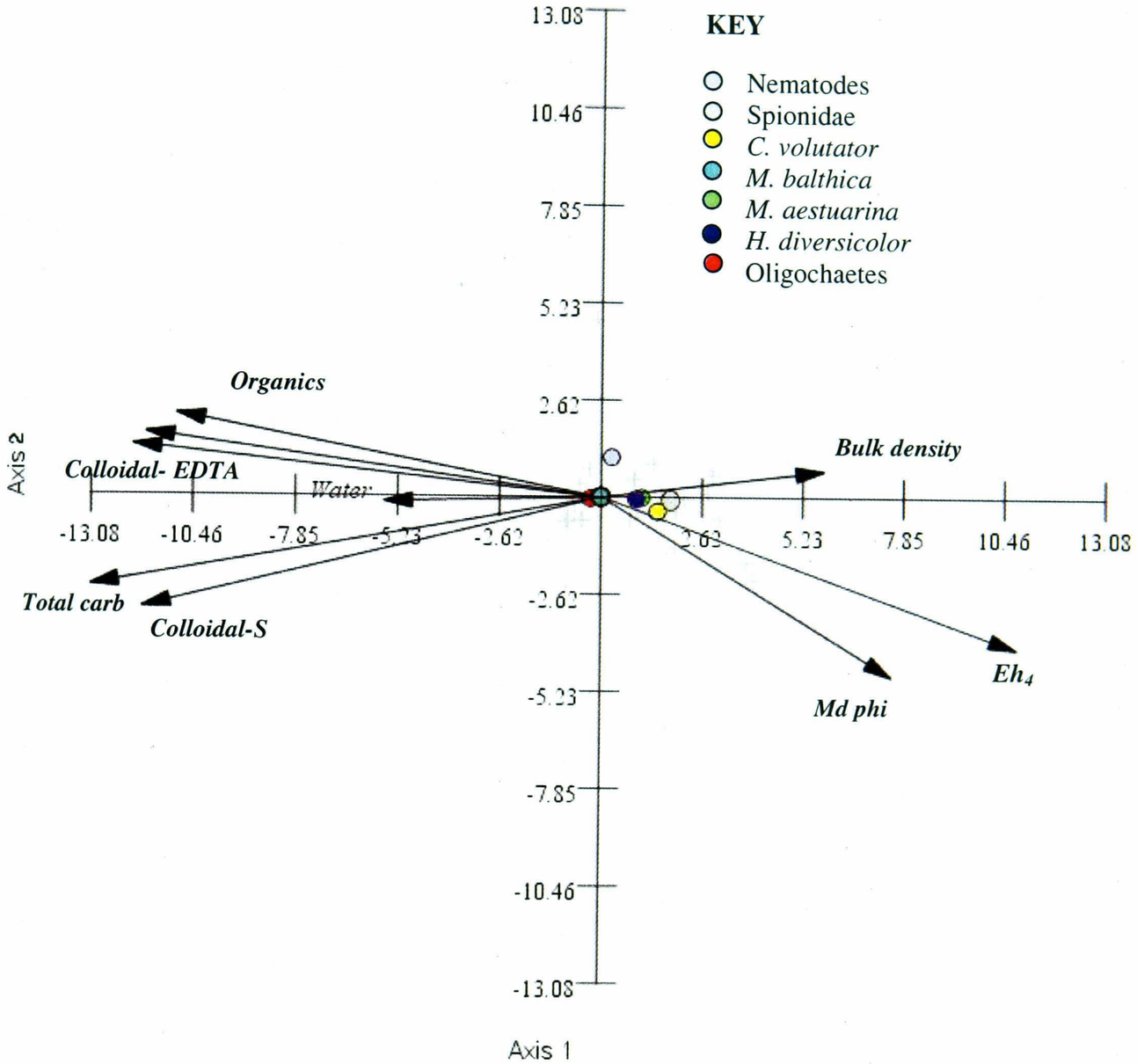


Figure 4.28. The relative importance of each environmental variable on individual species (CCA). The grey cross symbols indicate the positions of the sites, shown in Figure 4.29.

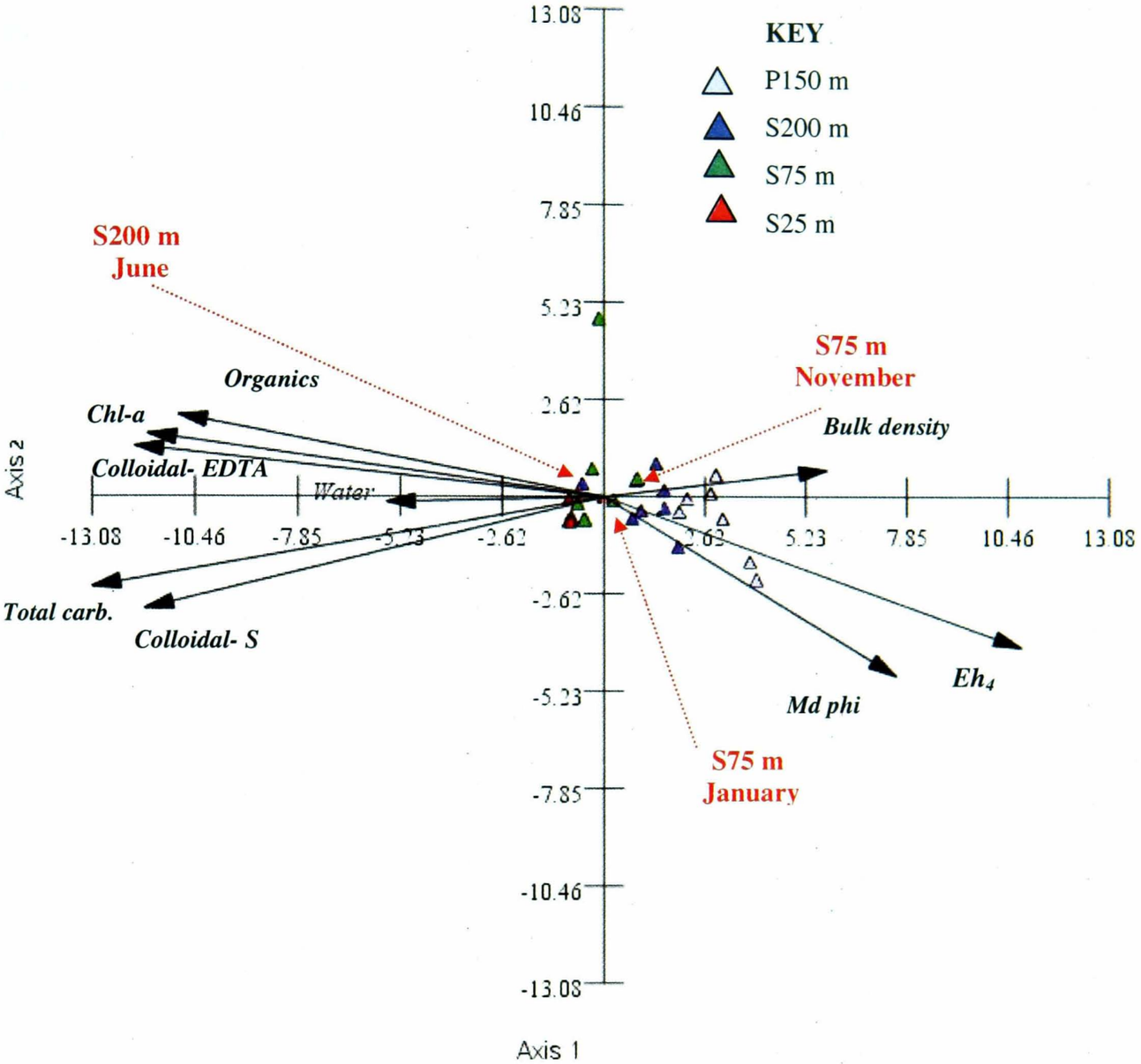


Figure 4.29. Ordination of the sites with reference to the measured environmental variables (CCA).

4.5. DISCUSSION

4.5.1. Communities present

The faunal communities of the mudflats of Paull and Saltend were found to be typical of estuarine habitats with low numbers of species but high abundance. Species present included high densities of oligochaetes (principally *Tubifex costatus* with smaller numbers of *Tubificoides benedeni* and enchytraeid worms) and *Hediste diversicolor* with *Manayunkia aestuarina, Corophium volutator, Macoma balthica,* spionid worms (*Streblospio shrubsolii* and *Pygospio elegans*) and nematodes being present in varying abundances, at certain sites. Other fauna found to be present at the Paull site in low abundances in the past include *Eteone longa, Nephtys hombergii* and *Pholoe* sp (Mazik & Elliott, 2000; Nikitik, University of Hull, pers. comm.). In addition, small *Carcinus maenas* were regularly seen on the surface of the mud, mainly at the Paull site but also occasionally at Saltend (Personal observation).

Three main community types were found, generally corresponding to distance from the discharge. The first included those sites closest to the outfall and was characterised by highly abundant oligochaetes with comparatively (in relation to other sites) low numbers of *H. diversicolor* and nematodes. The second community comprised much lower densities of oligochaetes with higher numbers of *H. diversicolor.* In addition, *C. volutator, M. aestuarina,* nematodes and spionid worms were present in low abundances. The third community contained all of the species listed above and differed from the second community in that the numbers of *C. volutator* and *M. aestuarina* were considerably higher. In addition, *M. balthica* was frequently recorded from these sites with *Hydrobia ulvae* also being present in low numbers at certain times of the year.

The intertidal benthic communities at Paull and Saltend have been studied by various authors with the findings generally being consistent with those of the present study with similar species, abundance and biomass values. The communities found in this study broadly correspond to the upper and mid-upper shore communities defined by Allen (2000a). Community types described by Allen (2000a) were correlated with tidal height with the lower shore communities containing larger numbers of *M. balthica, S. shrubsolii, P. elegans* and *M. aestuarina.* It is therefore likely that, whilst the absence of these species at some sites can be attributed to the discharge, absence or low abundance at sites such as Paull and S200 m is probably due to position on the shore rather than the discharge. Due to reasons of safety and practicality, it was not possible to conduct this study any further down the shore without the aid of a hovercraft.

Scott (1996) found the same three community types as Allen (2000a) although he found that the community within the vicinity of the discharge to be similar to that found in this study, with highly abundant oligochaetes but little else. Species compositions at the Paull site were also similar to those recorded in 1995 and 1996 by Mortimer *et al.* (1999) although there were some differences between abundances. This is most likely to be due to the fluctuating nature of estuarine communities. Scott (1996) compared the results of the Saltend survey to the results of five other surveys, spanning a period of twelve years. It was concluded that there had been no fundamental change to the communities, either in the immediate vicinity of the discharge or in the rest of the area, over that time. *H. diversicolor, M. aestuarina, S. shrubsolii, M. balthica* and two or three species of oligochaetes had remained the central components of the community over the mudflat as a whole with only *C. volutator* showing any significant fluctuation in abundance. Barnett (1984, in Nikitik, in prep.) stated that the populations of *C. volutator* in the Humber estuary were known to fluctuate widely and have been known to crash for no apparent reason. These findings are in close agreement with the findings of Allen (2000a) and those in the present study. Nikitik (University of Hull, pers. comm.) studied the communities along the banks of Old Fleet Drain extensively and also found similar patterns in terms of the species present and their distribution to those of Scott (1996), Allen (2000a) and the present study.

4.5.2. Seasonal effects on community structure.

Seasonal change in community structure is a common condition for assemblages of temperate marine and freshwater organisms (Boesch, 1973). Seasonal changes are to be expected as a result of larval recruitment, migration / immigration and mortality. Patterns of seasonal fluctuation among coastal invertebrates are not always simple or predictable and may vary with life habit, size of the area, hydrography, sediment properties and sampling design. In addition, predation and competition exert their own influence on seasonal patterns (Maurer *et al.*, 1979). Boesch (1973) stated that the gross nature of seasonal fluctuations in macrobenthic assemblages indicates that one-off surveys of temperate estuarine benthos may be of limited value.

The results of the present study have demonstrated a clear pattern of seasonality, in terms of abundance and biomass, at the majority of the sites sampled at Saltend and Paull. In general, both abundance and biomass reached a maximum during April and June (i.e. early summer) with minimum being in autumn / winter (November and January). This was demonstrated both at individual sites (although, with regard to abundance, was less pronounced at the Paull and S200 m sites) and when the data for each mudflat were averaged over the entire area. Abundance ratios (A/S) were consistently higher at the S25 m and S75 m sites, and were

notably high between April and September, indicating a large number of individuals belonging to very few species. In contrast, biomass ratios (B/A) were consistently higher at the Paull and S200 m sites, and were notably higher in April and June, indicating larger body size during these months.

At the Paull and S200 m sites, both mean and maximum recorded individual length and biomass were greater in June than in January with the largest animals being recorded in January from the S25 m and S75 m sites. This is thought to be due to large scale recruitment of small oligochaete worms and nematodes at the S25 m and S75 m sites which would have led to an overall reduction in mean individual length and biomass at these sites. Mean and maximum recorded length (considering all species within the community) were consistently lower at the S25 m and S75 m sites than at the S200 m and Paull sites. This, together with the difference in seasonal patterns between the two sets of sites, is considered to be related to pollution and is discussed in section 4.5.3. Mean and maximum length of *H. diversicolor* were generally greater at the Paull and S200 m sites, although, also demonstrated by the size frequency data, small numbers of large *H. diversicolor* were recorded from the S25 and S75 m sites. No seasonal patterns of length or biomass distribution were found for this species. In contrast, oligocheates showed a distinct increase in length and biomass between January and June at the Paull and S200 m sites whilst maximum values were recorded in January from the S25 m and S75 m sites. In addition, the length and biomass of the oligochaetes showed a distinct pattern of increase with increasing pollution. Individual nematode lengths also followed this trend, the reasons for which will be discussed in section 4.5.3. These differences in both size/biomass spectra and community biomass/abundance have important implications for bioturbation since larger organisms have greater potential to modify the sediments (Chapter 5).

Various studies which have been carried out in the past have demonstrated this seaonality within benthic communities. Studies by Beukema (1974), spanning five years, showed considerable seasonal variation in the animal abundance, biomass and the structure of the macrobenthic community at Balgzand in the western Wadden Sea. These fluctuations, although variable in magnitude, were found to follow a regular and more or less predictable annual course. Clear differences in the community biomass were found between summer (maximum) (June – September) and winter (minimum) (December – March) with intermediate levels in spring and autumn. Both numbers and individual animal weights were found to decrease between July and February. Hauser (1973, in Beukema, 1974) found biomass figures in winter to be approximately half those in summer on tidal flats in the western part of the German Wadden Sea. Maurer *et al.*,(1979) studied the benthic

communities at two sites off the Delaware coast over a period of one year. In general, a decrease in number of individuals was found between July and April with maximum mean densities being in July. A marked decline occurred in October, at the first site, after which density continued to decline more gradually until the following April. At the second site, density declined in the same manner but with a less dramatic decrease in October. Warwick & Price (1979) found seasonal variation in the numbers of nematodes on the mudflats of the Lynher estuary, Cornwall although species composition was found to be relatively stable. The highest densities were recorded in May with the lowest densities being in late autumn and early winter. Biomass also changed seasonally, following the same pattern as abundance, showing a peak in May.

Beukema (1974) associated this regular pattern of biomass increase during spring with changes in animal abundance (due to mortality, recruitment and migration) and changes in individual weight as a result of growth or weight loss. Weight reduction in bivalves during winter was found to be caused by consumption of reserve material. Beukema (1974) stated that in some species, recruitment of small animals may have contributed to the observed reduction of mean weight and a reduction in total biomass could have been due to individual weight reduction combined with a reduction in total abundance. However, in summer, total biomass increased largely as a result of the growth of the larger, long lived species and the increased abundance of juveniles following recruitment did not significantly contribute to the biomass in the Wadden Sea (Beukema, 1974).

Beukema (1974) found individual weight reduction to be of greater importance in the larger, long lived species (e.g. *Mya, Macoma* and *Arenicola marina*) and found this to account for a greater part of the biomass loss than mortality. Chambers & Milne (1979) also found the greatest variation in individual biomass to be associated with the largest organisms. In contrast, for the short lived species *Corophium volutator,* mortality and reduction in abundance contributed more to the overall decrease in biomass than did changes in individual weight (Beukema, 1974). This study therefore emphasised the importance of changes in individual body weights of larger, long lived species being responsible for changes in the biomass whereas in smaller, short lived species, mortality and change in abundance were thought to be the cause of reduced biomass in autumn and winter.

Chambers & Milne (1979) studied seven species from the Ythan estuary in Scotland and found all to exhibit seasonal changes in biomass, calorific value and body condition (dry weight of flesh in relation to body or shell length) factor. Peak weights were recorded in May for *Cerastoderma edule* and *C. volutator* and in June and October for *H. diversicolor.*

M. balthica showed maximum biomass in July whilst peak weights for *Hydrobia ulvae*, *Littorina littorea* and *Mytilus edulis* were recorded in September and October. The data suggested that a peak in body condition occurred at times when the animals contained ripe gametes and for *H. diversicolor* and *M. balthica*, peak weights were recorded prior to the onset of spawning. It was also suggested that an increase in the condition of *C. volutator* during the winter may be due to the development and maturation of gametes. Larger individuals of *H. ulvae* showed a loss in condition during their main breeding period (April to July) and this was attributed to the release of eggs (Anderson, 1971, in Chambers & Milne, 1979). Given that reproduction is usually seasonal, it is not surprising that corresponding body weight changes are seasonal. Ansell & Trevallion (1967, in Chambers & Milne, 1979) studied body weight changes in *Tellina tenuis* and found an increase in body weight immediately prior to spawning and a decline just after spawning. A second decline was recorded in during winter and was attributed to metabolism of the tissues (glycogen, protein and lipids). Ansell (1975, in Chambers & Milne, 1979) recorded similar patterns for *Astarte montague, A. elliptica* and *A. sulcata*. Changes in body weight in relation to the reproductive cycle are therefore reasonably well documented.

Furthermore, changes in the body condition of invertebrates are also likely to be related to their nutritional status and Chambers & Milne (1979) recorded high body conditions at times of peak food abundance. Estuarine invertebrates are predominantly deposit feeders, feeding on organic matter, micro algae and in some cases, other organisms. Cadee (1971, in McLusky, 1989) showed a clear seasonal pattern of microphytobenthos production on intertidal mudflats in the Wadden Sea which appeared to be closely linked to temperature. Maximum production was found to be in June and July when production was 10-12 times higher than that in December and January. The microphytobenthos can play an important role in the mudflat estuarine ecosystem, often contributing to a major part of the primary production. It is therefore not surprising that highest values of both community and individual biomass have commonly been recorded during the summer months. Baird & Underwood (1989) studied the seasonal dynamics of macrofaunal communities in Chesapeake Bay and found a dominant seasonal cycle in the activities of all sub-communities which was greatest in summer and least in the cold season. Biological activity was found to peak in summer, decrease throughout autumn and winter and rise again in spring. Nutrient inputs were considered to trigger the onset of increased biological activity which persisted throughout spring and summer.

In the present study, individual biomass was found to increase at the Paull and S200 m sites between January and June whereas the opposite was true for the S25 m and S75 m sites.

However, community biomass, together with abundance, increased at all sites during the summer. Given the findings of Beukema (1974), it is thought that changes in biomass at the S25 m and S75 m sites were associated with the large scale changes in abundance (presumably due to recruitment) which occurred at these sites. This statement is supported by the fact that at these sites (particularly the S25 m site), the abundance ratio (A/S) was notably higher between June and September (indicating more individuals / species) whilst the biomass ratio (B/A) was lower, indicating smaller individual body size. The fact that the maximum A/S value and minimum B/A value were both recorded in June suggests that recruitment may have taken place during this month. Garlo *et al.* (1975, in Maurer *et al.*, 1979) also found the spawning of *Mytilus edulis* and the subsequent disappearance of the spat to have a large impact on community biomass.

In contrast, the large increase in biomass at the Paull and S200 m sites was disproportionate to the relatively small increase in abundance. This together with the increase in individual biomass in June suggests that high biomass values during this month were due to changes in body weight and, perhaps, spawning condition although there is no direct evidence for the latter. However, Grant *et al.* (1990, in Scaps, 2000) reported spawning of *H. diversicolor* in the mid and upper reaches of the Humber estuary to take place in June or July. During the present study, sampling was carried out in early June and it is possible that individuals of *H. diversicolor* could have been collected immediately prior to spawning when their biomass may have been elevated. Again, this is supported by the biomass ratio which indicated higher mean individual biomass during the summer (April and June).

In addition, Beukema (1974) found minimum populations of predatory birds to coincide with maximum abundance and biomass of macrobenthic species. Predatory bird populations not only vary in density but also vary the number of prey items they consume in order to meet their minimum ingestion requirements (Chambers & Milne, 1979). Beukema (1974) stated that all bivalve molluscs of a given size contain less meat in winter than in summer which means that birds must either increase the number of prey items or actively select the larger organisms in order to fulfil their nutritional requirements. The Humber estuary is of major importance for overwintering populations of migratory birds with maximum densities generally occurring between late September - October and January - February (Cutts, 2001). This coincides with the lowest abundance and biomass values recorded from Paull and Saltend although bird numbers only correlate with abundance and biomass values for the Paull site (r^2 = 0.9, $p<0.01$). This is because the birds use the S25 m and S75 m sites primarily for roosting and very few birds feed in these areas (N. Cutts, University of Hull. pers. comm.). Marshall (1995) studied spatial and temporal variations in the fish

communities within the Humber estuary. Maximum abundances of flounder (one of the four dominant species) were recorded during the winter months and intertidal trawl sampling revealed that the juveniles were most abundant between August and November. Therefore, it is possible that increased predation contributes to the loss of abundance and biomass during the winter but the primary cause is thought to be the patterns of recruitment and mortality, together with seasonal changes in individual body size.

It is accepted that, due to moisture content and sediment present in the guts of animals, recording wet weight biomass does not give a precise representation of the actual biomass. Ideally, ash free dry weight should be recorded although in the present study, many of the individual organisms were so small that this was not feasible since, following combustion, their weights were too low to register on the balance. Several authors have derived formulae for the conversion of wet weight biomass to ash free dry weight for the various groups of marine organisms (e.g Ankar & Elmgren, 1976; Rumohr *et al.*, 1987; Ricciardi & Bourget, 1998). These formulae have generally been developed for entire groups of organisms (e.g. errant or sedentary polychaetes) and they do not account for seasonal variation in body weight, spawning condition or preservation method. Whilst the 95% confidence intervals were relatively narrow for most species, those associated with the oligochates (the dominant group at some sites sampled during the present study) ranged from 9.2-24.2%. It was therefore considered that using such conversion factors would add to the errors associated with recording wet weight. This was considered to be particularly important for the S25 m and S75 m sites where the oligochaetes were considerably larger than those at the Paull or S200 m sites. It is unlikely that the published conversion factors have taken account of the effects of enhanced growth due to pollution. Similarly, fixing in formalin and subsequent preservation in alcohol leads to weight loss of invertebrate samples (Rees *et al.*, 1990). However, as with the biomass conversions, conversion from preserved to fresh weight was considered to be a further source of error and was therefore not carried out. In the present study, the method used to determine biomass was consistent throughout, allowing direct comparison between samples. In addition, it was also consistent with the method used by Scott (1996) and Allen (2000a) who both studied the macrobenthic communities of the Saltend and Paull mudflats.

During the present study, diversity values fluctuated throughout the year at all sites although no seasonal patterns were observed. This is thought to be due to the fact that whilst abundances changed (which would result in changes to evenness) the actual species present did not. Therefore, there was no evidence for inward or outward migration of certain species from the community as has been observed by some authors (e.g. Boesch, 1973). This lack of

seasonality in diversity was also observed by Maurer *et al.* (1979). In the case of the present study, any increase in diversity was attributed to the random appearance of some of the rarer species. For example, *M. balthica* is known to be present at Paull all year round (personal observation) yet its distribution was patchy and therefore it did not always appear in samples. On occasions when it was recorded, diversity values increased (e.g., January, 2000). The slight increase in diversity at the S75 m site in January was thought to be due to the reduced number of oligochaetes and the greater importance of *H. diversicolor, M. aestuarina* and nematodes.

However, Boesch (1973) found that a minimum of 16 species groups was needed to adequately describe spatial and temporal patterns in diversity in the Hampton Roads area (over 7 months). Considerable variations in abundance patterns by habitat or season were apparent within the species groups with over 60% of the 93 species clearly showing seasonality either in terms of abundance or presence. These patterns were largely reflections of the markedly seasonal spawning cycles of the benthic invertebrates with the loss of individuals from the community being explained by physiological, interspecific biological factors, migration and predation (Thorson, 1966, in Boesch, 1973). Species which were present on a seasonal basis were thought to have been recruited from allochthonous sources. Other species responded primarily to habitat factors (mainly sediment).

4.5.3. Effects of pollution

The discharge at Saltend has had a clear impact on the intertidal communities which was evident throughout the year. This impact does, however, appear to be extremely localised with communities only being affected within 100 - 150 m of the discharge and then only affected in an extreme sense within 50 - 75 m of the discharge. Furthermore, cluster analysis showed the extent of this impact appears to be seasonally influenced. Whilst the S0 m and S25 m (high impact) sites remain different (in terms of their community structure) to the other sites throughout the year, similarity between the S75 m and S200 m sites increased during November and January suggesting a lower impact at the S75 m site during the winter months. In contrast, during the summer, the community at the S75 m site becomes more similar to the S25 m site. This was also demonstrated using ABC (abundance-biomass comparison) curves where Clarkes' *W* statistic indicated a higher degree of stress during the warmer months and a similar pattern of similarity between sites (using cluster analysis) was found when the sediment characteristics were examined (Chapter 2).

Statistically significant correlations were found between various environmental parameters and the faunal community structure and/or the abundance of individual species and these correlations were generally supported by the CCA analysis. However, not all of these parameters appear to influence the community structure to the degree that is indicated by these analyses. Firstly, water content, bulk density and particle size did not change consistently between sites or show any strong relationship to shore height or proximity to the discharge. Furthermore, the differences between Md phi values between sites were small and it was considered unlikely that this parameter could be responsible for such large differences in the community structure. All three of these parameters were, however, included in the top ten combinations of variables explaining faunal community structure by the BIOENV analysis. The CCA analysis indicated that particle size (Md phi) was at least as important as the other variables (with the exception of water content and bulk density) in terms of its influence over species distribution.

Microalgal biomass (chl-a) and sediment carbohydrate (colloidal-S, colloidal-EDTA and total carbohydrate) concentration were also found to be related to both species abundance and community structure and these variables were commonly selected during the BIOENV analysis. However, the highest concentrations were recorded from sites where the faunal community was dominated by oligochate worms. Since oligochaetes live head down in the sediment and feed on the bacteria and organic detritus associated with sub-surface deposits (Birtwell & Arthur, 1980; Hunter, 1981), the enhanced food supply (in terms of microalgae and carbohydrate) at the surface will not be available to these organisms. In contrast, species such as *H. diversicolor* and *C. volutator* have often been found to reduce microalgal biomass as a result of grazing (Gerdol & Hughes, 1994). Whilst lower microalgal biomass at the less polluted sites may, in part, be due to grazing by organisms which feed on diatoms, these species also feed on the bacteria and detritus associated with the surface sediments and, in the absence of diatoms, would be able to feed on other components of the sediment or, indeed, the overlying water. Therefore, microalgal biomass and sediment carbohydrate concentration are not thought to be a limiting factor in terms of species abundance and distribution.

Therefore, based on the data presented here, the differences in Eh and, to a lesser extent, organic content, provide the most likely explanation for the differences in community structure between sites. The correlations between these variables and individual species abundance/community structure are not strong (although there is a large amount of variability in the data) and it should be noted that the sediments in this area are subject to a large number of persistent organic compounds which, although not measured, could also be influencing the community structure. Furthermore, the degree of contamination in the water and distance

from the discharge were not included in the analysis but could, as demonstrated in Chapter 3, affect the community. It should also be noted that the multivariate analyses carried out appear to be interpreting a small amount of variation across a relatively homogeneous area.

As already described, the communities along this pollution gradient showed an almost typical response (in terms of abundance and number of species), according to the Pearson & Rosenberg (1978) model, to organic pollution. Communities within the immediate vicinity of the discharge were impoverished, consisting of large numbers of oligochaete (principally tubificid) worms. With increasing distance from the discharge, the number of species and diversity increased whilst the abundance generally declined. Contrary to the model, the biomass was lowest at the Paull site (i.e. the control site), and often increased to a maximum at the most polluted sites (S75 m and S25 m). This is thought to be due to the high abundance of oligochaetes at these sites together with the fact that they were larger in terms of length and biomass than at the other sites. This is supported by the fact that the biomass ratio (B/A) was consistently lower at the S25 m and S75 m sites than at sites further away from the discharge. It should also be noted that the SAB model was developed in a fully marine area influenced by organic pollution. It does not account for macrobenthic responses associated with other forms of pollution (e.g. metals or petrochemicals) (Davies & Tomlinson, 1991, in Tapp *et al.*, 1993) and therefore complete adherence to the model by the communities present at Saltend and Paull would not necessarily be expected.

Nikitik (University of Hull, pers. comm.) also found the benthic communities at these sites to show some degree of similarity to the Pearson & Rosenberg SAB curve. Abundance and biomass were found to reach a maximum 100 m from the outfall, corresponding to the peak of opportunists. The number of species showed a general pattern of increase with distance from the discharge. Although the relationships were not strong, they indicated a successional change in the benthic community with distance from the discharge. This demonstrates that the communities in the immediate vicinity are subject to a higher level of stress than those further away. As indicated by the present study, Nikitik (University of Hull, pers. comm.) also noted an increase in diversity with increasing distance from the outfall. This response (in terms of number of species, abundance and biomass) has been reported by numerous authors in the past (e.g., Pearson & Rosenberg, 1978; Pearson *et al.*, 1982; Yokoyama, 2002)

The findings of this study are consistent with those of McLusky *et al.* (1976), McLusky (1982) and McLusky & Martins (1998) who examined the long term effects of a petrochemical discharge on the intertidal mudflats of the Forth estuary, Scotland. These authors reported a defaunated zone in the immediate vicinity of the outfall, which was not

found during the present study, and an impoverished community within 500 m of the discharge. Beyond 1500 m, the number of species increased with abundance and biomass decreasing to levels more typical of an estuarine community. Improvements in effluent quality have, over time, resulted in increases in the abundance of *Corophium volutator*, *Macoma balthica*, *Eteone* sp. and spionid worms and the disappearance of the defaunated zone. These changes were attributed to reductions in the concentrations of toxic compounds rather than organic material.

When examining all species combined, there was a trend of increasing mean and maximum individual length and biomass with increasing distance from the discharge. The length and biomass frequency distributions also indicated an increase in the frequency of larger organisms with increasing distance from the source of pollution. Mean and maximum length / biomass and the size frequency distributions for *H. diversicolor* also generally followed this trend. The dominance of small, opportunistic species at polluted sites, in comparison with more complex communities at unpolluted sites, containing annelids, molluscs and crustaceans with a wide range of sizes and life history patterns, is a typical response to organic pollution (Gray, 1982; Pearson *et al.*, 1982). Schwinghamer (1988) also demonstrated the way in which differences in size spectra between communities could, in part, be related to their proximity to pollution sources. However, the oligochaetes at the sites close to the discharge were considerably larger than at the other sites suggesting that these organisms are thriving as a result of exposure to the effluent. It should also be noted that whilst very few *H. diversicolor* were recorded at the S25 m site, those that were there were large.

Laughlin *et al.* (1981) found short term exposure to low concentrations of water soluble hydrocarbons to result in increased growth of crab zoeae (*Rhithropanopeus harrisii*). This was explained by hormesis whereby a low concentration of a substance acts as a stimulus through the enhancement of physiological and biochemical processes. However, there was no suggestion that this process would occur following long term exposure, such as that experienced by the organisms found during the present study. Saiz-Salina & Francés-Zubillaga (1997) examined the effect of anoxic sediments on the growth of juvenile *H. diversicolor* and found growth to be enhanced in anoxic sediments. Hormesis was, again, considered as an explanation for this but also the fact that the mucous lining of the burrows of Nereid polychaetes may reduce exposure to contaminants. However, this enhanced growth was thought to be better explained by the enhanced nutritive status of the sediment. According to Valiela (1984, in Saiz-Salina & Francés-Zubillaga, 1997) and Holmer (1999), anaerobic degradation of organic matter is less efficient at harnessing energy than is aerobic metabolism. Therefore, anaerobic sediments can contain a reservoir of organic matter with an

associated microbial biomass. Since *H. diversicolor* is able to ventilate its burrow with overlying aerated water, it is able to gain access to this store of nutrients which would otherwise be unavailable to aerobic organisms.

Burrow aeration promotes microbial growth at the boundary between the oxic and anoxic zones where organic carbon and nitrogen accumulate (Meyer-Reil, 1994, in Saiz-Salina & Francés-Zubillaga, 1997). Anderson & Meadows (1978, in Saiz-Salina & Francés-Zubillaga, 1997) also found high densities of heterotrophic bacteria and chlorophyll concentrations to be associated with the lining of the burrows of *H. diversicolor* in comparison with surrounding anoxic muds. de Zwann & Babarro (2001) also stated that anoxia promotes microbial growth. Clough & Lopez (1993) found that the capitellid polychaete *Heteromastus filiformis* was also able to exploit these reserves of organic matter in anoxic sediments.

There is no evidence in the present study to suggest that the growth of *H. diversicolor* is enhanced as a result of exposure to anoxic sediments. However, given the findings of Clough & Lopez (1993) and Saiz-Salina & Francés-Zubillaga (1997), it would not be unreasonable to assume that the growth of the oligochaetes present at the S25 m and S75 m sites might be enhanced as a result of the anoxic sediment conditions. The ability of oligochaetes to tolerate pollution is well known (e.g. Lindeman, 1942, in Theede, 1969; Hunter & Arthur, 1978; Coates & Ellis, 1980; Degn & Kristensen, 1981; Hunter, 1981; Bagheri & Mclusky, 1982; Giere & Pfannkuche, 1982; Dubilier *et al.*, 1995) and Pearson & Rosenberg (1978) classified several oligochaete species as indicators of organic pollution. Hauschildt-Lillge (1982) also found that the enchytraeid *Lumbricillus lineatus* could increase cocoon production to compensate for reduced hatching success resulting from long term exposure to petroleum hydrocarbons.

However, not all species are tolerant of exposure to petrochemical or organic effluents and sediment toxicity studies (Chapter 3) demonstrated reduced survival of *H. diversicolor*, *C. volutator* and *M. balthica* at the S0 m and S25 m and, in the case of *C. volutator* and *M. balthica*, the S75 m site. LT_{50} values suggested that long term survival of these species at these sites was unlikely and sub-lethal effects such as reduced growth and reproductive success could be expected. Whilst the concentrations of several metals were slightly elevated at sites close to the discharge, many exceed their respective sediment quality guideline and PEL (Probable Effects Level) values at all sites, including those where more diverse communities were found. The implications of elevated metal concentrations and their possible toxic effects are discussed in Chapter 3. Stark *et al.* (2003) stated that oil in sediments represents a chemical and physical disturbance that could result in primary toxic

effects and secondary changes in sediment properties such as organic enrichment and increased anoxia. For example, de Zwann & Babarro (2001) found mortality to increase in *M. balthica* exposed to conditions of anoxia as a result (in part) of bacterial infection of the clams due to an increase in bacterial concentration following the onset of anoxia. These authors suggest that bacterial interference may also have an impact on the survival time of other bivalve and other invertebrate species under anoxic conditions in their habitat.

Despite its general high toxicity, several species are able to survive in sulphidic environments for a limited period of time. This can be the result of exclusion of sulphide from the body, detoxification (Powell & Somero, 1986 in Llansó 1991) or by switching to anaerobic respiration thus avoiding the inhibition of respiritory enzymes (i.e. cytochrome-C-oxidase) (Degn & Kristensen, 1981; Llansó, 1991; Hagerman, 1998; Bagarinao, 1992, in Modig & Ólafsson, 2001). Detoxification by oxidation is thought to be the most important adaptation to tolerating sulphide (Jahn & Theede, 1997). Sulphide tolerant animals may protect themselves from sulphide by the binding of metal-sulphur precipitations within the vesicles in the mantle edge (Windhoffer & Jahn, 1994; 1995, in Jahn *et al.*, 1997). Dubilier *et al.* (1995) found the oligochaete *Tubificoides benedeni* to tolerate high concentrations of sulphide through both anaerobic respiration and the deposition of iron sulphides in the mucous layer above the cuticle of the worm. These iron sulphides are either shed through moulting or reoxidised. However, Jahn (1997, in Jahn & Theede, 1997) suggested that this method of sulphide detoxification is only temporary since metal stocks in the tissues are quickly diffused. It was therefore suggested that the oxidation of sulphides to thiosulphate (main oxidation product) and small amounts of sulphite and elemental sulphur (latter two of only minor importance) is a more important alternative to precipitation. In addition, some species display avoidance behaviour (Modig & Ólafsson, 2001).

Detoxification mechanisms may, in part, explain why some species were found to thrive and certain species were periodically found at the more polluted sites (e.g., the occasional appearance of *C. volutator* at the S75 m site and the low abundance of *H. diversicolor* at the S25 m site). Wieser & Kanwisher (1961, in Theede *et al,* 1969) found that several nematode species could survive anoxia for more than 60 days and Lindeman (1942, in Theede *et al,* 1969) found freshwater *Tubifex* species to survive for more than 120 days under anaerobic conditions. Theede *et al.* (1969) found that lamellibranchs (*Scrobicularia plana* and *Mya arenaria*) to survive short term exposure to anoxia by closing their shells and that these species could tolerate anoxia for longer than *H. diversicolor*. However, *H. diversicolor* is considerably more tolerant of anoxia and high sulphide concentrations than other Nereid polychaetes (Theede *et al.*, 1969; Vismann, 1990; Sampou & Oviat, 1991, in Miron &

Kristensen, 1993a; 1993b). It has been suggested that this tolerance may have evolved at the expense of competitive ability, allowing the species to exploit habitats in which other Nereid polychaetes could not survive (Miron & Kristensen, 1993a; 1993b). Miron & Kristensen (1993a; 1993b) stated that respiration could be impaired as a result of exposure to sulphide and that burrow ventilation (and thus, sulphide removal) was directly proportional to body weight. Jensen (1986) found nematodes to have a higher length:body radius ratio in sulphidic habitats and this, in addition to the nutritive status of the sediment, may explain why the oligochaetes at the S25 m site are considerably larger than those at sites further away from the discharge (with a lesser degree of anoxia). Furthermore, whilst the abundance was low, large individuals of *H. diversicolor* were periodically recorded at the S25 m site but relatively few small ones. Given that *H. diversicolor* is classed as an omnivorous species, inward migration of larger worms to the more polluted sites may have occurred with the worms feeding on the abundant oligochaetes. Their large body size may have allowed them to survive for short periods (e.g. a few hours to a few days, as reported by Llansó (1991)) before migrating away from the site. Jahn & Theede (1997) and Jahn *et al.* (1997) also found the degree of sulphide tolerance to be positively correlated with body size in *Macoma balthica*.

Llansó (1991) found exposure of the polychaete *Streblospio shrubsolii* to anoxia or anoxia and sulphide to result in reduced feeding and burrowing followed by death within 2-3 days. It was also demonstrated that *S. shrubsolii* is a mobile, opportunistic species which can vacate its tube and rapidly colonise a more favourable environment. Therefore, the absence of this, and many of the other species present at greater distances from the discharge, from the S25 m site could either be due to the inability to survive and reproduce or to deliberate outward migration. Gamenick *et al.* (1996) found *C. volutator* to he highly intolerant of sulphide and Meadows *et al.* (1961) found this species to actively avoid sulphidic sediments. It is thought that seasonal changes in community structure at the S75 m site, and its seasonal increase in similarity to the S200 m or the S25 m sites, is related to the inward and outward migration of certain species. For example, during the autumn and winter, when the degree of anoxia was alleviated by cooler temperatures, low numbers of the species *Corophium volutator* and *Manayunkia aestuarina* were present, increasing the similarity between the S75 m and S200 m sites. This was also demonstrated in terms of the sediment properties. In contrast, these species were absent during the warmer months, when the sediment was more anoxic, making the site more similar to the S25 m site. Brooks *et al.* (2003) monitored the recolonisation of sediments beneath a salmon farm following chemical and biological remediation. Polychaetes and crustaceans were the first groups of organisms to colonise the sediment following significant reductions in organic content and sulphide concentrations. This suggests that these groups are either more tolerant of organically polluted sediments and can

colonise sediments in an earlier stage of remediation than can bivalves or it may illustrate that these groups are more opportunistic than the bivalve species which followed. This may provide an explanation for the fact that, along the pollution gradient at Saltend, polychaetes and amphipods were found closer to the discharge than bivalves were.

Warwick (2001) found *Streblospio, Pygospio, Manayunkia aestuarina* and oligochaetes (principally tubificidae) to be more abundant in the heavily metal contaminated Fal estaury than in the less contaminated surrounding estuaries. *Corophium volutator* was completely absent and *H. diversicolor* was reduced in abundance. However, despite the comparatively elevated metal concentrations at the S25 m site, all of these species are absent with the exception of the oligochaetes. This could be related to interspecific differences in metal (or other pollutants) tolerance or due to some ecological interaction effect. For example, large assemblages of burrowing deposit feeders may prevent the settlement of tube building species by disruption of sediments (Woodin, 1976) and Brenchley (1981, in McCann & Levin, 1989) showed that burrowing and feeding activities of deposit feeders to inhibit less mobile fauna. McCann & Levin (1989) found oligochaete (*Monopylehorus evertus*) densities of 8200 to 25,000 m^{-2} (1 and 3 times the field density) to decrease both survivorship and growth of juveniles of the polychaete *Streblospio benedicti*. It was anticipated by these authors that a reduction in growth would result in a reduction in reproductive success since fecundity in this species is positively correlated with body size (Levin, 1986, in McCann & Levin, 1986).

4.5.4. Critique of data analysis techniques.

Individually, the use of diversity indices, SAB analysis (Pearson & Rosenberg, 1978), Abundance - biomass comparison curves (Warwick, 1986) and cluster analysis has demonstrated the differences between those communities which are strongly influenced by pollution and those which are not at Saltend and Paull. Furthermore, each technique gave a similar result so that the output of one supports the output of the others, hence denoting robust patterns. Despite this, a number of techniques commonly used for the detection of change in marine communities are not considered appropriate for the analysis of estuarine community data and have, in the past, been heavily criticised.

The ABC method has been successfully applied to a number of subtidal, fully marine environments and Warwick (1986) found that in only one of 22 cases (Loch Linnhe and Loch Eil) did the results give a false impression of the pollution status of the community. This was thought to be due to failure to collect a sufficiently representative sample of the rarer biomass dominants. In the present study, the ABC method not only indicated increasing stress with increasing proximity to the outfall but also showed higher stress during the warmer months

(as indicated by Clarkes' *W* statistic). However, Nikitik (University of Hull, pers. comm.) used the same technique and did not find any indication that the communities within the immediate vicinity of the discharge were stressed. It was therefore concluded that this technique is of little use in estuarine studies.

There are several examples of studies where the ABC method failed to adequately describe the pollution status of intertidal and, particularly, estuarine communities. Based upon the assumption that intertidal areas would naturally be more stressed than subtidal areas due to the continually fluctuating environmental conditions, Beukema (1988) tested the suitability of the ABC method for the analysis of intertidal community data. In general, the results were inconsistent with several unpolluted sites being classified as severely polluted or disturbed. This was found to be due to high numbers of small organisms such as *Corophium volutator* and *Hydrobia ulvae* which did not contribute proportionally to the abundance and biomass and therefore gave the impression of a disturbed community. There is, however, no evidence to suggest that these species are indicative of pollution or even found in any significant abundance in such highly stressed areas as the curves were implying.

Dauer *et al.* (1993) attempted to apply the ABC method not only to an estuarine area (lower Chesapeake Bay) but also areas subjected to industrial pollution and seasonal anoxia / hypoxia. It was noted that intense recruitment events by one species could cause the abundance curve to lie above the biomass curve, falsely indicating a high level of stress. Inappropriate classification of stress may also result from the presence or absence of several or even single dominant species (in terms of the biomass). A solution to this may be ensuring adequate replication to collect the rarer biomass dominants although the occurrence of these species in sediments containing high levels of contaminants may also give a false indication of the level of stress. This problem was also highlighted by Warwick (1986). Similarly, a dense recruitment event in an unstressed system may give the impression of a high level of stress due to the large numbers of small individuals (Beukema, 1988; Dauer *et al.*, 1993). Dauer *et al.* (1993) found that recruitment of high numbers of the small polychaete *Marenzelleria* (*Scololepides*) *viridis* to indicate stress in the community. However, this species was classed as an indicator of organic pollution by Pearson & Rosenberg (1978) and its presence therefore suggests that the community is, in some way, disturbed.

Warwick & Clarke (1994) stated that a change from higher biomass dominance with increasing levels of disturbance results from a change in the proportions of different phyla, some phyla having larger body sizes and a change in the relative distributions of abundance and biomass among the species within the Annelida (specifically Polychaeta) but no other

major phyla (Mollusca, Crustacea, Echinodermata). It is well known that small polychaetes predominate among the pollution indicator species (species which increase in abundance under conditions of pollution, particularly or organic enrichment) (Warwick & Clarke, 1994). In cases where the ABC method has not succeeded in providing a measure of the pollution status of a community, it is because non-polychaete species have been dominant. These small non-polychaete species are not necessarily indicative of pollution. It was therefore suggested that the results be treated with caution if the species responsible for the polluted configurations were not polychaetes (Warwick & Clarke, 1994).

Meire & Dereu (1990) applied the ABC method to the unpolluted Oosterschelde (Netherlands) and the grossly polluted Westerschelde (Belgium) estuaries and found that whilst increased levels of stress were indicated as a result of long tidal exposure of mussel fishing, the analysis showed the Oosterschelde to be unstressed. This suggested that the ABC method was reasonably reliable. However, in the Westerschelde, the method also indicated that the community was unstressed, despite the level of pollution in the area. This was explained by the fact that there were very few species present and in such cases, the numerically dominant species also dominate the biomass. It was concluded that the success of the method for detecting pollution was largely dependent on the availability of reference data and on an adequate sampling programme. These studies highlight the fact that the very nature of estuarine communities (i.e., low species diversity and high abundance) makes it difficult to separate the effects of pollution on community structure from the effects of population dynamics and recruitment.

Warwick & Clarke (1994) stated that all measures of the biological effects of pollution are subject to error, particularly because of the immense variability inherent in real population dynamics and 'spot' sampling strategies. Dauer *et al.* (1993) concluded that no single method of analysis was likely to provide pollution stress classifications without unacceptable misclassifications, particularly in an estuarine environment. Therefore, it is necessary to use several methods for robustness (Dauer *et al,* 1993). Whilst the ABC method is not necessarily more sensitive than the calculation of diversity indices and is certainly less sensitive than multivariate techniques, it has the advantage of providing absolute rather than a comparative measure of pollution induced disturbance (Warwick, 1993 in Warwick & Clarke, 1994). The fact that the outcome of the ABC analysis has led to the same conclusions as that using other techniques demonstrates that, in the case of the present study, the output is reasonably reliable.

4.6. SUMMARY AND CONCLUSIONS

- Community structure was found to be influenced both by the discharge and by season.

- The communities found at Saltend and Paull in the present study were consistent with those of previous studies (Scott, 1996; Allen, 2000a; Nikitik, University of Hull, pers. comm.), being primarily composed of oligchaete worms within the vicinity of the discharge with numbers of *Hediste diversicolor, Manayunkia aestuarina, Corophium volutator, Macoma balthica* and spionid worms increasing with decreasing levels of pollution. Three main community types were found, corresponding to distance from the discharge, the first including the most contaminated sites between 0 and 75-100 m from the discharge which were characterised by large numbers of oligochaetes and some *H. diversicolor,* the abundance of which increased with distance form the discharge. Communities within the second cluster (predominantly including sites between 100 and 200 m from the discharge but also some closer sites at certain times of the year) were dominated by *H. diversicolor* with increasing numbers of spionid worms, *M. aestuarina* and decreasing numbers of oligochaetes in comparison to communities within the first cluster.

- The benthic community was found to broadly follow the pattern of the Pearson and Rosenberg (1978) SAB) model with high abundance, low biomass and low numbers of species within the immediate vicinity of the outfall. Univariate statistics (including diversity indices, abundance and biomass ratios and ABC analysis), despite their drawbacks, all indicated a higher level of stress at sites adjacent to the discharge. The level of stress was found to be greatest during the summer months, due to higher temperatures and more anoxic conditions within the sediment.

- Patterns of abundance were seasonal and were most pronounced at sites adjacent to the outfall (S25 m), with the highest values being recorded in summer. The number of species present varied very little over time. Biomass was also highest during the summer although in contrast to abundance, this was most pronounced at the Paull and S200 m sites. The magnitude of this difference in disproportionate to the change in abundance indicating individual growth rather than increased abundance. At the S25 m site, low biomass in June coincided with the highest abundance indicating that the biomass is made up by sheer numbers of organisms.

- The size and biomass frequency distributions indicated an increase in the frequency of larger organisms with increasing distance from the source of pollution. Furthermore, these distributions demonstrate the way in which the community changes from one dominated by small organisms to one containing all size classes as the level of pollution declines.

- Mean and maximum recorded length and biomass were generally greatest at the Paull and S200 m sites when all species were considered. However, the oligochaetes at the sites close to the discharge were considerably larger than at the other sites suggesting that these organisms are thriving as a result of exposure to the effluent.

CHAPTER 5

BIOTURBATION STUDIES

5.1. INTRODUCTION

As stated in Chapter 1, the effect of the sediment characteristics on benthic fauna has been relatively well studied but much less appears to be known about the effect of the organisms on the sediment properties, particularly with reference to sediment transport. The activity of benthic animals has a profound effect on their environment (Meadows & Campbell, 1988; de Wilde, 1991; Wheatcroft *et al.*, 1994), resulting in significant alteration of the sediment properties (Rhoads, 1974; Jones & Jago, 1993; Winston & Anderson, 1971). As stated in Chapter 1, the processes leading to such modifications are collectively termed biomodification or biogenic reworking which was defined by Rhoads (1967) as the result of sediment ingestion, manipulation (e.g. tube construction) and displacement as the animal passes through or over the sediment. The term biomodification encompasses the processes of (1) bioturbation (sediment mixing or disturbance) which generally results in bioresuspension and destabilisation of the bed, (2) biodeposition as a result of suspension feeding and the trapping of sediment particles within features such as tube mats, (3) bioirrigation through burrow ventilation and (4) biodiffusion where organisms move sediment particles randomly over short distances. A more detailed description of these processes is given in Chapter 1. The present chapter focuses primarily on bioturbation, examining the effects of burrowing and deposit feeding organisms on the sediment properties. This term is therefore used throughout.

Bioturbation has been shown to be dependent upon temperature (Rhoads, 1967) and particle size (Wheatcroft, 1992) and shape (Whitlatch, 1974, in Wheatcroft, 1992). However, the factors most highly correlated with bioturbation are feeding method and level in relation to the sediment-water interface, organism size and degree of mobility, population density, burrowing depth and the density of and spacing between animal tubes. To this, community structure should be added since different compositions of organisms with varying behaviours will have significantly different effects on the sediment properties (Rhoads, 1974; Lee & Swartz, 1980, in Mahut & Graf, 1987; Rhoads & Boyer, 1982). Fauchald & Jumars (1979) and Barnes (1987) provide a good description of invertebrate diets and feeding behaviour and Dauwe *et al.* (1998) defined a number of functional feeding groups, based on the dominant food source of each particular organism. These included carnivores, omnivores and detritivores / bacteriovores, suspension feeders, interface feeders (capable of alternate

suspension and surface deposit feeding), surface deposit feeders and sub-surface or head down deposit feeders. Of these broad modes of feeding, deposit feeding has been postulated to be the dominant transport mechanism (Thayer, 1893, in Wheatcroft, 1992).

In general, four classes of bioturbation have been described. Biodiffusive mixing describes random mixing of the sediment, leading to the uniform distribution of particles / organic matter within the bioturbated layer of the sediment (Gerino *et al.*, 1993; Boudreau, 1986a, in Dauwe *et al.*, 1998). Sub-surface, or head down, deposit feeding species, such as *Arenicola marina* and oligochaetes, which transport sediment from some depth within the sediment to the surface are termed 'conveyor belt' species (Gerino *et al.*, 1993; Wheatcroft *et al.*, 1994). Conveyor belt transport has also been termed 'active transport' (Gerino *et al.*, 1993). 'Reverse conveyor belt' transport describes the downward movement of sediment particles from the surface to some depth within the bed (e.g., *Hediste diversicolor*) (Benninger *et al.*, 1979; Boudreau & Imboden, 1987, in Wheatcroft *et al.*, 1994). Gerino *et al.* (1993) referred to reverse conveyor belt species as 'regenerators' which dig burrows and actively transport particles (e.g. in the form of faecal pellets) sediment to some depth within the bed. This activity may also result in the passive downward transport of particles as they fall into burrows although Wheatcroft (1992) stated that the downward percolation of fine particles in cohesive sediment would be unlikely where the gravitational forces on the particles was balanced by cohesive or adhesive forces. Finally, Jahnke *et al.*(1986, in Dauwe *et al.*, 1998) described the process of surface deposition where particles are deposited on the sediment surface as faecal pellets. All modes of transport have important ramifications for the redistribution and chemical transformation of sediments and particle subduction rates depend strongly on bioturbation mode. Therefore, identifying bioturbation mode is as important as identifying the rate (Wheatcroft *et al.*, 1994).

5.1.1. Effects of bioturbation on the physical and chemical properties of sediments.

Sediment bioturbation has important impacts on a wide range of phenomena in the coastal ocean with physical, chemical and biological consequences. The effects of benthic organisms on the physical properties of the sediment include changes in water content, grain size distribution, bed roughness, compaction, adhesion and rate of deposition (Rhoads, 1974; Rhoads & Boyer, 1982, Reichelt, 1991; Hall, 1994). In turn, this alteration of the sediment properties as a result of bioturbation can lead to changes in sediment stability and erosion potential (Winston & Anderson, 1971; Rhoads, 1974; Rhoads & Boyer, 1982; Jumars & Nowell, 1984a; 1984b; Meadows & Meadows, 1991; Jones & Jago, 1993; Widdows *et al.* 1998a; 1998b).

5.1.1.1. Water content.

The combined effect of burrowing and faecal pellet production causes the formation of void spaces within the sediment and therefore leads to an increase in water content (Hall, 1994). Rhoads & Boyer (1982) found that intensely worked muds generally contain around 60% water and can contain as much as 70%. Organisms such as *Macoma balthica, Yoldia limatula and Arenicola marina* can cause complete liquification of the sediment which is then ejected, into the water column, from their burrows. Water is injected into the sediment by some organisms in order to facilitate movement thus causing an increase in pore water pressure (Rhoads & Boyer, 1982) and the effect of muscular contractions by moving organisms also causes dilation of the sediment (Rhoads, 1974). In cohesive sediments, the effects of this may remain until the sediment is processed again. In contrast, the lack of cohesion between the particles in sandy sediments causes large voids, resulting from feeding, to collapse (Hall, 1994) which together with the improved drainage means that these sediments usually contain less water than muds.

Burrows, bound by mucous, constructed by benthic organisms create vertical and horizontal channels through which water may flow and therefore affect permeability (Meadows & Meadows, 1991). Meadows and Tait (1989) and Meadows & Hariri (1991) found that the vertical burrows constructed by *Hediste diversicolor* increased permeability whilst the 'U' shaped burrows of *Corophium volutator* reduced permeability. This was thought to be due to increased compaction at the burrow walls and the fact that mucous fills the interstitial spaces, thus preventing the passage of water (Meadows & Meadows (1991). These authors also suggested that permeability generally decreases with increasing organic input, increasing microbial activity and decreasing redox potential.

5.1.1.2. Particle size.

Fenchel *et al.* (1975) and Wheatcroft (1992) both showed that some deposit feeding species actively select the range of particle sizes they ingest. Taghon (1989, in Wheatcroft, 1992) demonstrated how the preferential ingestion of fine particles by head down deposit feeders could lead to biogenic grading of the bed with finer particles, ejected as pseudofaeces, being concentrated at the surface. Faecal pellets, which can also be ejected at the surface, have higher deposition rates than their constituent particles and therefore settle out close to the site of production (Rhoads, 1974). In areas of intensive reworking and relatively low sedimentation rates, the surface layers of the sediment can be dominated by faecal pellets, the concentration of which generally decreases with increasing depth (Rhoads, 1974). Not only is particle size distribution altered by this activity but also the shape and cohesive / adhesive properties of the particles (Rhoads, 1974).

The production of faecal pellets together with the secretion of mucous, increased depth of oxygen penetration and increase in the surface area of the sediment-water interface (through the construction of burrows) by burrowing macrofauna encourages the growth of bacteria, benthic diatoms and meiofauna. These beneficial effects of bioturbation, known as 'gardening' (Hylleberg, 1975), are described in Chapter 2.

5.1.1.3. Oxygen penetration and redox conditions.

Burrowing animals also significantly affect the chemical conditions within the sediments and one particularly important consequence of bioturbation is the introduction of relatively oxygen rich bottom water into the sediments (Libes, 1992; Mortimer *et al.*, 1999). Berner (1972, in Rhoads, 1974) found the rate of diffusion of oxygen into the sediments of the Long Island Sound to be several orders of magnitude greater in the presence of benthic fauna. Bioturbating organisms actively pump oxygenated water into their burrows and oxygen may reach considerable depth, thus altering the spatial distribution of biogeochemical zones within the pore water (Mortimer *et al.*, 1999). In the absence of burrowing animals, the diffusion of oxygen is restricted to the top few millimetres of the sediment whereas, in highly reworked sediments (e.g., with equilibrium communities), the anoxic layer is considerably deeper than in sediments with a lower level of bioturbation (Rhoads, 1974; Meadows & Campbell, 1988). The presence of burrows increases the surface area of the sediment-water interface and therefore oxygen or redox potential gradients extend horizontally within the sediment as well as vertically (Aller, 1982). Aller (1982) also stated that chemical gradients exist around faecal pellets because during microbial degradation, the oxidation of carbohydrates by heterotrophic bacteria and bacterial nitrification increase the oxygen demand within the sediment.

Mixing by burrowing infauna results in the transportation of black, sulphide rich mud, from below the RPD (redox potential discontinuity) to the oxidised layer which may result in an increased oxygen demand close to the surface and a subsequent reduction in pH of the near bottom water (Rhoads, 1974). Thorough mixing also prevents the formation of other chemical gradients that would otherwise be found in the sediment and has some impact on the rate of diagenetic reactions (Libes, 1992). The majority of bioturbation occurs in the oxic layer, above the RPD and therefore, chemical distributions in the oxic zone are relatively homogeneous. In contrast, anoxic sediments tend to have well defined concentration gradients. The chemical gradients within sediments are summarised in Chapter 2.

5.1.1.4. Nutrient cycling.

Bioturbation increases the surface area available for chemical exchange and, through modification of the redox conditions, alters the adsorption-desorption characteristics of the sediment. Bioirrigation of burrows also greatly enhances solute transport and nutrient cycling (Rhoads, 1974; Mortimer *et al.*, 1999). Berner *et al.* (1972, in Rhoads, 1974) found that mixing by benthic infauna greatly accelerated diffusion and transport of chemical substances and nutrients. Davey & Watson (1995) found that fluxes of soluble ammonia and silicate were 100 times greater in the presence of *Hediste diversicolor* in the Tamar estuary and established that this was due to burrow irrigation which caused nutrient enriched pore waters, diffusing through the burrow walls, to be flushed into the water column. Mortimer *et al.* (1999) found a clear pattern of decreasing phosphate efflux from the sediment with increasing density of *Hediste diversicolor*. This was explained by the higher redox values in the surface sediment, caused by the activity of *H. diversicolor*, which favours the adsorption of these substances onto iron oxides. Rhoads (1974) stated that head down deposit or conveyor belt species played an important role in the recycling of buried nutrients by reintroducing them to the active sediment layers from below the RPD. This influences the provision and distribution of microbial substrates (Yingst & Rhoads, 1980, in Wheatcroft & Martin, 1996; Dauwe *et al.*, 1998).

Macrofauna directly affect the cycling of carbon, nitrogen, sulphur and phosphorus by assimilating these elements from detritus. A large proportion of this is respired or excreted back into the environment as metabolites, with the rest being incorporated into the biomass and released following death, due to bacterial decay (Rhoads, 1974). Both aerobic and anaerobic decomposition of organic matter can be stimulated through bioturbation (Aller, 1982; Andersen & Kristensen, 1991; Dauwe *et al.*, 1998). The increased oxygen penetration provides the necessary electron acceptors for aerobic decomposition in the surface layers. The downward transport of sulphate and nitrate provides the necessary electron acceptors for anaerobic decomposition in the anoxic zone whilst the reduced metabolites (sulphide and ammonium) are simultaneously removed (Andersen & Kristensen, 1991). This enhanced flux of solutes in and out of the sediment, together with enhanced microbial growth, can significantly increase the rate of benthic metabolism (Andersen & Kristensen, 1991).

5.1.1.5. Contaminant flux.

This activity may also be important in the reintroduction and resuspension of pollutants (Wheatcroft & Martin, 1996). Metals (and other pollutants) become incorporated into the sediment through precipitation, adsorption and the formation of complexes with sediment particles (Rhoads, 1974). Mixing by animals may either cause these to be resuspended or

incorporated into the sediment fabric (Wheatcroft & Martin, 1996). Following ingestion by animals, metal complexes may be broken down by the acidic conditions in the gut and the metals may then be absorbed by and become concentrated within the animal (e.g., *Nucula* sp., as described by Rhoads, 1974). These are then released following the death of the animal. According to Friedman & Dingan (1968, in Rhoads, 1974) algae are also effective at concentrating metals.

Rasmussen *et al.* (1998) and Petersen *et al.* (1998) found *Arenicola marina* to increase the rate of incorporation of cadmium into the sediment by a factor of 1.5 – 2 and Petersen *et al.* (1998) found *Hediste diversicolor* and *Corophium volutator* to have a similar effect. Petersen *et al.* (1998) suggested that the downward transport of cadmium was caused by adsorption to metal oxides (magnesium and iron) and to the mucous lining of the burrows. The effect of bioturbation on the fate of hydrocarbons was studied by Gilbert *et al.* (1994) and Gilbert *et al.* (1996). *Hediste diversicolor* was found to initially enhance the rate of incorporation of hydrocarbons into the sediment as a result of burrow construction. However, the major influence of polychaetes on hydrocarbons was found to be their removal from the sediment. Schaffner *et al.* (1994; 1997) found particle resuspension by macrofauna to enhance the loss of hydrophobic organic carbon compounds from the sediment. The extent of this loss was found to increase with temperature suggesting higher rates of loss during the summer with the possible retention of these compounds during the winter (Schaffner *et al.*, 1994; 1997). Lee *et al* (1979) and Gilbert *et al* (1996) also highlighted the importance of polychaete worms in the degradation of hydrocarbons.

5.1.2. Measurement of bioturbation

According to Wheatcroft *et. al* (1994), one of the reasons behind the lack of understanding of the biology of bioturbation is the lack of suitable methods and materials for its quantification. Tracers used for this purpose should ideally be inert; detectable at low concentrations in order to allow for large scale dilution within the sediment; of a similar size, shape and density to the sediment into which it is to be placed so as not to interfere with the transport kinetics of the sediment and permanent (Wheatcroft *et al*, 1994). Several methods have been employed in the past, using a variety of techniques applicable both to the laboratory and the field.

5.1.2.1. Natural Radionuclides.

Natural radioisotopes have been used by several authors (e.g., Smethie *et al*, 1981; Wheatcroft & Martin 1996) in order to measure bioturbation rates. For example, Smethie *et al.* (1981) used Radon-222 distribution in sediment cores in order to determine the degree of irrigation and mixing of the sediments. This method was based on the fact that radium-226 is

associated with sediment particles and is therefore enriched within the sea bed in comparison with the overlying water. ^{222}Rn ($t^{1/2}$ 3.83 days) is the decay product of ^{226}Ra ($t^{1/2}$ 1600 years) and diffuses into the pore water also resulting in enrichment in comparison with the overlying water. The concentration gradient causes the ^{222}Rn to move upwards by molecular diffusion, forming a radon deficit (i.e., the difference between actual ^{222}Rn concentration and that predicted from ^{226}Ra decay), a process which is enhanced by biological activity. ^{222}Rn deficits were found to be greatest in areas of greater levels of biological activity. Other radionuclides used include ^{234}Th, ^{137}Cs and ^{210}Pb (Wheatcroft *et al.*, 1994, 1996; Gerino *et al.*, 1998).

The use of natural radionuclides as tracers of biological sediment reworking has yielded some useful results but their use is limited by the fact that the input function of natural radionuclides cannot be controlled and they can therefore not be used to directly track particle mixing of a particular size or type and it is not possible to distinguish between bioturbation modes. It is also difficult to demonstrate particle mixing by animals with life spans of a few months to a few years when the process has been occurring for centuries (Wheatcroft *et al*, 1994). In a comparison of various tracers used to study bioturbation, Gerino *et al.* (1998) found that the use of ^{234}Th resulted in far lower estimates of overall particle transport than did the use of chlorophyll-a or exotic particles (luminophores).

5.1.2.2. Exotic Particles.

The use of deliberately introduced tracers is the only way to quantify the movement of particles within the sediment column (Mahut and Graf, 1987). Mahut & Graf (1987) successfully used coloured sand grains (luminophores), of different size classes, to quantify bioturbation in the Hausgarten area of the Baltic Sea. Luminophores were produced by staining the sand grains with various colours of fluorescent dye, visible under ultraviolet illumination. This method has also been used by Gerino (1990) who studied bioturbation in the Mediterranean. However, information on the source of this dye and the staining procedure is not available as the technique is protected by patent (Mahut and Graf, 1987; Wheatcroft *et al*, 1994).

The long lasting nature of the dye, the fact that it is fluorescent and the ease with which the particles can be manipulated are all advantages of this relatively simple technique although there are also drawbacks. Although the attachment of the dye to the particle may be chemically stable, the dye is still likely to come off in the field, perhaps due to energetic grain-grain collisions (Olmez *et al.*, 1994). Furthermore, manual counting under a UV light is tedious and time consuming but necessary because other constituents of the sediment may

also show some fluorescence (e.g. shell fragments of molluscs) (Mahut & Graf, 1987). A major criticism of this method is that the particles are exotic (Olmez *et al*, 1994; Wheatcroft *et al*, 1994) and their physical and chemical properties may affect their mixing kinetics (Carey, 1989; Wheatcroft *et al.*, 1994). Mahut and Graf (1987) also stated that the dye fills the crevices on the surface of the particle, therefore restricting microbial attachment (Meadows & Anderson, 1966; Weise *et. al,* 1978, in Wheatcroft *et. al*, 1994). Given that deposit feeding organisms are more likely to select particles with some nutritional value, this could be a problem. Ideally, tracers of biological sediment transport must not differ in their surface topography as the feeding rate of many organisms is sensitive to particle surface properties (Olmez *et al.*, 1994). Wheatcroft (1992) deduced that different deposit feeding organisms actively select certain particle sizes when feeding. Therefore, although changes in size distribution, morphology and surface chemistry in particles used as tracers may not necessarily be a problem when studying the physical transport of sediments, it is potentially important to biological studies of particle mixing.

Wheatcroft (1991) measured rates of bioturbation in the near surface sediments of the Santa Catalina Basin (eastern Pacific) using spherical plastic beads ranging from 50 - 125μm in diameter. Wheatcroft (1992) used spherical glass beads ranging from 8 - 420μm in diameter in order to determine particle size dependant bioturbation. The beads can be detected either by eye or by light or scanning electron microscopy, depending on size (D. Paterson, University of St Andrews. Pers. comm.). Although reasonably effective, this method shares many of the disadvantages of using luminophores, particularly the length of time required for counting. When mixed with the sediment, smaller beads are also easily masked (Wheatcroft, 1992).

5.1.2.3. Labelled particles.

The simplest approach to labelling sediment particles would be to soak the particles in a solution containing a tracer and allowing adsorption to take place (Krezoski *et al.*, 1995). However, on exposure to changing chemical conditions, such as salinity and pH, it is unlikely that the tracer would remain attached to the particle due to competition for adsorption sites by other ions. Therefore it would be difficult to distinguish between labelled particles and simple loss of tracer from the substrate (Krezoski *et al.*, 1995; S. Suzuki, University of Hull. Pers. comm.).

Komarneni & Roy (1988) developed a technique whereby phlogopite, a form of mica, was labelled with caesium in such a way that the caesium became incorporated into the crystal lattice structure of the phlogopite, thus producing a tracer stable enough not to be affected by

changing chemical and physico chemical conditions in the environment. This was achieved by first depleting the phlogopite of its K^+ ions (using sodium tetraphenylboron). This causes expansion of the spacing between the minerals sheet like aluminosilicate layers which allows the entrance of caesium ions into the lattice, on exposure to caesium chloride. Following this, the lattice collapses slightly, trapping the Cs^+ which is then not exchangeable with other ions, even at high salinities (Krezoski *et al.*, 1995). This property makes the method suitable for use in marine and estuarine environments. Komaneri and Roy (1988) found Neutron Activation Analysis (NAA) to be the most effective way of detecting the bound caesium. X-Ray Fluorescence (XRF) can also be used but only at concentrations greater than 100 $\mu g\ g^{-1}$. Krezoski *et al.* (1995) successfully applied the technique to the measurement of sediment reworking in Lake Erie.

Olmez *et al.* (1994) developed a technique for labelling sediment particles based on the thermal diffusion of noble metals into the crystalline matrix of ambient sediment particles. Silver and gold were chosen (in order to label two different particle size classes) for (1) their low detection limits, allowing their concentrations to be accurately measured following dilution within the sediment; (2) the ease with which they could be reduced to their metallic state thus facilitating their diffusion into mineral matrices; (3) they are only present in trace amounts in most environments and (4) they are of no environmental concern if released at low levels. Labelling of particles was carried out by first exposing pre-combusted (at 800°C) sediment particles to solutions of silver nitrate in distilled water or gold, diluted with xylene, followed by low temperature drying and subsequent heating to reduce the metals to their metallic state. Diffusion of the metals into the sediment structure took place during heating to approximately 1100°C and any surface adsorbed metal was removed by acid etching. Detection of the metals was carried out by neutron activation analysis (NAA) although atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICPMS) would also be suitable. However, these methods require dissolution and preconcentration in order to obtain sufficient concentrations for detection and quantification. According to S. Suzuki (University of Hull, pers. comm.) preparing samples for this type of analysis would be difficult due to the way in which the metals were incorporated into the sediment particle. This method proved to be successful in the bioturbation studies performed in a subtidal area of Massachusetts Bay by Wheatcroft *et al.* (1994). Here there were clear differences in the vertical distributions of the two particle size classes during the summer months and differences between the depth of transport between the summer and autumn months. These results suggested that some degree of particle size selection was taking place and that bioturbation was seasonally influenced.

Although the analysis may be expensive, the labelling procedure is relatively cheap and the method appears to be reasonably accurate. Possible problems include the fact that density changes to the sediment particles may occur as a result of the labelling procedure. However, scanning electron microscopy confirmed that the temperature and acid treatments did not affect the morphology of the ambient sediment. Particle size distribution was slightly affected in one case where, for reasons which are not known, agglomeration of some of the particles labelled with gold occurred. In general, following maximum diffusion, density changes in sand particles (labelled with silver) were less than 8% and density changes in the silt particles (labelled with gold) were negligible (Olmez *et al.*, 1994). Exposure to strong acids did not induce leaching of the metals into solution it is therefore unlikely that any tracer would have been lost during use in sea water. Since the heat and acid treatments remove any microbial film on the sediment particles, soaking in unfiltered sea water prior to use was recommended (Olmez *et al.*, 1994; Wheatcroft *et al.*, 1994).

5.1.2.4. Sediment displacement.

Rate and depth of sediment reworking in intertidal and subtidal areas of Buzzards Bay, Massachusetts, were determined by measuring the displacement of several coloured sand layers within the sediment by Rhoads (1967). Sediment was excavated from a small area and the hole refilled with five alternate layers of sand and frozen, coloured sand sheets. Reworking rates were recorded as the time taken to disturb or completely obliterate the coloured layers. This method was also employed by Winston and Anderson (1971) in order to examine bioturbation along pollution and salinity gradients in the Great Bay estuarine system of New Hampshire. The technique was found to be effective although not all the organisms were able to reconstruct their burrows and so their activities could not be recorded.

Laboratory techniques for measuring sediment displacement include the use of thin section, glass aquaria filled with sediment and Rhoads (1967) quantified bioturbation by periodically siphoning faecal pellets from the sediment-water interface and measuring them volumetrically. A variation on this method involved placing organisms into plastic tubes (6.5 cm in diameter) and separating the sediment-water interface with a piece of filter paper or fine nylon mesh through which the posterior end of the organism protruded. Again, egested sediment was siphoned off and measured volumetrically (Mangum, 1964, in Rhoads, 1967). Thin section tanks have also been used to measure changes in sediment profiles resulting from the activity of various types of organism exposed to various levels of environmental stress (Hickson, 1994; Jennings, 1995; Bates, 1997; O'Brien, 1997).

Other methods of measuring sediment displacement include the collection of sediment ejected into the water column (Bender & Davis, 1984), the measurement of the overlying water turbidity (Davis, 1993), rate of faecal cast production (Retraubun, 1996) and photographic techniques (O'Brien, 1997). The latter involved placing a layer of calcium carbonate on the sediment surface which was photographed periodically. Changes in the proportion of sediment covered by the calcium carbonate, with time, were used as an indication of the degree of bioturbation. Finally, several authors have looked at burrow depth, morphology, and density as an indication of bioturbation. Methods include resin casting (Davey, 1991; Gerino, 1991; Nickell & Atkinson, 1995), dye staining (Pantin, 1960), X-ray radiography (Rhoads & Stanley, 1966) and infra red photography (Hamblin, 1962).

5.1.2.5. Classification techniques.

Swift (1993) suggested that the degree of bioturbation should intuitively be proportional to the total faunal abundance and its activity within the sediment. A scheme was devised where organisms were assigned scores according to their mobility and mode of feeding and burrowing (Table 5.1). The sum of scores for each species can be ranked to give an indication of which organisms are likely to have the greatest effect on the sediment.

Table 5.1. Scoring system used for classifying bioturbatory activity. (Swift, 1993).

Attribute		Score
Mobility	Sedentary or only moving within fixed tube structure	0
	Limited free movement, i.e., withdrawal into sediment when disturbed	1
	Slow movement within sediment with non-permanent burrow formation	2
	Freely mobile within sediment in a permanent, excavated burrow system.	3
Feeding	Carnivore of filter feeder.	0
	Sub-surface sediment ingestion; egestion at same level.	1
	Detritus or surface sediment ingested; egested at surface.	2
	Detritus or surface sediment ingestion; egestion below surface.	3
	Sub-surface ingestion; egestion at surface	4
Burrowing	No burrowing activity.	0
	Construction of simple surface hole or pit or covering the body in sediment as camouflage.	1
	Burrowing by displacement of particles without net transport.	2
	Burrowing with selective particle transport to surface	3
	Burrowing extensively horizontally and / or vertically with net transport to surface.	4

5.2. AIMS

It is clear that the relationship between feeding strategy and bioturbation has an important influence over the physical, chemical and biological properties of the sediment and that these properties may have important implications for sediment transport. Given that benthic community structure is seasonally influenced (Chapter 4), it is likely that the degree of bioturbation will also vary according to season. Furthermore, Rhoads (1967) highlighted the importance of faunal composition and suggested that it had more influence over the rate of sediment reworking than did faunal density. The impact of the discharge from BP Chemicals (Saltend) Ltd. was clearly demonstrated in Chapter 4 with faunal density decreasing with increasing distance from the outfall and individual body size and species diversity increasing. With changing species diversity, a change in the diversity of the functional groups present might also be expected.

It is hypothesised that the rate of bioturbation in polluted communities, characterised by large numbers of small organisms may be greater than that of communities in cleaner sediments, characterised by lower numbers of organisms. However, this is expected to be restricted to the surface with the overall effect of pollution being a reduction in the depth of bioturbation and the volume of sediment reworked. Furthermore, sub-lethal effects of the effluent are expected to cause a reduction in the bioturbation rate of individual species.

The present chapter details techniques for measuring bioturbation in the field and the laboratory which were developed and tested. These techniques were then used to determine the following:

- Differences in bioturbation by different community types from polluted and unpolluted areas.
- Examination of the effect of pollution on the bioturbation potential of individual species.
- Functional grouping of the species present in each community (in terms of feeding and sediment modification behaviour) and examination of the effects of pollution on the diversity of these groups.

The study was carried out at Paull and Saltend although due to the low species diversity of these mudflats, further experimental work was carried out on the Skeffliing mudflat at the more seaward end of the estuary. A description of the faunal community at Skeffling is therefore also given.

5.3. METHODS

5.3.1. Laboratory techniques

5.3.1.1. Thin section

The thin section technique (Rhoads, 1967) was used to examine the burrowing habits of individual species and to determine differences in the amount of sediment each species could displace. Plastic partitions were placed inside glass tanks (14 x 25 x 15 cm) to make a 0.5 cm thin section along each side of the tank. Samples of estuarine mud were collected from Paull, passed through a 300 μm sieve to remove the macrofauna and large meiofauna and mixed with clean seawater (20 psu). The sections were then filled with the slurry to achieve a sediment depth of 2 cm, left to settle for three days and the excess water siphoned off. A 1 cm thick layer of fine sand (212-300 μm) was added and covered with a further 2 cm of the sediment slurry. This was repeated, creating alternate layers of coloured sand (212-300 μm) (1 cm deep) and estuarine mud (2cm deep), with a green layer at 6-7 cm and a blue layer at 3-4 cm, until a sediment depth of 9 cm was achieved. A final 3 cm deep layer of estuarine mud was then added. Sand samples were acid washed, thoroughly rinsed in distilled water and soaked for five days, stirring regularly, in solutions of malachite green or methylene blue to achieve the colour. The sand was then rinsed to remove excess dye and soaked in seawater for 5 days in order to allow bacteria to colonise the particles.

Four tanks were set up, one control with no animals and the remaining three containing *Hediste diversicolor*, *Corophium volutator* and *Macoma balthica*. The four sides of the tank allowed four replicates for each species. It is accepted that the experimental set up was not random. However, separation of the species between the tanks prevented individuals of one species from moving into sections occupied by other species. In order to allow for the different sizes of the sections, *H. diversicolor* and *C. volutator* were added in abundances to give 1 individual / cm (i.e., 25 animals in the 25 cm section or 15 animals in the 15 cm section) and *M. balthica* were added in abundances to give 1 animal / 2 cm. Animals of similar sizes were chosen with individuals of *H. diversicolor* being 45-47 mm long, *C. volutator* being 10-12 mm long (telson-rostrum) and *M. balthica* being 3-4 mm long (shell length).

Strips of triple layered 500 μm mesh were glued to the inside of the tank and taped over the top of each section to prevent the animals from escaping into the water reservoir or into any of the other sections. The tanks were filled with clean seawater (20 psu), aerated with one airstone and maintained at 10°C. The profile of the sediment surface, the position of the coloured layers and the outline of any visible burrows was traced onto an acetate sheet at the beginning of each experiment and after 15 days. From this, changes in the sediment surface

profile could be determined. Sediment depth measurements were made at 5 mm intervals across the entire width of each section and the volume (mm^3) of reworked sediment was calculated by multiplying the sediment depth by the width of the section (5 mm) and the width of each interval (i.e. 5 mm). Total volumes were divided by the length of the thin sections in order to account for size differences between the sections resulting from the rectangular shape of the tanks.

The actual volume of sediment reworked within the coloured layers could not be calculated since the layers were broken up and the sediment dispersed around the tank. Therefore, the percentage change in the total visible area of each coloured layer was recorded. It is appreciated that the total visible area may not differ over time and that one continuous coloured layer may become a number of small randomly spaced coloured areas. Therefore, the average area of the remaining coloured sections was also recorded. These two techniques, in combination with graphical representation of the profiles, were considered to be an adequate means of demonstrating the degree of bioturbation caused by various organisms. The area of the coloured sections was calculated using MapInfo version 6.5.

In order to determine the effects of pollution, the experiment was repeated using effluent concentrations of 16% and 32%.

5.3.1.2. Lithium clay tracer

Due to the expense associated with the labelling or staining of sediment particles and that analytical techniques used in their detection, the use of many of the techniques described in section 5.1.2 was not considered feasible. Initially, attempts were made to use fine sands, using various stains to allow differentiation between the added and existing sand particles. However, for reasons discussed in section 5.5.3, this proved to be unsuccessful. Bentonite clay, however, is composed of particle sizes similar to those of estuarine muds and, being clay, is cohesive in nature. The lithium content is high (approximately 4000-5000 ppm) and small quantities of the clay should therefore be detectable within estuarine sediments. Attempts were made to examine the effect of bioturbation by different communities (i.e. between S25 m and S200 m), exposed to different effluent concentrations, using lithium as a tracer.

Samples of bentonite clay were obtained from Redland Aggregates. Prior to use, the actual lithium concentration of the clay, together with the background lithium concentration in sediment samples from Paull and Saltend were determined. Samples of approximately 0.15 g (bentonite clay) and 0.5 g (sediment) were weighed into 7 ml capacity Teflon microdigestion

vessels and 0.5 ml Aqua Regia (3:1 $HCl:HNO_3$) and 0.1 ml hydrofluoric acid added. The vessels were sealed and a digest blank prepared in the same way. The microdigestion vessels were placed in pairs inside 125 ml Teflon vessels containing 10 ml UHQ grade water, using small spacers to prevent immersion. The vessels were then heated in a microwave oven under pressure control to 90 psi and allowed to cool. They were then diluted to 10 ml using water and allowed to settle.

Lithium concentration was determined using a Perkin Elmer Plasma 40 emission Inductively Coupled Plasma (ICPOES) instrument, using a wavelength of 670.78nm with detection limits of 0.1 ppm. Prior to analysis, a calibration curve using a set of calibration standards with a concentration range of 0-100 ppm. The relationship was linear (r^2 = 0.99) and a calibration solution of 40 ppm gave readings of 39.48 and 39.49 ppm.

Sediment samples were taken from the key sites at Saltend, to represent different community types, using 22 cm plastic cores (68 mm i.d.). Fifteen 1 cm diameter holes were drilled, at 1 cm intervals between 2 and 17 cm, and the cores surrounded by a stainless steel sheath. Coring was carried out so that the sediment surface was level with the top of the first hole (2 cm from the top of the core). The cores were placed in tanks containing clean, aerated seawater (20 psu) and allowed to settle for 48 hours at 10°C. The tops of the cores were covered with 500 μm mesh lids to prevent the animals from escaping. Following settlement, the cores were separated into two tanks, one containing clean seawater and the other containing 32% effluent, with each tank containing three replicate cores from each site. 10 ml Bentonite clay was poured over the sediment surface inside each core using a plastic pot with a 500 μm mesh lid to ensure even spreading. The cores were then left for six weeks, with the water in the tanks being replaced every three days.

Following removal of the metal sheaths, sub-samples of sediment were taken at 1cm depth intervals using a metal spatula. The samples were oven dried for 48 hours at 85°C and their lithium content determined as above.

5.3.1.3. Photographic techniques

Clean sediment was collected from Paull, passed through a 300 μm sieve to remove the macrofauna and allowed to consolidate (at 10°C) for 7 days. Following settlement, the excess water was siphoned off and 75 plastic cores (10 cm i.d.) were filled to a depth of 15 cm and allowed to settle for a further 10 days. The cores were checked regularly for signs of drying and a 3 cm depth of clean seawater (20 psu) was maintained within each core. Fifteen cores were then placed in each of 5 plastic tanks. Fifteen individuals of *H. diversicolor* and *C.*

volutator were added to the cores together with 10 individuals of *M. balthica* and one individual of *Scrobicularia plana* to give three replicate cores of each species and three replicate cores containing no animals (controls) within each tank. The total wet weight biomass of the animals to be added to each core was recorded prior to adding them to the sediment (Table 5.2.) and animals of similar sizes were chosen (as in section 5.3.1.1). The aim of the experiment was to examine inter-specific differences in bioturbation potential and therefore the number of animals in each core should have been the same. However, availability restricted the numbers of *M. balthica* and *S. plana* used.

Glass beads (15 ml 10-50 μm diameter and 15 ml 45-85 μm) were spread over the surface of each core to produce a white layer. The cores were photographed and a 500 μm nylon mesh lids was placed over each core to prevent the animals from escaping. The five tanks were filled with sea water (20 psu) and the appropriate volume of effluent to give concentrations of 0, 4, 8, 16 and 32% and a low level of flow was maintained in each tank using a small aquarium pump to recirculate the water. Care was taken to direct the inflowing water towards the bottom of the tank to avoid disturbance of the sediment surface. The tanks were maintained in these conditions for 21 days with the effluent being replaced every three to four days. Every 7 days, the tanks were completely drained, the mesh lids removed and the sediment surface of each core photographed. At the end of the 21 day period, the cores were sieved and the number of remaining live animals recorded.

The change in proportion of dark (i.e. bioturbated mud) and white areas visible on the surface of the core was used as a means of determining the degree of bioturbation in each set of conditions. Sketch maps were drawn of the surface of each core so that actual features of bioturbation could later be distinguished from discolouration of the beads due to settlement of any sediment resuspended as a result of circulation and the underlying sediment showing through the bead layer. The images were scanned, geo-referenced and changes in the proportion of the black and white areas on the surface calculated using MapInfo version 6.5.

Table 5.2. Abundance (A) and mean (± SE) biomass (B) (g) of animals added to each core.

	Effluent concentration				
Species	**0**	**4**	**8**	**16**	**32**
M. balthica (A)	10	10	10	10	10
M. balthica (B)	4.62 (±0.17)	5.3 (±0.14)	5 (±0.12)	4.48 (±0.19)	4.91 (±0.09)
H. diversicolor (A)	15	15	15	15	15
H. diversicolor (B)	2.2 (±0.06)	2.2 (±0.12)	2.19 (±0.04)	2.13 (±0.06)	2.2 (±0.06)
C. volutator (A)	15	15	15	15	15
C. volutator (B)	0.05 (±0.006)	0.03 (±0.004)	0.05 (±0.003)	0.03 (±0.005)	0.05 (±0.008)
S. plana (A)	1	1	1	1	1
S. plana (B)	5.08	5.07	5.24	5.65	5.43

5.3.2. Field techniques

5.3.2.1. Particle tracer techniques

Samples of glass beads were obtained from Vacu-blast (International Surface Preparation Corporation), their advantages being (1) their spherical nature which allowed distinction between the beads and the sediment particles; (2) their size was representative of the range of particle sizes on the mudflats and (3) the low volume of beads required kept the cost to a minimum.

A series of 22 cm long plastic tubes (68 mm i.d.) with fifteen 10 mm diameter holes drilled at 1 cm intervals between 2 and 17 cm were pushed into the sediment, surrounded by a stainless steel sheath. The top 5 cm of the tube (without any holes) was left protruding from the sediment so that the first hole was level with the surface. 10 ml of glass beads (5 ml 10-50 μm diameter mixed with 5 ml 45-85 μm diameter) were poured onto the sediment surface, inside the tubes, using a plastic pot with a 500 μm mesh lid to ensure even spreading. Three cores were placed at each site and covered with a plastic bucket with a 500 μm mesh lid to protect the beads from being washed away, to prevent excessive sedimentation and to prevent the escape of macrofauna. The tubes were left *in situ* at the four key sites for six weeks (8 July – 20 August, 1998 and 10 January - 20 February, 1999) and checked periodically for signs of destruction and anoxia. A further set of cores was placed at Skeffling during July / August 1998. Macrofaunal samples (5 replicate cores) were taken from each site and analysed in the laboratory, as described in Chapter 4.

On return to the laboratory, the steel sheaths were removed and sub samples were taken at 1 cm intervals, using a curved metal spatula, freeze dried and stored until further analysis. The freeze dried sediment samples were gently disaggregated using a mortar and pestle, 1 g sub-samples were placed in a solution of 50 ml 6% hydrogen peroxide and the organic material was removed (Buchanan, 1984). 10 ml sodium hexametaphosphate (6.2 gl^{-1}) was then added

as a deflocculant and the samples were allowed to stand overnight, following vigorous stirring. Samples were then re-stirred and five 2 ml sub samples taken. These were allowed to settle overnight and the overlying water removed by pipette. Six drops of sediment were mixed with an equal amount of 40% glycerol (in order to increase the viscosity of the sample on the slide) and mounted on microscope slides. Following drying, the beads were counted under a light microscope at 10x magnification. Prior to use, the beads were examined under a scanning electron microscope in order to ensure that they were spherical and distinguishable from the sediment grains. Since different numbers of beads were transported into the sediment cores, tracer profiles were plotted as the proportion (relative to the total number retrieved from the core) of beads at each depth as described by Wheatcroft (1992).

5.3.2.2. Burrow depth and volume

The depth of animal activity and burrow volume within the sediment were determined using a modification of the resin casting technique used by Nickell & Atkinson (1995). Casts were made in rectangular plastic containers (14.5 x 12.5 cm) using a low density polyester resin (AP101PA, Trylon) together with a styrene thinner and a liquid catalyst hardener (MEKP). Sediment samples were taken in triplicate using a rectangular corer of the same size as the boxes, to a depth of 15 cm. A number of small holes were made in the bottom of the containers to allow expulsion of the air as the sample was allowed to slide out of the core into the container, thus minimising damage to the sediment sample and the burrow structures within it. The cores were then stored at 10°C for at least 72 hours to allow the burrows to open up and to allow excess water to drain away. This also allowed the animals to reform their burrows following damage during sampling.

Approximately 400 ml resin was used for each core, containing 15% (v/v) thinner and 1.5% liquid catalyst hardener which was sufficient to fill the burrows and leave an adequate hydraulic head to ensure that the burrows were completely filled. The resin was injected slowly into each burrow using a 5 ml syringe. When all the air and water had been expelled from each burrow (i.e., bubbling ceased), the resin was poured over the surface of the sediment to a depth of approximately 3 cm. The casts were left to dry for at least 48 hours under fume extraction, before being removed from the cores and cleaned of sediment. Cleaning was carried out over a 300 μm sieve, using a small hose to gently wash away the sediment. Due to its cohesive nature, the sediment tended to fall away from the cast in lumps with large lumps being sufficiently heavy to break the burrow structures away from the cast. Therefore, in order to minimise damage, the casts were inverted and the bottom of the containers was cut away. The sediment inside was gradually fluidised using the hose and

washed away. As sediment removal proceeded, the sides of the container were cut away. Any broken pieces of burrow were retained in the sieve.

Due to their delicate nature, damage to the casts was difficult to avoid. Therefore, intact casts were photographed to give a visual representation of changes in burrow volume and density in relation to pollution and, where possible, burrow length was measured. The burrows were then removed from the base of the cast and their volume determined by weight in comparison to the known weight of 1 cm^3 dried resin.

5.3.3. Classification techniques

Species were divided into feeding groups based on the observations of Fauchald & Jumars (1979) and Barnes (1987) according to their diet and their feeding mechanism. Feeding classes, to indicate diet, included carnivores (**CAR**), omnivores (**OM**), detritivores (**DET**) (including species feeding on benthic diatoms) and bacterivores (**BACT**). Classes of feeding mechanism included sub-surface deposit feeders (**SUBDF**), surface deposit feeders (**SDF**) and suspension feeders (**SUSP**). Organisms were also classified in terms of their bioturbation and sediment modification potential, using the following four classes: (1) ***biodiffusers*** which mix at random with the sediment; (2) ***conveyor-belt species*** which actively transport sediment from some depth to the surface as a result of sub-surface deposit feeding or burrow excavation; (3) ***regenerators*** which transport sediment from the surface to some depth (reverse conveyor-belt transport) and (4) ***surface depositors*** which both feed and deposit faeces at the surface. Species which are known to switch between feeding modes were classified by their most common feeding mechanism. These modes of feeding and bioturbation are described in more detail in section 5.1. Community bioturbation scores were calculated according to the feeding, burrowing and motility of each species, using the scheme in Swift (1993).

Cluster analysis was used (Ludwig & Reynolds, 1988) to group sites in terms of the feeding groups and the bioturbation potential of the communities present. The cluster analysis was carried out using faunal community data from Paull and Saltend together with faunal community data collected from three sites at Skeffling in July 1999. The field and laboratory methods used for the collection and identification of macrofauna are described in Chapter 4.

5.4. RESULTS

5.4.1. Laboratory studies

5.4.1.1. Thin section

Changes in the sediment profile at the sediment-water interface were found to be dependant upon species and effluent concentration (Figure 5.1). One-way ANOVA tests, together with Tukeys' test, showed that at all concentrations, the volume of sediment displaced in the control sections was significantly lower than that displaced in those sections containing animals ($p<0.01$). The volume of sediment displaced decreased with increasing concentration ($p<0.01$) although for *Hediste diversicolor,* there was little difference between the 0% and 16% concentrations. Volumes of sediment displaced were in the range of 39-155 mm^3 for *Macoma balthica*, 72-117 mm^3 for *H. diversicolor* and 20-97 mm^3 for *C. volutator* at 32% and 0%, respectively. The impact of *C. volutator* on the sediment was consistently less that of *M. balthica* or *H. diversicolor* whilst *M. balthica* had the greatest impact at the 0% concentration ($p<0.05$) but *H. diversicolor* had the greatest impact at the 32% concentration ($p<0.01$). This suggests that whilst *M. balthica* appears to have the greatest bioturbation potential of the three species, its activity is more sensitive to the effects of the effluent than that of *H. diversicolor.* During the ANOVA test, homogeneity of variance could not be achieved and the above results were therefore verified using the Kruskal-Wallis test.

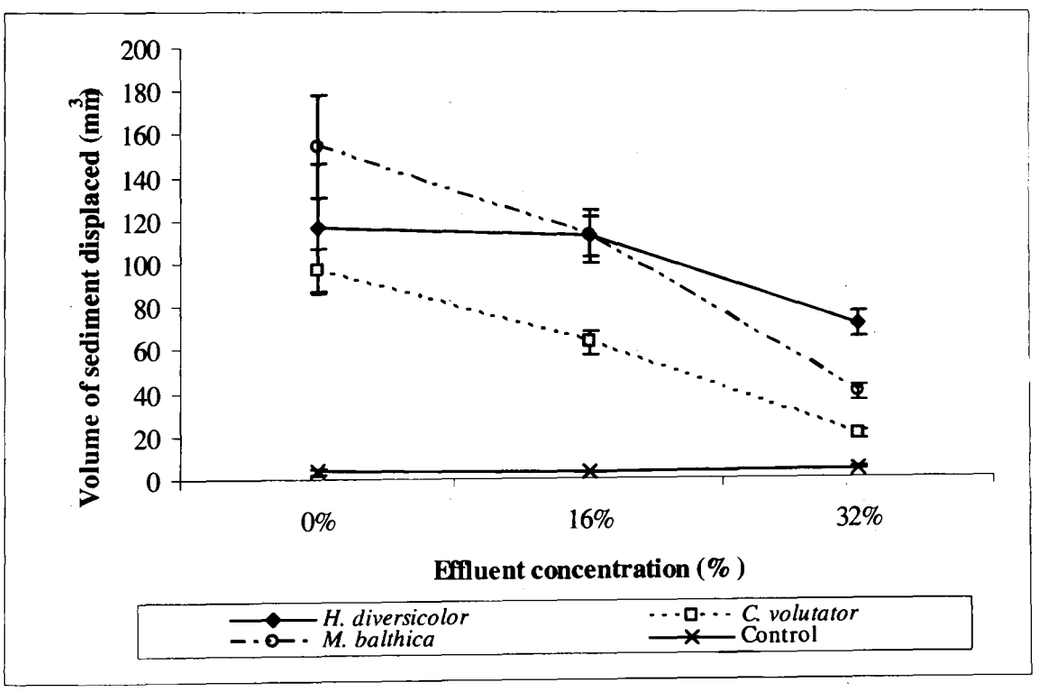
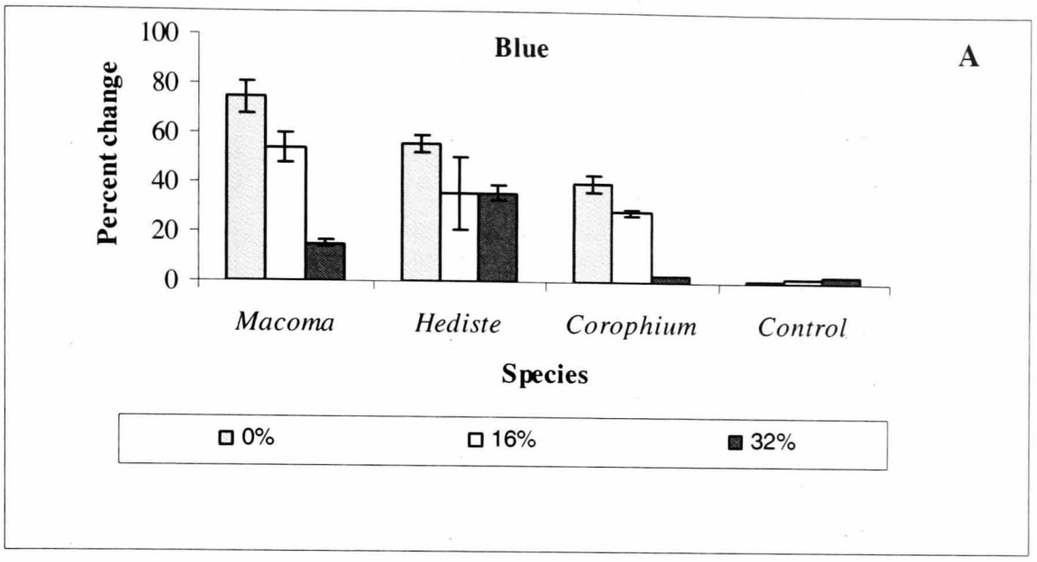


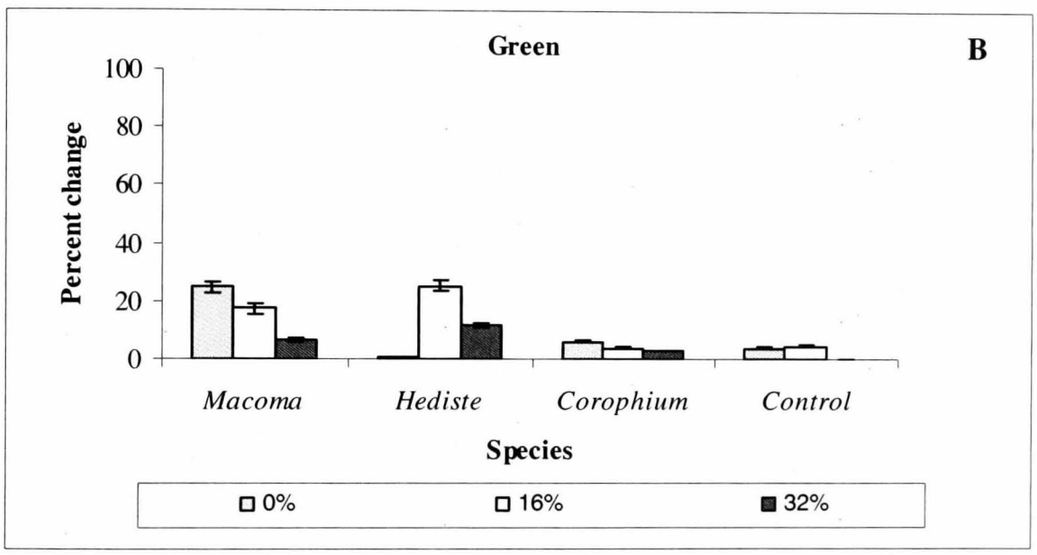
Figure 5.1. Volume (mean ± SE) of sediment displaced by each species at each effluent concentration.

The degree of bioturbation at depth within the sediment was also found to be affected by species and effluent concentration (Figure 5.2 A-C). Again, the degree of sediment disturbance (as indicated by the percentage change in the area of the coloured layers and the average area of the remaining coloured sections) was significantly lower in the control sections than in those containing animals ($p<0.01$). The blue layer (at 3-4 cm depth) was disturbed to the greatest degree with *M. balthica* causing the greatest degree of disturbance (16% at 32% effluent concentration to 75% at 0% effluent concentration) and *C. volutator* causing the lowest degree of disturbance (2% at 32% effluent concentration to 40% at 0% effluent concentration). Both differences between species and effluent concentration were found to be statistically significant ($p<0.01$).

In the green (6-7 cm) and sand coloured layers (9-10 cm), the greatest degree of bioturbation was, again, found to be caused by *H. diversicolor* and *M. balthica*, indicating that these species are capable of burrowing deeper than *C. volutator*. Differences between species and concentrations were, again, found to be statistically significant ($p<0.05$). It should be noted that the percentage change in area of the green layer caused by *H. diversicolor* at 0% effluent concentration is considerably lower than that caused by the other species at the other concentrations. This implies that there was comparatively little bioturbation by this species. However, as stated in section 5.3.1.1, it is possible for the coloured layer to be broken into smaller components without any change to the total area. This can be seen in Appendix 5 which shows examples of the acetate tracings and provides visual representation of the differences in bioturbation between the three species.

In general, it was observed that a high degree of bioturbation resulted in the coloured layers being broken into a greater number of sections with smaller areas in comparison to lower levels of bioturbation. Figure 5.3 shows the reduction in mean area of the blue, green and sand coloured layers between 0 and 15 days and also shows the area of these layers to be greater at 32% effluent concentration than at 0%. One-way ANOVA tests showed the effect of concentration to be statistically significant for all species in all layers ($p<0.05$) and also showed significant differences between species ($p<0.05$) with *C. volutator* consistently causing the lowest level of bioturbation.





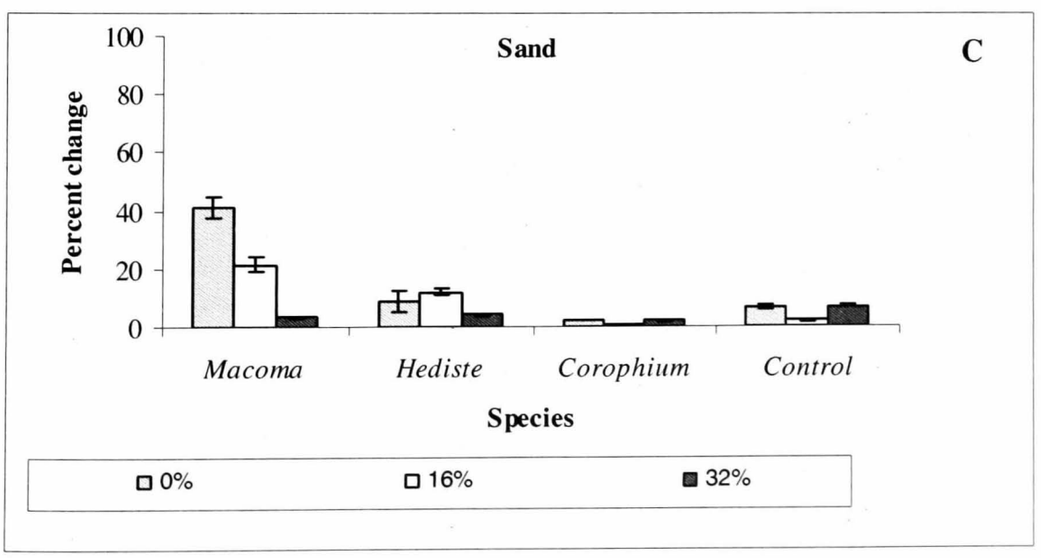
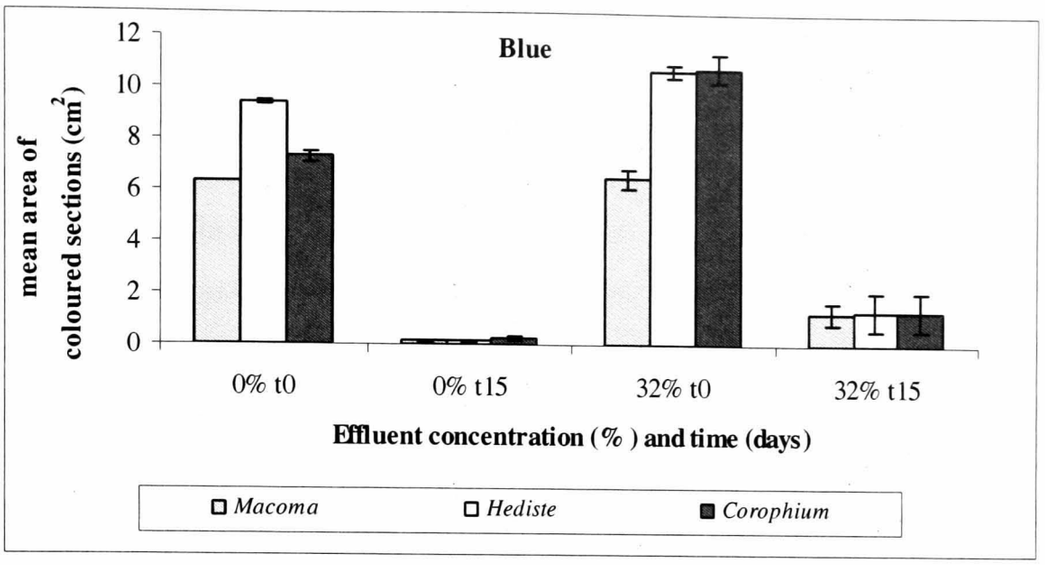
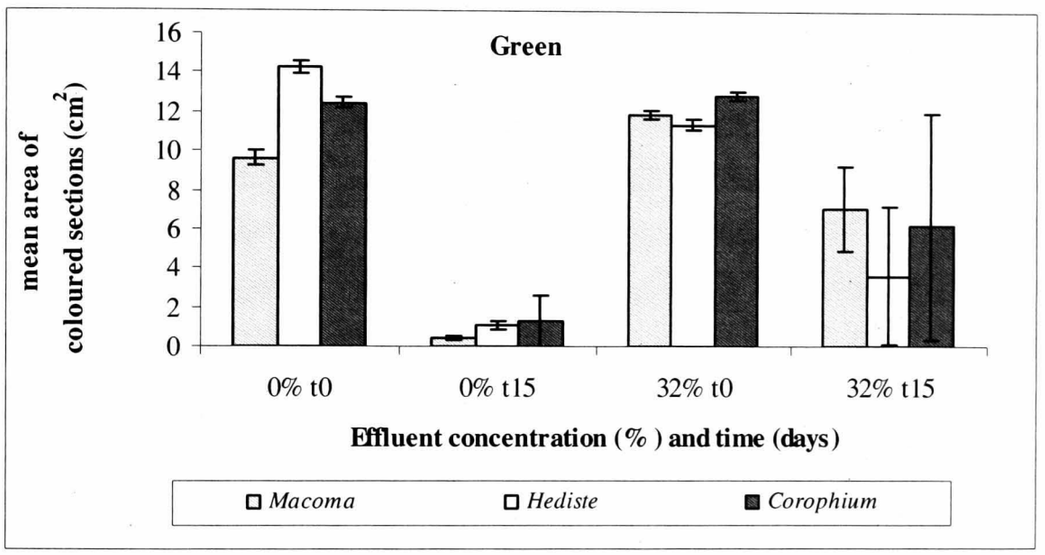


Figure 5.2. Percentage change (mean ± SE) in the area of the coloured sediment layer in relation to species and effluent concentration. A = Blue (3-4cm), B = Green (6-7 cm), C = sand coloured layer (9-10 cm).





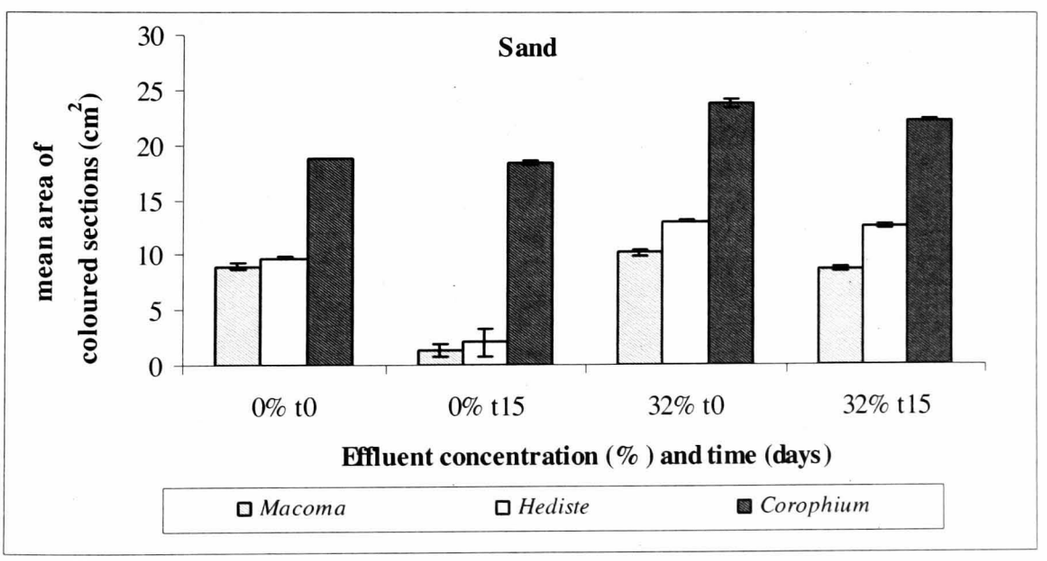


Figure 5.3. Difference in area of coloured sections (mean ± SE) between species at 0% and 32% effluent concentration.

5.4.1.2. Lithium clay tracer

Background lithium concentrations ranged from 54 - 59 ppm and differences between sites and depths were not found to be significant. The lithium concentration of the bentonite clay was 4414 - 4550 ppm. The lithium concentration of the sediment was found to decrease with depth (Figure 5.4) although concentrations were higher than background concentrations (as indicated by their positive values) suggesting that some lithium had been transported into the cores. Figure 5.4 (B and C) suggests that at 0% effluent concentration, a greater proportion of the lithium was transported into the sediment in cores taken from the S200 m site than from the S25 m site. In contrast, at 32 % effluent concentration, a greater proportion of the lithium appears to have been transported into the cores from the S25 m site. This indicates a greater degree of bioturbation in cores taken from the S200 m site at 0% effluent concentration but suggests that bioturbatation by the organisms in these cores is inhibited upon exposure to an effluent concentration of 32%. The activity of the communities in the cores taken from the S25 m site appears to be less sensitive to the effluent. The proportion of lithium transported into the cores at 0% effluent concentration was also slightly higher than that in cores exposed to 32% effluent. Despite these general trends, no statistical differences were found between sites at any depth and no significant effect of concentration was found (using one-way ANOVA tests).

Data expressed as percentages do not always follow a normal distribution (one of the assumptions of the ANOVA test) and the arcsine transformation is therefore commonly used (Dytham, 2003). One-Way ANOVA tests using arcsine transformed data did not reveal any statistical differences and therefore the results of the initial ANOVA test were accepted. Furthermore, the Shapiro-Wilk test ($n<50$) showed the data to be normally distributed ($p>0.05$) and Levenes' test showed homogeneity of variance ($p>0.05$).

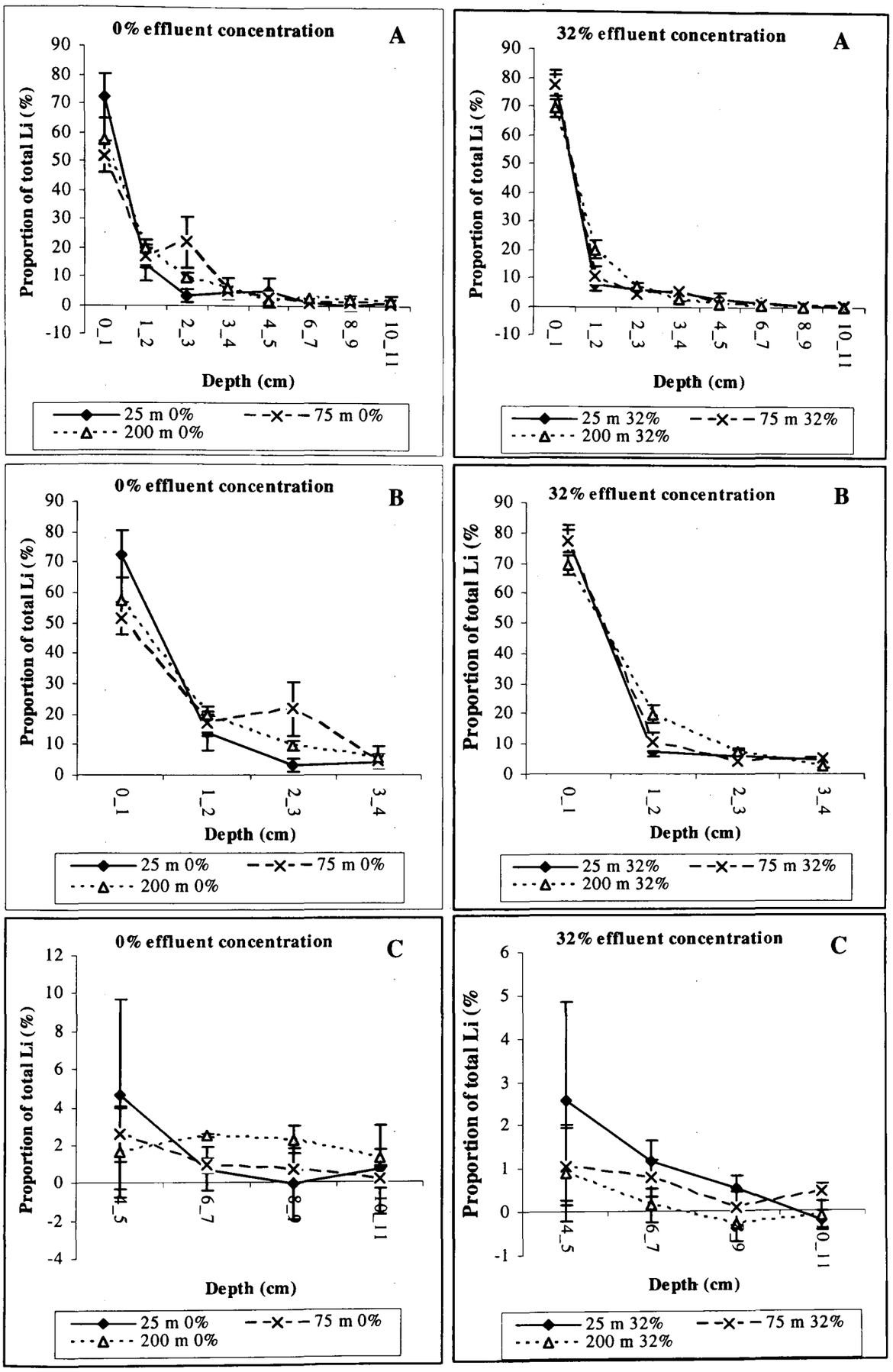


Figure 5.4. Proportion (mean ± SE) of Li transported to each depth within the sediment cores by three different community types exposed to 0% and 32% effluent concentration (A = whole profile, B = top 4 cm, C = 5-10 cm).

5.4.1.3. Photographic techniques

A distinct increase in bioturbation over time was demonstrated by the use of time lapse photography with the degree of bioturbation being dependant both upon species and effluent concentration (Figure 5.5). The area of black (mud) visible in the control cores ranged from 0.2% at t_0, 32% effluent concentration, to 7% at 21 days, 0% effluent concentration, showing an increase over time but no consistent pattern in relation to effluent concentration. This increase in percentage black over time was attributed to the deposition of resuspended sediment (by organisms in the other cores in the tank and as a result of water circulation within the tank) together with the escape of organisms from other cores into the control cores. The generally greater degree of bioturbation (as indicated by the greater area of black) at 0% effluent concentration was attributed to the greater level of activity of the organisms in this tank. Both *Corophium volutator* and *Hediste diversicolor* showed a steady increase in bioturbation (as demonstrated by the increase in visible sediment) over time with the greatest increase being associated with the lowest effluent concentrations. The area of black visible in cores containing *C. volutator* ranged from 1.9% at t_0 (0% effluent concentration) to 65% at 21 days (0% effluent concentration) with the visible black area in cores containing *H. diversicolor* ranging from 4.7% at t_0 (4% effluent concentration) to 93% at 21 days (0% effluent concentration).

Cores containing *Macoma balthica* showed a rapid increase in the visible black area, reaching 76% at 7 days and a maximum of 92% after 14 days (0% effluent concentration). There was little difference between the visible black area of cores at all other effluent concentrations after 14 days. Cores containing *Scrobicularia plana* showed a similar trend with the percent black reaching almost 100% at all effluent concentrations after the first seven days. These results suggested that, despite only containing one animal, the size of the cores was insufficient for an organism with such high bioturbation potential. Therefore, no assessment of the effect of the effluent on the bioturbation potential of this species can be made. Examples (one replicate only) of photographs taken at each time interval, for each species at each concentration are presented in Appendix 6.

Data were tested for homogeneity of variances and normality (Shapiro-Wilk test, $n<50$) and were found to meet the assumptions of the ANOVA test. Three-way ANOVA (Table 5.3) tests revealed that the degree of bioturbation was significantly affected by time ($p<0.01$), effluent concentration ($p<0.01$) and species ($p<0.01$). Furthermore, the interactive effects of all combinations of variables were statistically significant ($p<0.01$). Further one-way ANOVA tests, together with Tukey tests, showed the greatest degree of bioturbation to be associated with the lowest concentrations for all species except *S. plana* ($p<0.01$). In general,

there was little difference in bioturbation potential between the 0% and 4% concentrations and the 4% and 8% concentrations during the first seven days with the effect of the 8% concentration becoming more pronounced over time. Bioturbation potential was consistently significantly lower at the 16% and 32% concentrations than at all other concentrations throughout the experiment.

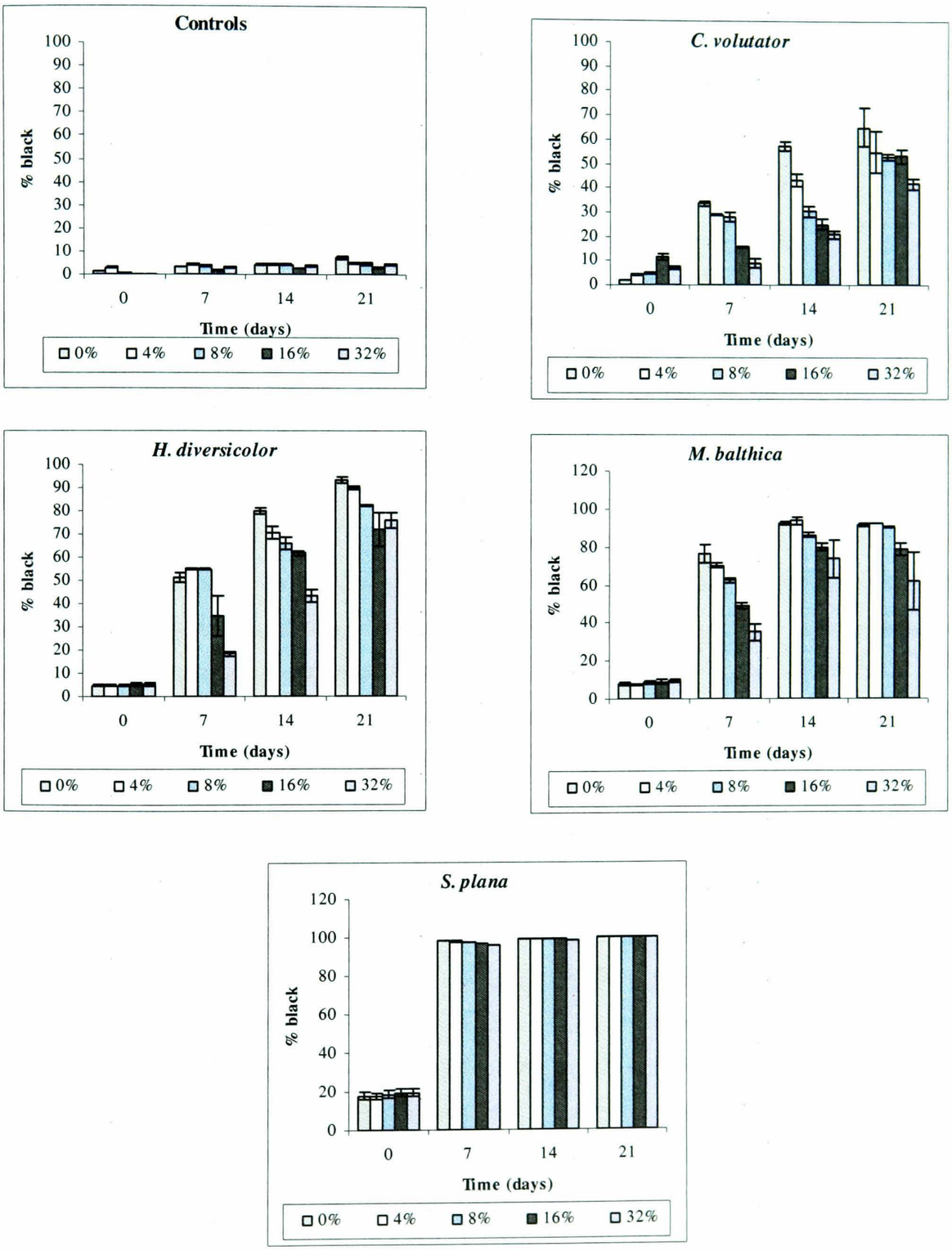


Figure 5.5. Percentage change (mean ± SE) in the area of visible (black) sediment in relation to time, species and effluent concentration.

Table 5.3. 3-way ANOVA output showing the individual and interactive effects of time (T), concentration (C) and species (S) on bioturbation potential.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	388807.8	99	3927.352	290.234	0.00
Time	133731.5	3	44577.15	3294.283	0.00
Concentration	5656.064	4	1414.016	104.4968	0.00
Species	193390.5	4	48347.63	3572.924	0.00
T*C	3081.717	12	256.8098	18.97842	0.00
T*S	51021.46	12	4251.789	314.2101	0.00
C*S	3660.908	16	228.8068	16.90898	0.00
T*C*S	3070.719	48	63.97331	4.727672	0.00
Error	2219.194	164	13.53167		
Total	855922.9	264			
Corrected Total	391027	263			

5.4.2. Field studies

5.4.2.1. Glass bead tracer

The proportion of beads retrieved from each core was found to differ significantly ($p<0.01$) between depths for the Paull and Saltend sites in July and January with the proportion of beads generally decreasing with increasing depth (Figure 5.6). In July / August 1998, the S75 m and S200 m cores showed a pattern of decreasing bead concentration with increasing depth with beads being transported down the full length of the core. Within the top 5-6 cm, the proportion of beads transported into the S75 m core was significantly higher ($p<0.01$) at each depth than the S200 m core although a significantly ($p<0.01$) greater proportion of the beads in the S200 m core were transported to depths between 6-7 cm and 14-15 cm (Figure 5.6 B, C). This suggests a greater degree of bioturbation to greater depths by communities at the S200 m site. There was no apparent trend in bead distribution within the top 5-6 cm in cores from the S25 m site and no beads were transported below this depth. This again indicates an increase in bioturbation potential with decreasing levels of pollution. These results are explained by the comparatively large number of *Hediste diversicolor* (18,999 m^{-2}) and the presence of *Macoma balthica* at the S200 m site (Table 5.4). The cores at the Paull site were found to have been tampered with during the last week of the experiment. They could therefore not be retrieved intact and no data were available from this site for July / August 1998.

As in the July cores, the proportion of beads transported into the cores from the S25 m site in January 1999 peaked between 1 and 3 cm and very few beads are transported below 6-7 cm (Figure 5.6 D, E). The proportion of beads transported below this depth was marginally (but not statistically significant) greater in January 1999 than in July 1998 (Figure 5.6 C, F) possibly suggesting that the degree of bioturbation may be seasonally influenced. However, two-way ANOVA tests did not reveal any significant effect of season or any interactive effect between site and season. The proportion of beads transported into cores (below 6 cm) from the S25 m site remained consistently lower than that in cores taken from the other sites, again suggesting that the bioturbation of communities in polluted sediments is lower than that of communities in cleaner sediments.

Within the top 5-6 cm, there was little difference ($p>0.05$) between the proportion of beads transported into cores from the S75 m, S200 m and P150 m sites (Figure 5.6. D, E). Whilst the proportion of beads transported to a depth of 9 cm into the S200 m was less than that transported into the S75 m and P150 m cores, there was no difference between these three sites in terms of bioturbation at depths of 9-15 cm (Figure 5.6 F). There was no consistent pattern of increasing bioturbation with increasing distance from the outfall between these three sites during January / February 1999. Two-way ANOVA tests did not show any significant effect of season, although the degree of bioturbation by the community in the in the S200 m core was lower in January than in July. This is attributed to the large reduction in the number of *H. diversicolor* present and the absence of *M. balthica* in January 1999 (Table 5.4). Table 5.4 also shows a greater degree of similarity between the communities at the S200 m and the S75m sites.

Homogeneity of variance and normality could not be achieved by transformation of the data (arcsine) and therefore the results of the ANOVA test were confirmed using a Kruskal-Wallis test.

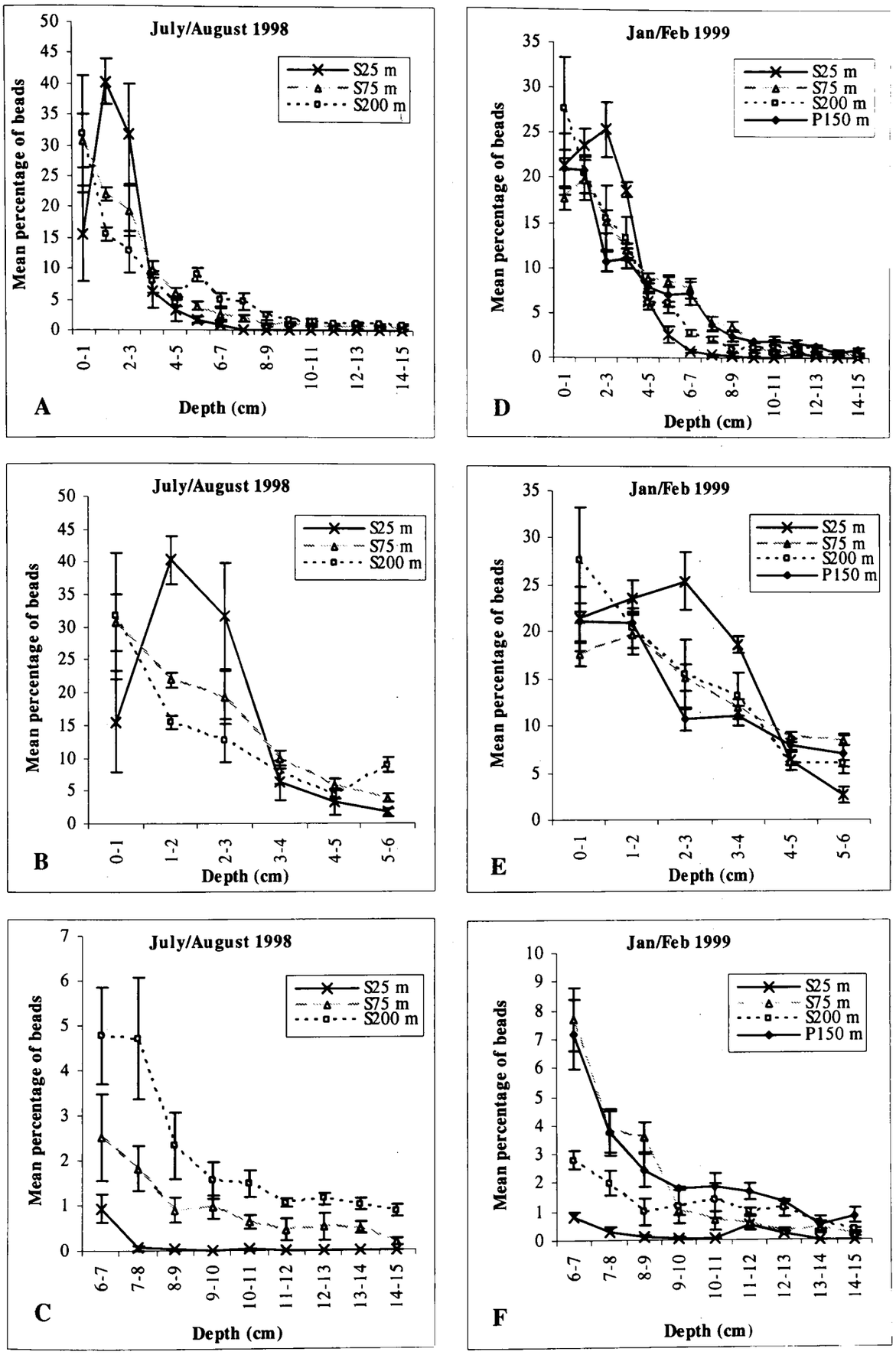


Figure 5.6. Bead transport (mean ± SE) as an indication of bioturbation for the entire core (A, D), 0-6 cm (B, E) and 6-15 cm (C, F) at Saltend and Paull in July / August 1998 and January / February 1999.

Table 5.4. Mean abundance (A) (No. m^{-2}) and biomass (B) (g m^{-2}) of the macrofauna present at Saltend and Paull during the bioturbation studies.

	S25 1998	S75 1998	S200 1998	S25 1999	S75 1999	S200 1999	P150 1999
Hediste diversicolor	A= 734 B= 36	A=1707 B= 111	A= 18999 B= 199	A= 2368 B= 27	A= 4240 B= 40	A= 6057 B= 32	A= 4543 B= 16
Manayunkia aestuarina	-	-	A= 87 B= 0.8	-	A= 55 B= 0.001	A= 550 B= 0.01	A= 1790 B= 0.04
Corophium volutator	-	A= 139 B= 0.8	A= 367 B= 3.3	-	-	A= 386 B= 0.6	A= 1652 B= 0.6
Macoma balthica	-	-	A= 92 B= 10	-	-	-	A= 344 B= 11
Spionidae	-	-	A= 275 B= 0.18	-	-	A= 386 B= 0.07	A= 1996 B= 0.11
Oligochaetes	A= 58191 B= 49	A= 38181 B= 23	A= 598 B= 4	A= 18669 B= 6	A= 37007 B= 4	A= 5507 B= 1	A= 2754 B= 0.2
Nematodes	A= 3579 B= 0.1	A= 5782 B= 0.14	A= 121 B= 0.02	A= 110 B= 0.002	A= 220 B= 0.01	A= 771 B= 0.01	A= 757 B= 0.02
TOTAL	**A=62,504 B= 85**	**A=45,809 B= 135**	**A=20,539 B= 217**	**A=21,147 B= 33**	**A=41,522 B= 44**	**A=13,657 B= 34**	**A=13,836 B= 28**

The proportion of beads transported into the Skeffling cores was found to differ significantly ($p<0.01$) with depth although there was no trend of decreasing bead concentration with increasing depth into the cores for any of the Skeffling sites. Furthermore, there was generally no similarity in patterns between sites although there was a peak in the proportion of beads retrieved from depths between 2 and 5 cm at all sites (Figure 5.7). This was also observed at the S25 m site.

One-way ANOVA tests between sites for each depth generally showed a greater degree of bead transportation to greater depths in cores from the Skeffling sites than in cores from the Paull and Saltend sites ($p<0.01$). However, statistical differences were not found at all depths. Bioturbation, as indicated by the comparatively large proportion of beads found in the deeper layers of the cores, at the Skeffling sites was consistently greater than that at the S25 m (July 1998 and January 1999) and S75 m site (July 1998). In addition, the proportion of beads found in the upper layers of the Skeffling cores was consistently lower than in cores taken from the S25 m and S75 m sites, indicating that more of the beads had been transported to some depth within the Skeffling cores.

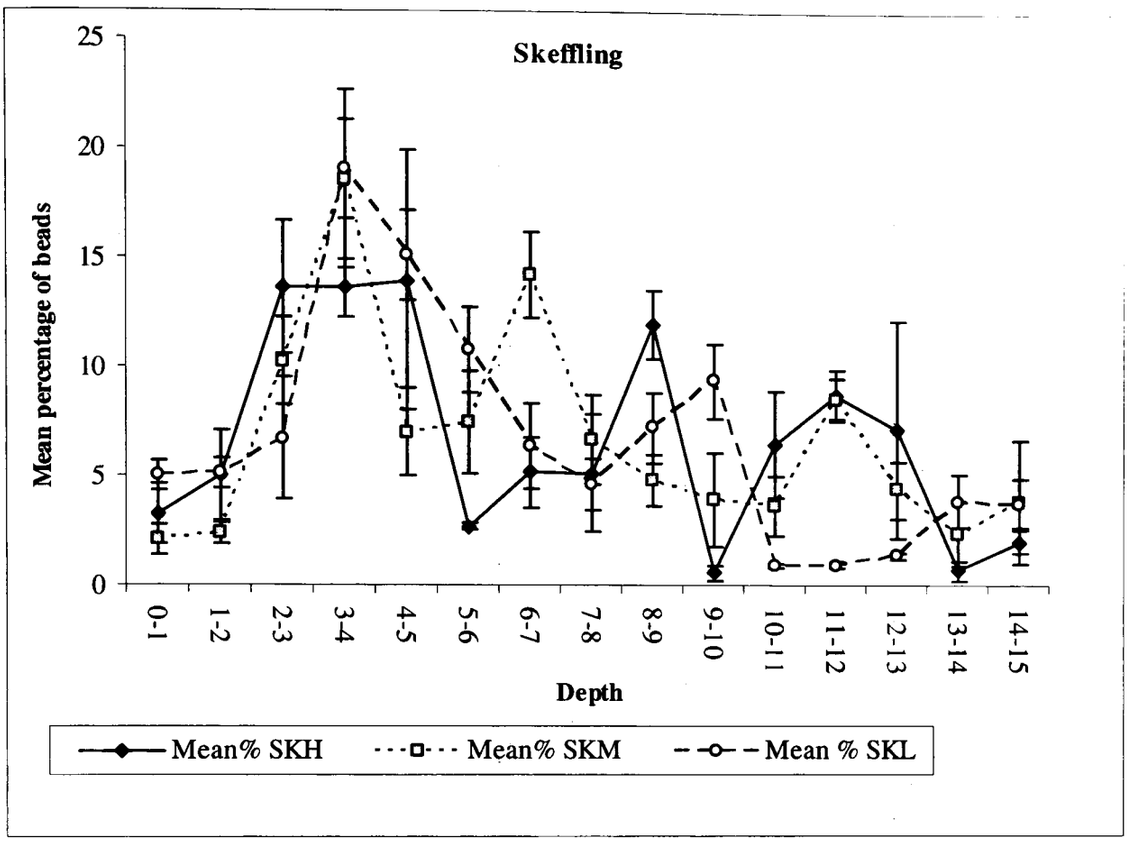


Figure 5.7. Bead transport (mean ± SE) as an indication of bioturbation for the entire core at Skeffling.

The abundance of *H. diversicolor* and oligochates present at Skeffling was considerably lower than that at the S75 m, S200 m and P150 m sites. However, species diversity at Skeffling was comparatively high with larger bodied, deeper burrowing animals such as *A. marina* and *C. edule* being present. The abundance of *M. balthica* at Skeffling was also much greater than that at any of the Saltend or Paull sites (Table 5.5).

Table 5.5. Mean abundance (A) (No. m^{-2}) and biomass (B) (g m^{-2}) of the macrofauna present at Skeffling during the bioturbation studies.

	SKL	SKM	SKH
Macoma balthica	A= 2386 B= 178	A= 3304 B= 467	A= 3579 B= 290
Cerastoderma edule	A= 275 B= 1052	A= 275 B= 624	
Retusa obtusa	A= 2202 B= 12	A= 551 B= 3	A= 2662 B= 17
Hydrobia ulvae	A= 2203 B= 18	A= 275 B= 4	A= 643 B= 7
Hediste diversicolor	A= 92 B= 0.9	A= 367 B= 36	A= 92 B= 3
Nephtys hombergii	A= 275 B= 6		A= 92 B= 2
Arenicola marina	A= 92 B= 10	A= 275 B= 4	
Eteone longa / flava	A= 1193 B= 0.2	A= 275 B= 0.04	A= 184 B= 0.05
Manayunkia aestuarina	A= 184 B= 0.01		
Spionidae	A= 551 B= 0.3	A=275 B= 0.28	A= 918 B= 1.1
Oligochaetes	A= 7434 B= 2	A= 7893 B= 3	A= 5323 B= 2
Nematodes	A= 92 B= 0.002	A= 184 B= 0.005	A= 92 B= 0.001
Carcinus maenas	A= 275 B= 5	A= 275 B= 7	
TOTAL	**A= 17,257 B= 1284**	**A= 13,674 B= 1148**	**A= 13,493 B= 322**

5.4.2.2. Resin casting

Maximum burrow depth ranged from 3.5 cm at the S25 m site (Figure 5.8 A) to 12 cm at the Paull site (Figure 5.8 D) with intermediate depths at the S75 m and S150 m sites being 7 and 7.5 cm respectively. It is also of note that the large *H. diversicolor* burrow present in the S25 m cast is horizontal within the sediment rather than vertical as is the case at all the other sites. The S200 m cast was badly damaged and could therefore not be photographed. Although no precise quantitative data are given, Figure 5.8 clearly shows a reduction in burrow depth and volume with increasing pollution and therefore supports the statement that bioturbation potential is reduced by proximity to a pollution source.

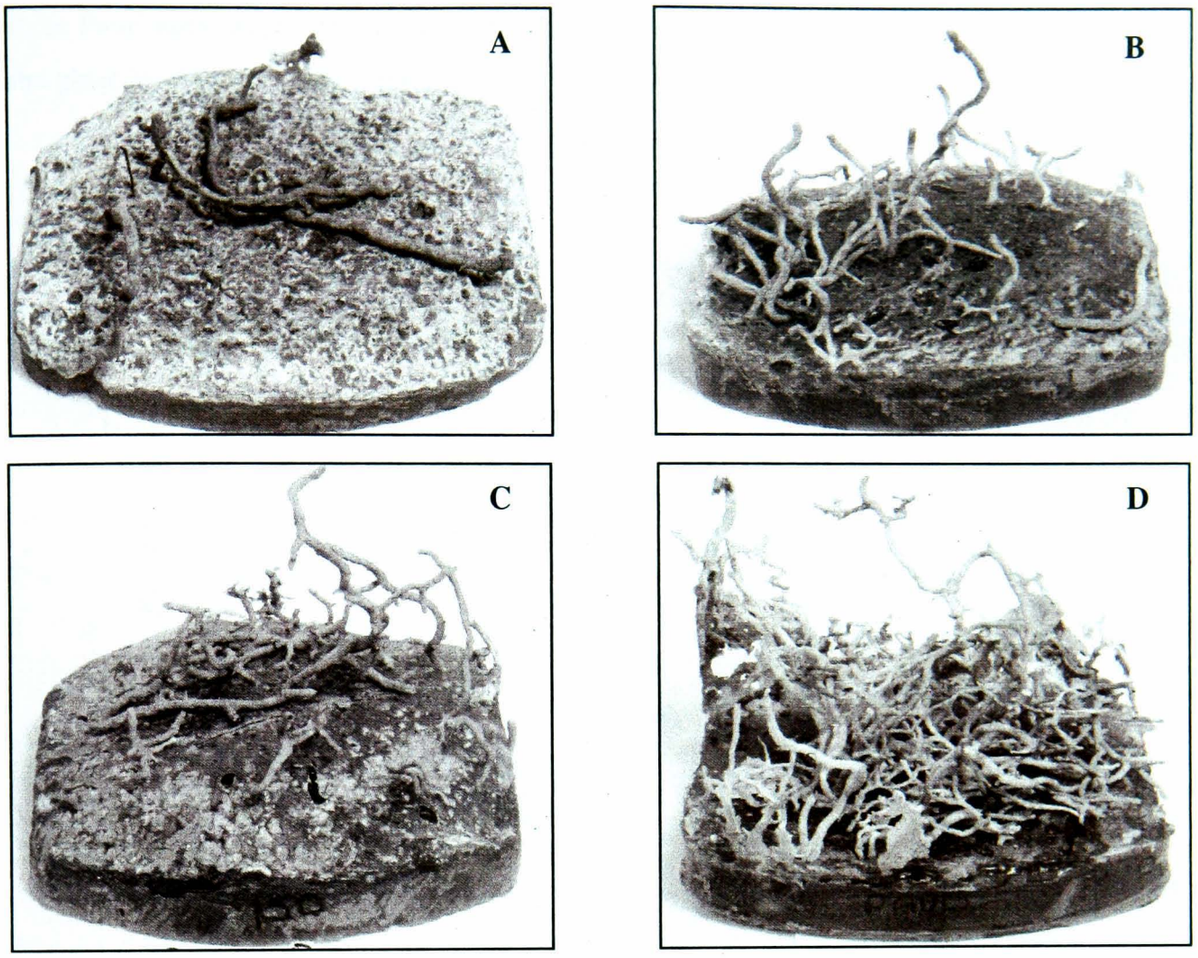


Figure 5.8. Resin burrow casts from S25 m (A), S75m (B), S150 m (C) and P150 m (D)

Attempts were made to quantify burrow volume by calculating the weight of 1 cm^3 dried resin, removing the burrows from the base of the cast and converting their collective weights into a volume for each site. Burrow volumes at the S25 m and S75 m were significantly lower ($p<0.01$) than at the Paull or S200 m site in July 1998 and January 1999. The volume of the burrows at the S25 m site was also significantly lower ($p<0.01$) than at the S75 m site for both months (Figure 5.9). No significant differences were found between the S200 m and the Paull site.

Bioturbation, as indicated by burrow volume, was greater in January than in July for the S25 m and S75 m sites but was found to be reduced in January at the Paull and S200 m sites. A two-way ANOVA (Table 5.6) test showed a statistically significant differences between sites, months and a significant interaction between the two. However, further one-way ANOVA tests showed that there was only a statistical difference between months at the S75 m site.

No significant differences were found between the burrow volumes of casts made at the Skeffling sites. Furthermore, burrow volume at these sites is lower ($p<0.01$) than at the S200

m or Paull sites despite the apparent higher level of bioturbation at Skeffling as indicated by the glass bead tracer study (section 5.4.3.1).

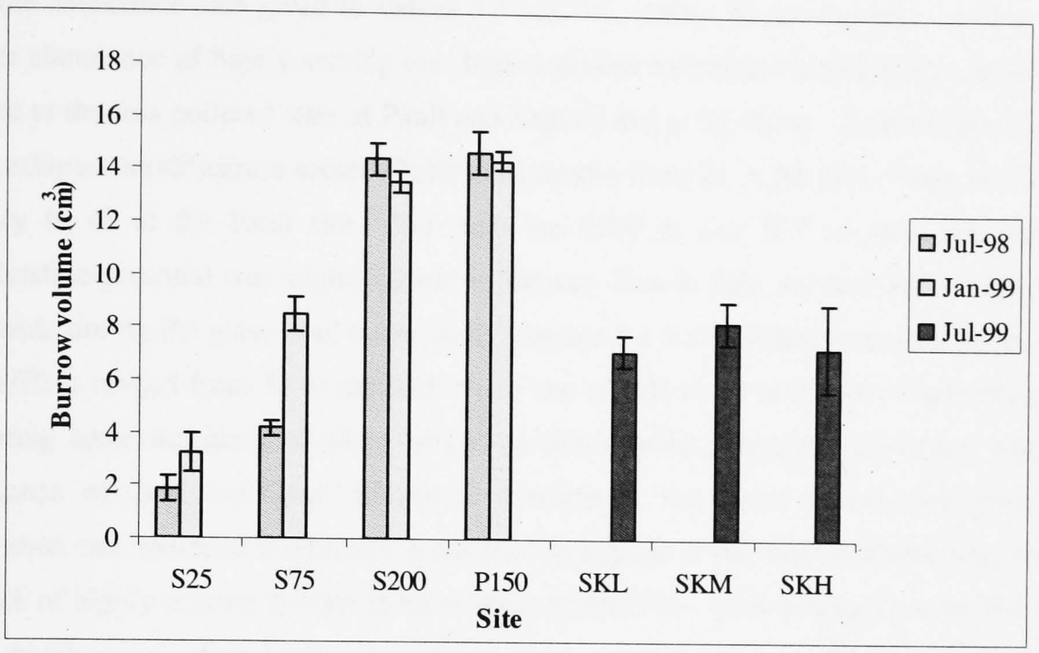


Figure 5.9. Burrow volume (mean ± SE), calculated from resin casts, for Paull and Saltend in July 1998 and January 1999.

Table 5.6. Two-way ANOVA output showing the individual and interactive effects of site and season on burrow volume.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	653.85	7	93.41	95.51	0.00
Intercept	2096.27	1	2096.27	2143.60	0.00
SITE	621.88	3	207.29	211.98	0.00
SEASON	7.59	1	7.59	7.77	0.00
SITE * SEASON	24.37	3	8.12	8.31	0.00
Error	15.65	16	0.98		
Total	2765.77	24			
Corrected Total	669.5	23			

5.4.3. Classification techniques

Table 5.7 gives details of the feeding strategy, sediment modification mode and sediment modification score for each species present at Saltend, Paull and Skeffling. This, together with the abundance data given in Tables 5.4 and 5.5, clearly shows a greater number and a greater abundance of highly scoring (i.e. high sediment modification potential) species to be present at the less polluted sites at Paull and Saltend and at Skeffling. At Paull and Saltend, total sediment modification scores (Table 5.8) ranged from 21 at the S25 m site in July and January to 46 at the Paull site. For both the S200 m and S75 m sites, the sediment modification potential was slightly lower in January than in July, an observation which was also made during the glass bead tracer study (section 5.4.3.1). Sediment modification scores at Skeffling ranged from 53 at the high shore site (SKH) to 80 at the low shore site (SKL) indicating some influence of shore height on bioturbation potential. However, when the abundance of each individual species is considered, this trend is reversed giving the impression that sediment modification potential is highest at the more polluted sites, despite the lack of highly scoring species in these areas (Table 5.8). This is thought to be due to the large numbers of oligochaetes present at these sites, together with the fact that total abundance decreased with increasing distance from the discharge. In addition, the abundance of the highly scoring species at the unpolluted sites was low (by up to 2 orders of magnitude) in comparison with the abundance of oligochaete worms at the polluted sites. Therefore, despite their high scores in terms of sediment modification, they do not impact so highly upon the total sediment modification score as the more abundant species.

The cluster analyses (Figure 5.10 A-C) show three distinct groups with the S200 m (January 1999) and P150 m sites being more than 70% similar in terms of species, more than 80% similar in terms of the feeding grouped present and approximately 70% similar in terms of sediment modification potential. The communities at these sites were dominated by *Hediste diversicolor* with *Corophium volutator, Manayunkia aestuarina,* spionids, oligochaetes and nematodes also being present. In addition, low numbers of *Macoma balthica* were also present at Paull. The majority of these organisms are surface deposit feeders which are also capable of suspension feeding and are classed as 'regenerators', which cause the downward movement of surface sediment particles to some depth within the bed, in terms of their sediment modification mode. *M. balthica* is also a surface deposit feeder which is classed as a destabilising 'conveyor belt' species in terms of its sediment modification mode.

The second major cluster includes The S25 m and S75 m sites which are approximately 60% similar in terms of species with the S75 m 1998 and 1999 groups being 85% similar. There is approximately 80% similarity in the feeding groups these sites contain although the S25 m

1999 site is grouped with the Skeffling site, possibly because of the greater abundance of *H. diversicolor* and much lower abundance of oligochaetes in comparison with the 1998 values. There is also over 60% similarity between the sites in terms of sediment modification. In general, there is approximately 20-30% similarity between this group of sites and the P150 m / S200 m group for species, feeding groups and sediment modification potential. The communities at these sites were dominated by large numbers of oligochaete worms which are classed as head down deposit feeders and 'biodiffuser' / 'conveyor belt' species. There were also comparatively low numbers of *H. diversicolor.* The majority of the species present at the Paull and Saltend sites generally feed on detritus and bacteria with *H. diversicolor* being classed as an omnivore.

The three Skeffling sites show 75-80% similarity in terms of the species and feeding groups present and the sediment modification potential of these organisms. In contrast to Saltend and Paull, the communities at Skeffling were dominated by *M. balthica* but contained relatively few *H. diversicolor* and, in comparison with the S25 m and S75 m sites, relatively few oligochaetes. Furthermore, a number of large, deep burrowing, destabilising species such as *Cerastoderma edule* and *Arenicola marina* were present. In addition to the detritivores and omnivores present at Saltend and Paull, a number of carniverous species were also present at Skeffling (e.g. *Carcinus maenas, Nephtys hombergii, Eteone longa/flava, Retusa obtusa*).

In general, the diversity of species and feeding strategies and the sediment modification potential increased as a result of decreasing stress (i.e. pollution or salinity stress due to location within the estuary). At the 'stressed' end of the scale (S25 m and S75 m), the community was dominated by non-selective head down deposit feeding, biodiffusive / conveyor belt species (S25 m and S75 m sites) which may occur in high abundances but do not burrow to great depths within the sediment. Intermediate communities (S200 m and P150 m) were composed of larger numbers of selective and non-selective subsurface and surface deposit feeders with the presence of omniverous species. Although these communities contained some biodiffusive species, regenerators and, to a lesser extent, conveyor belt species, were the dominant groups. This suggests a greater potential for sediment modification at these sites. At Skeffling, the community contained a greater number of relatively large bodied conveyor belt species (*A. marina, M. balthica, C. edule*) with a greater diversity of feeding strategies including the presence of carnivores.

Table 5.7. Feeding strategy and sediment modification potential of the species present at Saltend (S), Paull (P) and Skeffling (SK). Secondary feeding mechanisms are given in lower case. Sediment modification scores were calculated based on the dominant modes of feeding and sediment modification.

SUBDF Subsurface deposit feeder; **SDF** Surface deposit feeder; **SUSP** Suspension feeder; **CAR** carnivore; **OM** Omnivore; **DET/BACT** Detritivore / bacteria; **B** Biodiffuser; **C** Conveyor belt species; **R** Regenerators; **S** Surface depositors.

Species	Feeding	Sediment modification	Sediment modification score	Location
Hediste diversicolor	SDF / susp OM	R	9	S, P, SK
Manayunkia aestuarina	SDF / susp DET/BACT/om	R	3	S75, S200, P, SK
Nephtys hombergii	CAR	B/R	5	SK
Arenicola marina	SUBDF DET/BACT	C	10	SK
Eteone longa/flava	CAR	B/R	5	SK
Spionidae	SDF / susp DET/BACT/om	R/S	3	S75, S200, P, SK
Corophium volutator	SDF DET/BACT	R/S	8	S75, S200, P
Carcinus maenas	CAR	B/R	3	SK
Macoma balthica	SDF / susp DET/BACT	C/S	11	S200, P, SK
Cerastoderma edule	SUSP DET/BACT	C/S	10	SK
Hydrobia ulvae	SDF DET/BACT	B/R	5	SK
Retusa obtusa	CAR	B/R	3	SK
Oligochaetes	SUBDF DET/BACT	C/B	6	S, P, SK
Nematodes	SUBDF DET/BACT	C/B	6	S, P, SK

Table 5.8. Total sediment modification scores for sites at Saltend, Paull and Skeffling (scores which account for abundance are shown in brackets).

	S25 m	S75 m	S200 m	P150 m	SKL	SKM	SKH
January	21 ($1.3x10^5$)	24 ($2.6x10^5$)	35 ($9.4x10^4$)	46 ($9x10^4$)			
July	21 ($3.7x10^5$)	29 ($2.8x10^5$)	43 ($1.8x10^5$)		80 ($1.1x10^5$)	72 ($9.9x10^4$)	53 ($8.7x10^4$)

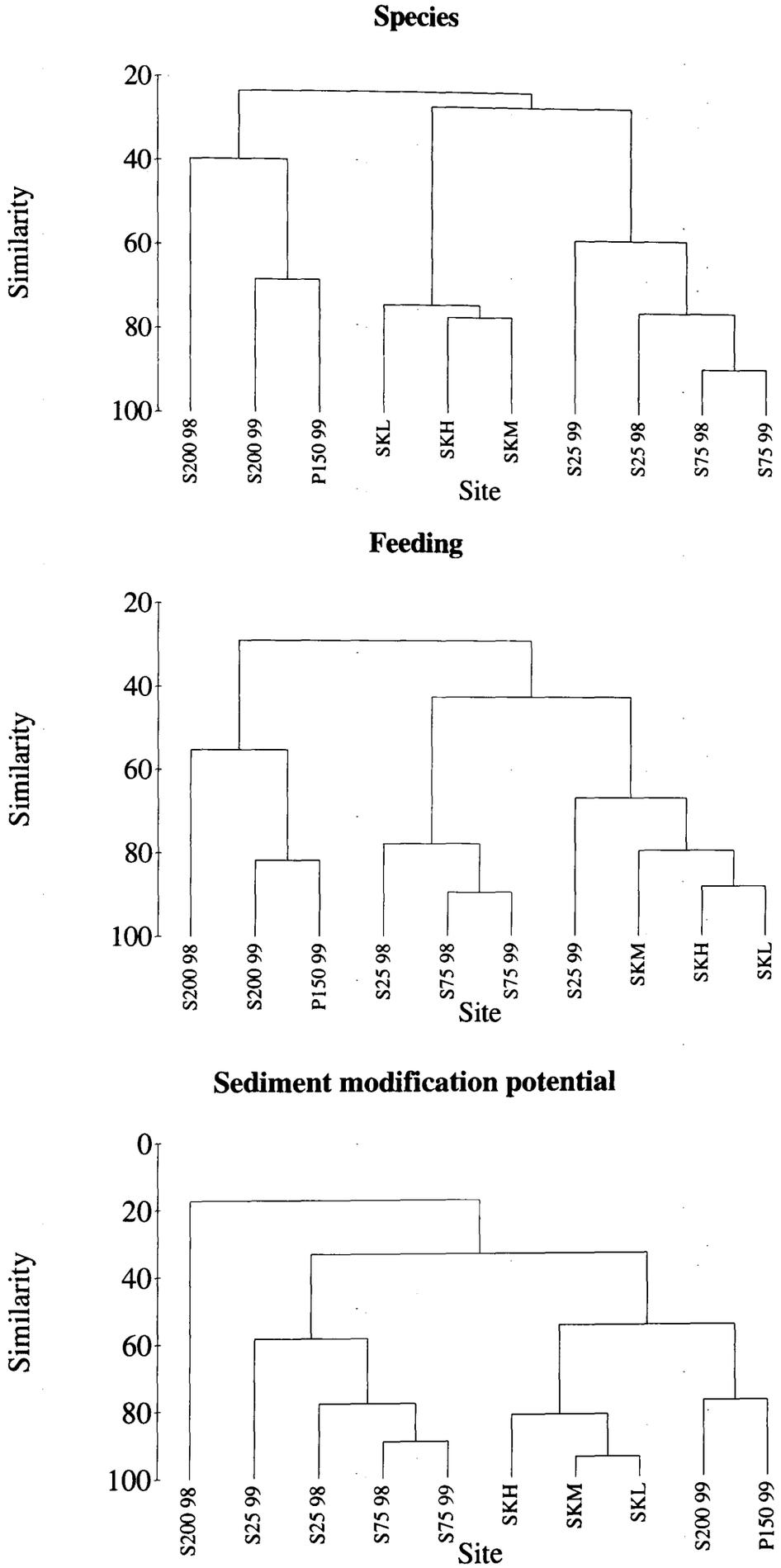


Figure 5.10. Cluster analyses of species (A) and feeding guilds (B) present and of sediment modification potential (C) at Saltend, Paull and Skeffling.

5.5. DISCUSSION.

5.5.1. General observations

Both laboratory and field based studies have demonstrated a clear reduction in bioturbation with increasing levels of pollution. Field studies, carried out along a pollution gradient, highlighted the importance of community structure with lower levels of bioturbation being associated with communities composed of large numbers of individuals but low biomass and low diversity (in terms of both structure and function). Furthermore, there were apparent sub-lethal effects of the effluent on individual species as demonstrated by the reduction in bioturbation by *M. balthica, H. diversicolor* and *C. volutator.* Inter-specific differences in bioturbation potential were also recorded with *M. balthica* and *H. diversicolor* showing a greater degree of mixing than *C. volutator.* No experiments were carried out using oligochaete worms (the dominant organism at the most polluted site) due to the difficulties associated with their collection and maintenance in the laboratory. However, the absence of the three above species from the most polluted sites is thought to be the reason for the comparatively low level of bioturbation (in terms of depth) at the most polluted site.

Numerous studies have been carried out on the effects of bioturbating organisms on the flux of contaminants in and out of the sediment (e.g., Gilbert *et al.*, 1994; Schaffner *et al.*, 1994; Gilbert *et al.*, 1996; Schaffner *et al.*, 1997; Petersen *et al.*, 1998; Rasmussen *et al.*, 1998) but there have been relatively few studies examining the effects of pollutants on bioturbation. However, those studies which have been carried out provide evidence that bioturbation potential can be reduced by increasing levels of pollution or some other form of physico-chemical stress. For example, Winston & Anderson (1971) demonstrated the way in which the degree of bioturbation decreased with increasing distance into an estuary as a result of salinity induced changes in faunal density and diversity. Winston & Anderson (1971) also found the effects of an oil spill to reduce bioturbation potential as a result of changes to the macrofaunal community structure.

Nickell *et al.* (2003) examined the relationship between benthic community structure, benthic fluxes and bioturbation in relation to a salmon farm. The degree of bioturbation (rate and depth) was found to increase with increasing distance from the fish farm and to be related to community structure (species present and their abundance and biomass). Increased bioturbation potential was also found to be associated with increasing animal size, an observation also made by Zwarts & Wanink (1989) and Zwarts *et al.* (1994) who found the burrowing depth of *Macoma balthica* and *Scrobicularia plana* to increase with body size. Bioturbation was also found to increase with increasing organic content of the sediment, apart from the sites beneath the fish farm where bioturbation was similar to that at sites with low

organic content. It was suggested that bioturbation potential is greatest in places with intermediate levels of organic matter. Wheatcroft & Martin (1995; 1996) examined levels of bioturbation along a pollution gradient (primarily organic carbon, metals and DDT) on the Palos Verdes Margin, South California where bioturbation rate was found to be twice as high at a site 5 km from an outfall in comparison to that at the site of the outfall. No structural differences in the macrofaunal community were found and differences in bioturbation rate were therefore explained by sub-lethal responses (i.e. reduced burrowing activity) to the elevated concentration of DDT and organic content of the sediment at the site of the outfall.

Nikitik (in prep) demonstrated a reduction in bioturbation (measured as the time taken to burrow) with increasing levels of contamination by examining the burrowing rate of *Macoma balthica* exposed to sediments collected from various distances from the BP outfall at Saltend. Burrowing rates, expressed as median effective times (ET_{50}), ranged from 0.48 hours in sediments collected 100 m from the discharge to 2.99 hours in sediments collected 50 m from the discharge. Whilst there were some inconsistencies, there was a definite trend of decreasing burrowing time with increasing distance from the discharge, indicating that sediment quality can influence the bioturbation potential of this species. Gilbert *et al.* (1994) found 15 day exposure to oil to cause a 77% reduction in burrowing by *Hediste diversicolor* in comparison with the controls. Levell (1976, in Gilbert *et al.*, 1994) and Gordon *et al.* (1978, in Gilbert *et al.*, 1994) also found the presence of oil to reduce the 5-day sediment reworking time by *Arenicola marina* by 70%. O'Brien (1997) also noted a reduction in the rate of cast production by *Arenicola marina* in sediment samples collected within close proximity to the BP discharge at Saltend.

Bartsch *et al.* (1999) exposed nymphs of the burrowing mayfly *Hexagenia bilineata* to cadmium spiked sediments and monitored the turbidity of the overlying water as an indication of sediment resuspension through bioturbation. Turbidity and, hence, the level of bioturbation, were found to decrease with increasing cadmium concentration. In contrast, Briggs *et al.* (2003) found *C. volutator* activity to cause increased turbidity in the overlying water when exposed to contaminated sediments and used this increase in turbidity as a means of rapid sediment toxicity assessment. During the first two days of exposure to sediments spiked with concentrations of copper (10-day LC_{50} 129 $\mu g\ g^{-1}$) or total petroleum hydrocarbons (10-day LC_{50} 231 $\mu g\ g^{-1}$) slightly higher than the LC_{50}, turbidity increased rapidly for both contaminants but then declined as animal abundance decreased due to death. At concentrations close to the LC_{50}, turbidity remained higher than that in the lower concentrations and the controls. It was concluded that exposure to moderate concentrations of toxicants causes a behavioural response (i.e. increased bioturbation) in *Corophium volutator*

which results in increased sediment resuspension. This increased bioturbation may be due to enhanced burrow irrigation in an attempt to remove the source of contamination.

5.5.2. Laboratory studies

The thin section and photographic techniques demonstrated both the reduction in bioturbation potential with increasing levels of pollution and the inter-specific differences in bioturbation potential. O'Brien (1997) found increasing effluent concentration to reduce the bioturabtion potential of communities composed of *H. diversicolor, C. volutator* and *M. balthica*, using a photographic technique similar to that used in the present study. Using the thin section technique, O'Brien (1997) also showed the bioturbation potential of *M. balthica* to be reduced by increasing effluent concentration, as demonstrated by differences in changes in surface roughness at the sediment-water interface.

With reference to the Paull and Saltend communities, both techniques showed *M. balthica* and *H. diversicolor* to be more important bioturbators, in terms of depth and rate, than *C. volutator*. However, under the influence of the effluent, the activity of *H. diversicolor* became more important than that of *M. balthica* suggesting the lower sensitivity of this species to the effluent. Given these results and due to its abundance in comparison to that of *M. balthica*, *H. diversicolor* is considered to be the most important species in terms of bioturbation at the Paull and Saltend sites. Mugnai *et al.* (2003) also found *H. diversicolor*, together with the spionid polychaete *Streblospio shrubsolii* (present at Paull and the S200 m site), to be one of the most important species in terms of bioturbation in the Venice Lagoon. Winston & Anderson (1971) found *H. diversicolor* to be an important bioturbator in the Great Bay estuarine system, New Hampshire, and found that its removal following an oil spill resulted in considerably lower levels of bioturbation. In contrast to the present study, Winston & Anderson (1971) found that *M. balthica* had relatively little impact on the sediment and suggested that the species was of relatively minor importance in terms of sediment reworking. However, it should be noted that this observation was made in contaminated sediments, following an oil spill, and as demonstrated in the present study and by Nikitik (in prep), the burrowing activity of *M. balthica* is reduced by petrochemical pollution.

The photographic study showed that a single individual of the bivalve *Scrobicularia plana* could rework the sediment at such a rate that the entire white surface of the core was covered with sub-surface sediment, deposited by the animal, in less than 48 hours. This occurred at all effluent concentrations and therefore no conclusions could be drawn about the effect of pollution on this animal. Hughes (1969) found that *S. plana* was a deep burrowing species,

able to migrate vertically within the sediment to exploit both surface and subsurface food supplies. It was also found to eject faecal and pseudofaecal material at a high rate, often with force. It is therefore clear that this species is of importance, in terms of its bioturbation potential, at Skeffling, particularly in upper shore areas where it was seen in its greatest abundance. It would be useful to determine the effect of pollution on such large species although a much larger area of sediment / animal than used in this study would be required and observations would need to be made at more frequent time intervals, possibly over a shorter time period.

Both the photographic technique and the thin section technique proved to be particularly useful in providing good visual evidence for differences in bioturbation potential between species and between effluent concentrations. By using both techniques, it was possible to examine the effects of bioturbation both at the sediment surface and at depth. However, quantification of the degree of bioturbation was, to some extent, subjective, particularly in the case of the photographic study. Distinction between light and dark areas was largely dependent upon the quality of the photographs and, from the point of view of the software used in analysis, could be obscured due to shadows and reflection. Care was taken to avoid this by using adequate lighting and by completely draining the tanks before the photographs were taken. However, because the surface of the mud was wet, reflections were still present on a small number of images. Furthermore, there was a gradient of increasing darkness rather than an absolute colour change from white to black as bioturbation progressed. In some cases, the difference between features of bioturbation and discoloration of the white surface (e.g. due to the underlying sediment showing through the bead layer) could be seen easily but in other cases, the difference was uncertain. The differentiation between bioturbated sediment undisturbed sediment was therefore subjective.

Changes in the roughness of the sediment surface, caused by bioturbation by different species, were relatively easy to measure and quantify using the thin section technique. Changes to the deeper sand layers were more difficult to quantify because the sand did not remain as one continuous layer and parts of the layer became completely obliterated as the sand particles became mixed with the mud. For this reason, measurement of the change in total visible area was considered to be an appropriate means of showing the effects of bioturbation. However, it became apparent that whilst the initial continuous layer may be broken up into smaller components, the overall visible area may not necessarily change. Therefore, measurements of the average area of the visible coloured sections were made. This, together with the above technique, gave a reasonable measure of the degree of bioturbation.

In the present study, bioturbation studies involving individual species were restricted to the use of those species which were abundant, easy to collect and easy maintain under laboratory conditions. At the S25 and S75 m sites, oligochaetes are the dominant organism and it would have been valuable to examine their effect on sediment mixing and the effect of pollution on the rate of mixing by this group. It is possible that whilst their activity in terms of depth may be reduced in comparison to that of other species (Hunter & Arthur, 1978; Hunter, 1981), their rate of activity, coupled with their abundance, may be greater with an increase in bioturbation rate allowing more rapid removal of sulphide from their burrows (Miron & Kristensen, 1993b; Nickell *et al.*, 2003). However, oligochaetes are easily damaged during sieving, particularly when the sediment has a tendency to form oily clods, and collecting sufficient numbers of intact animals was difficult. In addition, the animals ability to escape made it difficult to maintain them in the laboratory. It is suggested that experiments using oligochaetes should be carried out but on a smaller scale (in terms of the size of the sediment cores used) than that used in the present study.

The lithium clay tracer study was carried out using sediment cores taken from 25, 75 and 200 m from the outfall, containing macrofaunal communities characteristic of the area from which they were collected. There was some indication that, when exposed to clean sea water, the degree of bioturbation was greatest by communities found at the lowest levels of contamination. When exposed to an effluent concentration of 32%, the greatest proportion of the lithium was transported into the cores by communities from the polluted S25 m site suggesting that these organisms have a higher tolerance to the effluent. However, differences in the lithium concentration at each depth between sites were generally not statistically significant. Given the expense of the lithium analysis, together with the fact that, at most depths, the lithium concentration was only slightly elevated above background levels, repeating this experiment was not considered to be worthwhile. It should also be considered that with a lithium concentration of over 4000 ppm, the bentonite clay may have some toxic effect on surface deposit feeders. However, the toxicity of lithium to the species used in this study is not known.

5.5.3. Field studies.

Both of the field techniques used were successful in demonstrating the reduction in bioturbation potential with increasing levels of pollution. The most striking evidence was provided by the resin casts which clearly show an increase in burrow depth and volume with increasing distance from the source of pollution. It is appreciated that quantification of burrow volume by this method is not precise since air bubbles can become trapped within the resin, water and animals may prevent the penetration of resin into the burrows and removal of

an entire burrow from the base of the cast is difficult. Damage to casts during washing was difficult to avoid with the smaller burrows being particularly susceptible to breakage. These problems were also encountered by Davey (1994). However, this provides good visual evidence for differences in the degree of bioturbation between sites. Differences in burrow volume between sites can be related to the macrofaunal data presented in Chapter 4 which show the greater abundance of large, deeper burrowing organisms (*H. diversicolor*) at the low impact sites in comparison the S25 m site where the community is dominated by large numbers of shallow burrowing oligochaete worms. It is also of note that the large *H. diversicolor* burrow present in the S25 m cast lies horizontally rather than vertically as in casts from the other sites. This may have been due to the low redox potential, and probably high sulphide concentration, of these sediments which could have discouraged the animal from burrowing any deeper.

Davey (1999) also used this technique to demonstrate the reduction in burrow volume caused by arsenic pollution at East Clough (Humber estuary) in comparison with that at the relatively unpolluted Paull site. Burrow volumes at East Clough were found to be between 1 and 3 cm^3 in comparison with volumes of 4-5 cm^3 at Paull. The addition of 100 *H. diversicolor* to the sediment resulted in an increase in burrow volume to 12-13 cm^3 at Paull but only 5 cm^3 at East Clough. Whilst no data regarding the faunal communities at each site were presented, these results indicate a lower level of burrowing activity at the East Clough site.

The results of the resin casting study agree well with those of the glass bead tracer study, providing further evidence that pollution induced community changes can reduce bioturbation potential. Despite the general trends, the data were quite variable with no two replicate tracer profiles being the same, an observation which was also made by Mugnai *et al.* (2003) Whilst the maximum bead concentration in the S25 m cores was within the top 5 cm and concentration declined steeply at depths greater than this, no apparent trends in bead distribution were found within this region. This is the depth zone of greatest bioturbation (Rhoads, 1974) and it is possible that the tracer profiles have been flattened by the high numbers of oligochaetes and nematodes present which act as biodiffusers (Dauwe *et al.*, 1998). Tracer distributions with a maximum at the surface, followed by a sharp decrease with depth, are typically generated by biodiffusive mixing (Wheatcroft *et al.*, 1990). Oligochaetes are sub-surface deposit feeders which deposit faecal pellets at the sediment surface, an activity which has a similar effect to that of a high sedimentation rate (Rice, 1986; Mugnai *et al.*, 2003). This explains the sub-surface peak in the number of beads recovered from the S25 m cores. Gerino *et al.*, 1994 also noted that subsurface peaks were generated by head down deposit-feeding organisms or 'conveyor belt species' which deposit faecal material at the

surface. This sub-surface peak was also recorded in the S75 m cores where the abundance of oligochaete worms is also high.

Hunter (1981) examined the vertical distribution of oligochaetes in the intertidal areas of the Thames estuary and found the greatest proportion of worms to be in the top 2 cm of the sediment although the greatest proportion of breeding worms and their cocoons was found in the 2-4 cm layer. This layer is often black and anoxic but the cocoons are better protected against the effects of erosion and fluctuating salinity and temperature. Newly hatched worms, however, were found to be intolerant to conditions of anoxia and moved up into the superficial layers of the sediment following hatching. This explains why no, or very few beads, were recorded from depths greater than 5 cm at the most polluted site.

In the S75 m, S200 m and P150 m cores, the beads were transported down the full length of the core with some small peaks in bead concentration at deeper levels. Comparatively high numbers of *H. diversicolor* were present at these sites, particularly the S200 m and P150 m sites. This species is classed as a 'regenerator' and such species can cause deep subsurface peaks in tracer concentration though ingestion at the surface and deposition as faecal pellets at some depth within the sediment. Fresh, surface material can also mechanically drop into their burrows (Wheatcroft, 1990; Mugnai *et al.*, 2003). Mugnai *et al.* (2003) found *H. diversicolor,* together with *Streblospio shrubsolii*, both abundant at the P150 m and S200 m sites, to be the most important species, in the Venice Lagoon, in terms of non local (regeneration) transport associated with deep tracer peaks. Winston and Anderson (1971) also found small polychaetes, such as *S. shrubsolii*, to be important bioturbators. These findings are also consistent with those of Nickell *et al.* (2003) who studied the effects of deposition from a fish farm on benthic community structure and bioturbation potential. At the most organically enriched site, the dominant mixing process was biodiffusive (i.e. similar to the S25 m site). The tracer profile had a subsurface peak with a steep decline in tracer concentration with increasing depth. With reducing organic content, the tracer profiles become shallower, indicating that mixing is taking place to a greater depth, with sub-surface peaks indicating the activity of 'regenerator' species. Community biomass and animal abundance were greatest at the most organically enriched sites although mean individual size and biomass was greater in sediments with lower organic content.

The communities present at Skeffling were similar to those recorded by Davey & Partridge (1998) and Widdows *et al.* (1998a) being dominated by *M. balthica* with significant numbers of *H. diversicolor, Manayunkia aestuarina,* spionid worms (*S. shrubsolii* and *Pygospio elegans*), *Retusa obtusa, Hydrobia ulvae* and oligochaetes. Low numbers of *Nephtys*

hombergii, Eteone longa/flava and *Cerastoderma edule* were also present, particularly towards the low shore. *Arenicola marina* was recorded in low numbers, although numerous casts were seen on the sediment surface at two sites sampled during the present study, but was not recorded by Davey & Partridge (1998) or Widdows *et al.* (1998a). *Scrobicularia plana* (shown to be an important bioturbator using photographic techniques) was abundant, particularly in upper shore areas and *Mya arenaria* was also seen on several occasions but neither species was recorded in any of the core samples. This is thought to because these species are capable of burrowing to depths beyond the range of the corer. This was also suggested by Beukema (1974; 1976) and Zwarts & Wanink (1989) who found *S. plana* to be capable of burrowing to depths of 20-30 cm. Green (1968) and Hughes (1969) also found that *M. arenaria* could burrow to depths of 60 cm.

At Skeffling, there were no trends of decreasing bead concentration with increasing depth with multiple sub-surface peaks being present. As at the S25 m site, there was a peak in tracer concentration immediately below the surface at all three of the Skeffling sites. These patterns of tracer distribution are thought to be due to the higher diversity of species and also of functional groups at these sites in comparison with the Paull and Saltend sites. Firstly, *A. marina*, *C. edule* and *M. balthica* are know to be important bioturbators (e.g.Cadée, 1976; Retraubun *et al.*, 1996; Davey & Partridge, 1998; Widdows *et al.*, 1998b) through the excavation of feeding pits, ejection of faecal pellets at the surface, movement within the sediment and the creation of voids into which tracer particles may fall. There were also significant numbers of 'conveyor belt' organisms such as oligochaetes and nematodes and of 'regenerator' species such as *H. diversicolor* and spionid worms. It is the combined activity of the various functional groups of organisms present which is thought to have resulted in this relatively random distribution of beads throughout the cores, together with the greater abundance of larger organisms, capable of transporting the beads to greater depths. The inclusion of the Skeffling results demonstrates the change in bioturbation potential along a salinity gradient, as was demonstrated by Winston & Anderson (1971).

Attempts were made to dye sediment particles (63 - 150μm) using methylene blue and malachite green in order to create luminophores similar to those used by Mahut & Graf (1987). However, this technique proved unsuccessful for a number of reasons. Whilst the sediment particles could be successfully stained, the colour was not sufficiently bright to allow easy distinction between the coloured sand particles and the ambient sediment under the microscope. Furthermore, fine grained sediments with a high water content do not appear to be able to support sand particles. Sand grains were found throughout the entire depth of the core. A study by Rhoads (1974) showed that sand particles will sink through the surface

layers of the mud and concentrate at a depth where the sediment is able to support them. This 'passive' particle transport was found to occur with or without the presence of burrowing animals.

Whilst glass beads are considered to be an appropriate tracer due to the fact that they are inert and within the size range of the sediment particles they were used to simulate, the technique has its drawbacks. Wheatcroft (1992) and Wheatcroft *et al.* (1994) suggested that variability could be introduced as a result of uneven bead spreading, coring and counting. These studies were carried out in a subtidal area where 200 ml glass beads were spread over 1 m^2 of the sea bed and therefore, uneven spreading of beads is highly likely and possibly unavoidable. It is also likely that subduction of beads occurred during coring although the effects of this were minimised by sectioning the cores and discarding the outer ring of sediment at each depth interval. In contrast, in the present study, beads were only spread over an area of 36 cm^2 and care was taken to ensure that the entire sediment surface was covered with beads. In a small area such as this, uneven distribution of beads on the surface is less likely to be a source of variability. Subduction of beads during coring was avoided by placing the cores in the sediment before the beads were added. The entire core was then removed and sub-samples taken from holes drilled along the length of the core. Therefore, the only significant sources of variability are considered to be those associated with counting, due to masking of the beads, particularly at low concentrations and the exotic nature (shape, inertness and density) of the beads may affect their mixing kinetics (Wheatcroft *et al.*, 1994).

Tracer studies clearly indicated a greater degree of bioturbation at the Skeffling sites than at the Saltend and Paull sites. However, the burrow volumes, calculated from the resin casts, indicated a lower level of bioturbation at Skeffling. Davey & Partridge (1998) examined the depth distribution of macrofaunal species at this site and found the majority of organisms to occur within the surface layers of the sediment. For example, 92% of the *M. balthica* and 70-90% of *Nephtys hombergii* recorded were found within the top 4 cm. Of the *H. diversicolor* recorded, 40% were found in the 0-4 cm and 5-8 cm depth intervals with 20% being found at depths greater than 8 cm. However, the numbers of *H. diversicolor* present at Skeffling were considerably lower than at Paull or Saltend. During casting, individuals of *H. diversicolor* and *C. volutator* were seen to emerge from their burrows in an attempt to escape the resin but no bivalves emerged. It is possible that bivalve species (*M. balthica, C. edule, S. plana* and *M. arenaria*) would prevent the passage of the resin into their burrows thus giving the impression that what could effectively be a large burrow or void did not exist. In some cases, individuals of *M. balthica* were found within the resin. In addition, these species can liquefy the sediment, in order to facilitate their movement through it, and the voids they create may

become infilled. The burrows of *N. hombergii* were found to be horizontal and the majority of burrowing activity by this species occurs within the superficial layers of the sediment (Davey & Partridge, 1998). In addition, *Nephtys* burrows are transient and become infilled with sediments of higher water content than that of the surrounding material. Therefore, this species was not considered to have a great effect on sediment mixing (Davey & Partridge, 1998)

These studies support the statement by Rhoads (1967) that the degree of sediment reworking to be more closely related to the faunal composition than to abundance. Intensive reworking suggests but does not require high densities of benthic organisms as indicated by the high abundance of organisms at the S25 m site, but low level of bioturbation, in comparison with the relatively low abundance of organisms at Skeffling but the high level of bioturbation. Bender & Davis (1984) also highlighted the importance of animal size, which generally increases with increasing distance from the discharge. Whilst the frequency of sediment expelled by the sub-surface deposit feeder *Yoldia limatula* decreased with increasing body size (i.e. a higher rate of bioturbation), a clear positive relationship was found between the actual amount of sediment expelled and animal size suggesting that larger deposit feeding animals are more important in terms of bioturbation.

Further evidence for increasing bioturbation with increasing levels of pollution is provided by the total sediment modification scores for each community. The S25 m community scored only 21 in comparison with the communities at the S200 m and P150 m sites which were exposed to a lower level of pollution and scored between 35 and 46. A further reduction in stress (through increased salinity and a reduction in the magnitude of salinity fluctuations) resulted in a more diverse community at Skeffling, scoring between 53 and 80. Whilst the differences in bioturbation potential indicated by these scores agree with the results of the field and laboratory studies, the scoring system could be greatly improved if abundance and/or animal size were considered. For example, at Skeffling, *Nephtys* and *Eteone* contribute significantly to the total sediment modification score yet their impact on the sediment may be minor, particularly as they do not occur in particularly high densities. Davey & Partridge (1998) suggested that the effects of *Nephtys*, in terms of sediment mixing, may be small and mediated only slowly over time.

However, when the sediment modification scores were calculated, accounting for abundance, the above trend was reversed indicating higher sediment modification potential at the more polluted sites. This supports the hypothesis given in section 5.2 that the rate of bioturbation in polluted communities, characterised by large numbers of small organisms may be greater than

that of communities in cleaner sediments, characterised by lower numbers of organisms. However, bioturbation by the communities present at these sites was largely restricted to the surface layers (as demonstrated by the glass bead tracer and resin casting studies). It has also been demonstrated that whilst small organisms may rework the sediment at a higher rate than larger organisms, the actual volume of sediment reworked by larger organisms is much greater (Bender & Davis (1984). Therefore, despite their high sediment modification potential score (when abundance is considered) the bioturbation potential of the communities in the more polluted sediments is considered to be less than that of the communities in the cleaner sediments.

In general, the reduction stress, either as a result of pollution or salinity stress due to location within the estuary, resulted in an increase in species diversity. Coupled with this was an increase in the diversity of feeding strategies, moving from a community dominated by non-selective, sub-surface deposit feeders to one composed of selective and non-selective surface and subsurface feeding detritivores, omnivores and carnivores, leading to a greater potential for sediment modification. In terms of bioturbation, there was also an increase in diversity from a community containing biodiffusers only (S25 m) through those being dominated by regenerators (S200 m and P150 m) to those containing all groups (Skeffling). Given that bioturbation and feeding are inter-related (Rhoads, 1974; Rhoads & Boyer, 1982), it is not surprising that pollution also results in a reduction in the diversity of bioturbation guilds, a feature reflected in the use of the Infaunal Trophic Index to denote pollution status (Elliott, 1993).

As described in Chapter 4, the patterns of community change recorded in this study were typical of those found along organic gradients, as documented by Pearson & Rosenberg (1978). In addition, these authors also demonstrated a progressive simplification of trophic variety in response to increasing organic enrichment and found a general decline in carnivorous and selective surface deposit feeding species to be coupled with an increase in non-selective sub-surface deposit feeders. They also suggested that communities at the more polluted end of the gradient were dominated by detritus feeders. This simplification of trophic variety was also observed by Horne *et al.* (1999) who recorded a change from a community with an evenly distributed percentage of surface and sub-surface deposit feeders in an uncontaminated area, to a community dominated by sub-surface feeders in an area contaminated with mercury and PCBs. The abundance of carnivores also declined with increasing levels of contamination. Furthermore, Pearson & Rosenberg (1978) also found changes in trophic structure to be accompanied by a change in the depth of the sediment occupied by the macro fauna and in the physical size of the species. Bonsdorff & Pearson

(1999, in Rosenberg, 2001) found that the number of functional groups (in a subtidal environment) increased with increasing salinity whilst Dauwe *et al.* (1998) found differences in the composition of trophic groups to be related to food availability.

Pearson & Rosenberg (1978) stated that trophic structures are particularly influenced by organic gradients and are therefore considered fundamental to the analysis of community change in relation to organic inputs. Rosenberg (2001) highlighted several studies where benthic organisms had been divided into numerous functional groups based on the source of their food, their mode of obtaining it and their degree and mode of motility. The number of groups defined ranged from 12 (Lee & Swartz, 1980, in Rosenberg, 2001) to 22 (Fauchald & Jumars, 1979). However, many of the studies which have examined functional groups of benthic organisms have been carried out in subtidal, marine areas where faunal diversity is rich in comparison to that of a mid-estuarine intertidal area. Therefore, in the present study, the number of functional groups was kept to a minimum due to the low species diversity. Three major factors were considered: source of food (carnivore, omnivore, bacteria / detritus feeders), the mode of obtaining food (deposit or suspension feeding) and the mode of bioturbation (conveyor belt species, regenerators, biodiffusers, surface depositors) giving rise to a total of ten functional groups. This allowed easier interpretation of the data and prevented actual differences in functional groups from being obscured by an over-complicated classification system.

Although the functional classification of benthic communities has been widely used, it has been subject to considerable criticism. According to Jumars & Nowell (1984a), several attempts have been made to classify benthic organisms into functional groups. This has largely been unsuccessful since organism behaviour may change depending upon the environmental conditions and an organism may have various simultaneous effects on its environment through performing a single activity. For example, deposit feeders such as *H. diversicolor* (Nielsen *et al.*, 1995) and *M. balthica* (McLusky & Elliott, 1981) are known to be capable of suspension feeding and may change between the two modes over a single tidal cycle. *Pygospio elegans*, *Streblospio shrubsolii* (Fauchald & Jumars, 1979), *Scrobicularia plana* (Hughes, 1969) and *Mya arenaria* (Rasmussen, 1973) are also able to alternate between deposit and suspension feeding and there is increasing evidence for this plasticity in feeding (Pearson & Rosenberg, 1978; Snelgrove & Butman, 1994). Given that deposit feeding is generally classed as a stabilising activity and suspension feeding encourages sediment deposition, a change in feeding behaviour will also mean a change in sediment modification potential. This was demonstrated by Widdows *et al.* (1998b) where *C. edule* was found to cause significant bioturbation but was also found to cause significant biodeposition.

However, despite these problems, analysis of the changing importance of the various feeding groups along organic gradients provides a readily interpretable guide to changing ecological structure in response to environmental factors. The broad, functional classification of benthic communities is therefore generally considered to be valuable in describing some of the dominant processes in a community (Pearson & Rosenberg, 1978; Dauwe *et al.*, 1998).

Several studies have suggested that bioturbation may be seasonally influenced. Grant & Daborn (1994) found the activity of *Corophium volutator* to increase during summer and Winston & Anderson (1971) postulated that many benthic organisms were more active during the summer months and therefore the rate of sediment reworking was greater. Aller & Cochran (1976), Martin & Sayles (1987, in Wheatcroft *et al*, 1994) and Wheatcroft *et al*, (1994) all found elevated mixing rates during the warmer months. Wheatcroft *et al* (1994) suggested that this may be the result of temperature driven metabolic changes, alteration of deposit feeding rates due to changes in the redox conditions (e.g. may increase burrow irrigation to overcome effects of sulphides (Cadée, 1976; Miron & Kristensen, 1993b; Nickell *et al.*, 2003)) or a response to increased food availability (Cadée, 1976). It was also suggested that changes in feeding mode (e.g. from deposit to suspension feeding) may occur in relation to seasonal variation in food quantity and quality. Cadée (1976) also found reworking by *Arenicola marina* to be greater in summer than in winter and Retraubun *et al.* (1996) explained this by the fact that laboratory experiments showed the activity of *A. marina* to be temperature dependent. Bender & Davis (1984) found feeding, and hence bioturbation, to increase with increasing temperature providing further evidence for a seasonal influence on bioturbation. Mugnai *et al.* (2003) recorded bioturbation to greater depths in the autumn compared with spring, again suggesting a seasonal influence.

In contrast to all of these studies, Birtwell & Arthur (1980) reported movement of individuals of *Tubifex costatus* (an abundant species at the S25 m site) and *Limnodrilus hoffmeisteri* towards the sediment surface during the warmer months, probably to avoid anoxic sediments which are closer to the surface during summer. This implies a decrease in the depth of bioturbation during summer. These seasonal migrations were not noted in *Tubificoides benedeni*, possibly due to the greater tolerance of this species to anoxia. (Hunter & Arthur, 1978, in Hunter, 1981).

Zwarts & Wanink (1989) found siphon weight in *M. arenaria, S. plana, M. balthica* and *C. edule* to be positively related to shell size and that burying depth increased with increasing siphon and, hence, body size. They also found the deposit feeder *S. plana* to burrow twice as deep during the winter and switch to suspension feeding. *M. balthica* also increased its

burrowing depth during winter but this did not occur in the suspension feeding species *C. edule* or *M. arenaria*. Zwarts & Wanink (1989) suggested that burrowing depth was more variable in deposit feeders than in suspension feeders. Zwarts & Wanink (1989) attributed this to avoidance of low temperatures and / or predator evasion since the risk of being taken by a predator decreases with increasing depth in the sediment. Species which are able to switch between feeding modes may vary their burrowing depth to a greater extent than those which rely on one feeding mode only. Goss-Custard (1966, in McLusky, 1968a) stated that *C. volutator* increased its burying depth as the temperature decreased below 4°C. This activity not only has important ramifications for predator evasion but also for bioturbation and impacts on the sediment properties.

Whilst there was only weak evidence for any seasonal influence over bioturbation in the present study, the glass bead tracer study did indicate the deeper transport of beads during the winter months in comparison to the summer months at the S25 m and S75 m sites. This would be consistent with the findings of Birtwell & Arthur (1980) since the degree of anoxia was found to be much greater at these sites during the summer months. Furthermore, the abundance of *H. diversicolor* was greater at the S25 m site during the colder months which would increase the level of bioturbation, assuming that sub-lethal effects of the sediment did not prevent burrowing. This difference between seasons at the S25 m and S75 m sites was also reflected in the burrow volumes and was most noticeable at the S75 m site where the abundance of *H. diversicolor* was comparatively high. However, winter burrow volumes at the S200 m and P150 m sites were slightly lower than summer volumes, possibly reflecting the lower abundance of animals. Despite this suggestion, no statistical differences were found between months and the difference in burrow volume was negligible.

5.6. SUMMARY AND CONCLUSIONS

Through the use of a combination of laboratory and field studies, it has clearly been demonstrated that pollution can reduce bioturbation potential both through its effects on community structure (in terms of both the taxonomic and functional groups present) and as a result of sub-lethal effects which reduce the rate of bioturbation. In addition, interspecific differences in bioturbation potential were identified with the absence of particular species from certain communities providing an explanation for inter-community differences in bioturbation potential. In summary, this study has led to the following conclusions:

- Laboratory studies showed *M. balthica* and *H. diversicolor* to be important species in terms of bioturbation with the relative importance of *H. diversicolor* increasing with increasing effluent concentration (within the range of concentrations used in the present study) due to the lower sensitivity of this species to the effluent. Due to its abundance, *H. diversicolor* is considered to be the most important bioturbator at the Paull and Saltend sites, with the exception of those sites within the immediate vicinity of the discharge which were dominated by oligochaetes. At Skeffling, the activity of *M. balthica*, together with other burrowing bivalves which, whilst not recorded in great abundances during core sampling (due to their burying depth), are known to be present in considerable numbers, is considered to be of greatest importance. The rapid bioturbation rate, in comparison to all other species, of *S. plana* was clearly demonstrated under laboratory conditions.

- The removal of such non-tolerant species from the community as a result of pollution resulted in a reduction in bioturbation potential in terms of depth of activity. Therefore, as demonstrated by the use of tracers and resin burrow casts, bioturbation potential was found to be lowest at the most polluted, S25 m site and highest at Skeffling where pollution-induced stress is absent and the stress associated with low and fluctuating salinity is reduced. However, the rate of bioturbation by organisms in polluted communities (i.e. large numbers of oligochaetes) needs to be determined.

- In general, the reduction in stress, either as a result of a reduction in pollution or salinity stress due to location within the estuary, resulted in an increase in species diversity. Coupled with this was an increase in the diversity of feeding strategies, moving from a community dominated by non-selective, sub-surface deposit feeders to one composed of selective and non-selective surface and subsurface feeding detritivores, omnivores and carnivores. Since feeding and bioturbation are closely related, this gives rise to an increase in diversity from a community containing biodiffusers only (S25 m) through

those being dominated by regenerators (S200 m and P150 m) to those containing all groups (Skeffling). This change in diversity of feeding and bioturbation guilds, together with animal size, explains the difference in the degree of bioturbation between sites. These results demonstrate that community structure is of greater importance to bioturbation than animal abundance. Whilst there is no direct evidence from the present study, it is possible that the increase in both individual and community biomass with increasing distance from the discharge (Chapter 4) may also explain the increase in bioturbation.

- Despite their associated problems, the techniques used to quantify bioturbation in the present study have given valuable results. The fact that some of them gave contrasting results (e.g. the tracer and resin cast studies at Skeffling) highlights the fact that a combination of several techniques is required in order to understand fully the bioturbation processes in any one area.

CHAPTER 6

INTRODUCTION TO EROSION AND SEDIMENT DYNAMICS

6.1. INTRODUCTION

The general characteristics of estuarine sediments are described in Chapter 2. Sediment characteristics are largely dependent on the hydrodynamic conditions of an area and muddy sediments commonly occur in areas where both currents and waves exert only weak forces on the bed (Elliott *et al.*, 1998; Whitehouse *et al.*, 2000). Such conditions tend to occur in areas with a medium-large tidal range (>3 m) which are sheltered from wind driven waves (Pethick, 1984). Weak hydrodynamic forces allow the settlement of fine particles and intertidal mudflats are predominantly composed of clay and silt particles and, to a lesser extent, fine sands (Elliott *et al.*, 1998). Deposits of fine material tend to have low permeability and therefore, the upper part of the bed is usually very soft, with a high water content (sometimes >100%), and in a state of partial consolidation (Parchure & Mehta, 1985).

Very fine particles may be kept in suspension by Brownian motion until flocculation and deposition occur as a result of electrostatic attraction (Whitehouse *et al.*, 2000). Consolidation of a deposit involves the gradual expulsion of water by the weight of the overlying sediment, together with an increase in both density and strength of the bed (Whitehouse *et al.*, 2000). Water content decreases with increasing depth into the sediment causing stratification within the bed (Mehta, 1989). Therefore, the sediment profile includes (1) boundary layer (Section 6.3.1) material suspended in the water column, (2) a mobile fluid mud layer from which sediment may easily be resuspended, (3) a stationary mud layer and (4) a fully consolidated layer. The upper, mobile fluid mud layers of the bed are separated from the fully suspended material by the lutocline although in cohesive sediments, the distinction between the two layers is not as clear as in non-cohesive sediments. Shear strength (resistance to the frictional forces exerted by moving water) increases with depth and therefore, erosion potential is reduced. Figure 6.1 shows these layers together with velocity and concentration gradients above the bed. The processes of sediment deposition, consolidation and erosion are only partly understood and as yet, it has not been possible to predict the behaviour of cohesive sediments from their physical and chemical properties.

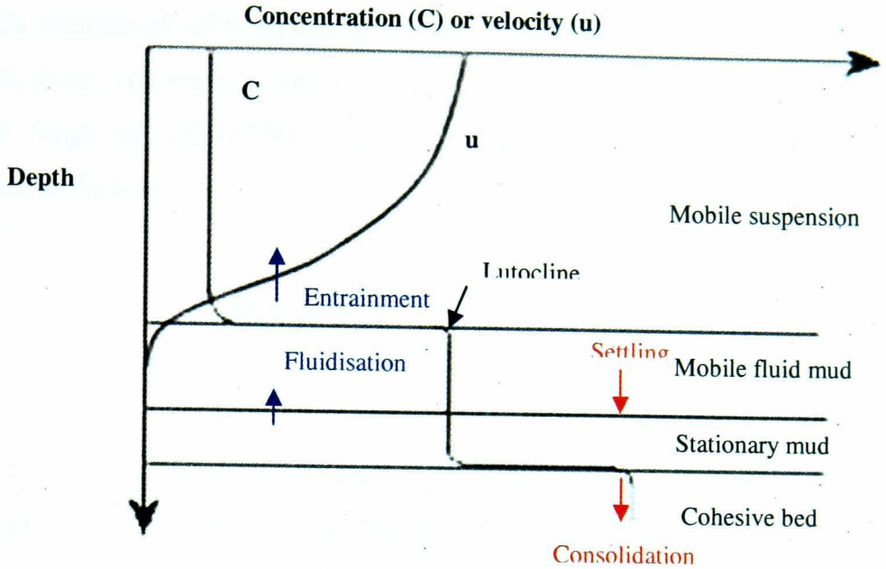


Figure 6.1. Velocity and suspended sediment concentration gradients above the bed (Mehta, 1989). The lutocline differentiates the mobile, well mixed suspension layer from the high concentration, mobile fluid mud layer.

6.1.1. Erosion processes

Water flow over the bed may be laminar, turbulent or transitional. In laminar flow, water molecules move in straight, parallel lines and do not mix (Open University, 2002). Laminar flow occurs in very slow moving, shallow water and can generally be regarded as a laboratory artefact since its occurrence in natural systems is so rare (Paterson & Black, 1999). In contrast, turbulent flow is chaotic and irregular with water molecules moving in all directions (including backwards) causing mixing of the flow layers, the term 'turbulence' referring to the high variation in flow velocity and direction around a mean value (Open University, 2002; Massey, 1989, in Paterson & Black, 1999). The transition between laminar and turbulent flow can be sudden and is influenced by various factors. As the velocity or density of the water increases or as viscosity decreases, the transition to turbulent flow is promoted (Paterson & Black, 1999). Turbulence plays a key role in sediment transport since it tends to make sediment particles easier to transport and keeps them moving for longer. The advent of turbulent flow can be seen as the dominance of inertial forces (the resistance to change in motion) over the more sedate viscous forces (viscosity is a measure of resistance to flow, i.e. how easily deformed the fluid is). Inertial forces describe the friction within a substance and keep water flowing if it is misdirected from the main flow direction. Viscous forces suppress turbulence by damping down the variations in motion through friction (Open University, 2002). The Reynolds number (*Re*) is the ratio of inertial : viscous forces acting on the bed

and is a fundamental descriptive character of the flow. *Re* can be calculated in order to predict the extent of turbulence from experimental data and is dependent on the speed and geometry (width and depth) of the flow and the fluid's density and viscosity. In general, a Reynolds number of <500 indicates laminar flow whilst values above 2000 are indicative of turbulent flow. However, Massey (1989, in Paterson & Black, 1999) suggested the more cautious range of 2000-4000 to indicate turbulent flow. The Reynolds number can be calculated as follows:

$$\mathbf{Re = uD / \nu} \qquad (1)$$

where:

u	=	mean velocity (m s^{-1})
D	=	depth (m)
ν	=	ρ/η (kinematic viscosity)
ρ	=	water density (kg m^{-3})
η	=	dynamic or molecular viscosity (assumed to be 0.001) (Dyer, 1997)

Erosion is the process of sediment removal from the surface of the bed as a result of water movement above it and transport is the movement of suspended sediment and high concentration layers on or near the bed by flow (Whitehouse *et al.*, 2000). Frictional forces exerted by the surface over which water is flowing causes retardation of the near bed flow in comparison with that in layers above the bed, resulting in a profile of increasing velocity with increasing height above the bed (Open University, 2002). This results in a shear stress which is a measure of the force exerted on a particle resulting from the difference in the speed of the flow above and below it (Pethick, 1984; Dyer, 1986; Hall, 1994; Open University, 2002), which acts upon the bed and, depending on its magnitude, leads to erosion and sediment transport (Hall, 1994). The region in which frictional forces with the bed cause this retardation of the flow is termed the boundary layer (Pethick, 1984; Dyer, 1986; Hall, 1994; Paterson & Black, 1999), the characteristics of which are described in greater detail in section 6.3.1. The characteristics of the flow within the boundary layer determine the amount of bed shear stress with the highest levels of stress being associated with the steepest velocity gradients (Pethick, 1994). In thick boundary layers, the difference in velocity between layers in the flow (i.e. between the top and bottom of a particle) is small and therefore bed shear

stress is low. In thin boundary layers, these differences in velocity are much greater, bed shear stress is greater and therefore, sediment transport is greater (Paterson & Black, 1999).

Two types of fluid force act upon particles in a flow - those due to drag (acting mainly on the upper, exposed surface of the particle) which act parallel to the bed and those due to lift, as a result of unequal pressure distribution around the grain, acting normal to the bed (Middleton & Southard, 1984; Soulsby, 1997). Drag has two components. *Skin drag* (or skin friction) arises from shear (change in velocity with height or depth causing friction between layers in a flow or between the flow and the surface over which it is flowing) between the fluid and the particles surface and the frictional force acts directly upon the surface of the grains. According to Paterson & Black (1999), skin friction is of greatest importance where the surface of the bed is smooth (e.g., the surface of a mudflat) and can be the dominant force responsible for the erosion of muddy sediments. *Pressure or form* drag arises from pressure differences caused by the diversion of flow around the particle and results in movement of the particle parallel to the bed. Form drag occurs when the bed is uneven, for example, when ripples or worm casts are present (Soulsby, 1997). The force of lift becomes increasingly important with increasing particle size (Soulsby, 1997). High velocity at the top of a particle (in relation to that at the bottom) creates lower pressure and thus results in lift when the pressure difference is great enough to overcome the effects of gravity. This is known as the Bernoulli effect (Middleton & Southard, 1994). Soulsby (1997) also described how acceleration occurring under oscillatory waves could cause increased shear stress and lead to particle entrainment. However, according to the Open University (2002), because of the large tidal range in some estuaries and the low gradient of the mudflats, each particular area is exposed to the breaking waves for only a short period of time. Therefore, sediment transport by the flood and ebb currents dominates over that by wave action.

The movement of sediment particles is initiated when the combined forces of lift and drag produced by the fluid become large enough to overcome the force of gravity and the frictional, cohesive and adhesive forces holding the particles in place (Middleton & Southard, 1984; Hall, 1994). The velocity or shear stress at which particles begin to move are termed the critical shear velocity (u_{*crit}) and the critical shear stress (τ_{0crit}), respectively (Dyer, 1986; Open University, 2002; Hall, 1994). Four modes of sediment transport in water have been described (Middleton & Southard, 1984; Open University, 2002). *Sliding or rolling* particles remain in continuous contact with the bed whereas particles transported by *saltation* are forced to jump along the bed. Sediment particles transported by these processes are termed the bedload. Particles transported in suspension (suspended particulate matter (*SPM*)) are supported by turbulence and travel at the same velocity as the water (Hall, 1994). They rarely

come into contact with the bed unless the flow slackens. In general, fast flowing water causes the transport of sediment particles by saltation and as suspended particulate matter (Open University, 2002). Sternberg (1972, in Nowell *et al.*, 1981) suggested that the ratio of settling velocity : entrainment velocity is commonly used to indicate that particles with diameters of less than 100μm move as suspended load.

6.1.2. Factors influencing the erodibility of sediments

Sediment transport processes are better understood for non-cohesive sediments (sands) than for cohesive muds and clays (Soulsby, 1997). This is because the grains of cohesive sediments behave in a collective rather than an individual manner so that the number of variables influencing the nature of the erosion process is increased. In sandy sediments, particle size is the major influence over sediment mobility and transport, whereas in muds, sediment mobility is determined by its bulk properties (Whitehouse *et al.*, 2000). These include factors such as porosity / water content and bulk density together with pH, Eh, changes in the electrostatic field surrounding clay minerals, ionic composition, organic content, temperature (and hence, viscosity of the eroding fluid), particle size and rheology (the stress and strain in a sediment-water mixture under the influence of some external loading (shear) (Paterson & Black, 1999; Whitehouse *et al.*, 2000). In addition are the chemical and biological processes acting upon sediments (both cohesive and non-cohesive) which are discussed in Chapter 7.

Sediment chemistry appears to be the least well understood factor involved in sediment stability. For example, Montague (1984) stated that the erodibility of sediments generally increases with increasing pH, possibly due to changes in the electrostatic properties of the sediment particles, and Liou (1970, in Montague, 1984) found that the critical shear stress required to erode sediments at pH 5.6 was 14 times higher than that required to erode the same sediments at pH 8. Photosynthetic reactions, in areas of dense algal populations, are known to increase pH (perhaps to values as high as pH 10 (Montague, 1984) during periods of low tide, thus potentially increasing erosion potential. On the other hand, Montague (1984) stated that high rates of respiration at high tide or at night, may result in pH values becoming as low as 5.5 which could potentially reduce erosion potential. Whilst pH is buffered in saline waters, erosion potential might be expected to change diurnally and seasonally and also spatially.

Redox potential and electro-chemical changes are central to the understanding of sediment transport although little is known of how changes in Eh affect sediment stability (Montague, 1984). According to this author, this lack of knowledge is due to the difficulties involved

with establishing a redox gradient in the laboratory. Salinity also affects sediment erodibility although this is thought to be unimportant at salinities of above 10 psu (Parchure & Mehta, 1985). Metabolism within sediments often produces gas bubbles (e.g., carbon dioxide, ammonia, methane, hydrogen sulphide and hydrogen) (Montague, 1984). These are generally dissolved but where they do occur, they could potentially decrease sediment stability by forcing individual particles apart. These gas vacuoles are particularly prevalent in fine, organically enriched sediments.

Position on the shore is also an important determinant of sediment stability since sediments with high water content are generally more easily eroded than sediments with low water content. Sediments on the high shore, which may only be inundated at high water during spring tides, will have a longer period to dry out and consolidate than sediments on the low shore which are only exposed during spring tides (Parchure & Mehta, 1985). They are also exposed to lower shear stress, for shorter periods of time and less frequently than sediments on the low shore, and are therefore less likely to erode.

Considering the effects of the physico-chemical and biological properties of sediment on erosion, two main types of erosion have been identified (Mehta, 1991, in Paterson & Black, 1999). Surface (Type I) erosion occurs when particles are eroded from the surface as flocs and appears to be the dominant mode of erosion under natural conditions (Amos *et al.*, 1998). Erosion peaks rapidly then decreases over time as the fine material is removed from the surface, exposing the more consolidated sediments underneath. Type II (bulk) erosion occurs when the stresses on the bed greatly exceed the erosion threshold and larger particles or larger clods are removed. A combination of these two processes may occur where erosion begins as Type I erosion and as shear stress increases, Type II erosion dominates so that erosion rate continues to increase.

6.2. AIMS.

It has long been recognised that a variety of biological processes are dependent upon near bed water movement and sediment dynamics (Nowell & Jumars, 1987). These processes include the distribution of larvae and food particles, the ability of an organism to settle and maintain a burrow or tube within the sediment and the ability to tolerate conditions of high flow or high turbidity (Muschenheim *et al.*, 1986). More recently, the importance of the influence of benthic communities and the activity of the organisms they contain on sediment properties has been recognised (e.g., Rhoads, 1974; Jumars & Nowell, 1984a; 1984b; Hall, 1994; Snelgrove & Butman, 1994; Paterson & Black, 1999). The study of sediment transport is therefore of ecological relevance and various devices have been developed to measure erosion both in the laboratory and the field. Erosion measurement generally involves the use of some sort of flume where water is transported, through a channel, over the sediment surface although, as described in section 6.3.2., various other devices have also been developed.

The present chapter gives an explanation of the boundary layer processes which influence sediment transport together with a brief review of some of the devices which have been used to quantify erosion. Based on the theory regarding fluid dynamics and erosion processes, the design, construction and calibration of a straight channel laboratory flume are described and an evaluation of its suitability for making erosion measurements carried out. The aim has then been to use this flume to study differences in the erosion characteristics of sediments taken from areas exposed to different levels of pollution and containing different infaunal communities (Chapter 7).

6.3. INTRODUCTION TO FLUID DYNAMICS AND EROSION MEASUREMENT

6.3.1. The benthic boundary layer

As water flows over the sea bed a frictional force is exerted upon the water such that the flow has an average velocity in the middle but friction with immobile surfaces (i.e. the bed and air at the surface) slows down the flow right at these surfaces. Therefore, the flow at the sediment – water interface is zero and the effect of the friction extends into the water column, gradually reducing with height above the bed so that water in the upper layers flows more quickly than that close to the sea bed (Dyer, 1986; Open University, 2002; Hall, 1994; Snelgrove & Butman, 1994; Paterson & Black, 1999). This region of deviation from free stream velocity is known as the benthic boundary layer, the depth and form of which are dependant on factors such as bed roughness, current velocity and turbulence. In turbulent flow, mixing is high with high speed water being mixed with low speed water and the boundary layer therefore tends to be thin. In laminar flow, there is little mixing and the boundary layer therefore tends to be thicker. With regard to surface roughness, a very smooth surface such as mud does not deflect the flow to a great extent so mixing does not occur to a high degree. Therefore, the boundary layer is comparatively thick. In contrast, rough surfaces deflect the flow to a greater degree and increase mixing, resulting in a thinner boundary layer. In deep sea areas, the boundary layer may occupy 1 - 10m of the water column whereas in shallower areas (such as intertidal areas) it may occupy the whole depth (Open University, 2002). Sheng (1986, in Wymer, 1997) suggested that the boundary layer associated with the slowly varying tidal currents of estuaries can be of the order of 1m thick. According to Seitze (1976, in Nichols, 1984) boundary layer flow is one of the least understood geophysical scale flows, largely because there are so few observational data.

The boundary layer consists of two regions, depending on the type of flow (Figure 6.2). Under conditions of smooth turbulent flow (or hydraulically smooth flow), a viscous sub-layer exists, extending a few millimetres into the water column from the bed (Open University, 2002; Hall, 1994). Due to frictional forces, the thin layer of water which is in contact with the bed will be stationary (Open University, 2002). Above this, the velocity of each layer of water will increase with height above the bed. In the viscous sub layer, this velocity increase is linear and the flow is termed hydraulically smooth (Muschenheim *et al.*, 1986). The presence of this layer is dependent upon grain size current velocity and viscosity and the smooth turbulent conditions are generally broken down if the grain size exceeds one third of the depth of the viscous sub-layer. The flow is then termed 'hydraulically rough' (or rough turbulent flow) and the viscous sub-layer no longer exists (Muschenheim *et al.*, 1986). Above the viscous sub-layer (or down to the bed in conditions rough turbulent flow), velocity increases logarithmically to reach free stream velocity above the boundary layer. The terms

hydraulically rough and smooth refer to the roughness or relief of the surface over which the water is flowing and the extent to which the surface interferes with the flow (Paterson & Black, 1999).

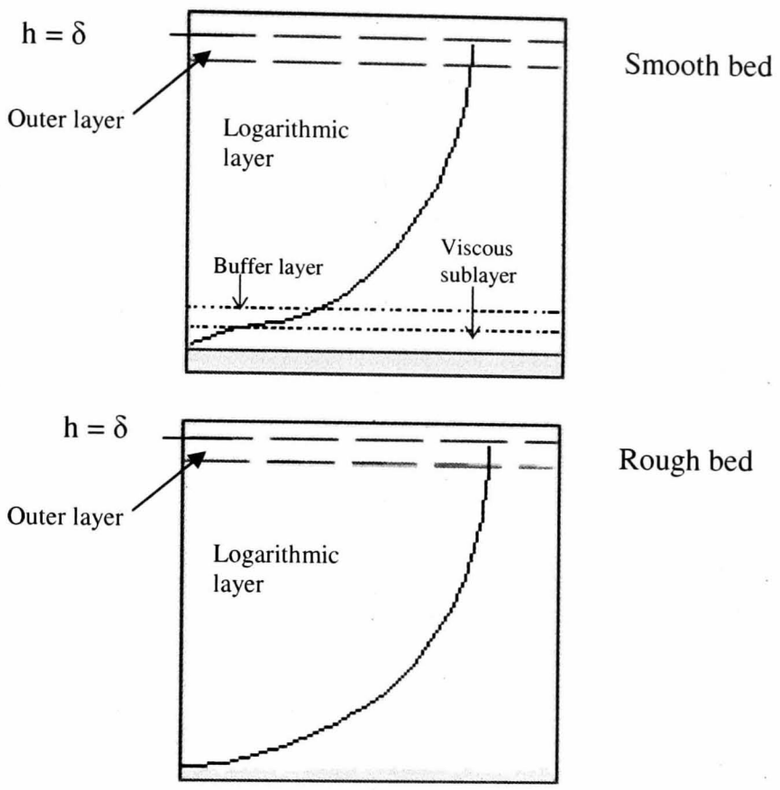


Figure 6.2. Velocity profiles for (a) smooth turbulent flow and (b) rough turbulent flow. (Redrawn from Hall, 1994). δ denotes the height of the boundary layer.

As velocity increases with height above the bed, a shearing force is produced as upper layers of water move over those directly below them. This force, arising from momentum transfer between layers in the flow creates a tangential drag force which acts on the bed, creating a shear stress (τ_0, in N m^{-2}) (Hall, 1994). The force or stress acting upon the bed as a result of flow is related to the rate of change in velocity above the bed within the boundary layer, the greatest stress being associated with the steepest velocity gradients (Paterson & Black, 1999). In order to cause sediment transport, this force must overcome the effects of gravity and the frictional and cohesive / adhesive forces keeping the particles on the bed. Shear stress is greatest at the sediment - water interface since this is the region of greatest friction and therefore, the viscous sub-layer (if it exists) is an important area for sediment transport and the transport of organic matter to and from the benthos (Muschenheim, 1987a; 1987b, in Snelgrove & Butman, 1994; Paterson & Black, 1999). The shear stress at the bed is termed τ_0

and is a function of water density (ρ) in kg m^{-3} and the friction or shear velocity (u_*) in m s^{-1}, as demonstrated by equation 2.

$$\tau_0 = \rho(u_*^2) \quad (2)$$

(Nowell & Jumars, 1987; Open University, 2002)

However, bed shear stress (τ_0) cannot be directly measured and so has to be derived from other variables (Paterson & Black, 1999). In addition to turbulence velocity, the velocity gradient close to the bed also depends upon the mixing length (κz). Shear stress at the bed is related to the rate of change in velocity over the whole profile. Since τ is directly related to u and $\tau \propto \delta u / \delta z$, the following equation applies.

$$\delta \bar{u} / \delta z = u_* / \kappa z \quad (3)$$

where:

$\bar{u}$ = mean velocity

z = height above the bed

κ = von Karmanns' constant ($\cong$ 0.4 Wimbush (1976); Nowell *et al.*, (1981)).

κz represents the eddy size contributing to the momentum transfer at height z (Nowell & Jumars, 1987; Hall, 1994)

Integration of equation 3 gives the von Karmann-Prandtl 'law of the wall', from which the shear velocity and therefore, bed shear stress can be calculated (Jumars & Nowell, 1984a; Muschenheim *et al.*, 1986; Nowell & Jumars, 1987; Hall, 1994).

$$u_{(z)} = u_*/\kappa \ln(z/z_0) \quad (4)$$

where:

$u_{(z)}$ = mean velocity at height z above the bed

u_* = friction or shear velocity

κ = von Karmanns' constant (0.4)

z_o = roughness length (height above the bed where the extrapolated current velocity becomes zero)

This equation describes the velocity profile above the bed (i.e. the region close to 'the wall') where the momentum is transferred by turbulent stresses (Nowell & Jumars, 1987). According to Hall (1994), this provides the simplest way of determining friction or shear velocity and, therefore, bed shear stress. If mean velocity at height z (i.e. $\bar{u}_{(z)}$) is plotted against ln z (Figure 6.3), a straight line will be obtained, the intercept being equal to the roughness length (z_o) and the slope (dy/dx) being equal to κ/u_*. Rearranging this equation gives:

$$u_* = \kappa/(dy/dx) \qquad \text{Or} \qquad u_* = \kappa(dx/dy) \qquad (5)$$

such that τ_0 can be calculated from equation 2. Figure 6.3 and equation 4 show that when $z = z_0$, $u = 0$, indicating that z_0 is the height above the bed at which flow becomes zero. According to the laws of logarithms, ln ab = ln a + ln b and ln a/b = ln a - ln b (Bostock & Chandler, 1990). Therefore, equation 4 can be rearranged to give:

$$u_{(z)} = u_*/\kappa \, (\ln z - \ln z_0) \qquad (6)$$

Experimental studies have demonstrated that, under conditions of smooth turbulent flow, z_0 is proportional to the thickness of the viscous sub-layer (Hall, 1994). In the more usual conditions of rough turbulent flow, z_0 has been shown to be a function of bed roughness (k_s). If the bed is smooth, ks is termed Nirkurdase roughness and is a function of grain size (**D**) so that $k_s = 2.5D_{50}$ and $z_0 = k_s / 30$. When the bed is rippled, k_s is a function of ripple steepness and is termed equivalent Nirkurdase roughness (Hall, 1994).

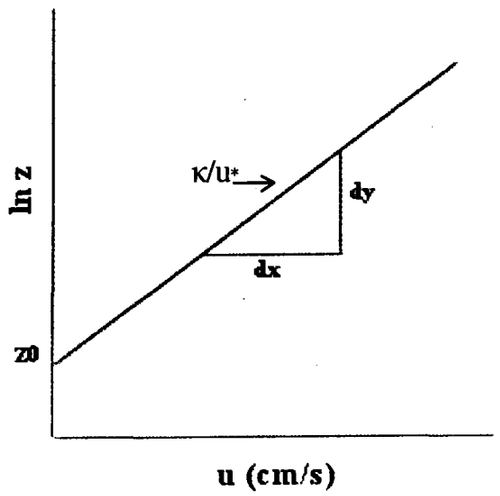


Figure 6.3. The von Karmann-Prandtl velocity profile (Dyer, 1986).

Nowell & Jumars (1987) stated that equations 4 and 5 only apply at heights above the bed sufficient for molecular viscosity not to be the main mechanism responsible for momentum transfer. Therefore, in the viscous sub-layer, the velocity profile is as follows:

$$U / u_* = z u_* / \nu$$

or,

$$u_z = z u_*^2 / \nu \qquad (7)$$

(Muschenheim *et al.*, 1986; Nowell & Jumars, 1987)

where:

ν = Kinematic viscosity ($\nu = \mu / \rho$ where μ is the dynamic viscosity)

6.3.2. Methods of quantifying sediment transport.

Various techniques are available for studying sediment transport both in the laboratory and the field. The majority of these involve the use of some sort of flume where water is pumped through a channel, producing a fluid flow over the sediment water-interface of sufficient strength to induce sediment erosion. Erosion is determined as changes in turbidity over time or by determination of the critical shear stress (τ_{crit}) required to induce erosion (Black & Paterson, 1997). Flumes have been extensively used in sedimentary geology and civil engineering studies (Nowell & Jumars, 1987). However, whilst water flow is of importance to sediment transport rates, it also strongly influences the functioning of benthic communities (e.g. in terms of habitat characterisation and food distribution) (Muschenheim *et al.*, 1986; Nowell & Jumars, 1987). In addition, as will be described in greater detail in Chapter 7, benthic organisms can also influence the sediment properties, boundary layer flow and hence, erosion characteristics of the sediment. According to Muschenheim *et al.* (1986), flumes provide an essential tool for the study of boundary layer processes. A good review of in-situ techniques is given by Black & Paterson (1997). These include various flow-through flumes which can be used both sub and intertidally (e.g., Scoffin, 1967; Young & Southard, 1978, in Black & Paterson, 1997). The sediment eroded in these flow-through flumes is transported and lost out of the end and Cowgill (1994, in Black & Paterson, 1997) used differences in the turbidity of the inflowing and outflowing water as a measure of erosion rate.

Recirculating flumes, from which the sediment and water is not lost, may be annular or of a form with a header tank and overflow tank connected by a straight channel through which water is pumped. Recirculating, annular flumes are continuous parallel walled circular channels and are the most commonly used flumes for field studies. Widdows *et al.* (1998a; 1998b; 1998c; 2000) used such a flume to measure erosion rates, on the Humber estuary, in

relation to macrobenthic communities, current velocity and position on the shore. Critical erosion velocities were found to be lowest in pools and gullies, where the water content was highest, and with increasing numbers of deposit feeding bivalves. Flow within this flume was induced using a rotating plate on the surface of the water. However, due to the circular nature of the flume, secondary fluid motions (i.e., perpendicular to the direction on the primary flow), resulting in both horizontal and vertical velocity profiles, may be a problem (Sheng, 1989, in Black & Paterson, 1997; L. Frostick, University of Hull. Pers. comm.). Another criticism is that, since the current velocity is higher at the inner wall, bed shear stress is also higher here and consequently, erosion rates are not uniform across the bed. However, Black & Paterson (1997) stated that the constant channel geometry and the infinite flow length of the flume allow the full development of the boundary layer.

Because sediment is not lost from a recirculating flume, the water becomes progressively more turbid as erosion proceeds. According to Best and Leeder (1993) concentrations of suspended sediment as low as 2 g l^{-1} result in lower current velocities at the bed and therefore erosion rates may be lower than expected. Hence, this factor should be considered when using recirculating flumes. Black & Paterson (1997), however, stated that this is likely to bear more relation to natural erosion processes than the conditions in a flow through flume.

An alternative method of assessing erosion potential is to use a vertical jet system. Various types exist, e.g. the cohesive strength meter (CSM) which was used by Paterson (1989). Pulses of water are directed at the sediment surface, at different forces which are determined by the height above the bed. Sediment resuspension is determined by measuring turbidity changes which are monitored by changes in light transmission through the test chamber. Variations on this technique include the in-situ simple shear test (INSIST, e.g., Christian & Daborn, 1990; Faas et al., 1992, in Black & Paterson, 1997) and the instrument for shear stress in-situ (ISIS. Williamson & Ockenden, 1996).

Field measurements have the advantage that the sediment can be studied in its natural state. However, laboratory flumes are good for experimentally manipulating and maintaining uniform test conditions and have the advantage of allowing measurement of bedload, as well as suspended load, transport. Muschenheim *et al.* (1986) designed a straight channel flow through flume (Dalhousie flume) which was used for studying the effects of various organisms (faunal and algal) on erosion and deposition processes. Grant & Daborn (1994) used a similar flume (although in this case, the sediment sample could be lowered and raised to be flush with the bed) for studies involving *Corophium volutator*. Other recirculating flumes which have been used for laboratory studies include the straight channel flume used by

Nowell *et al.* (1981) and the annular flume used by Davis (1993). The design of a flume is dependent on its purpose and is usually a compromise between what is ideally required and what is feasible (in terms of cost, labour and operation). Black & Paterson (1997) indicated that each type of flume is designed for specific purposes and has its own merits and drawbacks in relation to its particular use and no one flume is free from disadvantages and problems.

6.3.3. Theoretical considerations in the design of flumes.

a) General flume characteristics and layout

A recirculating laboratory flume (Figure 6.4) generally consists of a header tank and an overflow tank, joined by a channel through which the water flows and erosion measurements are made. The water is pumped round the system and enters the channel, through a screen or baffle which diffuses the flow. The water then passes through the channel and exits, generally over a weir, into an overflow tank and is pumped back to the header tank before re-entering the channel. Sediment samples can be placed in the bottom of the channel at a suitable distance from the entrance to ensure boundary layer development and reproducible flow conditions.

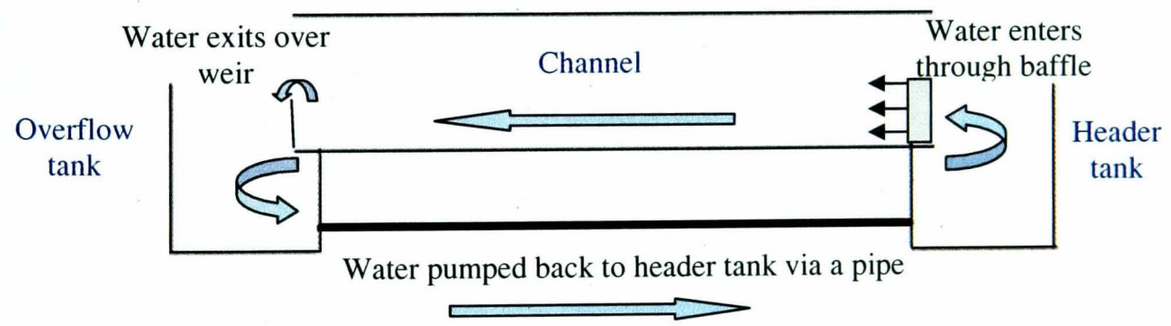


Figure 6.4. Generalised characteristics of a linear recirculating laboratory flume

b) Entrance conditions.

The general design and features of a linear recirculating flume are given in Figure 6.4. According to Muschenheim *et al.* (1986) and Nowell & Jumars (1987), the entrance conditions in a flume are important to the development of smooth flow and smooth, reproducible flow is desirable for carrying out controlled experimental erosion measurements. In a recirculating flume, the water has generally just exited a pipe, possibly with several bends, and the flow entering the channel is therefore rather turbulent. In addition to this, exit

from the pipe causes a jet and the flow must diverge as it enters the flume. According to these authors, the effects of this may take several pipe diameters to decay and as a general rule, a length of channel 20 times the width of the pipe is required to overcome this problem.

Turbulence can be controlled by forcing the flow through some sort of screen or diffuser which breaks the flow into smaller scales, spreading it across the whole width of the channel (Williams, 1971; Laws & Livsey, 1978; Muschenheim *et al.*, 1986; Nowell & Jumars, 1987). The flow then enters the flume with uniform velocity and distribution (vertically and laterally) although pressure changes occur as the water passes through the screen, the effects of which are dependant on the screen density. For example, a long, very dense screen will cause the propagation of waves downstream (Nowell & Jumars, 1987). Therefore, these authors recommend that the length of the tubes comprising the screen should be at least 20 times greater than the diameter in order to apply a sufficiently strong strain on the fluid to reduce the incoming turbulence, without causing major changes in pressure.

Muschenheim *et al.* (1986) used nylon furnace baffling together with industrial lighting diffuser to straighten the flow. Other useful materials include the ceramic honeycomb grids from catalytic converters. Nowell & Jumars (1987) pointed out that such a screen would help to improve an already well cut flow but cannot generate a smooth boundary layer velocity profile from exceptionally turbulent flow. Following exit from the screen, the flow then requires a sufficient length of flume (dependant on the channel roughness and the velocity of the water) to adjust to the conditions (Williams, 1971). According to Williams (1971), the thickness of the boundary layer increases with distance downstream. Its development may be determined by velocity profile measurements and by using equation 8:

$$\delta / x = \frac{0.38}{(V_0 x / \nu)^{0.2}} \qquad (8)$$

Albertson *et al.*, (1960, in Williams, 1971).

Where:

δ = thickness of the turbulent boundary layer (m)

x = distance from start of channel (m)

V_0 = free stream velocity (m s^{-1})

ν = Kinematic viscosity (1.08×10^{-5} ft^2 / sec at 20°C. Chow, 1959)

($\approx 3.56 \times 10^{-6}$ m s^{-2} in freshwater)

If the above formula is rearranged, the distance over which the boundary layer would become fully developed can be calculated. Distances for various velocities, at a water depth of 10 cm are given in Table 6.1.

$$\frac{\delta^5 V_0 x}{x^5 \nu} = (0.38)^5$$

$$x / x^5 = \frac{(0.38)^5 \nu}{\delta^5 V_0}$$

$$x^{(1-5)} = \frac{(0.38)^5 \nu}{\delta^5 V_0}$$

$$x^{(-4)} = \frac{(0.38)^5 \nu}{\delta^5 V_0}$$

$$(x^{-4})^{-1} = ((0.38)^5 \nu) / (\delta^5 V_0))^{-1}$$

$$\therefore \quad x^4 = \frac{\delta^5 V_0}{(0.38)^5 \nu}$$

and

$$x = \sqrt[4]{\frac{\delta^5 V_0}{(0.38)^5 \nu}}$$

Table 6.1. Boundary layer formation at different velocities.

Velocity (ms^{-1})	x (m) when δ = 0.1m	x (m) when δ = 0.05m
0.01	1.37	0.578
0.05	2.05	0.87
0.1	2.44	1.027
0.15	2.72	1.137
0.2	2.91	1.211
0.25	3.07	1.29
0.3	3.21	1.35

c) Exit conditions.

The exit conditions, together with the channel slope and the flow rate, determine the depth of the flow (Muschenheim *et al.*, 1986). When water is allowed to flow freely over an edge, acceleration of the flow occurs as the overflow is approached (Nowell & Jumars, 1987) and the depth of the water is reduced. Chow (1959), Williams (1971), Muschenheim *et al.* (1986) and Nowell & Jumars (1987) all suggested placing tailgates or a weir at the end of the channel to maintain the water depth. However, the presence of a weir causes a sudden deepening of

the water and the flow may be diverged upwards from the bed (Henderson, 1966, in Nowell & Jumars, 1987) and the effects of this are noticeable for some distance upstream. The test section must be clear of this and it is recommended that erosion measurements are taken at least five weir depths upstream of the weir to avoid backup effects (Steeter & Wyllie, 1979, in Muschenheim *et al.*, 1986). Nowell & Jumars (1987) suggested that adjustable louvered gates provided a good compromise between the backup effects of a weir and the effects of freely flowing water.

d) Flume geometry.

The width and smoothness of the channel are important since the flume walls affect the flow, as well as the bed (Muschenheim *et al.*, 1986). According to Jumars & Nowell (1984b), measurements must be made far enough from the side walls for the bottom boundary layer to fully dominate the flow and in order to achieve this, a width : depth ratio of between 3 and 10 is required (Muschenheim *et al.*, 1986). Nowell & Jumars (1987) state that this ratio should be greater than 7 with 5 generally being accepted as an absolute minimum.

6.4. FLUME DESIGN AND CALIBRATION

6.4.1. Construction

The purpose of the flume was to provide a means of measuring sediment erosion under controlled conditions which are uniform to each sample. The design was based on a modification of the Dalhousie flume (Muschenheim *et al.*, 1986) and is shown in Figures 6.5 a and b and Plates 6.1 - 6.2. The flume consisted of two 330 L tanks (head tank and overflow tank) joined by a 3.5 m aluminium channel, 0.35 m wide and 0.3 m deep. Upon initial filling, the water depth was 0.45 m in the head tank, 0.2 m in the overflow tank and 6 cm in the channel. Two 25 L sand filled containers were placed in the head tank to reduce the volume of water required to fill the flume which gave a total volume of approximately 340 L of water circulating in the flume. Water was pumped round the system using two 750W central heating pumps (P1 and P2), each with a capacity of 210 gallons / minute, and a rubber air pipe (internal diameter 25 mm). According to Nowell & Jumars (1987) with this pipe diameter, a 3.5m channel should more than adequate to counteract the effects of a jet produced following flow through the pipe. The water will also have passed through the tank before entering the channel which will further dampen the effects of the pipe. Two 0.15 m long baffles, consisting of boxes (35x10cm) containing 5 mm internal diameter plastic tubes (drinking straws), were placed one behind the other at the entrance in order to reduce turbulence and to reduce the distance required for boundary layer development.

For the purpose of this study, the slope of the channel was fixed at 3° which was sufficient to maintain the depth and speed of flow in the test section without significantly reducing the depth at the head of the flume. The flow velocity and depth was controlled by two valves together with a system of weirs (one vane weir and one slotted weir with adjustable height positioned at the channel exit at 3.32 m and 3.45 m, respectively). The vane weir, as suggested by Nowell & Jumars (1987), consisted of three steel plates, the angles of which could be adjusted to slightly restrict the passage of water without resulting in large scale changes to the characteristics of the flow. The second weir consisted of a series of 3 cm high plastic slats which could either be added or removed to adjust the speed of the flow. This weir prevented the sudden acceleration and shallowing of the water as it exited the channel. Using the two weirs in combination allowed a more gradual change to the flow characteristics at the channel exit.

A circular sample box (0.22 m diameter, giving a total sample area of 0.038 m^{-2}), to accept undisturbed sediment cores from the field, was fitted between 2.15 m and 2.37 m along the length of the flume. This distance was determined following characterisation of the flow, as described in section 6.4.2.2 and according to the recommendation by Nowell & Jumars (1987) that the test section be placed at least five weir depths upstream of the weir. Assuming a maximum weir (and hence flow) depth of 9 cm, it was theoretically necessary to place the test section at least 0.45 m upstream of the weir. The test section was placed in the centre of the channel, 6 cm from the channel edge at its diameter. The sample box had a false bottom which could be moved up and down so that the top of the sediment core was always level with the bed of the flume. 0.2 m downstream of this (at 2.57 m), a 0.2 m diameter funnel was attached to the bottom of the flume so that sediment transported as bedload could be retained. It is accepted that this may have interfered with the flow although, given that the presence of the weir can diverge the flow away from the bed, it was considered more important to keep the funnel clear of the weir in order to ensure collection of material transported as bedload. A ramp was placed at the exit to prevent excessive turbulence which would have increased air intake by the pump and resulted in unsteady flow.

Williams (1971) found that the continued exposure to water causes the formation of aluminium hydroxide on the walls of aluminium channels. This can create problems when making erosion measurements and therefore, the inside of the flume was coated with an epoxy resin based paint.

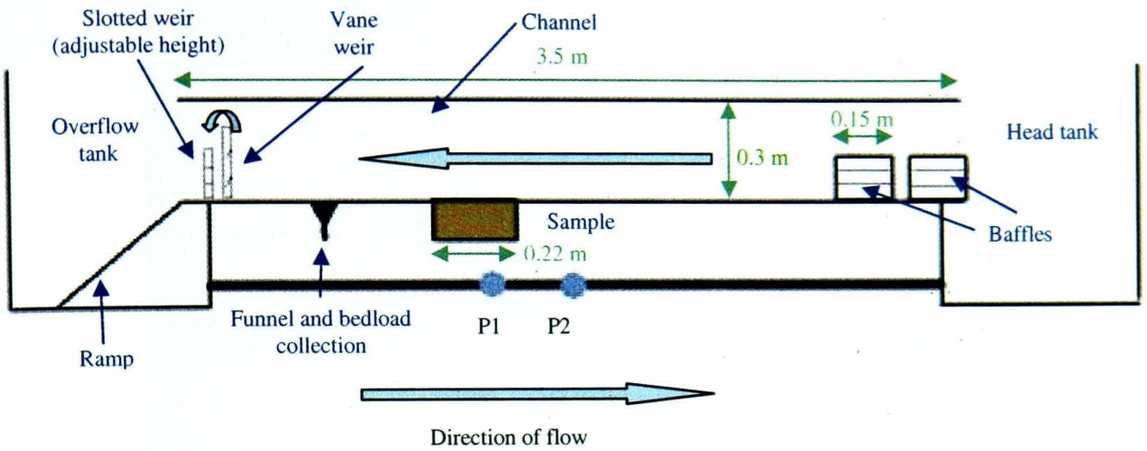


Figure 6.5a. Flume design - side view.

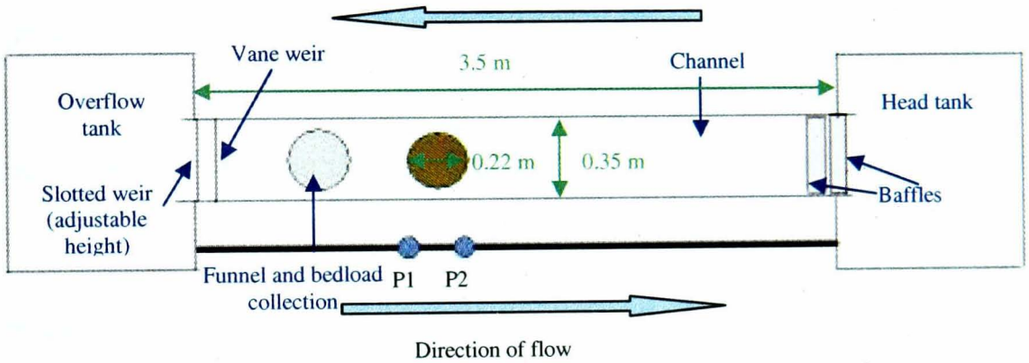


Figure 6.5b. Flume design - viewed from above.

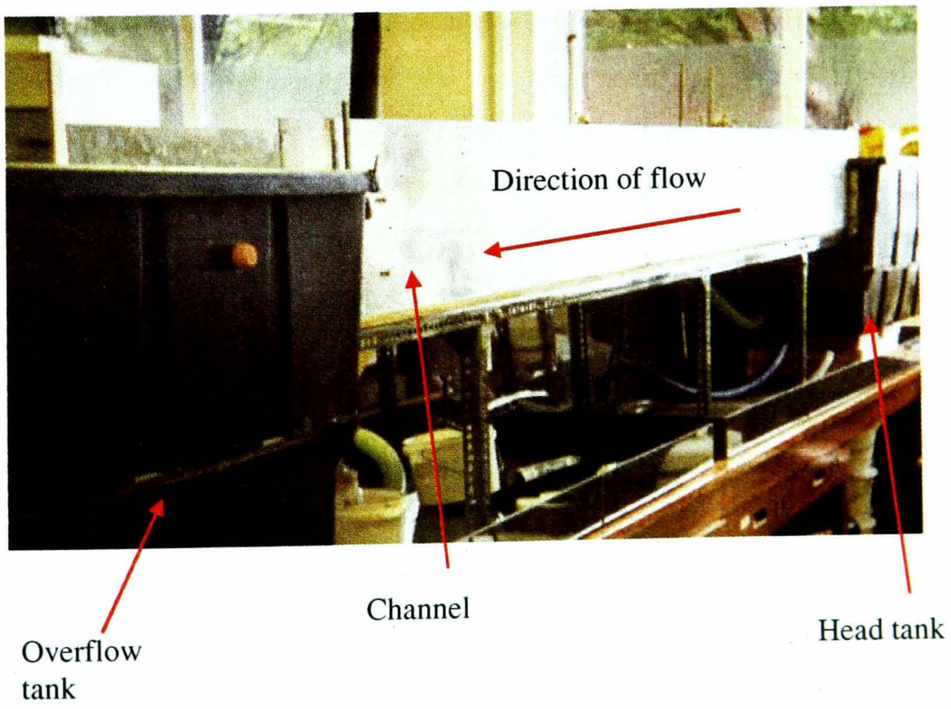


Plate 6.1. Side view of whole flume

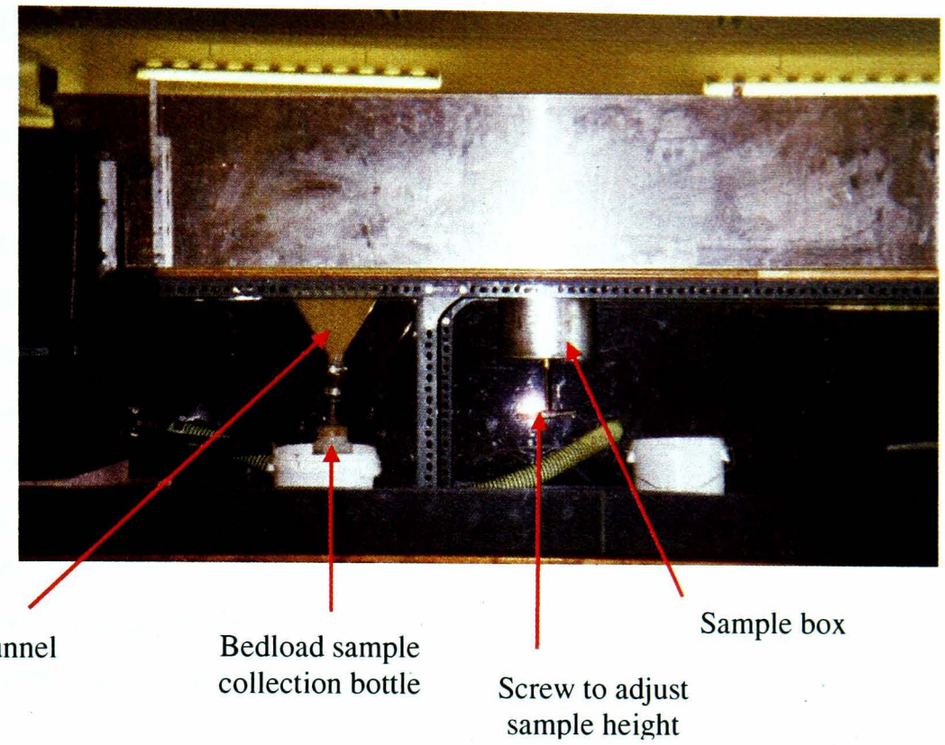


Plate 6.2. Side view of flume showing the sample box and bedload trap

6.4.2. Flume calibration

6.4.2.1. Pump speed calibration

Prior to any measurements, a scale of flow velocities was devised by recording the velocity profiles in the expected region of the test section of the flume using various combinations of weir height and pump speed (controlled by a valve). Pump speed could be increased by the opening of a valve with maximum current velocity being achieved using both pumps simultaneously with both valves fully open. Reducing the height of the weir (by removing one or more slats) also increased current speed. Velocity was recorded using a Kent Miniflo low speed probe suitable for measuring velocities within the range of 2.5-150 cm s^{-1}. Rotation of the probe's impeller was recorded in Hz and converted to cm s^{-1} using the regression equation derived from Figure 6.6. Velocity profiles were plotted for each pump speed and u_* and τ_0 calculated using equations 2 and 4. Water density was calculated according to Libes (1992), as follows:

$$\rho = (\sigma / 1000) + 1$$

At 20°C and 15 psu, $\sigma = 11.16$. Therefore, for this temperature and salinity, $\rho = 1.011$ g cm^{-3}.

6.4.2.2. Flow characterisation

Before the flume could be used for erosion measurements, the behaviour of the flow had to be examined to ensure that the baffle and weirs were effective in straightening the flow and controlling velocity. This was achieved by a combination of velocity measurements and visual observations using dyes, at different current speeds. A solution of potassium permanganate was introduced at various distances along the bed of the flume using a narrow glass pipette. The point at which mixing was reduced to a minimum and the dye flowed in straight lines was noted. In addition, small amounts of sand were placed at the entrance to the channel and deposition patterns noted following generation of the flow. Photographs were taken and deposition patterns were compared with those following introduction of the sample. Jumars & Nowell (1984) state that velocity measurements at one arbitrary height are insufficient to characterise the flow in the region of the bed. This is because the value of z_0 is estimated by extrapolation and its statistical confidence limits (and therefore those for the boundary shear velocity, u_*) are broad unless measurements are made at least six depths. Therefore, velocity profiles were plotted at various distances along the middle of the channel and at various current speeds. Using these methods, in combination with calculations using equation 8, an approximate estimate of the best part of the flume to introduce the sample was made. Within this area, detailed characterisation of the flow was carried out by making velocity measurements at depth intervals of 0.5 cm at 1 cm intervals across the width of the flume. Following introduction of the sample, velocity profiles were recorded across the entire width of the flume at 25 cm intervals along the length, with more concentrated measurements being made in the region of the sample. This allowed determination of the effect of both the weirs and the presence of the sample on the flow. u_* and τ_0 values were calculated from the gradient of the line using the actual gradient as a mean value and the 95% CL as the upper and lower limits for the calculation of u_*. This effectively gave three values of u_* which could be used to calculate the mean value of $\tau_0 \pm$ 95% CL. Because u_* was calculated in cm s^{-1} and ρ was in g m^{-3}, the calculated shear stress values were in dynes and had to be divided by 10 to give N m^{-2} (Dyer, 1997).

6.5. RESULTS

6.5.1. Pump speed calibration

Figure 6.6 shows the relationship between impeller speed and current velocity and the formula used to convert Hz to cm s^{-1}. Twelve pump speed settings were chosen to give a velocity range (at 0.5 cm above the bed) of 5.3 - 65 cm s^{-1} and a bed shear stress range of 0.18 - 1.78 N m^{-2} (Table 6.2 and Figure 6.7). A steady increase in current velocity was achieved by a combination of increasing the pump speed and reducing the weir height. However, once both pumps were running at maximum speed, current velocity could only be increased by changing the weir height which resulted in a much larger increase in current velocity. Therefore, the large increase in current velocity from 34 - 65 cm s^{-1}, as shown in Table 6.2, was unavoidable and current velocities between these values could not be achieved. The increasing error associated with the increasing current velocity suggests that the flow conditions at high velocities were less reproducible than those at lower velocities and that conditions of high speed flow were less stable. Shear velocity (u_*) and bed shear stress (τ_0) values were calculated from the von Karmann-Prandtl velocity profiles (Appendix 7), the relationship between ln z and velocity being highly significant in all cases ($p<0.01$) with r^2 values ranging from 0.82 to 0.97. Reynolds numbers calculated for each pump setting gave a range of values of 4805 at 5.3 cm s^{-1} to 423,920 at 65 cm s^{-1} (Table 6.2), indicating turbulent flow at velocities of 7.9 cm s^{-1} and above (according to Massey, 1989, in Paterson & Black, 1999).

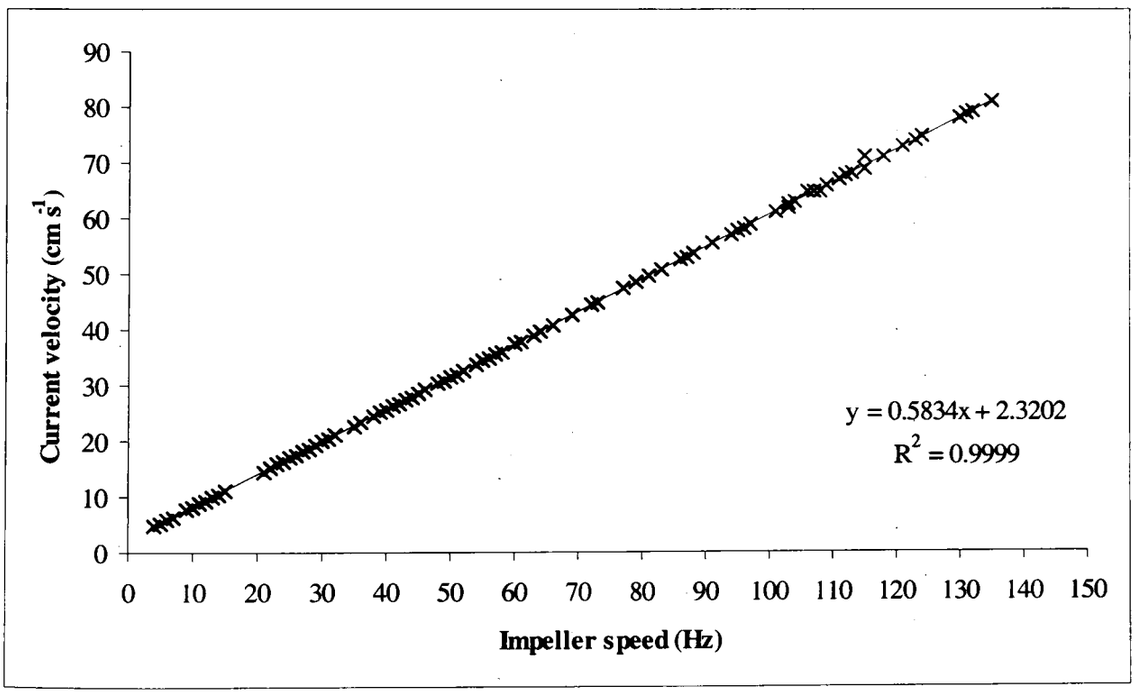


Figure 6.6. Calibration curve showing the relationship between impeller speed (Hz) and velocity in cm s^{-1}.

Table 6.2. Pump settings and associated velocities at a height of 0.5 cm above the bed. Measurements were made at a channel width of 17.5 cm and a length of 225 cm which represented the centre of the test section. The vane weir was set at an angle of 45° for pump settings 1-10 and was fully opened for pump settings 11 and 12. Values of u_* and τ_0 were calculated from velocity profiles. The Reynolds number (Re) was calculated using mean flow velocity.

Pump setting	Velocity (cm s^{-1})	U_*	τ_0	Re	SE (velocity)	P1 position	P2 position	Weir depth
1	5.3	1.73	0.18	4805	0.15	1	Off	6 cm
2	7.9	1.5	0.23	15,238	0.22	2	Off	6 cm
3	10.2	1.53	0.24	21,500	0.32	3	Off	6 cm
4	15.2	1.63	0.27	37,706	0.16	4	Off	6 cm
5	16.8	1.85	0.35	54,638	0.22	4	1	6 cm
6	19.3	2.32	0.54	72,251	0.24	4	2	6 cm
7	22.5	2.36	0.56	89,399	0.41	4	3	6 cm
8	26.0	2.9	0.85	123,054	0.25	4	4	6 cm
9	28.6	3.4	1.16	163,173	0.28	4	5	6 cm
10	31.5	3.69	1.38	192,978	0.27	4	6	6 cm
11	34.1	3.81	1.47	241,820	0.56	4	6	6 cm
12	65.1	4.19	1.78	423,920	1.39	4	6	3 cm

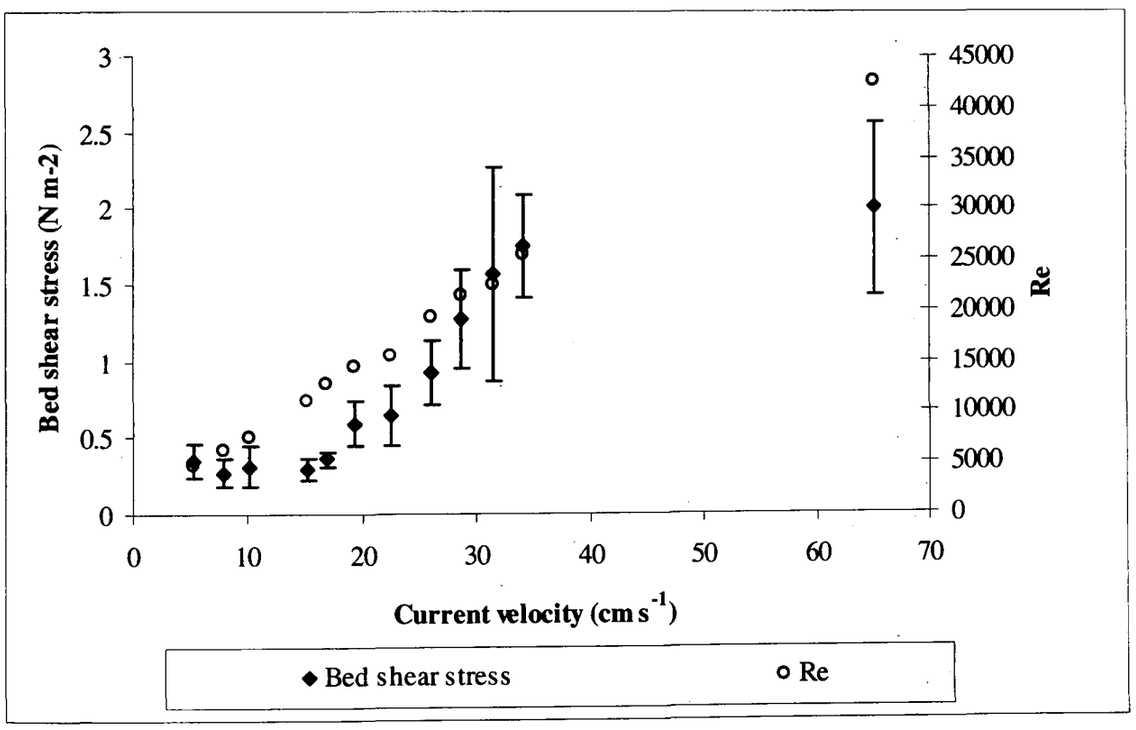


Figure 6.7. Bed shear stress values (mean ± SE) and associated Reynolds numbers for flow velocities generated by each pump setting.

6.5.2. Flow characterisation

Flow velocity was measured at 0.5 cm depth intervals from the flume bed to the surface of the water and at 1 cm intervals across the entire width of the flume. Measurements were made at various distances along the flume with concentrated measurements being made in the region of the sample. The data presented in the present chapter show that at 200 cm (Figure 6.8) along the length of the flume (before the sample), reasonable flow conditions were achieved for the pump setting used (pump setting 5, current velocity of 16.8 cm s^{-1}) with smooth contours of increasing velocity with increasing height above the bed and increasing distance from the wall of the channel. With the exception of those positions close to the wall of the flume, the majority of the velocity profiles show a good linear relationship with depth with r^2 values generally being between 0.8 and >0.9 (Appendix 8a). Maximum flow velocity was in the centre of the channel with a velocity of 13-14 cm s^{-1} being recorded at the bed and 18-19 cm s^{-1} being recorded at the surface. These data indicate that the sample was placed at a sufficient distance along the length of the flume not to be influenced by unsteady flow as the water exited the baffle.

At 210 cm, immediately upstream of the sample, the flow began to show signs of disturbance (Figure 6.9) with regions of lower velocity developing within the higher flow areas in the upper layers of the middle region of the flume. Figure 6.9 suggests that turbulence may be increasing slightly although there is no difference between the Reynolds numbers across the flume at the two positions (Figure 6.17). There also appears to be a slight reduction in flow velocity at the bed indicating that, at this point along the length of the flume, the presence of the sample and the associated change in bed roughness were beginning to impact upon the flow characteristics. Again, with the exception of measurements made close to the channel wall, the relationship between velocity and depth was good with r^2 values generally being around 0.8->0.9 (Appendix 8b)

The increase in turbulence and break down of flow was more obvious at 215 cm (the point of transition between the flume bed and the sample) and large variations in velocity, both with depth and width, can be seen (Figure 6.10). Plate 6.3 shows flow patterns (indicated by straight lines of sediment deposited on the channel bed) prior to addition of the sample box. This regular pattern of sediment deposition is compared to the random pattern of deposition following introduction of the sample (Plate 6.4), indicating that the flow was disturbed as it encountered the sample. In general, an increase in turbulence results in an increase in the Reynolds number (Livsey, 1995). However, Figure 6.18 shows that Reynolds numbers are lower at the point of the sample and after it. Average flow velocities in the middle of the channel ranged from 7-8 cm s^{-1} at the bed to 15-16 cm s^{-1} at the surface. This shows considerable deceleration of the flow in comparison with the 200 and 210 cm positions which

has resulted in the lower Reynolds number. Whilst the linear relationship between velocity and depth was maintained (r^2 = 0.7 - >0.9), there was a change in the gradient of the line, particularly in the region of the sample, which consistently occurred after the first three data points (Appendix 8c). This was also noted, although to a lesser extent, in the profiles for the 200 and 210 cm positions.

The flow remained unsteady, particularly close to the bed, with velocities remaining lower than upstream at the 225 and 235 cm positions (Figures 6.11 and 6.12). Reynolds numbers also remained comparatively low (Figure 6.18). The linear relationship between velocity and depth was maintained although the change in gradient after the first three data points was still present (Appendix 8d and 8e). At 250 cm (Figure 6.13), downstream of the sample, flow velocity increaseed slightly with values in the middle of the flume ranging from 11-12 cm s^{-1} at the bed to 17-18 cm s^{-1} at the surface. Flow velocity continued to increase at the surface at 275 and 280 cm with mid channel values as high as 19-20 cm s^{-1} at the surface (Figures 6.14 and 6.15). However, flow at the bed began to decelerate with mid channel values being between 8 and 10 cm s^{-1}. The flow also showed signs of becoming more turbulent with regions of lower velocity developing within the higher flow areas in the upper layers of the middle region of the flume. At 290 cm (the position of the weir), there was considerable deceleration of the near bed flow with the current velocity generally being 2-3 cm s^{-1} but as low as 0-1 cm s^{-1} close to the channel wall. In contrast, surface current velocity increased to a maximum of 30 cm s^{-1} (Figure 6.16). This indicates that the flow was being directed away from the bed towards the surface. The fact that near bed velocity increased between 235 (after the sample) and 250 cm, before decelerating in the region of the weir demonstrates that the presence of the weir was not impacting upon the flow conditions in the region of the sample. Calculation of bed shear stress downstream of the sample was not considered necessary and therefore velocity profiles were not constructed for this region of the flume.

Values of ln z_0 and z_0, together with their statistical significance, are presented in Tables 3 and 4 (respectively) in Appendix 9 with a summary being presented in Figure 6.17. It can clearly be seen that the value of z_0 increases in the region of the sample, indicating a roughening of the bed, with the highest value being in the middle of the channel at 215 cm. This is the point at which the flow first encounters the sample and coincides with the point of maximum shear stress (Figure 6.19).

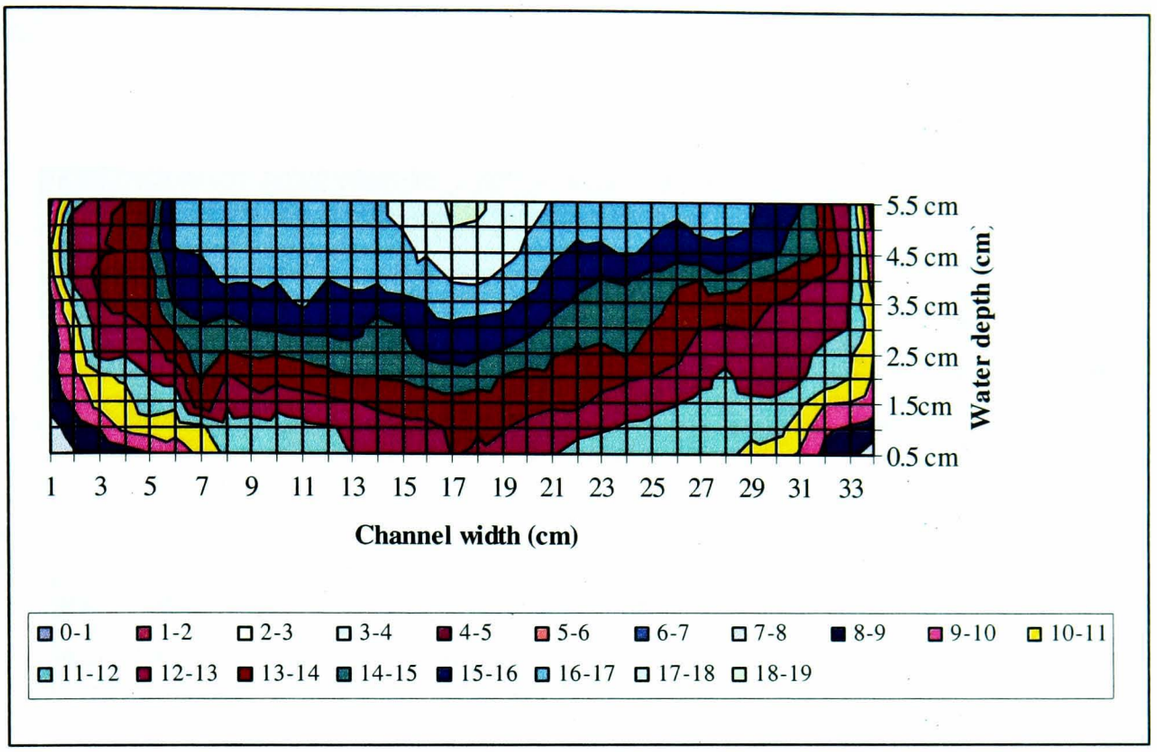


Figure 6.8. Velocity patterns at 200 cm (just before sample) with a weir height of 6 cm and a current velocity of 16.8 cm s^{-1} (pump setting 5).

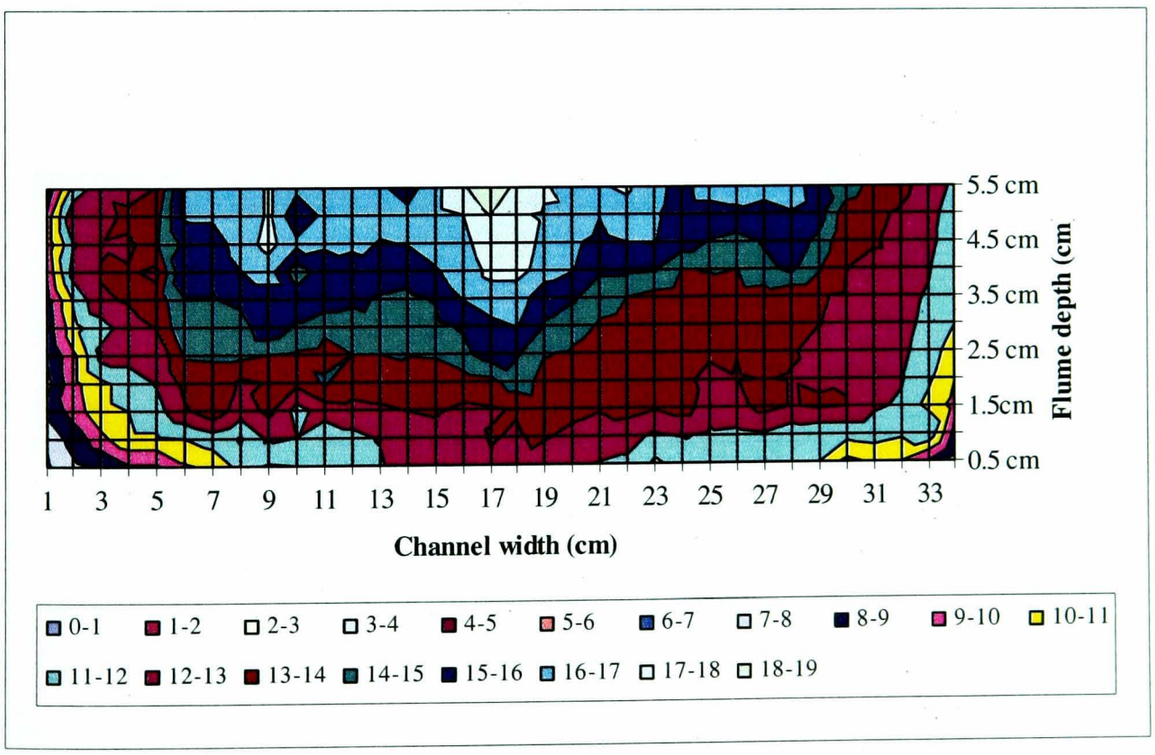


Figure 6.9. Velocity patterns at 210 cm (just before sample) with a weir height of 6 cm and a current velocity of 16.8 cm s^{-1} (pump setting 5).

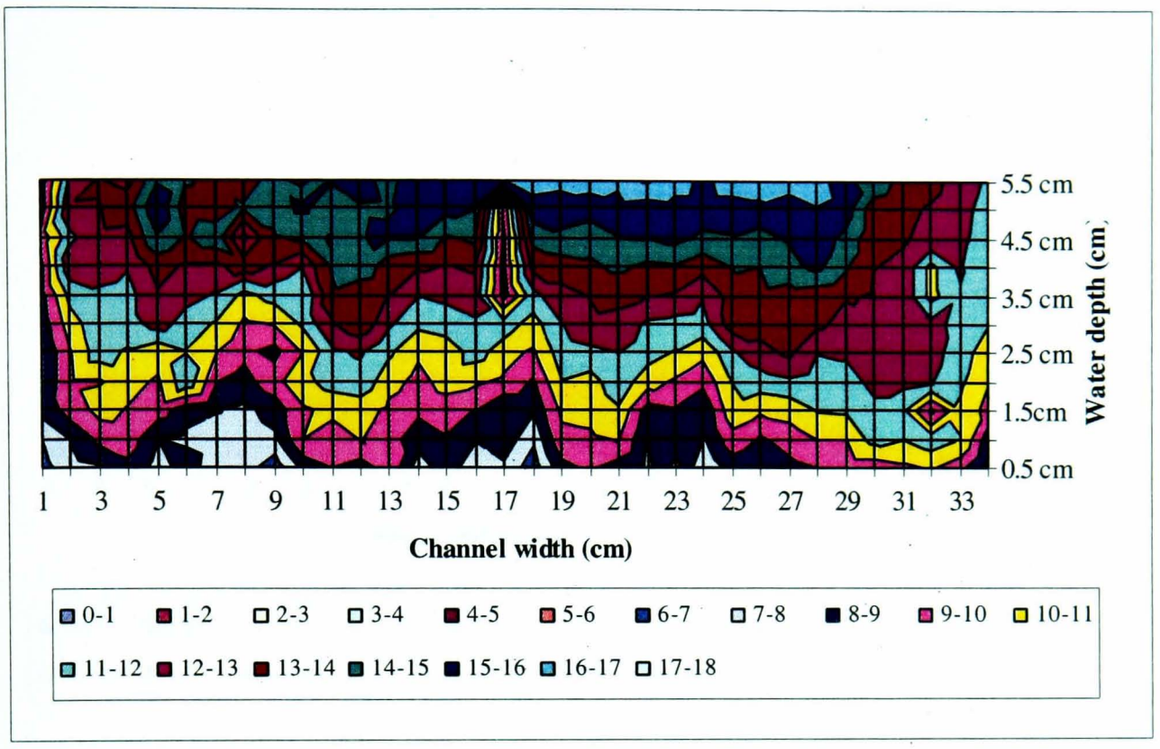


Figure 6.10. Velocity patterns at 215 cm (beginning of sample) with a weir height of 6 cm and a current velocity of 16.8 cm s^{-1} (pump setting 5).

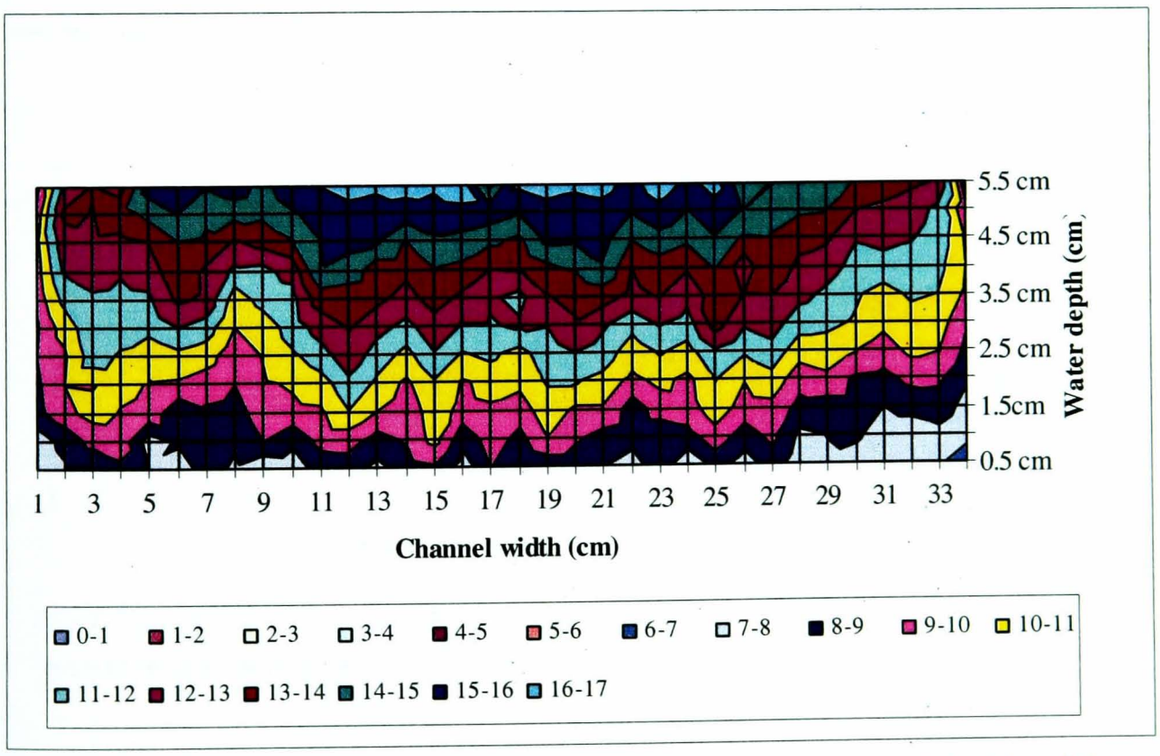


Figure 6.11. Velocity patterns at 225 cm (middle of sample) with a weir height of 6 cm and a current velocity of 16.8 cm s^{-1} (pump setting 5).

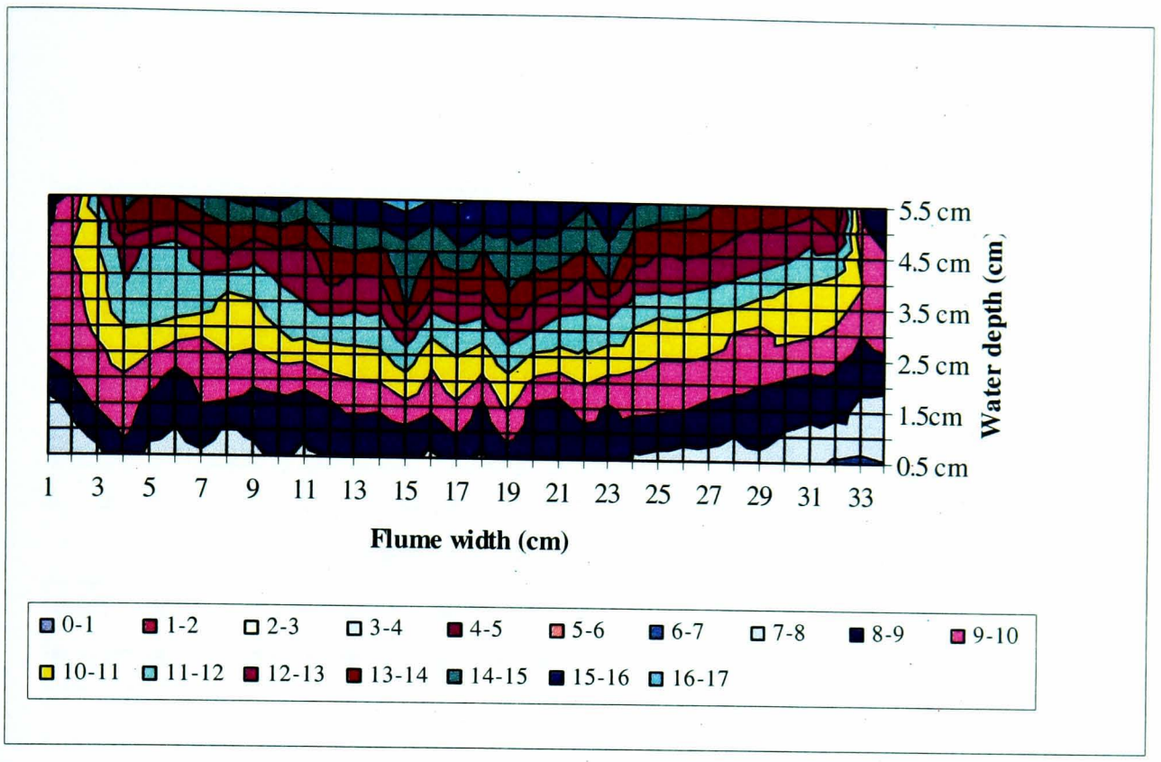


Figure 6.12. Velocity patterns at 235 cm (end of sample) with a weir height of 6 cm and a current velocity of 16.8 cm s^{-1} (pump setting 5).

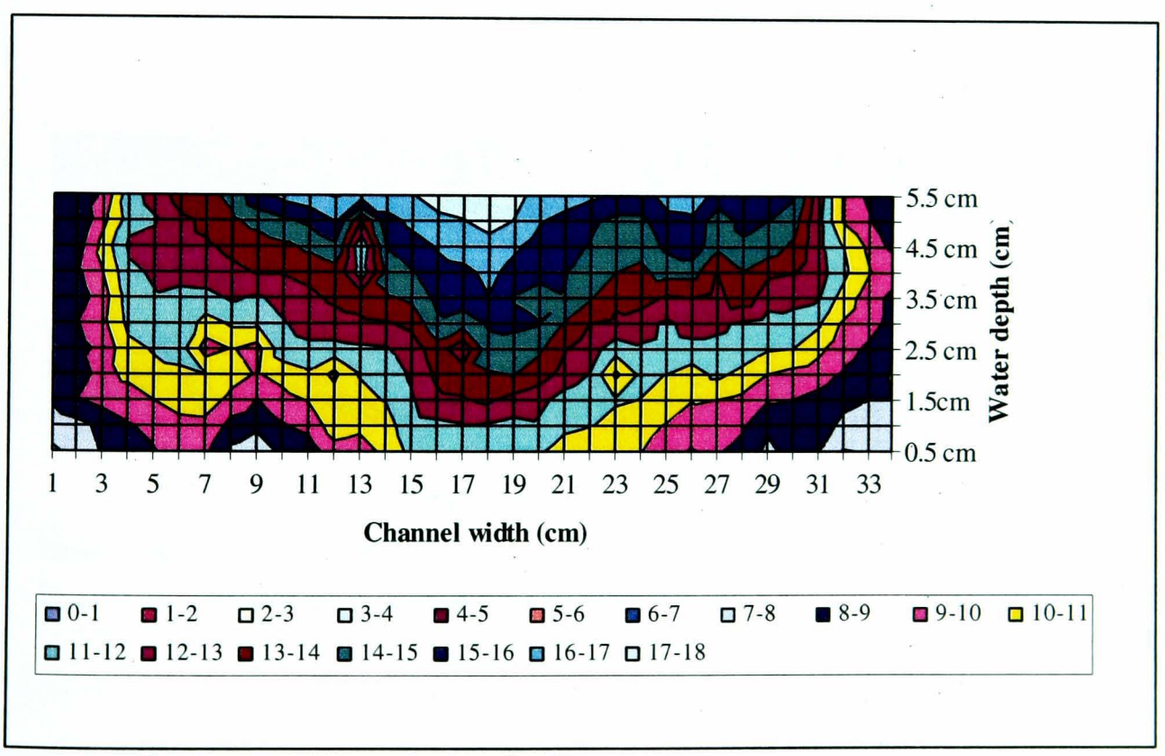


Figure 6.13. Velocity patterns at 250 cm with a weir height of 6 cm and a current velocity of 16.8 cm s^{-1} (pump setting 5).

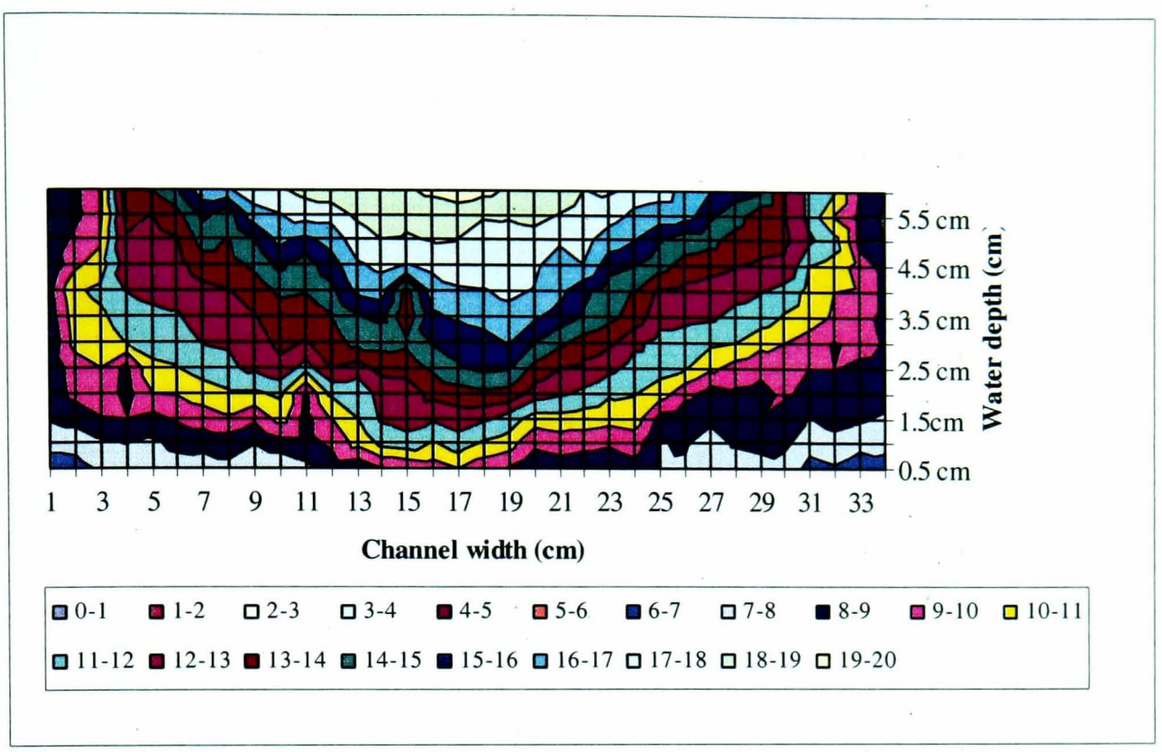


Figure 6.14. Velocity patterns at 275 cm with a weir height of 6 cm and a current velocity of 16.8 cm s^{-1} (pump setting 5).

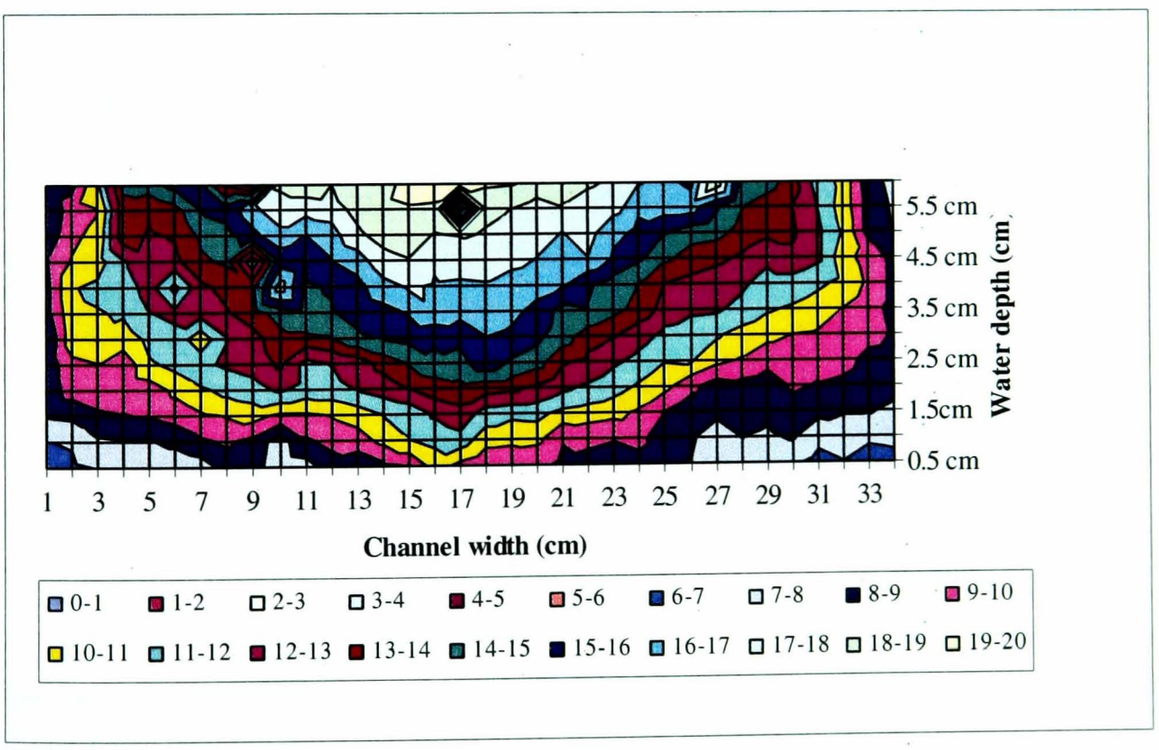


Figure 6.15. Velocity patterns at 280 cm with a weir height of 6 cm and a current velocity of 16.8 cm s^{-1} (pump setting 5).

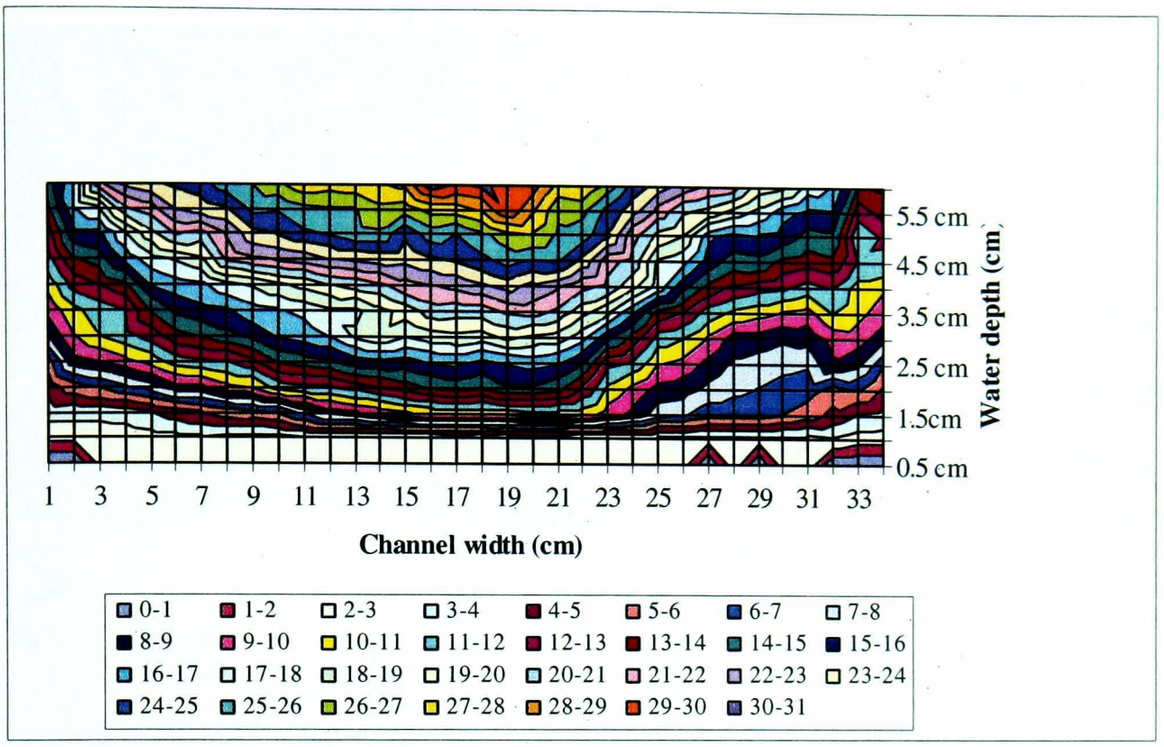


Figure 6.16. Velocity patterns at 290 cm (next to weir) with a weir height of 6 cm and a current velocity of 16.8 cm s^{-1} (pump setting 5).

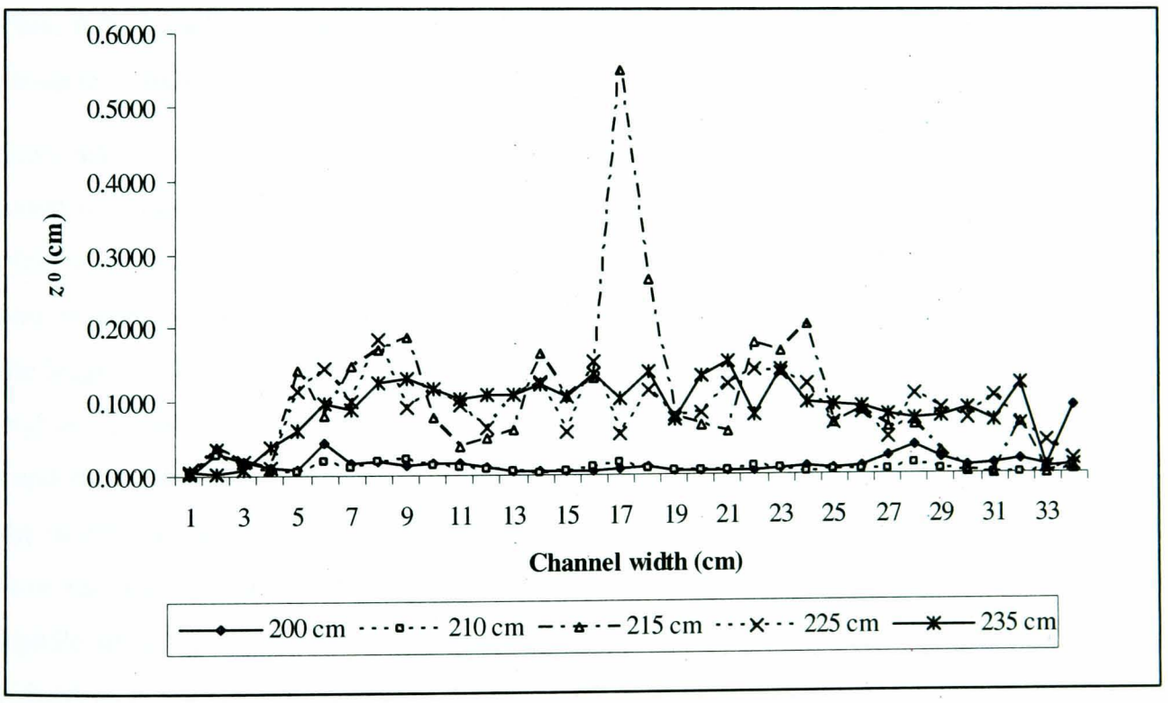
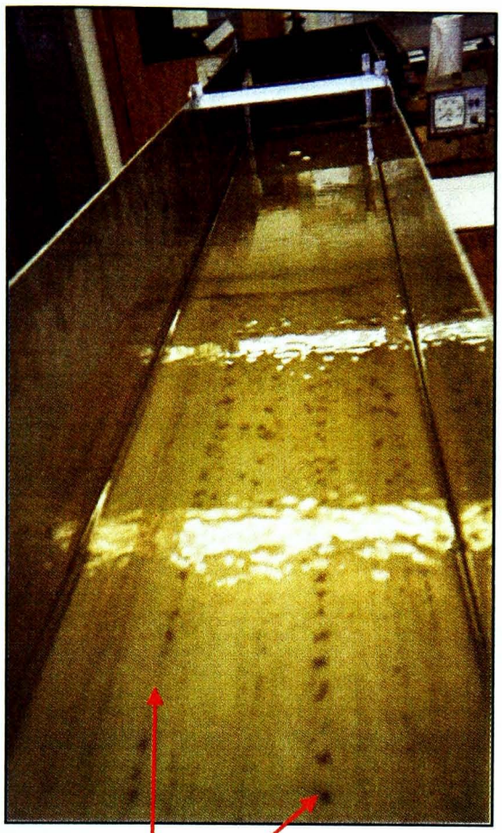


Figure 6.17. Variation in z_0 (derived from velocity profiles) across the channel and at various distances along the length, in the region of the sample



Steady flow, indicated by regular pattern of sediment deposition

Plate 6.3. Steady flow prior to the introduction of the sample (bed roughness is uniform along the channel)

Disturbed flow, indicated by random sediment deposition around the sample

Sample

Funnel

Plate 6.4. Disturbance of flow around the sample

Reynolds numbers (Figure 6.18) calculated for positions across the width of the flume and along its length (200-235 cm) show that, in all cases, values were above the critical 2000 - 4000 value which indicates turbulent flow where inertial forces dominate over viscous forces and erosion can proceed (Dyer, 1986; 1997; Paterson & Black, 1999). At all positions along the length of the flume, *Re* was lowest at the channel edges due to the reduced velocity in this region. Channel edge values were all below 6000 with values being below 5000 on the left hand side. Maximum turbulence (as indicated by the highest Reynolds numbers) occurred in the middle of the flume at the 200 and 210 cm positions and reached almost 9000. As the flow encountered the sample (215 cm), Reynolds numbers decreased to around 7000 in the middle of the channel and remained low at 225 and 235 cm. This was attributed to the reduction in current velocity as the water flowed from the smooth, painted bed of the flume to the rougher, more uneven sediment surface. Despite the reduction in *Re*, bed shear stress increased in the region of the sample (Figure 6.19). More detailed graphical representation of shear stress distribution is given in Figures 6.20-6.24.

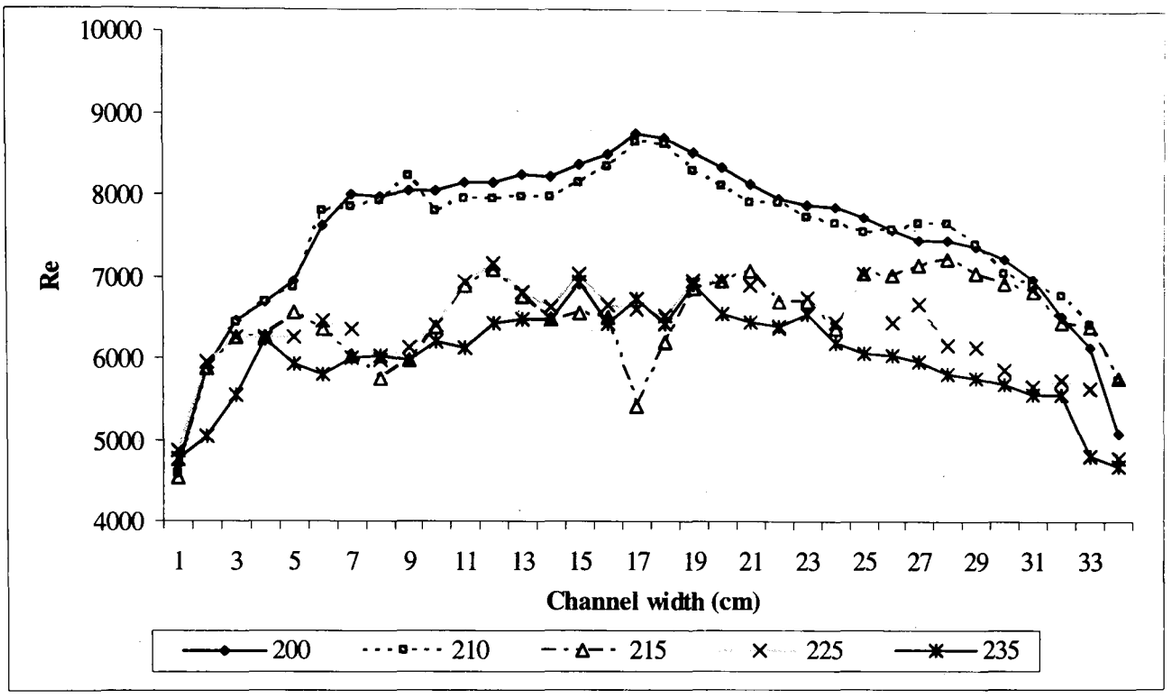


Fig 6.18. Changes in Reynolds number along the length and across the width of the flume in the region of the sample.

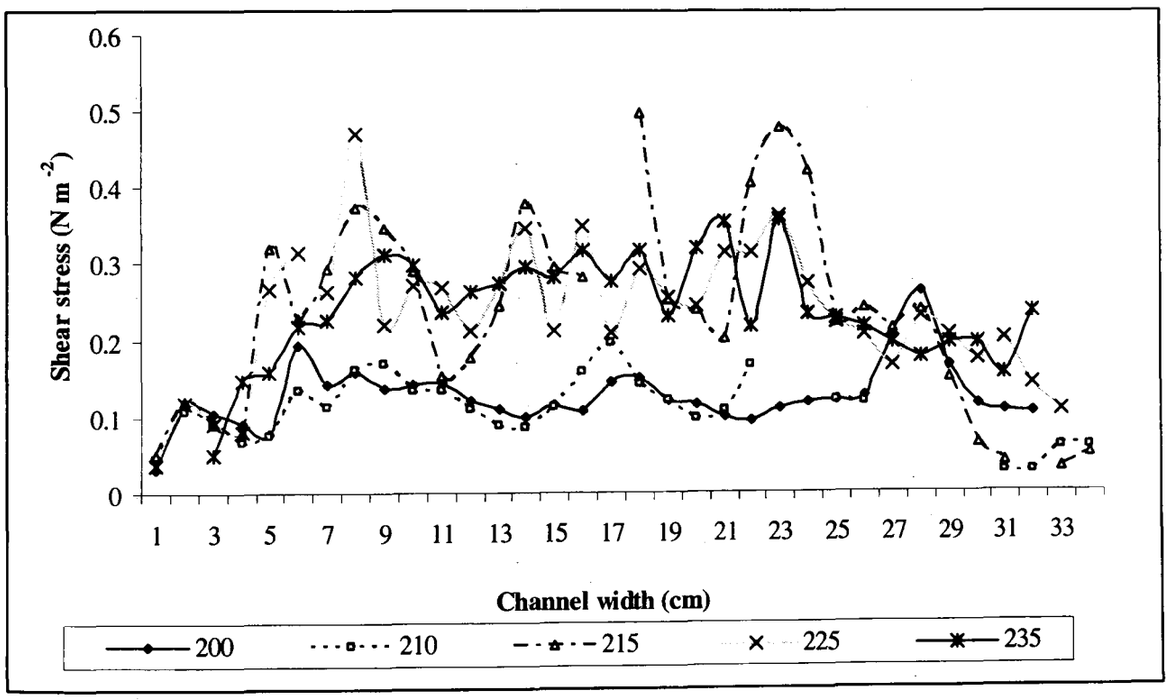


Figure 6.19. Changes in bed shear stress along the length and across the width of the flume in the region of the sample.

The velocity profiles presented in Appendix 8a-e were used to calculate values of u_* and τ_0. The statistical significance of the u_* values are presented in Appendix 9 and the distribution of τ_0 across the flume is shown in Figures 6.20-6.24. Shear stress values have not been calculated for velocity profiles which did not show a good linear trend (despite having high r^2 values) and have therefore been omitted from the Figures. Data are presented as bar charts to give a clear visual representation of cross channel variation in shear stress and as points with 95% CL to demonstrate that the variation is not as great as the mean values suggest. At 200 cm (Figure 6.20), two circulation cells appear to be present (S. McLelland, University of Hull. Pers. comm.), indicated by low values of shear stress close to the flume walls, increasing to a maximum in the centre of each cell and decreasing again towards the central region of the channel. The presence of these circulation cells (particularly on the right hand side of the flume) was less obvious at 210 cm (Figure 6.21) and it is possible that they did not exist. Shear stress values at 200 and 210 cm were similar with values ranging from 0.03 N m^{-2}, close to the channel wall, to a maximum of 0.3 N m^{-2}.

At 215 cm (Figure 6.22), shear stress distribution across the flume became more random, providing no evidence for the presence of different circulation cells. This was possibly due to interference caused by the step change from the smooth channel bed to the rougher surface of the sample and could be further complicated by the circular nature of the sample. This not only means that the sample was surrounded by a smooth surface but that the flow encountered it at different distances along the flume. No evidence of the two circulation cells was present at 225 or 235 cm (Figures 6.23. and 6.24). As already stated, shear stress increased as the flow encountered the sample with shear stress values ranging from 0.05 to 0.5 N m^{-2} at 215 and 225 cm and 0.05 to 0.35 at 235 cm.

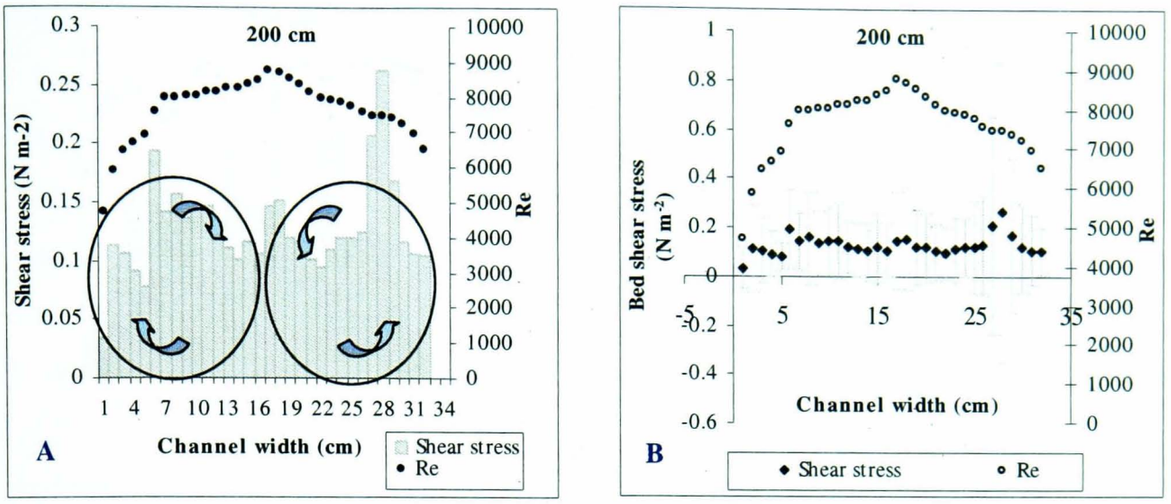


Figure 6.20. Cross channel variation in mean bed shear stress (A), mean bed shear stress ± 95% CL (B) and Reynolds number at 200 cm. The presence of two possible circulation patterns is indicated by the circles in diagram A (S. McLelland, University of Hull, Pers. comm.). Shear stress values have been omitted for positions where τ_0 could not be calculated due to the non-linear nature of the velocity profile.

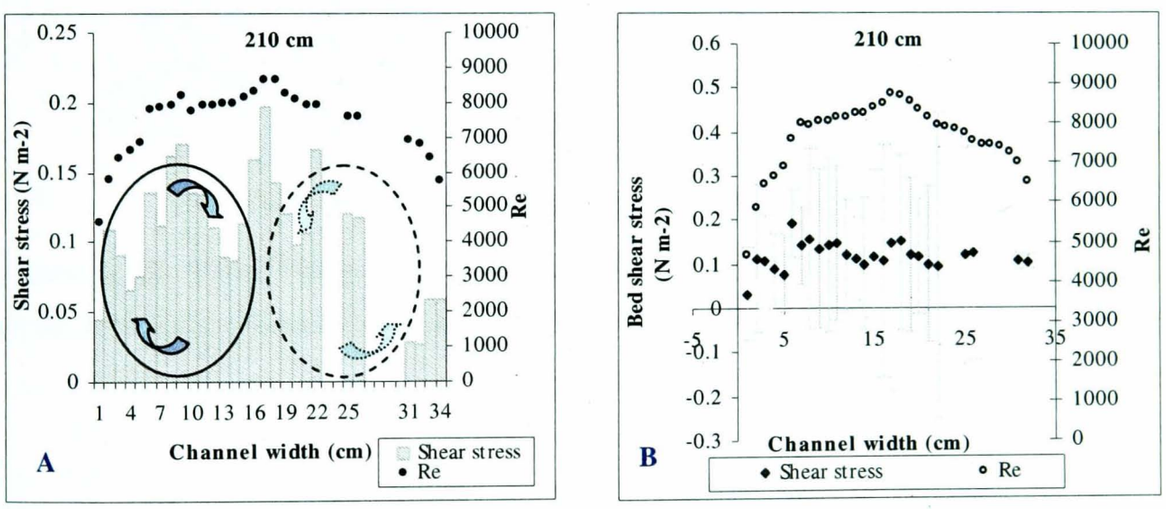


Figure 6.21. Cross channel variation in mean bed shear stress (A), mean bed shear stress ± 95% CL (B) and Reynolds number at 210 cm. The presence of two possible circulation patterns is indicated by the circles in diagram A, the dotted lines indicating that the presence of the second cell is uncertain (S. McLelland, University of Hull, Pers. comm.).

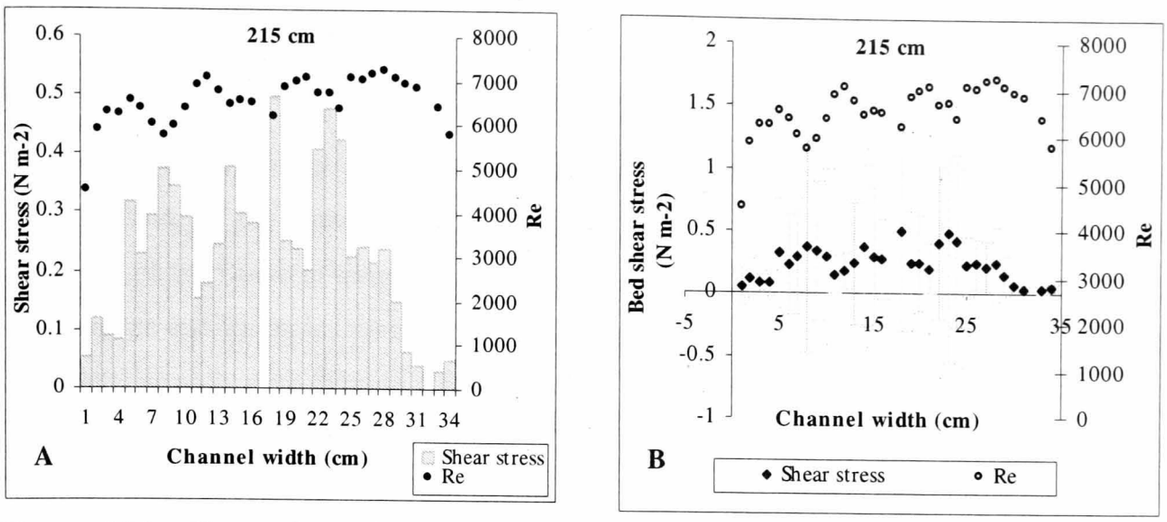


Figure 6.22. Cross channel variation in mean bed shear stress (A), mean bed shear stress ± 95% CL (B) and Reynolds number at 215 cm.

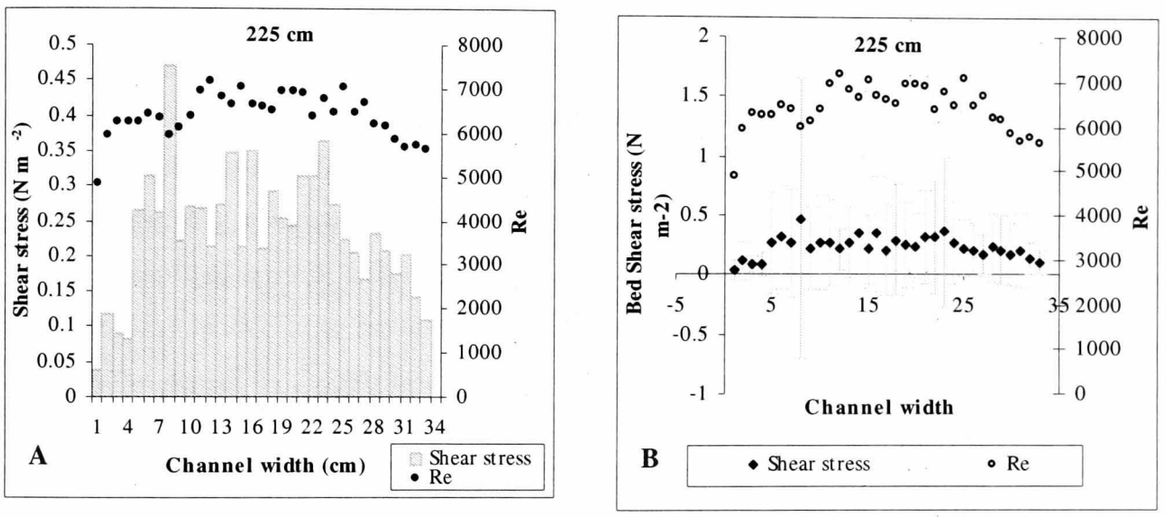


Figure 6.23. Cross channel variation in mean bed shear stress (A), mean bed shear stress ± 95% CL (B) and Reynolds number at 225 cm.

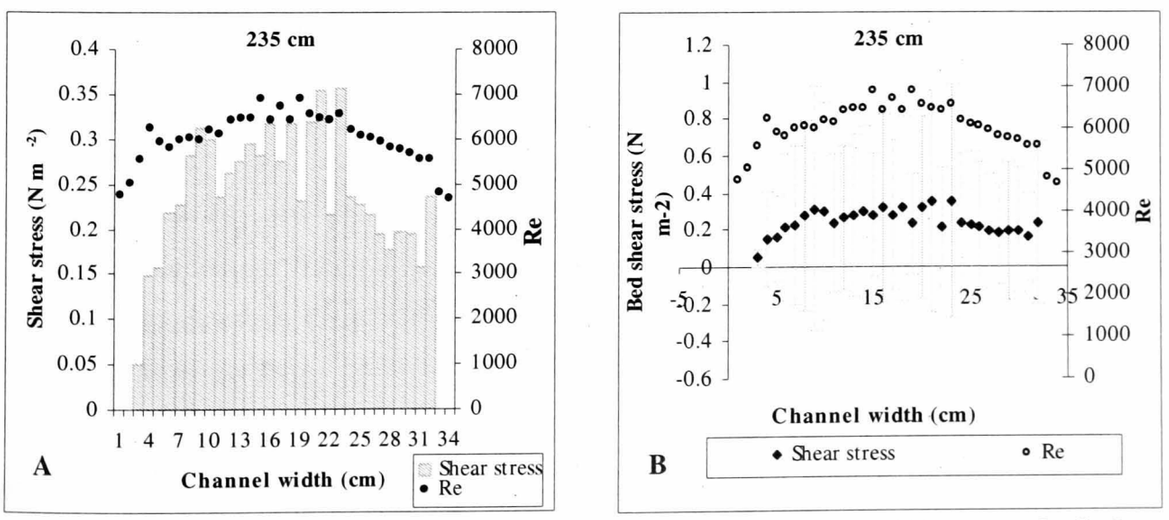


Figure 6.24. Cross channel variation in mean bed shear stress (A), mean bed shear stress ± 95% CL (B) and Reynolds number at 235 cm.

6.6. DISCUSSION

6.6.1. Current velocity and shear stress produced by the flume.

In general, the majority of authors give a general description, including diagramatic representation, of the flumes they have used in erosion studies but most do not state the properties of the flume in terms of the range of current velocities, shear velocities, bed shear stress values and Reynolds numbers that can be generated by the device. Those who do give these details rarely give them in a format which allows direct comparison between devices, a problem which was discussed in detail by Tolhurst *et al.* (2000a; 2000b). For example, Widdows *et al.* (1998a; 1998b; 2000) reported a free stream velocity range of 0 - 50 cm s^{-1}, resulting in a shear stress range of 0-2 Pa, for their benthic annular flume, but gave no indication of the range of shear stress values in N m^{-2}. Amos *et al.* (1992, in Black & Paterson, 1997) were able to achieve a maximum flow velocity of 0.9 m s^{-1} using the Sea carousel but, again, gave no indication of the bed shear stress values. The maximum current velocity which could be generated by the flume used in the present study was 65 cm s^{-1}. Higher velocities were achievable by decreasing the height of the weir although this resulted in a considerably shallower and more erratic flow which was considered unsuitable for making controlled erosion measurements. This velocity is similar to those generated in the flume used by Widdows *et al.* (1998a; 1998b; 2000) and is comparable with flows of 0.29-0.55 m s^{-1} reported by Le Hir *et al.* (2000) for the Humber estuary. Black (1998) recorded maximum current speeds of the order 40-45 cm s^{-1} during spring tides whilst current speeds of 10-12 cm s^{-1} were found to characterise neap tides. The range of velocities which can be generated by the flume used in the present study is therefore considered to be realistic for the study of erosion.

Other authors have recorded shear velocities (u_*), from which shear stress can be calculated. Blanchard *et al.* (1997) reported shear velocities (u_*) of 0.32 - 2.62 cm s^{-1}, calculated from logarithmic velocity profiles, using the laboratory flume 'HYCOBENTHOS'. Springer *et al.* (1999) recorded shear velocities of 0.12 - 0.48 cm s^{-1} for flows of 1 - 10 cm s^{-1}, using a linear recirculating laboratory flume. The range of pump settings used in the present study resulted in a shear velocity range of 1.5 - 4.2 suggesting that the shear stress values for all three devices would be similar. It also suggests that a higher range of shear stress values could be produced by the flume used in the present study than by the devices used by Blanchard *et al.* (1997) and Springer *et al.* (1999).

Shear stress values produced by the flume used in the present study ranged from 0.18 - 1.78 N m^{-2}. These values are considerably lower than those achieved by Schünemann & Kühl (1991) who reported a maximum shear stress of 3 N m^{-2}, using the EROMES device. Tollhurst *et al.* (1999) were able to generate bed shear stress values of 0.2-9 N m^{-2} using the Cohesive Strength Meter (Paterson, 1989) and Williamson & Ockenden (1996) were able to produce a shear stress range of 0.02-5 N m^{-2} using the ISIS device (instrument for measuring erosion shear stress *in situ*). However, the recirculating field flume used by Black and Cramp (1995, in Black & Paterson, 1997) could only generate bed shear stress values of 0.1 - 0.9 N m^{-2}. It should be noted that most authors report critical shear stress values (i.e. the shear stress required to initiate erosion) of less than 2 N m^{-2} (e.g. Christie *et al.*, 2000; Tolhurst *et al.*, 2000a; 2000b). However, Tolhurst *et al.* (2000b) also demonstrated that the erosion threshold of sediments which had been transported from the field to the laboratory could be as high as 17 N m^{-2}. The implications of transporting sediment samples for laboratory erosion measurements are discussed in Chapter 7 but it is worth noting here that the effects of transporting samples over short distances within Hull are unlikely to be as great as those associated with the transportation of samples between the Humber estuary and St Andrews (as in Tolhurst *et al.*, 2000b). Considering this, together with the fact that such high erosion thresholds are not reported by other authors, the fact that the flume cannot generate shear stress values of this magnitude, is not considered to be a problem. During calibration of the flume, considerable erosion of sediment samples exposed to this range of shear stresses was observed. Widdows *et al.* (2000) reported a maximum critical erosion velocity of 40 cm s^{-1}, during measurements made using an annular flume on the Skeffling mudflats (Humber estuary) which, again, is well within the range of velocities which can be produced by the flume used in the present study. Finally, Le Hir *et al.* (2000) calculated the distribution of shear stress over the Skeffling mudflat in the outer Humber estuary. Maximum spring tide values of 5 N m^{-2} were calculated for mid channel and coastal areas whilst shear stress over the intertidal areas only reached a maximum of 2 N m^{-2} and, on many parts of the mudflat, was less than 1 N m^{-2}. Therefore, the range of shear stress values which can be produced by the flume used in the present study is considered to be reasonably representative of the shear stress values recorded in the field.

Whilst the range of shear stresses produced by the flume is considered to be adequate, velocity and shear stress could only be increased in controlled increments to 34 cm s^{-1} and 1.47 N m^{-2}. These conditions were achieved with both pumps running to full capacity, a weir height of 6 cm and the vane weir being fully open. Velocity could only be increased by further reducing the weir height to 3 cm which resulted in a dramatic increase in flow velocity

and sudden shallowing of the flow. Since erosion is more likely to occur at higher velocities, it is important to have a good range of values at the upper end of the scale in order to obtain a more refined value of critical erosion velocity or critical shear stress. This could possibly have been achieved by dividing the weir slats into smaller sections (e.g. 0.5 or 1 cm) to allow a more gradual reduction of weir height. However, reducing the weir height below 6 cm resulted in erratic flow conditions which were not reproducible between samples. Therefore, calculated critical erosion velocity / critical shear stress values would not be comparable between samples.

The calculated Reynolds numbers ranged from 4805 at 5.3 cm s^{-1} to 423,920 at 65 cm s^{-1}, indicating turbulent flow in the flume. These values are similar to those given in Springer *et al.* (1999) who reported Reynolds numbers to range from 2000 - 20,000 between 1 and 10 cm s^{-1}.

6.6.2. Flow characterisiation.

The relationship between velocity and ln z was found to be linear and highly significant for all pump settings with z_0 (roughness length) values ranging from 0.3 cm at 5.3 cm s^{-1} to 0.002cm at 65 cm s^{-1}. In general, low values of z_0 are indicative of a smooth bed (Dr. S. McLelland, University of Hull. Pers. comm.). The values reported in the present study are considerably higher than those given in Springer *et al.* (1999) who reported values of 400, 6 and 1 μm for flow velocities of 1, 5 and 10 cm s^{-1}, respectively. Three possible explanations are offered for this. Firstly, the bed of the flume used in the present study was considerably rougher than that of the flume used by Springer *et al.* (1999). Whilst there were some small scale irregularities along the painted surface of the flume, they were not considered to be sufficiently large to cause a difference in the roughness length of an order of magnitude. A more realistic explanation could be the resolution with which velocity measurements were made. Springer *et al.* (1999) used a high precision acoustic-Doppler velocimeter with which measurements could be made very close to the bed and at mm intervals. In contrast, the flow meter used in the present study could only be used to make measurements at 5 mm intervals, at a minimum height of 5 mm above the bed due to the size of the impeller.

According to Dyer (1986), this displacement height causes slight curvature (depending upon its magnitude) of the log-velocity profile and the actual height of the logarithmic velocity

profile may differ from that indicated by measurements. Correction for this can be made by modifying the von-Karmann-Prandtl equation (equation 4 in section 6.3.1) as follows:

$$u / u_* = 1 / \kappa \ \ln ((z + d) / z_0)$$

Correction for this displacement height was made (using a programme provided by Dr. S. Mclelland, University of Hull) but the difference between the corrected and uncorrected values of u_*, τ_0 and z_0 was negligible. Amos *et al.* (2000) reported that their miniflume had fully developed boundary layer at 10 mm above the bed. This emphasises the importance of high resolution measurements and suggests that the low resolution of the measurements made in the present study could explain some of the problems interpreting the data. Finally, unsteadiness of the pumps could also have contributed to the error associated with the velocity measurements.

Velocity profile measurements (at 16.8 cm s^{-1}) across the flume channel showed that at 200 cm along its length, the flow was reasonably well developed with a good linear relationship between ln z and velocity. However, the presence of the sample was found to cause disturbance in the form of deceleration of the flow, instability of the flow and an increase in bed shear stress. Logan & Jones (1963, in Livsey, 1995) studied the effects of changes in bed roughness, from smooth to rough conditions, on flow characteristics and found turbulence to increase (i.e. an increase in the Reynolds number and Reynolds stress). In contrast, Makita (1968, in Livsey, 1995) found turbulence to decrease upon transition from a rough to a smooth bed. Observations of a smooth to rough surface change by Livsey (1995) showed that mean flow accelerated as a result of increased surface roughness and this acceleration was restricted to the region of the step. However, rapid deceleration occurred following this in response to the increased friction associated with the increased surface roughness. The velocity profiles in the present study suggest an increase in turbulence as the flow approaches and encounters the sample although, contrary to expectation, the Reynolds number was found to decrease indicating a reduction in turbulence. This was attributed to the fact that calculation of the Reynolds number involves dividing the mean velocity by viscosity which has a value of <1. Therefore, a reduction in velocity will result in a smaller Reynolds number.

The response of the flow to the change in bed roughness between the channel floor and the sample did not agree with the findings of Antonia & Luxton (1972, in Livsey, 1995), the reduction in turbulence implying that the change had been from rough to smooth. This is entirely possible since the development of microbial biofilms and the production of a gelatinous slime on the sediment surface could result in smoothing of the surface. In addition, the recirculating conditions in the flume resulted in the deposition of some of the heavier

particles on the flume bed. However, despite attempts to make the sample flush with the bed, a lip was always present and an increase in turbulence should have been noted at this point. As would be expected, a large increase in z_0 and an increase in shear stress were noted as the flow hit the sample. As erosion proceeded, any microbial biofilm would have been removed and the sediment surface became rougher due to the removal of both fine particles, transported as SPM and larger clods, transported as bedload. Therefore, it is unlikely that the surface of the sample was actually smoother than that of the channel floor. It is more likely, as suggested by Dr. S. McClelland (University of Hull. Pers. comm), that the reduction in velocity, probably caused by increased friction and gradual deepening of the flow (due to the angle of the flume), had affected the reaction of the flow to the change in bed roughness.

Mulhearn (1968, in Livsey, 1995) observed that if the change in roughness was depressed (i.e. the rough surface, in this case the sample, was lower than the smooth surface, the flume bed) turbulence and shear stress was reduced downstream of the roughness change. This was attributed to a sheltering effect which, in the case of the present study, could have been the lip of the sample box. Samples were generally slightly (frequently 2-3mm) lower than the flume bed due to sagging, progressive erosion and the fact that attempts were made to avoid protrusion of the sample above the flume bed (which would have resulted in scouring and increased erosion). This could also explain the unexpected reduction in turbulence and shear stress following transition from the smooth to the rough surface.

Upon transition from a rough to a smooth surface (i.e. from the sample to the painted surface of the flume bed), a reduction in turbulence would be expected. In the present study, the transition between the smooth and the rough surface resulted in a reduction in both velocity and turbulence but downstream of the sample, the transition between rough and smooth did not result in any change in turbulence (as indicated by the Reynolds number) although velocity increased slightly. Antonia & Luxton (1972, in Livsey, 1995) found that following a change from a smooth to a rough bed, 15 boundary layer thicknesses downstream of the roughness change were required for full adjustment of the flow. Given that the diameter of the sample was 22 cm, it is unlikely that the flow would have fully adjusted to the 'rough' bed in the region of the sample before encountering the change to the 'smooth' bed of the channel floor. Therefore, it is not surprising that the findings observed by Antonia & Luxton (1972, in Livsey, 1995) were not observed in the present study.

According to Livsey (1995), the structure of equilibrium, turbulent flow over smooth and rough boundaries is relatively well understood but much less is known about the effects of changes in bed roughness on the flow characteristics (Livsey, 1995). Elliott (1958, in Livsey, 1995) found that changes in surface roughness could lead to the formation of an internal boundary layer with a layer of modified flow propagating outwards from the boundary downstream of the roughness perturbation. An interface was found to exist between the internal boundary layer where velocity characteristics were determined by the new bed roughness and the outer layer which retained the characteristics of the upstream flow. The velocity profiles of the internal boundary layer and the outer layer were both found to be linear but had different gradients. The point at which the gradient changed (the intersection or 'knee point') was taken to be the interface between the two layers (Antonia & Luxton, 1971a, in Livsey, 1995). Antonia & Luxton (1972, in Livsey, 1995) found the internal boundary layer to develop at a much slower rate on transition from a smooth to a rough surface than from a rough to a smooth surface. It was suggested that the response of flow to the change from a rough to a smooth surface was controlled by advection of turbulence from the upstream rough surface and its downstream decay. Following transition from a smooth to a rough bed, on the other hand, the response of the flow was dominated by the production of turbulent energy over the rough surface. Robert *et al.* (1993) concluded that in the internal boundary layer, the turbulence structure was dominated by eddy shedding from coarse material whereas outside this layer, it was controlled by the decay of turbulent structures produced upstream of the roughness change.

Velocity profiles plotted in the present study showed a good linear relationship between ln z and velocity (Appendix 8). However, in the immediate region of the sample, the linear nature of the profiles changed so that the gradient of the first three points of the line was different to that of the remaining points. The fact that the position of this change in gradient was consistent across the flume and along its length (over the sample) suggests the possible formation of an internal boundary layer.

6.7. SUMMARY AND CONCLUSIONS

- Current velocities, Reynolds numbers and values of Z_0 in the flume are comparable to those of other authors. The range of shear stress values produced is low in comparison to that produced by some erosion devices although the maximum shear stress value is greater than critical shear stress values reported by a number of authors. In addition, the range of shear stress values which can be produced in the flume are representative of those recorded from intertidal areas in the Humber estuary suggesting that realistic field conditions can be simulated. The range of shear stress values produced in the flume is therefore considered to be satisfactory.

- Flow conditions in the region of the sample appear to be unaffected by the entrance or exit conditions suggesting that the sample has been positioned appropriately for making erosion measurements.

- There was a considerable reduction in current velocity and turbulence (as indicated by the lower Reynolds numbers) as the flow encountered the sample. Shear stress, however, increased. Despite this, the good linear relationship between ln z and u was maintained.

- Velocity profiles in the region of the sample indicate the possible development of an internal boundary layer at the transition between the smooth, painted channel floor and the less even, rougher surface of the sediment sample.

- At higher velocities, the flow appears to become more turbulent and less stable, as indicated by the large error bars. This was unavoidable given the available budget and equipment.

- Whilst the near bed flow velocity will be monitored during erosion experiments, it will not be possible to plot detailed velocity profiles in the region of the sample in the same manner as has been carried out here. It must therefore be assumed that the flow conditions presented in the present chapter will remain uniform between samples during experimental analysis.

- The flume is considered to be suitable for making erosion measurements for the purpose of this study. However, it is accepted that there are a number of problems with it, including unstable and non reproducible flow at high velocities. The erodibility of sediment samples will therefore be determined as the mass of sediment eroded rather than through the use of critical erosion thresholds, accuracy of which is considered to be spurious.

- The aim of the present chapter was to design and calibrate a laboratory flume and assess its suitability for use in erosion studies. It was not, however, to carry out a high resolution, detailed study of the behaviour of flow in channels. Characterisation of the flow has demonstrated the conditions to which a sample would be exposed during experimental erosion studies and the resolution is therefore considered to be adequate.

CHAPTER 7

EROSION MEASUREMENTS

7.1. INTRODUCTION

The effect of pollution-induced changes on bioturbation by different benthic communities was described in Chapter 5. Chapter 6 provided an explanation of the way in which the physical and chemical properties of the sediment may affect erosion and gave a description of erosion processes. The present chapter will focus on the way in which physical, biological and chemical modifications to the sediment properties, resulting from differences in bioturbation and animal activity, can affect the erosion characteristics of the sediment.

Over the past three decades, there has been increasing interest in sediment transport and the influence of animal activity on sediment properties (Austen, *et al.* 1999). Black & Paterson (1998) summarised the parameters and processes controlling the behaviour of intertidal cohesive sediments, emphasising the physical, chemical and biological interactions. Figure 7.1 demonstrates how atmospheric and oceanic forces (waves and tides) govern the hydrodynamic regime and how that, in turn, determines patterns of erosion and deposition and, ultimately the properties of the sediment. The sediment properties determine the biological communities (macrofaunal, meiofaunal and microbiological) which then influence the sediment properties through bioturbation, biostabilisation and bioirrigation. This biological activity impacts upon features such as particle size and particle size distribution, cohesion and adhesion, pore water pressure, physico-chemical properties and nutrient / contaminant fluxes, and organic content. In turn, these properties impact upon the processes of deposition, consolidation, and erosion of the sediment.

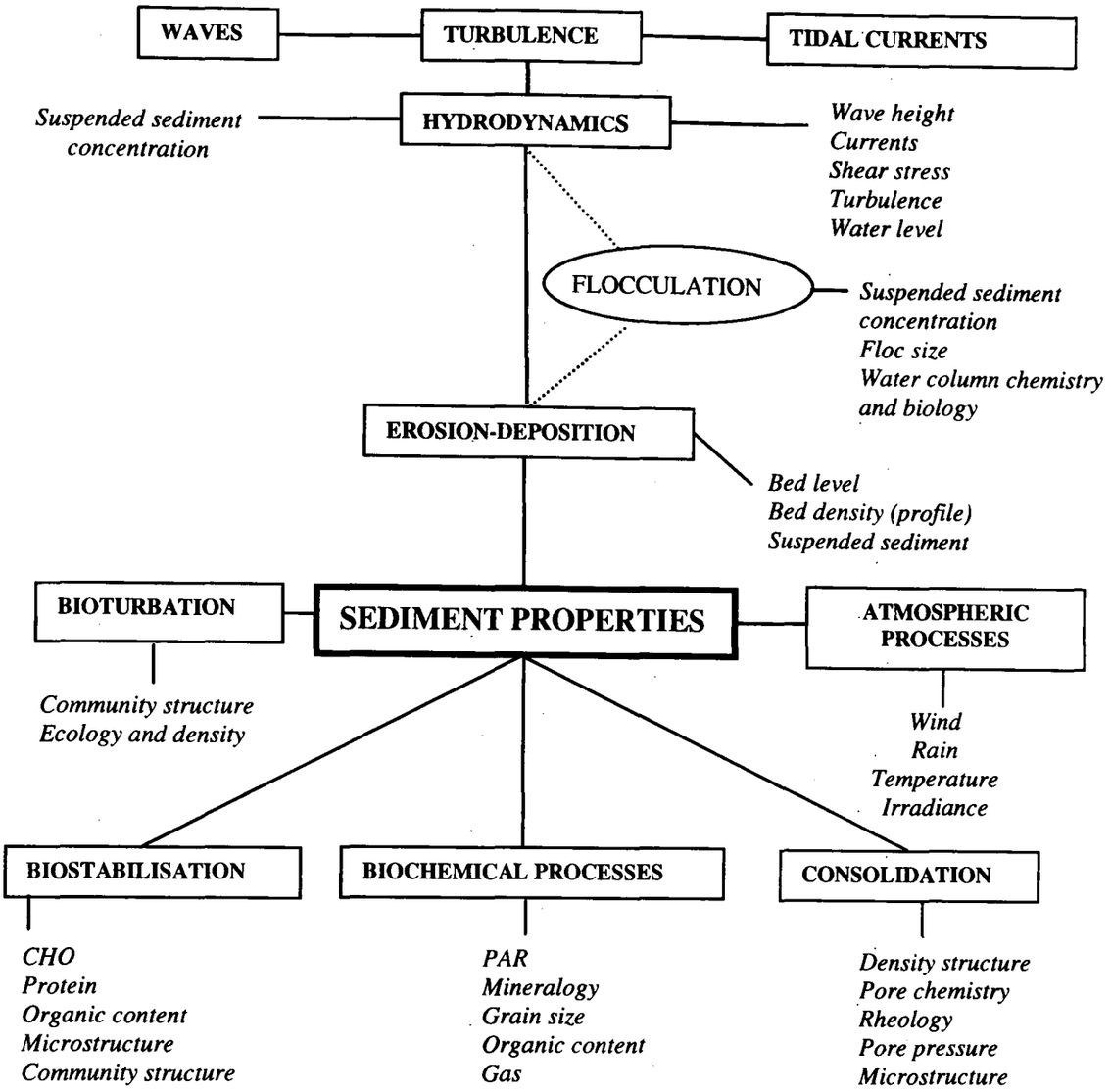


Figure 7.1. Schematic diagram of the parameters and processes which interact to control erosion and deposition of cohesive intertidal sediments (processes = bold; parameters = italics). (Black & Paterson, 1996, in Black & Paterson, 1998).

Nowell *et al.* (1981) and Jumars & Nowell (1984a) investigated the way in which various activities could enhance erosion or cause stabilisation of the sediment and found the following broad modes of organism effect:

1). Alteration of the properties of the flow

Firstly, biogenic structures alter the properties of the flow by altering the surface roughness of the bed (Nowell *et al.*, 1981). This may be the result of large macrofauna creating feeding pits, faecal mounds, tubes and tracks which increase surface roughness, or the activity of meiofauna and smaller macrofauna having a smoothing effect on the structures created by larger organisms (Rhoads & Boyer, 1982; Jumars & Nowell, 1984a). These features may either enhance resuspension or protect the bed from erosion by either increasing or decreasing the fluid momentum incident on the surrounding bed. Isolated features such as sparse worm

tubes or faecal mounds cause increased, localised turbulence and are therefore sites of initial erosion (Nowell *et al.*, 1981; Rhoads & Boyer, 1982; Hall, 1994). This was demonstrated by Nowell & Church (1979, in Jumars & Nowell, 1984a) who looked at the effect of different densities of Lego® blocks (small plastic bricks), in a flume, on erosion. At low densities, it was found that the maximum turbulence velocity was around the structure and therefore, the maximum amount of energy was dissipated at the bed and erosion occurred. In contrast, high densities of blocks resulted in the development of a skimming flow, with maximum turbulence on top of the blocks. The blocks therefore protected the sediment and the critical erosion velocity was comparatively higher than at lower densities. Eckman *et al.* (1981) also demonstrated this in flume studies using varying densities of the tubiculous Polychaete *Owenia fusiformis* and found that various tube densities spanned the range from stabilisation to destabilisation. In general it was found that tube coverage of a flume sample by up to 4% of the total area could enhance erosion. At higher densities, erosion was reduced and deposition enhanced protection of the bed, an observation which has also been made in seagrass beds (Scoffin, 1970, in Jumars & Nowell, 1984a) and in areas of dense macro algae (Frostick & McCave, 1979). However, Eckman *et al.* (1981) stated that in addition to density, this effect was dependent on tube roughness, geometry, the height : width ratio and the presence of microbial films. Nowell *et al.* (1981) concluded that any activity producing structures large enough to approach the thickness of the viscous sub layer (Chapter 6) would alter the fluid stresses on the bed both up and down stream of the structure, potentially affecting erosion.

The production of biogenic structures, for example the production of tracks or faecal mounds, also alters particle exposure to the flow. Fenton & Abbot (1977, in Nowell *et al.*, 1981) found that for coarse material (2.5mm diameter), a protrusion of half the particle diameter above the bed would reduce the critical erosion velocity by a factor of 1.7.

2). Alteration of adhesive and cohesive properties

As already stated, mucilage production by benthic organisms enhances stability due to changes in the adhesive and cohesive properties of the individual particles. According to Jumars & Nowell (1984a), the ratio of gravitational : cohesive / adhesive forces keeping particles together on the bed becomes increasingly important as grain size decreases. Although adhesive forces resulting from animal / microbial activity are of great importance in governing sediment erodibility, the relationship between the two is complex and poorly understood. For example, deposit feeding organisms must break the bonds between particles at the start of feeding which is potentially destabilising but following feeding, movement and excretion, these bonds are reformed as a result of mucous secretion. The suspension feeding

bivalve *Transenella tantilla* which produces crawling traces, thus altering particle exposure to the flow and enhancing erosion, also produces mucous which may help to stabilise their tracks (Jumars & Nowell, 1984a). Furthermore, animal activity enhances the growth of microbial populations (a phenomenon known as 'gardening', Hylleberg, 1975), as described in Chapter 2, which also secrete stabilising mucous. In addition to increasing particle adhesion, mucilages also increase particle deposition by reducing the probability of resuspension once a particle has come into contact with and stuck to the bed.

The importance of the influence of benthic microalgal populations, through secretion of mucous, on sediment erosion characteristics was mentioned in Chapter 2. The stabilising effect of biofilms has been extensively studied by various authors (e.g. Underwood & Paterson, 1993a; 1993b; Yallop *et al.*, 1994; Underwood & Smith, 1998; Austen *et al.*, 1999; Blanchard *et al.*, 2000; Tolhurst, 2000; deDekere, 2003) all of whom have found a reduction in critical shear stress to be associated with the abundance of diatoms and the concentration of carbohydrate in the sediment. Paterson (1997, in Paterson & Black, 1999) stated that the presence of mucous on the bed not only enhances particle cohesion but also reduces the roughness of the bed thus reducing the surface shear for a given flow.

3). Alteration of particle movement

Animal activity alters particle movement by, for example, the ejection of watery plumes of faecal and pseudofaecal material into the water column (bioresuspension), thus increasing the erosion potential (e.g., Tellinid bivalves). According to Rhoads (1974), this activity is an adaptation to life in muddy sediments which ensures that respiration is not impaired. In contrast, suspension feeding organisms occurring at high density (e.g., Mussel beds) will enhance particle deposition (including faecal and pseudofaecal material), thus reducing particle momentum and increasing stability (Graf & Rosenberg, 1997; Widdows *et al*, 1998a; 1998b). This process is termed biodeposition. Widdows *et al.* (1998a) found biodeposition rates (due to the activity of *Mytilus edulis*) to be 40 times higher than natural sedimentation rates and at densities of 1400 individuals m^{-2} and that this species could reduce sediment erodibility by an order of magnitude. Ahn (1993) found that suspension feeding by the Antarctic bivalve *Laternula elliptica* significantly increased the rate of sedimentation and estimated the rate of particulate organic carbon flux through biodeposition to be 95 mg C m^{-2} d^{-1}. Indirect biodeposition may be enhanced due to the secretion of adhesive substances by animal and microbial / algal populations which would effectively cause sediment particles to stick to the bed (Graf & Rosenberg, 1997).

4). Sediment fluidisation

Deposit feeding (both surface and sub-surface) bivalves such as *Macoma balthica, Yoldia limatula* and *Cerastoderma edule* have generally been found to reduce sediment shear strength as a result of ploughing beneath the sediment surface, increasing water content and pore water pressure, reducing compaction and cohesion and through the forceful ejection of liquefied faecal and pseudofaecal material into the water column (Widdows *et al.*, 1998a; 1998b). However, according to Rhoads and Boyer (1982), no consistent relationship has been found between measured geotechnical properties and sediment shear strength. This may be due to the complexity of the interactions between the numerous physical, chemical and biological factors which influence stability (Hall, 1994).

Work concerning the effect of these species on the sediment properties suggests that the destabilising effects far outweigh any stabilising effects (e.g., mucous secretion) that these organisms may have whilst feeding in this manner. Bender and Davis (1984) and Davis (1993) studied the behaviour of *Yoldia limatula* and *Macoma tenta* and found that both species strongly fluidised the sediment, ejecting plumes several centimetres into the water column. These authors calculated that one individual of *Y. limatula* could resuspend 440g (dry weight) of sediment / year and that natural population densities could resuspend between 15.8 and 24.6 Kg dry sediment / year. Bender & Davis (1984) also found a positive linear relationship between animal size and the weight of material ejected / hour for individuals of *Y. limatula* of up to 18 mm. These observations demonstrate the importance of organism size and density in determining the degree of bioturbation. During flume studies on the Humber estuary, Widdows *et al*, (1998b) found positive correlations between the density of bioturbating organisms, such as *M. balthica* and *Cerastoderma. edule*, and the critical erosion velocity and amount of suspended particulate matter. At densities of >1000 individuals m^{-2}, *Macoma balthica* was found to increase sediment erodibility 4 fold. In contrast, these authors also described *C. edule* as being one of the most important biodepositers in the Humber estuary as a result of its suspension feeding activity.

5). Particle size distribution.

Animals transport sediment particles both vertically and horizontally within the sediment, either through actively selecting specific particle sizes when feeding or through indiscriminately ingesting particles (Rhoads, 1974; Rhoads & Boyer, 1982; Meadows & Meadows, 1991; Hall, 1994). This results in the redistribution of the various particle size classes, often with a concentration of fine sand-sized pelletal particles being added to an unpelletized silt-clay matrix (Rhoads & Young, 1970). The relationship between particle size and critical erosion velocity is linear (Meadows & Tufail, 1986) and therefore mixing and

particle redistribution by benthic infauna will have some impact on the erosion characteristics of the sediment. In general, the critical erosion velocity is higher for consolidated, cohesive sediments and therefore it would be expected that unconsolidated faecal pellets would be more easily entrained than ambient sediments. This was indeed observed by Sternberg (1972, in Nowell *et al.*, 1981) who found the faecal pellets of Oligochaetes to have settling velocities which were twice that of other particles but the critical erosion velocities were much lower. Furthermore, this author also stated that the ratio of settling velocity : critical erosion velocity is commonly used to indicate that particles with diameters of less than 100μm move as suspended load and that the production of faecal pellets may cause changes in the mode of transport as well as the rate. However, this relationship is complicated by the presence of bacteria and microalgae which rapidly colonise faecal material. Rhoads & Boyer (1982) stated that the entrainment of faecal material is largely dependant on the binding effect of these micro-organisms and Nowell *et al.* (1981) found faecal mounds to be less easily eroded than the surrounding sediment. Therefore, before transportation may occur, current velocities must be sufficiently high to overcome both the cohesive attraction and to break the adhesive bonds between the faecal pellet and the bed. The transport and stability of faecal pellets is also dependent on shape, compaction, size and the composition of the food, all of which are species specific (Rhoads, 1974, Taghon *et al.*, 1984). Schafer (1972, in Nowell *et al.*, 1981) found cylindrical pellets to be rapidly destroyed by moving water whereas the discoidal pellets of species such as *Macoma balthica* were easily transported intact and could form sedimentary deposits. In muds where pioneer communities (dominated by opportunistic species) exist, the rate of sediment reworking is low and therefore, faecal pellets would be expected to remain at or close to the surface, particularly as the rate of pellet production will be high and most organisms do not reingest their own faeces until it has at least developed a sufficient coating of micro-organisms to be of nutritional value (Schafer, 1972, in Nowell *et al.*, 1981; Longbottom, 1973; Taghon *et al.*, 1984). In equilibrium communities, with a high rate of sediment reworking, a greater degree of particle subduction would be expected (Hall, 1994). In general however, the faecal pellet content of mud decreases with depth (Rhoads, 1974).

According to Jumars & Nowell (1984a), past attempts to classify organisms in terms of their sediment modification potential have been largely unsuccessful due to the fact that only two classes of behaviour were considered (stabilising or destabilising). There is now increasing evidence that a single organism could do both simultaneously or have one effect on one environment but another somewhere else by performing the same activity. For example, tracks produced by an organism crawling over a smooth sediment surface would potentially enhance sediment resuspension. The same organism, however, crawling over a rough surface

may have a smoothing effect, therefore potentially reducing erodibility (Jumars & Nowell, 1984a).

As a further complication, the activity of benthic organisms changes in relation to factors such as current velocity, turbidity, the presence of other species and food availability. As indicated in Chapter 1, many species thought to be deposit feeders have been found to be capable of suspension feeding and are known to switch between feeding modes over tidal cycles and with the seasons (e.g., *Hediste diversicolor, Macoma sp, Cerastoderma edule).* Many suspension feeding species are also able to switch to deposit feeding, for example, *Mya arenaria* acts as a deposit feeder during periods of low water. According to Snelgrove & Butman (1994), there is increasing evidence for this plasticity in feeding behaviour. Since deposit feeding is (broadly speaking) a destabilising activity and suspension feeding is usually associated with enhanced deposition, this switching between feeding modes also means a switch between sediment modification modes. Therefore most species cannot be classed as consistently stabilising or destabilising. Changes in bioturbation potential and sediment stability would be expected not only spatially, but also temporally, both on a seasonal scale and over a tidal cycle. According to Jumars & Nowell (1984a), as current speeds increase, the importance of bioturbation and animal activity probably reduces as biogenic structures disappear and transport becomes predominantly the result of high current speeds.

7.2. AIMS

Since sediment properties, in terms of stabilisation and destabilisation, differ with species and, hence, different communities, it is expected that changes in communtity structure and the degree of bioturbation, resulting from pollution, will lead to changes of the erosion characteristics of sediments. Chapter 5 showed that bioturbation potential was significantly reduced in polluted, oligochaete dominated, communities in comparison to those containing larger, deeper burrowing species such as *H. diversicolor.*

The aim of the present chapter is to determine the effects of this change in community structure and bioturabtion potential on sediment erosion potential. It is hypothesised that this reduction in bioturbation could lead to changes in the way the sediment erodes in terms of rate of transport, type and duration of erosion and critical erosion velocity. It is also hypothesised that chemical changes to the sediment as a result of pollution may alter the erosion characteristics. Chapter 6 described the various devices available for the measurement of sediment erosion and described the construction and calibration of a linear recirculating laboratory flume.

The primary objectives of the present chapter are therefore as follows:

- use the laboratory flume to investigate differences in the erodibility of sediment samples collected from sites at Paull and Saltend with different infaunal communities, as influenced by the discharge;
- validate the flume experiments with field measurements using the Cohesive Strength Meter (CSM);
- determine the impact of effluent concentration and individual species on sediment erodibility (using the CSM);
- determine a depth profile of sediment shear strength using a shear vane.

7.3. METHODS

7.3.1. Laboratory flume studies

7.3.1.1. Sample collection

Sediment samples were collected from the four key sites S25 m, S75 m, S200 m and P150 m) using plastic buckets (22 cm i.d.) with their bottoms removed. The buckets were upturned, undisturbed sediment cores removed (to a depth of 15 cm) and the lids placed on the bottom to prevent the core from slipping out. The samples were transported in large plastic tanks containing a small amount of seawater (2-3 cm deep) and were covered with polythene to minimise drying. Samples were collected in triplicate from each site stored at 10°C. Aerated seawater (20) was carefully poured over the surface of the mud twice daily and allowed to drain during storage to simulate tidal inundation. A depth of at least 2 cm water was maintained in the bottom of the tank to prevent drying of the samples. Attempts were made to ensure that samples were stored in the same conditions and that analysis took place as quickly as possible following sample collection.

In order to minimise disturbance through handling and manipulation, samples were introduced to the flume by removing the lids and pushing the lip of the bucket into the sample box. The false bottom inside the box was raised, to prevent the sample from sagging in the middle, and gradually lowered as the sample slid into the box. Once the entire core had been removed from the bucket, the bottom of the sample box was adjusted to that the sample surface lay flush with the bed of the flume. The sample was then loosely covered with bubble wrap (ensuring no contact between the plastic and the sample) secured with tape at the corners and the flume slowly filled (Widdows *et al.*, 1998a). Following filling of the flume, the bubble wrap was allowed to gently float off by loosening the tape, first on the downstream edge, and the sample allowed to settle for two hours. The bottom of the sample box was then adjusted again (if necessary).

7.3.1.2. Flume operation

Following initial measurements of suspended solids, the pumps were switched on in order to induce flow in the flume. Suspended solids were measured immediately and then the flume allowed to run for 15 minutes at each of the pump settings described in Chapter 6 with measurements being made end of the 15 minute period. Sediment transport was quantified using two methods. Suspended particulate matter (SPM) concentration was determined by direct measurements from water samples. Following each 15 minute period, three replicate 1 l water samples were removed and filtered though GF/C grade filters. The pre-weighed filters were then dried at 80°C and re-weighed to determine the amount of sediment retained. The amount of sediment transported as bedload was also determined at the end of each 15 minute

period. The sample bottle was removed from the bottom of the sediment trap in the flume and replaced with a clean one. The contents were then washed out into a beaker and the dry weight determined. Throughout the each experiment, temperature, salinity, dissolved oxygen and velocity at the bed were recorded. Prior to commencing each experiment, three 1 l water samples were filtered, dried and the pre-weighed filters weighed in order to determine a salinity correction factor.

7.3.1.3. Calculations

In order for the data generated through the use of the flume to be comparable to those generated using other devices, the data had to be standardised to time and area. This was achieved through converting measurements of suspended particulate matter into Kg and dividing by the area over which erosion was taking place (i.e. 0.038 m^2) to give a value SPMn in kg m^{-2}. The SPMn concentration was multiplied by the volume of water in the flume to give the actual SPM concentration in the whole flume (Widdows *et al.*, 1998a). The standardised erosion rate (ε_{st}) was then calculated as follows:

$$\varepsilon_{st} = (SPMn_{t2} - SPMn_{t1}) / (t_2 - t_1) \quad (1)$$

Where:

ε_{st}	=	standardised erosion rate
$SPMn_{t1}$	=	area and volume normalised SPM at time = 1...
t	=	time

7.3.2. CSM studies

7.3.2.1. Calibration and operation

The CSM allows small scale measurements of the spatial and temporal variation of sediment stability of exposed intertidal areas. The full details of the construction of the CSM are given in Tolhurst *et al.* (1999) and Tolhurst (2000). In brief, the device consists of a water filled chamber, containing a lamp, which is pushed onto the sediment surface. A vertical jet of water is directed at the sediment surface, in short pulses, the pressure of which is increased sequentially over the duration of the experiment. Erosion is determined by changes in light transmission across the test chamber with light transmission being reduced by increasing sediment resuspension. Compressed air is provided by a three litre diving tank pressurised to 210 bar.

As the CSM measures changes in light transmission, prior to any analysis, a calibration curve must be constructed in order to convert % light transmission to suspended particulate matter

(g l^{-1}). Fifteen 500 ml solutions of diluted sea water (20) with a progressively increasing concentration of dried estuarine mud were made up. Following thorough mixing, the test chamber of the CSM was filled and light transmission recorded. Concentrations ranged from 0 - 77 mg l^{-1} sediment and were sufficient to reduce light transmission from 91.6 to 23%. The following equation, derived from Figure 7.2, was used to convert % light transmission to SPM (in g l^{-1}):

$$\% \text{ light transmission} = -8.4302 \sqrt{\text{SPM}} + 94.836 \qquad (2)$$

($r^2 = 0.9885$, $p<0.01$)

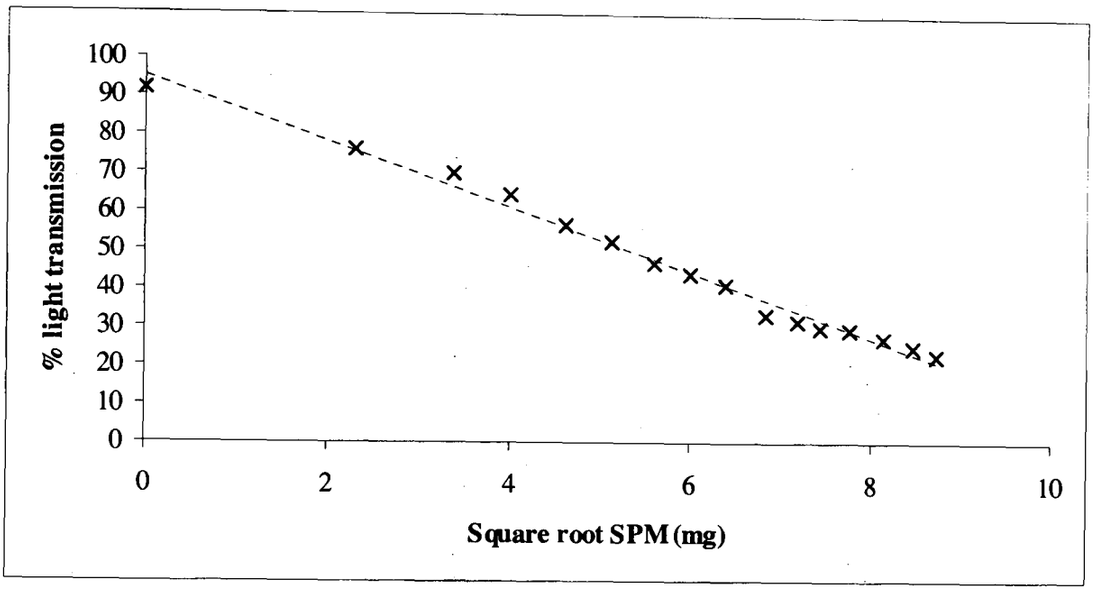


Figure 7.2. Calibration curve for the conversion of light transmission to suspended particulate matter.

Erosion measurements using the CSM were carried out both in the laboratory and the field. Following the advice of T. Tolhurst, (Gatty Marine Laboratory, University of St Andrews), settings for tests 'Sand 7' and 'Sand 9' were used. These were considered the most appropriate for estuarine cohesive sediments. The initial jet pressure of test Sand 7 was 0.3 psi with pressure increasing by 0.3 psi every 3 seconds to a maximum pressure of 12 psi. The Jet was fired for 1 s with data being logged every 0.15 s for 3 s. Test Sand 9 had an initial jet pressure of 0.5 psi, incrementing by 0.5 psi up to a pressure of 5 psi then by 1 psi to a final, maximum pressure of 30 psi. Again, the jet was fired for 1 s with data being logged every 0.1 s for 3 s. The test chamber was attached to a metal rod which was pushed into the sediment (to a depth of approximately 20 cm) and the test chamber lowered until it was secure in the sediment. The test chamber was then slowly filled with water of the same salinity as the surrounding pore water and the initial light transmission noted. Care was taken to avoid resuspending any sediment during filling of the chamber although this was sometimes

unavoidable. Therefore, in cases where the initial transmission was less than 80%, the test was rejected and repeated on a fresh, undisturbed area of mud. The test was allowed to run its full course with the pressure at which a 10% reduction in light transmission was observed being noted. Data were downloaded from the CSM using the CSM software supplied and light transmission data for each pressure increment being averaged over the 3 second logging period. Percentage light transmission was then converted to SPM using equation 2. Five replicate tests were carried out for each sediment type tested.

7.3.2.2. Field studies

CSM measurements were carried out, as described above, at the four key sites at Saltend and Paull in July 1999. A further set of measurements were taken at Skeffling. However, the CSM was available for a limited period only and insufficient data (in terms of quality and quantity) could be collected from this site. These data have therefore been omitted.

7.3.2.3. Laboratory studies

Sediment from Paull was sieved through a 300 μm sieve, using seawater (20), in order to remove the macro fauna and larger meiofauna. The sediment was then divided into three portions and stored at 10°C, with aeration, and allowed to consolidate for 7 days in large plastic tanks. Following settlement, the excess water was decanted with 1 portion of the sediment being mixed with 16% effluent, 1 portion being mixed with 32% effluent and the final portion being mixed with an equal volume of clean seawater (20), to act as a control, so that each portion was treated the same in terms of the proportion of liquid added. The sediment was then allowed to settle and consolidate for 50 days. The sediment surface was kept moist by adding either clean sea water or effluent at the appropriate concentration with a depth of approximately 2-3 cm water being maintained. Due to the biodegradable nature of the effluent under conditions of light and aeration, fresh effluent solutions were added every 7 days throughout the settlement period.

Following this settlement period, 36 plastic cores (20 cm deep, 10 cm i.d.) were filled to a depth of 15 cm with 12 cores being placed in each of three tanks. The tanks were filled with the appropriate clean seawater / effluent concentration and left to settle for 7 days. Following settlement, sufficient numbers of *H. divericolor* (45), *Macoma balthica* (5) and *Corophium volutator* (30) (of similar size) to simulate approximate field densities at Paull were added to the cores to give three replicate cores of each species and three replicate cores containing no animals within each concentration. The cores were covered with 500 μm nylon mesh to prevent the animals from escaping and a low level of flow was maintained in each tank using a small aquarium pump to recirculate the water. The animals were maintained under these

conditions for 28 days before erosion measurements were made using the CSM. The water level in the tank was reduced so that the sediment was exposed to the air for four hours prior to measurement (to simulate tidal exposure). The cores were then sieved and the number of remaining animals recorded.

7.3.2.4. Calculations

Erosion pressures applied by the CSM are recorded in psi (pounds / square inch) and are converted to KPa (Kilopascals) in Excel, using a macro provided by T. Tolhurst (Gatty Marine Laboratory, St Andrews). Following this initial conversion, jet pressure in KPa was converted to N m^{-2} to allow comparison with other erosion devices, using the following formula:

$$\tau_0 = y_0 + A1\,[\,1\text{-}\exp(-x/t1)] + A2^{*}[1\text{-}\exp(-x/t2)] \qquad (3)$$

Where:

τ_0	=	equivalent horizontal shear stress (Nm^{-2})	
y_0	=	zero	
x	=	eroding pressure (kPa)	
A1	=	67	
A2	=	-195	
T1	=	310	
T2	=	1623	(Tolhurst *et al.*, 2000a)

SPM values derived using equation 2 were standardised to time and area to give the value SPMn as described in Section 7.3.1. In this case, the area over which erosion was taking place was 0.0007 m^{-2}. Standardised erosion rates (ε_{st}) were calculated using equation 1

7.3.3. Shear vane measurements.

Sediment shear strength was measured in the field using a hand held Geonor H-10 field inspection vane. Measurements were made in July 1999 at the four key sites and in August 1999 at all sites.

7.3.4. Statistical analysis

Statistical differences in the total mass of sediment eroded, erosion threshold (critical shear stress) and sediment shear strength (shear vane data) were determined using one way ANOVA tests followed by *a posteriori* comparison of means (Zar, 1996).

Critical shear stress values were determined for the CSM data using linear regression (Sutherland *et al.*, 1998; Tolhurst *et al.*, 2000a). Scatterplots of the SPM data were visually examined and the point at which the slope of the erosion profile changed was noted. The data were then divided into two or more sets, depending upon the shape of the curve, and linear regression analysis carried out on each separate data set, the point at which the regression lines crossed being equivalent to the critical shear stress. Division of the data sets was verified by progressively adding or removing data points to obtain the optimum r^2 value for each line, thus reducing some of the subjectivity associated with visual examination of the data.

7.4. RESULTS

7.4.1. Flume studies.

Velocity was recorded at a height of 0.5 cm above the bed throughout each experiment. At approximately 22 cm s^{-1} (approximately 0.86 Nm^{-2}, 120 minutes), there was no difference in velocity between experiments ($p>0.05$) (Figure 7.3). However, at velocities above 25 cm s^{-1}, the pumps became unsteady and flow conditions were not as reproducible. At 135 minutes, flow velocities ranged from 27 cm s^{-1} (approximately 0.9 Nm^{-2}) for the S200 m samples to 49 cm s^{-1} (approximately 1.7 Nm^{-2}) for the S25 m samples. At 150 minutes, flow velocities were around 60 cm s^{-1} (approximately 2.2 Nm^{-2}) for all samples except the S75 m samples where velocity reached 77 cm s^{-1} (approximately 2.76 Nm^{-2}). This should be considered during interpretation of subsequent results since the effect of unsteady flow and differences between experiments may override the effects of any natural difference in sediment erodibility between samples. Values of shear stress (Nm^{-2}) were estimated by fitting a regression line to Figure 6.7 (section 6.5.1) ($r^2 = 0.88$, $p<0.05$) which shows the shear stress values calculated from velocity profiles at each current speed. The relationship is, however, not linear and the values given here are therefore only approximate.

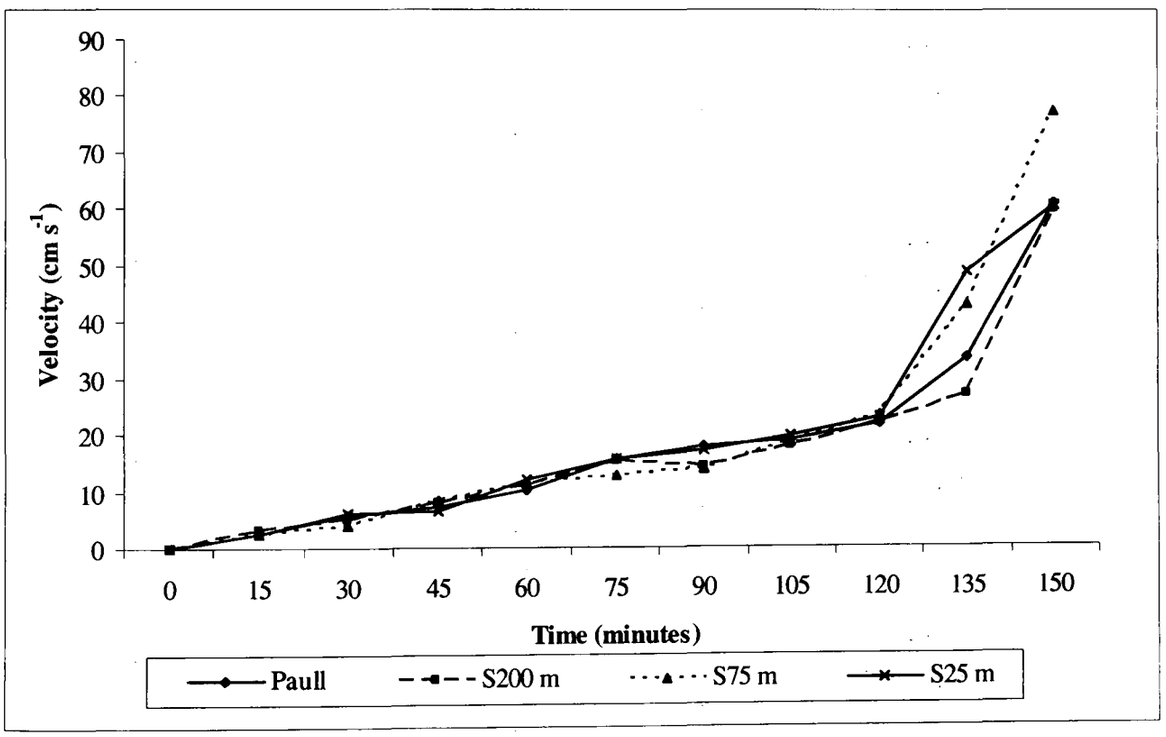


Figure 7.3. Mean velocity increase over time throughout each experiment.

Figures 7.4 and 7.5 show cumulative erosion over time in terms of suspended particulate matter (SPM) concentration and bedload transport. In terms of SPM concentration, there was no statistical difference in erosion between samples for the first 45 minutes of each flume run with erosion only increasing very slightly with increasing velocity. After this time (11 cm s^{-1}, approximately 0.42 Nm^{-2}) erosion of the S200 m, S75 m and Paull samples increased above

that of the S25 m sample with erosion of the S200 m sample increasing to the greatest extent. This trend continued as velocity increased. Erosion of the S75 m and Paull samples remained equal up to a velocity 23 cm s^{-1} (approximately 0.84 Nm^{-2}), after which erosion of the S75 m sample became comparatively high. However, it should be noted that mean velocity during these flume runs was also higher. Throughout the entire experiment, erosion (expressed as SPM in gm^{-2}) of the S25 m samples was consistently lower than that of any other samples. Bedload transport increased gradually with increasing velocity with erosion of the Paull samples generally being greater than that of the S25 m, S75 m and S200 m samples at all velocities above 16 cm s^{-1} (approximately 0.6 Nm^{-2}). At 38 cm s^{-1} (approximately 1.1 Nm^{-2}), erosion of the Paull and S200 m samples increased dramatically in comparison to that of the S25 m and S75 m samples.

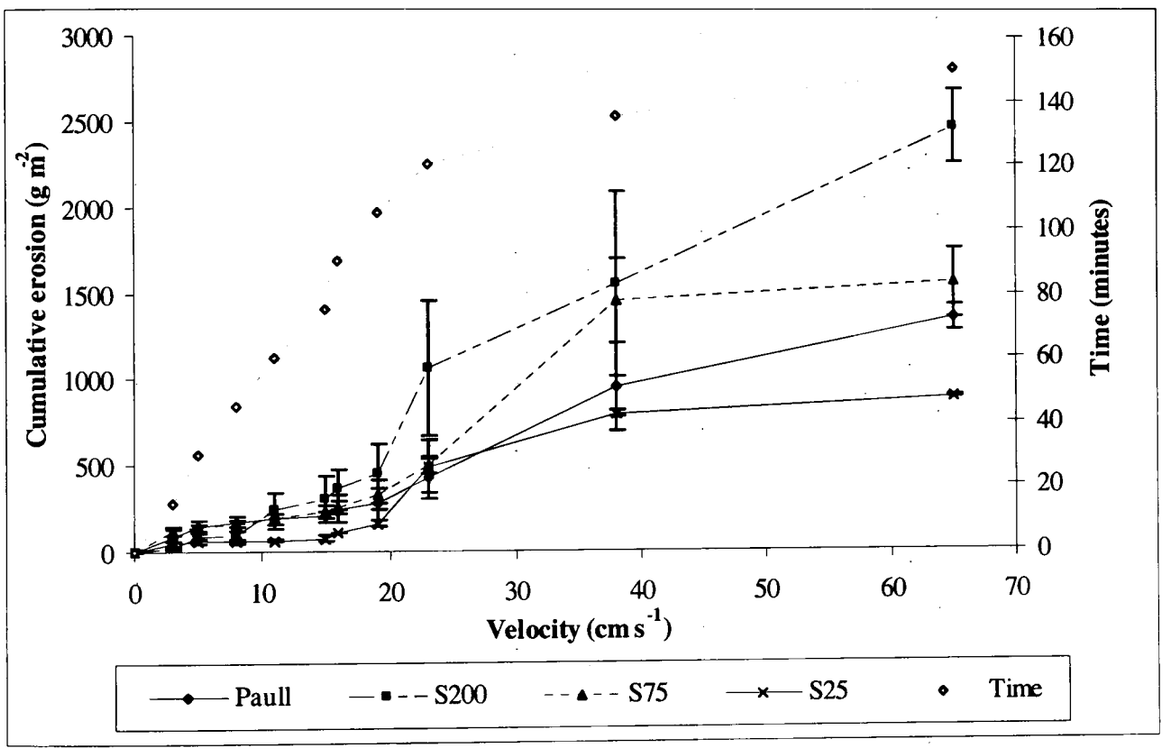


Figure 7.4. Cumulative suspended solid erosion (mean ± SE) (Filter data) profiles for the four key sites.

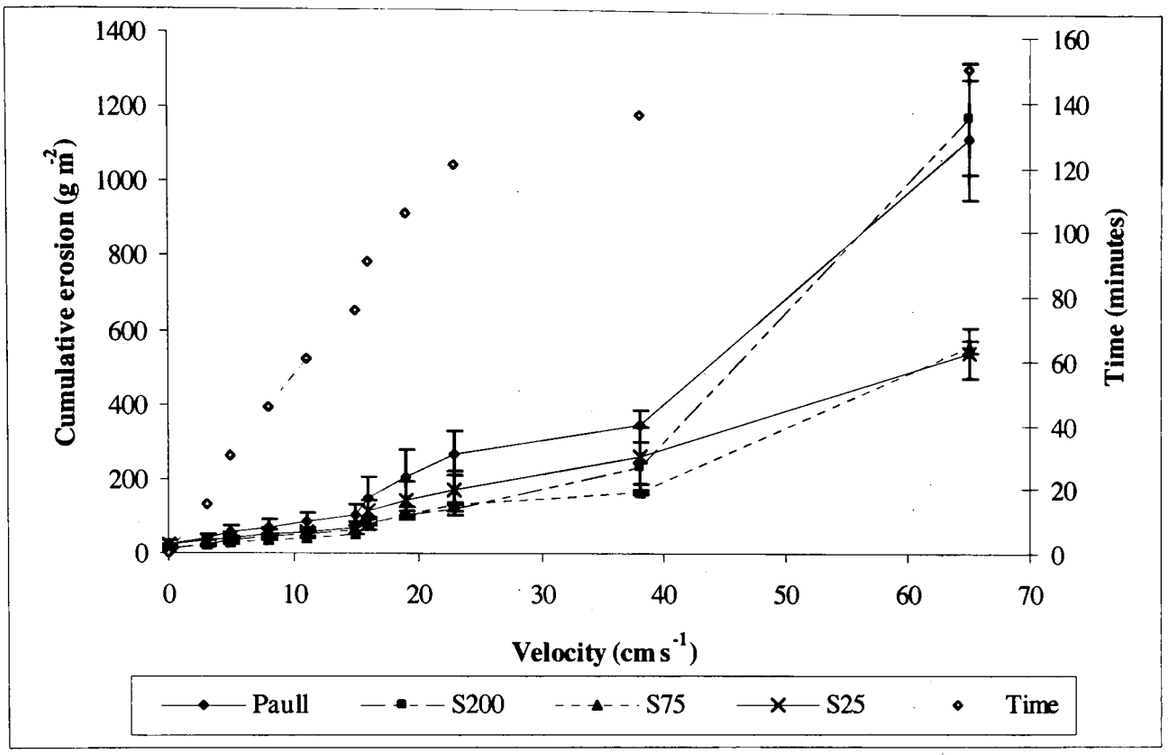


Figure 7.5. Cumulative bedload erosion profiles (mean ± SE) for the four key sites.

Maximum total erosion as suspended solids was recorded from the S200 m samples for both 135 and 150 minutes (38 and 65 cm s^{-1}) with total erosion of the Paull, S200 m and S75 m samples being considerably higher than that of the S25 m sample (Figures 7.6 and 7.7). However, one way ANOVA tests showed that these differences in erosion between samples were not statistically significant except for that between the S200 m sample and all others at 150 minutes ($p<0.05$). In terms of sediment transported along the bed of the flume, total erosion of the S200 m and Paull samples was considerably greater than that of the S75 m and S25 m samples ($p<0.05$) after 150 minutes but remained relatively similar between samples at 135 minutes with mean erosion of the S75 m samples being lowest.

Despite differences in erosion as bedload and suspended material generally not being statistically significant, it should be noted that erosion of the S25 m samples was consistently low and that the higher velocity during flume runs for the S75 m samples does not appear to have caused particularly high levels of erosion in comparison to the S200 m and Paull samples as would be expected if the characteristics of each sample were the same. Therefore, it may be inferred that the sediments at the S25 m and S75 m sites were less easily eroded than those at the S200 m and Paull sites. This is particularly apparent when the bedload transport data are examined.

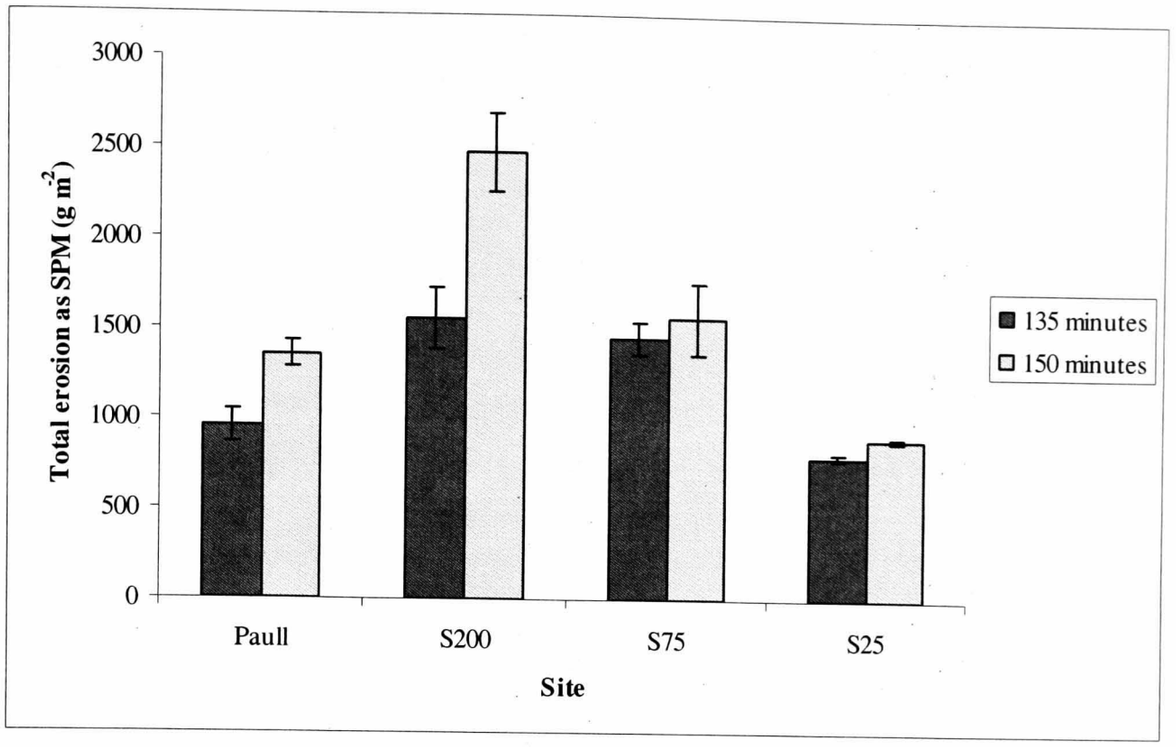


Figure 7.6. Total erosion (mean ± SE) as suspended solids (filter data) after 135 and 150 minutes.

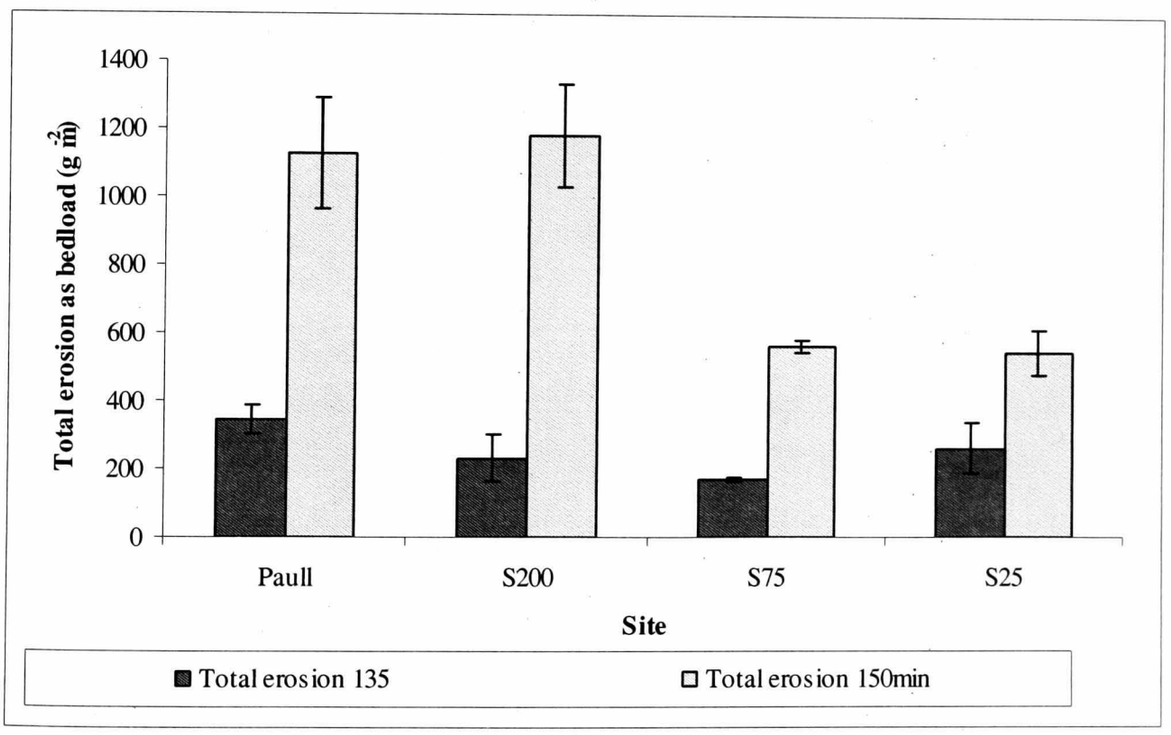


Figure 7.7. Total erosion (mean ± SE) as bedload after 135 and 150 minutes.

Erosion thresholds (as critical erosion velocity) were derived for SPM and bedload transport using regression analysis, as described in section 7.3.4. (Figure 7.8). The limited number of data points, together with the large incremental increase in current velocity made it difficult to fit the regression lines so that an accurate estimation of the erosion threshold could be made. Therefore, regression analysis was only carried out using mean data and statistical analysis to determine differences between the sites could not be carried out. However, Figure 7.8 indicates that the erosion thresholds for the S200 m and P150 m sites were slightly lower than those for the S25 m and S75 m sites for both suspended material and bedload transport. This was particularly the case for the P150 m site where considerable erosion (as bedload) initially commenced at 18 cm s^{-1} (initial threshold) with a second peak at 37 cm s^{-1} (second threshold).

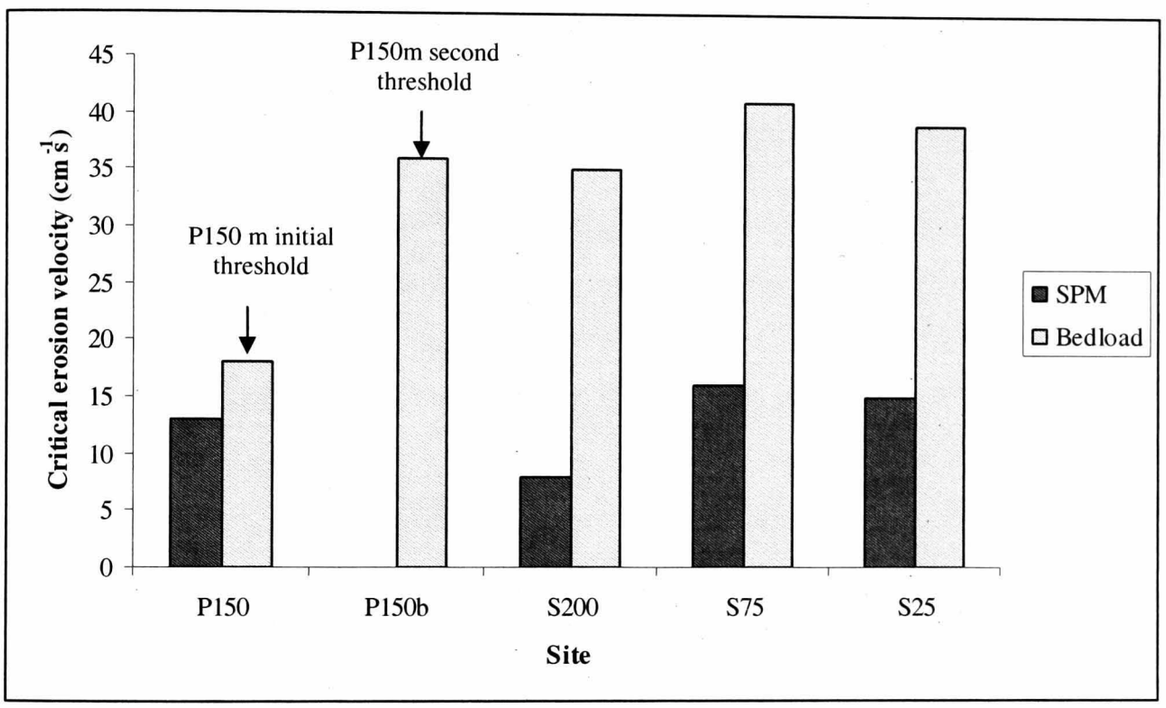


Figure 7.8. Critical erosion velocities for material transported as SPM and bedload (derived from mean erosion profile data, therefore SE could not be calculated).

All samples showed an initial pulse of surface sediment resuspension as the flow was initiated (3 cm s^{-1}, approximately 0.13 Nm^{-2}) (Figure 7.9). Following this initial resuspension, erosion rates decreased and, for the S25 m, S75 m and Paull samples, began to rise again steadily as the velocity reached 15 cm s^{-1} (approximately 0.56 Nm^{-2}). The S200 m sediment showed alternate increases and decreases in erosion rate as the velocity increased. Between 19 (approximately 0.7 Nm^{-2}) and 23 cm s^{-1} (approximately 0.8 Nm^{-2}), the rate of erosion of all samples increased sharply, the maximum increase being the S200 m sample, the erosion rate of which then decreased slightly before increasing to a maximum at 65 cm s^{-1} (approximately 2.3 Nm^{-2}). In contrast, the erosion rate of the S25 m sample declined as the velocity increased through 38 (approximately 1.4 Nm^{-2}) and 65 cm s^{-1}. The erosion rate of the Paull and S75 m

samples continued to increase to 38 cm s^{-1}, most noticeably for the S75 m sample although this was the point at which velocity was also high in comparison to all other samples. Erosion rates then declined as the velocity increased to 65 cm s^{-1}. It should, again, be noted that despite the high velocity during flume runs with the S75 m samples, the erosion rate at 65 cm s^{-1} decreased to a much greater extent than that of the Paull sediment. This reduction in erosion rate as the velocity increases to a maximum suggests that erosion of the fine material, which is easily resuspended, was coming to an end and that any further erosion would occur as bedload transport rather than as suspended solids.

There was no statistical difference (possibly due to the large standard error) in the rate of erosion (in terms of bedload transport) between samples at any velocity except at 65 cm s^{-1} where the erosion rate of the S200 m sediment was higher than that of all other sediments. As with the suspended sediment transport, erosion rate only really began to steadily increase as the velocity reached 15 cm s^{-1} (Figure 7.10).

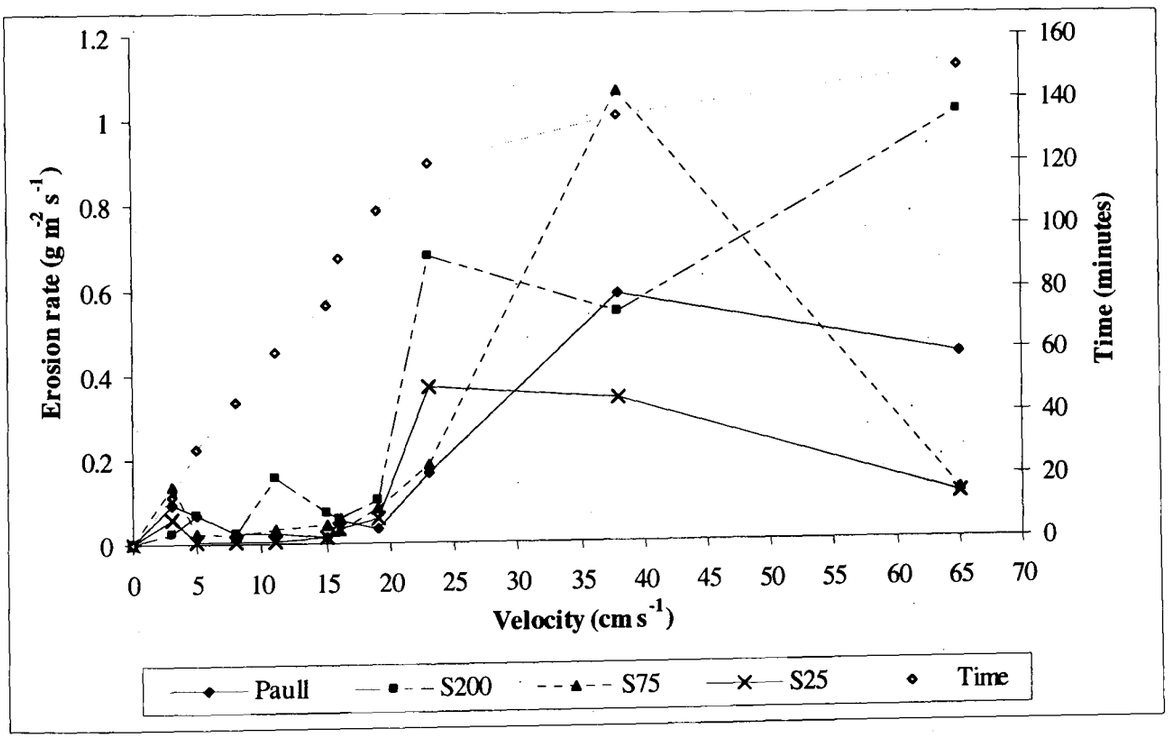


Figure 7.9. Erosion rates of sediment samples from the four key sites in terms of suspended solid transport (filter data) (SE omitted for clarity).

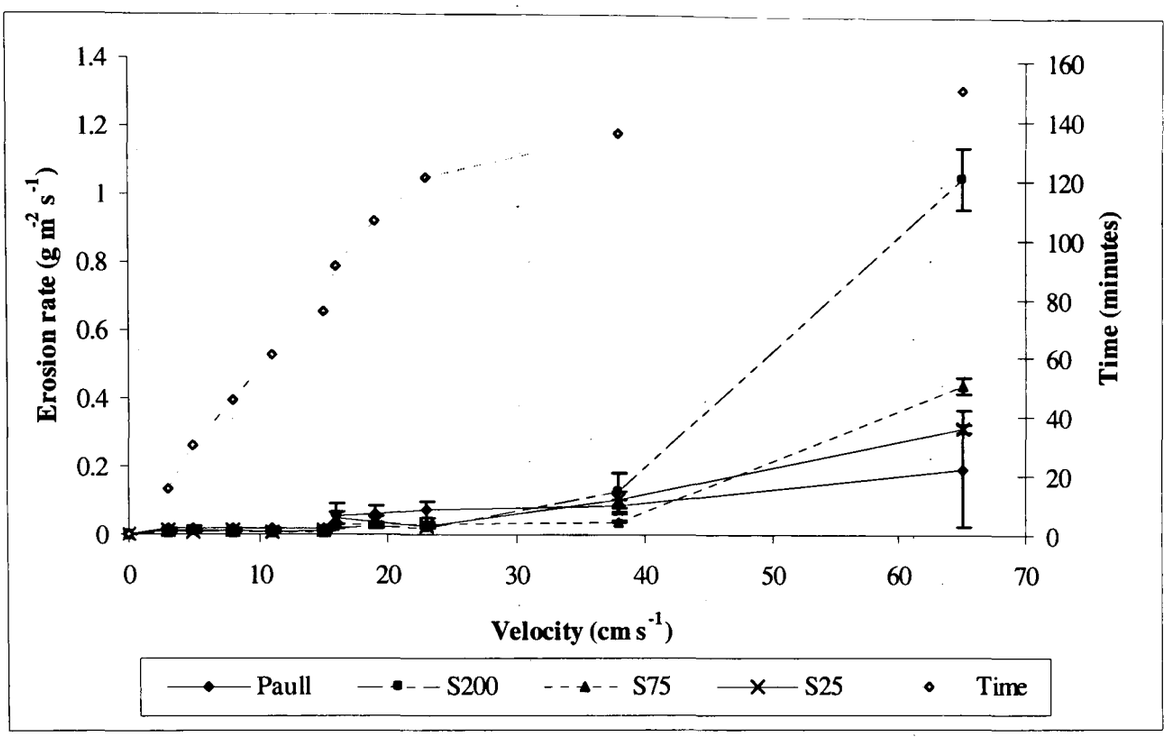


Figure 7.10. Erosion rates (mean ± SE) of sediment samples from the four key sites in terms of bedload transport.

7.4.2. CSM - field studies

Comparison of erosion profiles plotted for Paull and Saltend sites suggests that the sediments at the Paull (upper and mid shore sites) and the S200 m site were more easily eroded than those at the S75 and S25 m sites (Figure 7.11). Despite the large error bars associated with the S200 m and the P25 m data, statistical differences ($P<0.05$) were found between the three unpolluted sites (S200 m, P25 m and P150 m) and the S75 and S25 m sites for a number of shear stress values, particularly as erosion progressed. It can clearly be seen that erosion began at lower shear stress values at the two Paull sites and at the S200 m site than at the S75 and S25 m sites.

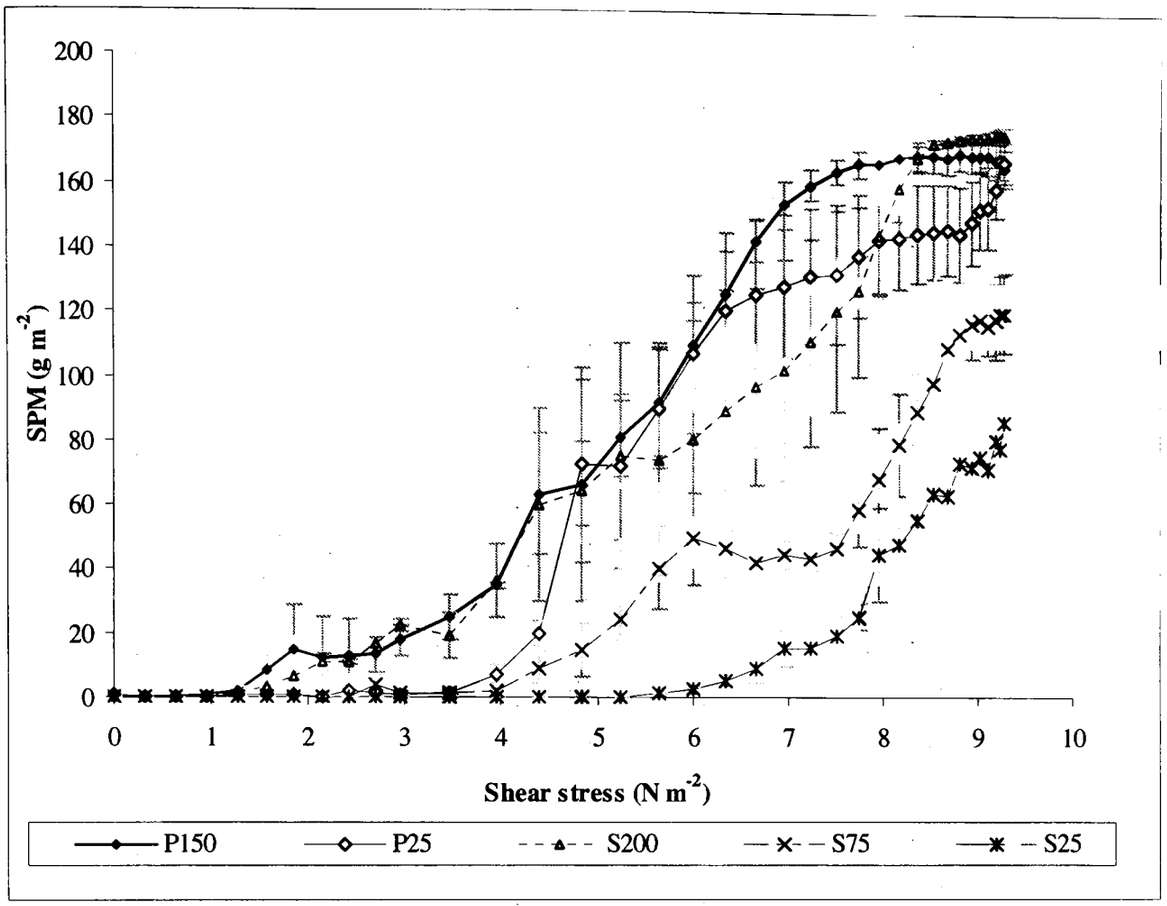


Figure 7.11. Erosion profiles (mean ± SE) for Paull and Saltend sediments.

In order to make a direct comparison between the erosion characteristics the different sediments, both critical shear stress and total erosion (expressed as g m^{-2}) at 9.29 N m^{-2} (i.e. the end of the test) were determined. Critical shear stress was defined using regression analysis as described in section 7.3.4 and the results of the analysis are presented in Appendix 10. Critical shear stress values for the S200 m, P150 and P25 m sites were 1.9, 2.4 and 2.7 N m^{-2}, respectively. These values were significantly ($p<0.05$) lower than those determined for the S75 and S25 m sites (4.4 and 6.0 N m^{-2}, respectively) (Figure 7.12). Furthermore, the total erosion was also significantly ($P<0.05$) greater at the S200 m and Paull sites with the lowest total erosion value being recorded from the S25 m site (Figure 7.13). The fact that these parameters do not differ statistically between the two Paull sites suggests that the differences in erosion characteristics of these sediments are not wholly attributable to shore height. However, the erosion threshold of the P25 m site was slightly higher than that of the P150 m site suggesting that the greater tidal height, and hence, drying time, may influence the erosion characteristics to some extent.

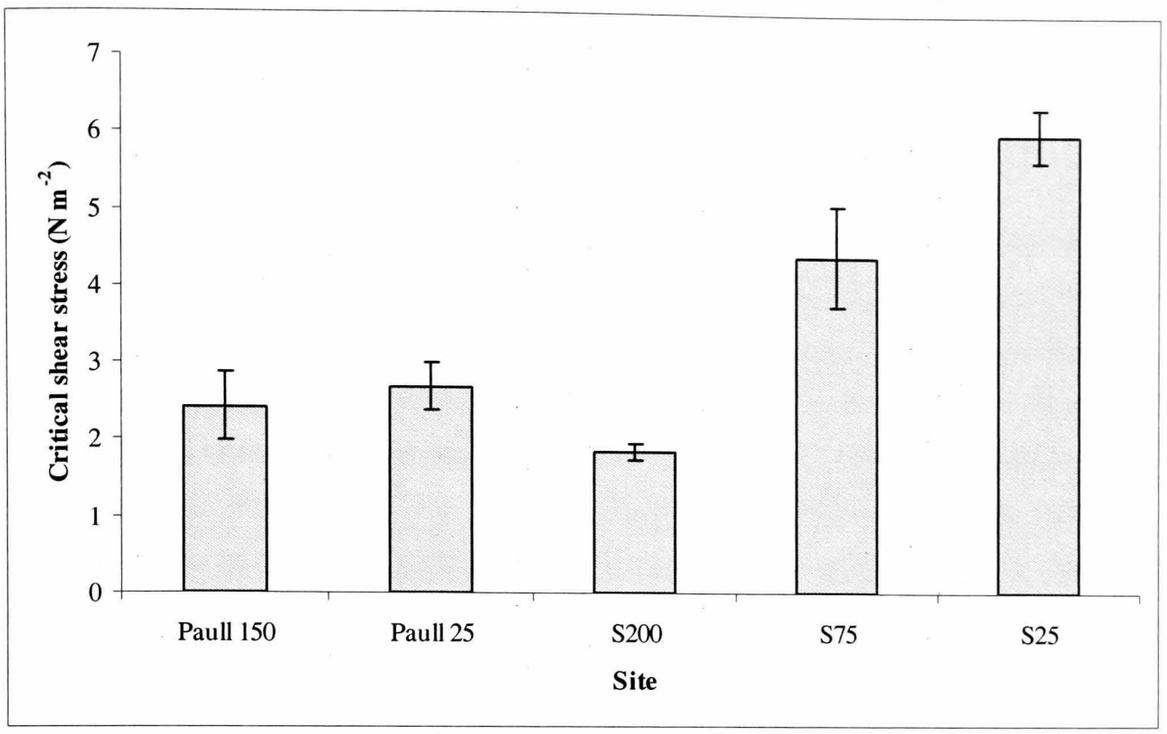


Figure 7.12. Critical shear stress (mean ± SE) of Paull and Saltend sediments.

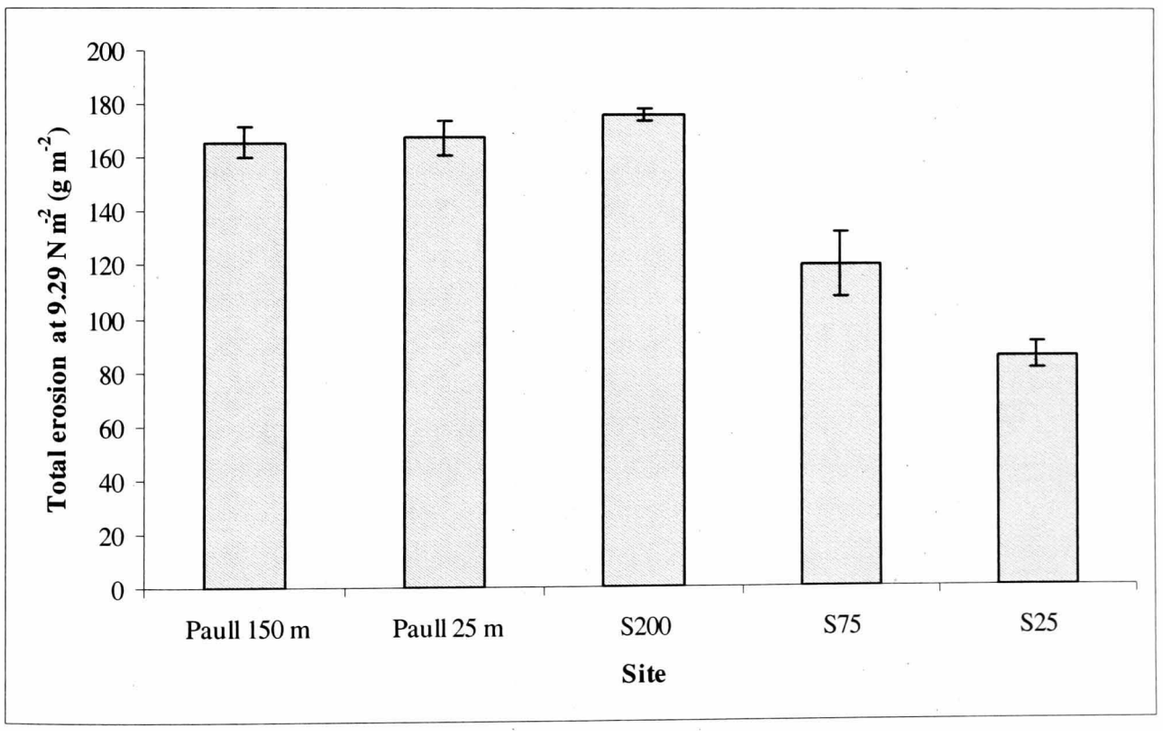


Figure 7.13. Total erosion (mean ± SE) at 9.29 Nm^{-2} of Paull and Saltend sediments.

Erosion rates (Figure 7.14 A-E) differed between sites with the maximum rate of erosion occurring at 4.4 N m^{-2} at the P150 and S200 m sites and 4.8 N m^{-2} at the P25 m site. Erosion rates at the two Paull sites showed a general (but not steady) pattern of decline following these peaks, becoming close to zero at approximately 8 N m^{-2}. Erosion at the S200 m site declined, following an initial peak, but increased to a second peak at 8 N m^{-2} before declining

to approximately zero at 8.6 N m^{-2}. Maximum erosion rate at the S75 m site occurred at 5.6 N m^{-2} with a second peak between 7.8 and 8.7 N m^{-2} before. The maximum erosion rate at the S25 m site occurred at 7.9 N m^{-2}, after which the pattern of erosion was erratic.

At the maximum applied shear stress, erosion at the S200 and P150 m had ceased and SPM concentration remained constant, although it is possible that had shear stress been increased further, a second pulse of erosion may have occurred (Figure 7.14). In contrast, erosion was still occurring and SPM was continuing to increase at the maximum shear stress for the S25, S75 and P25 m sites. The flume data indicated that as erosion as SPM decreased, erosion as bedload material increased rapidly. It is possible that whilst the CSM study indicated a decrease in erosion rate at the S200 m and P150 m site at the higher shear stress values, the bed may have begun to erode as large flocs or clods, rather than as a fine suspension. These flocs could either have been too heavy to become resuspended inside the CSM chamber or may not have caused a reduction in light transmission in the same manner as a suspension of fine material would. Erosion of this nature may therefore not be recorded by the CSM. Despite the large variability in the data, the results of the flume and the CSM experiments agree reasonably well in that both indicate that the erosion potential of the polluted S25 m sediments is less than that of the cleaner sediments at Paull and the S200 m site.

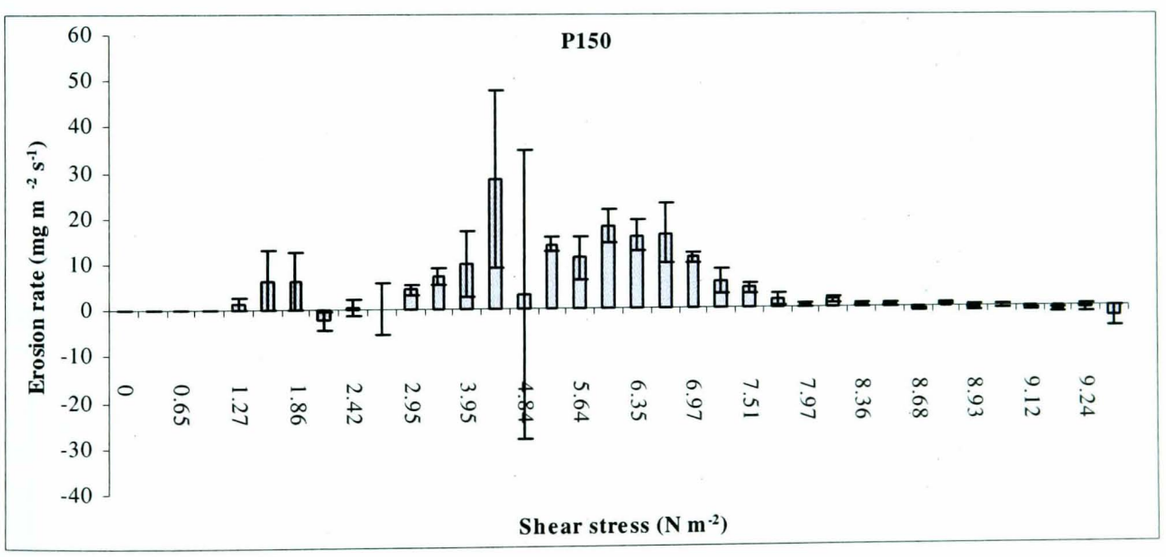
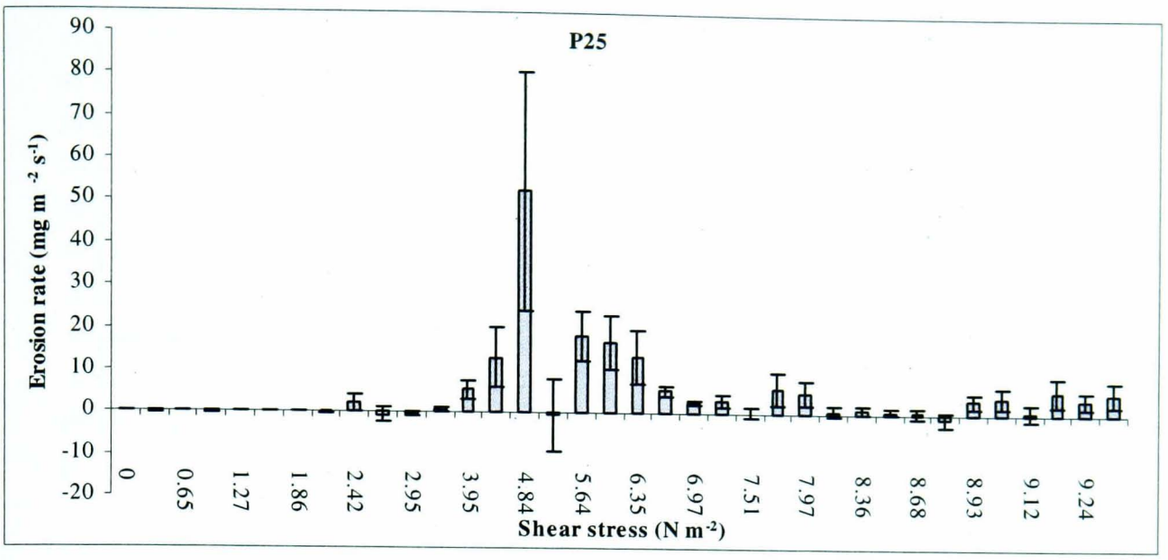
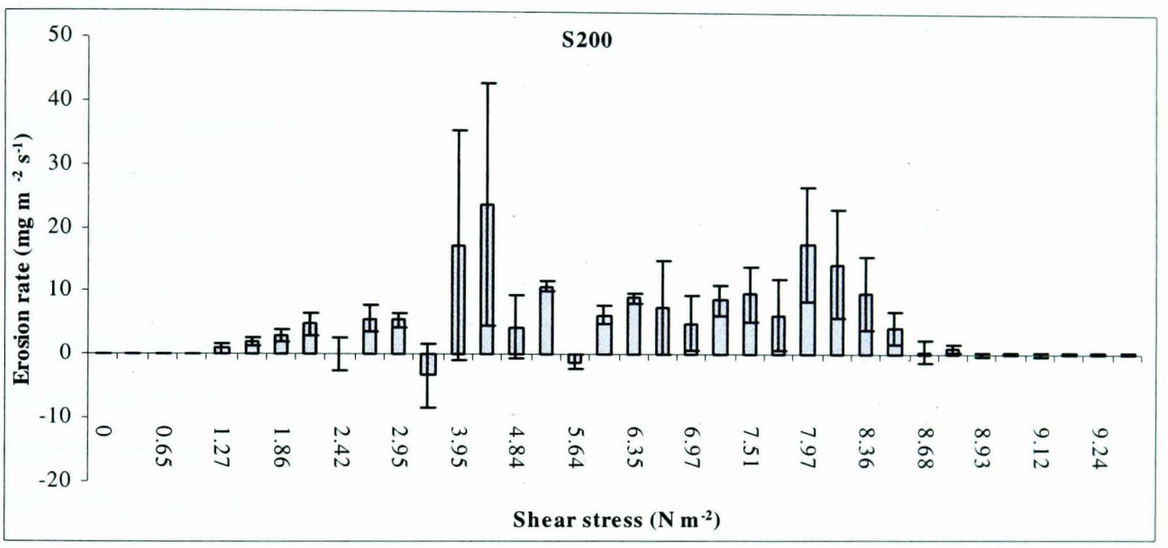


Figure 7.14. Erosion rates (mean ± SE) of Paull and Saltend sediments.





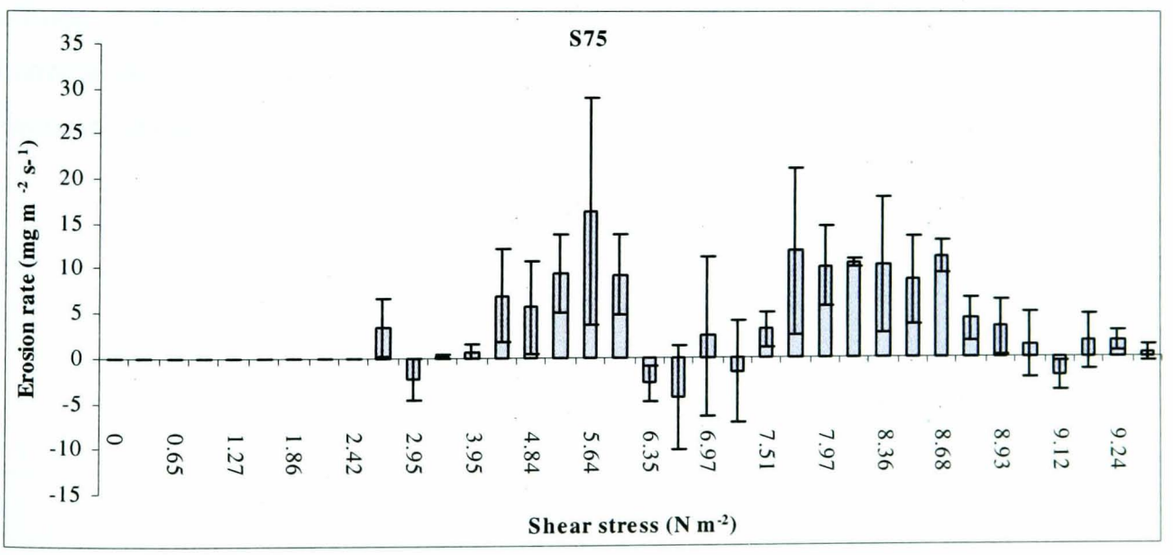


Figure 7.14 (cont).

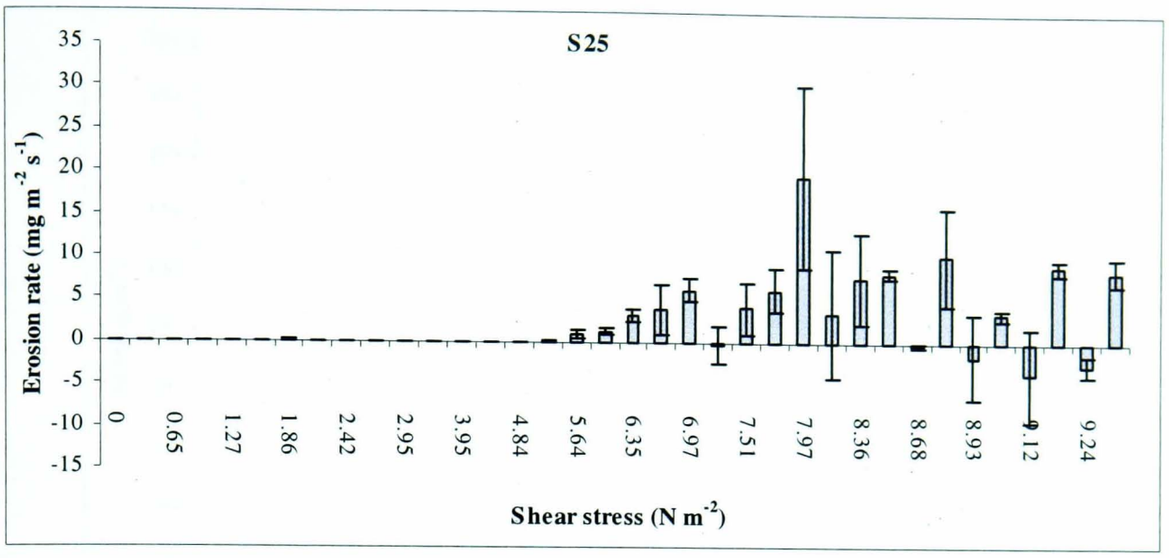


Figure 7.14 (cont).

7.4.3. CSM - laboratory studies.

Erosion profiles for *Hediste diversicolor*, *Macoma balthica* and *Corophium volutator* are compared to those of control (no animals) sediments at 0, 16 and 32% effluent concentration in Figures 7.15 – 7.17, respectively. Standard error bars have been omitted from these figures for clarity but are displayed in Appendix 11. Differences in the range of applied shear stresses are due the different test settings used. That is, the setting 'Sand 7' (0-6 N m^{-2}) was initially used until it was realised that at the maximum applied shear stress, erosion was still proceeding and it was decided that the setting 'Sand 9' with a range of 0-9.3 N m^{-2} would be more appropriate. There was a general pattern of decreasing erosion potential with increasing effluent concentration for all species, including the controls although statistical differences between the erosion profiles were not found. As with the field data, critical shear stress values were calculated using regression analysis (Appendix 11).

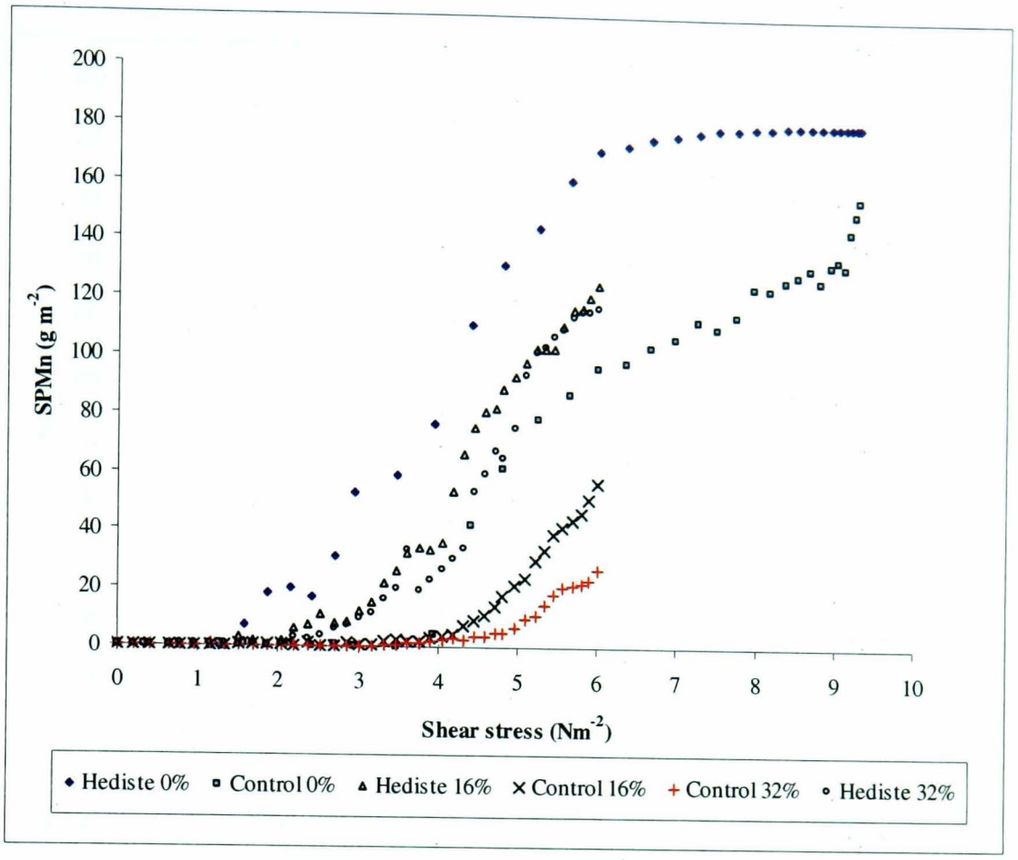


Figure 7.15. Comparison between the erosion profiles (mean ± SE) for *H. diversicolor* and controls at 0, 16 and 32% effluent concentration.

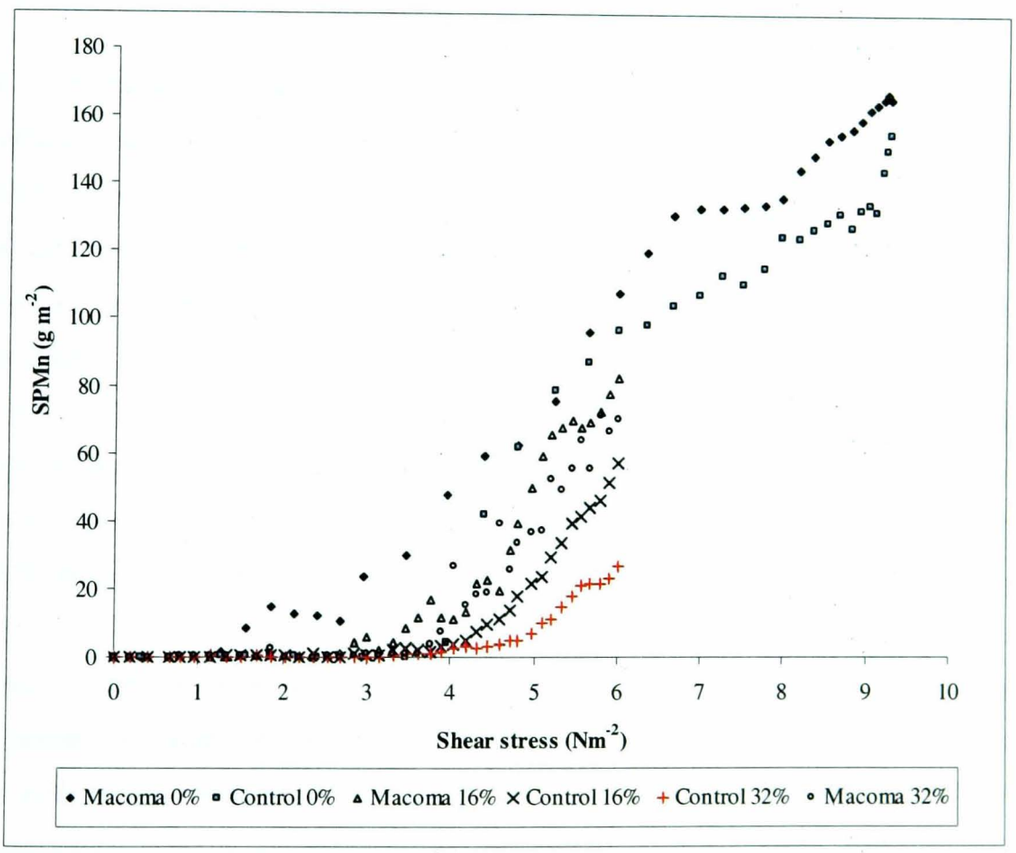


Figure 7.16. Comparison between the erosion profiles (mean ± SE) for *M. balthica* and controls at 0, 16 and 32% effluent concentration.

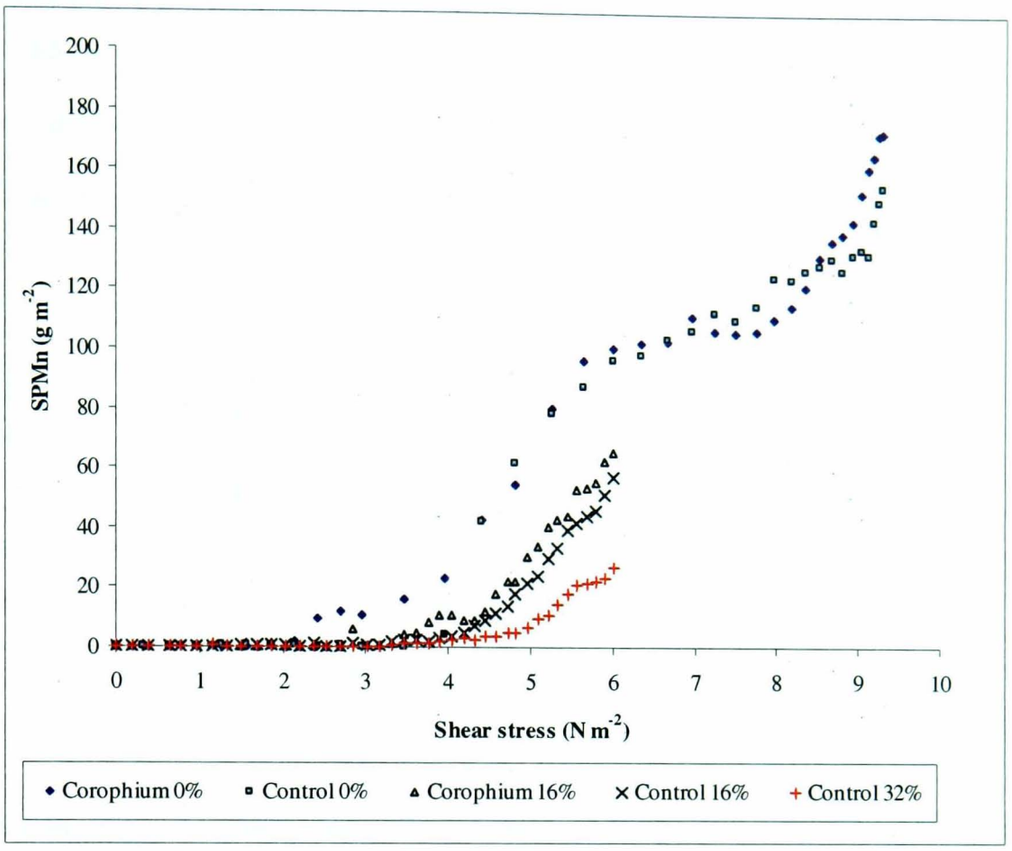


Figure 7.17. Comparison between the erosion profiles (mean ± SE) for *C. volutator* and controls at 0, 16 and 32% effluent concentration.

Figure 7.18 shows a trend of increasing critical shear stress with increasing effluent concentration, suggesting that pollution of this type may reduce erosion potential. However, statistical differences between concentrations were not found. It can also be seen that the critical shear stress for *H. diversicolor* was consistently lower (i.e. greater erosion potential) than that for all other species and differed significantly from the controls at all effluent concentrations. Critical shear stress values for this species ranged from 1.6 N m^{-2} at 0% to 2.7 N m^{-2} at 32% effluent concentration in comparison to 2.9-4.04 N m^{-2} for the controls for the same effluent concentrations. Critical shear stress values for *M. balthica* and *C. volutator* were also lower (although not statistically significantly) than those for the controls with values for *M. balthica* being consistently lower than those for *C. volutator*.

Total erosion was reduced with increasing effluent concentration for all species although, again, statistical differences were not found between concentrations (Figure 7.19). The highest total erosion value was recorded from sediments containing *H. diversicolor* at all concentrations. Erosion of sediments containing *M. balthica* and *C. volutator* was only greater than that of the controls at 16 and 32% effluent concentration. One way ANOVA tests showed that statistically significant differences between treatments only existed at the 32% effluent concentration.

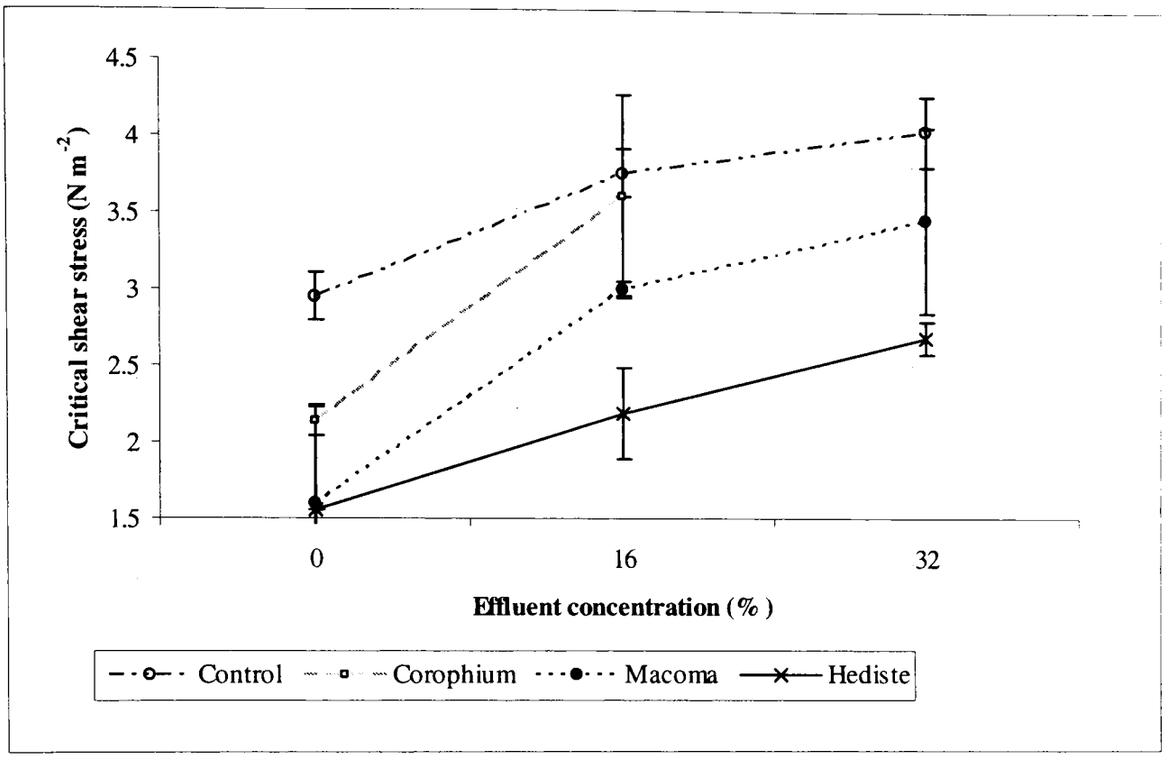


Figure 7.18. Critical shear stress values (mean ± SE) for *H. diversicolor, M. balthica, C. volutator* and controls at 0, 16 and 32% effluent concentration.

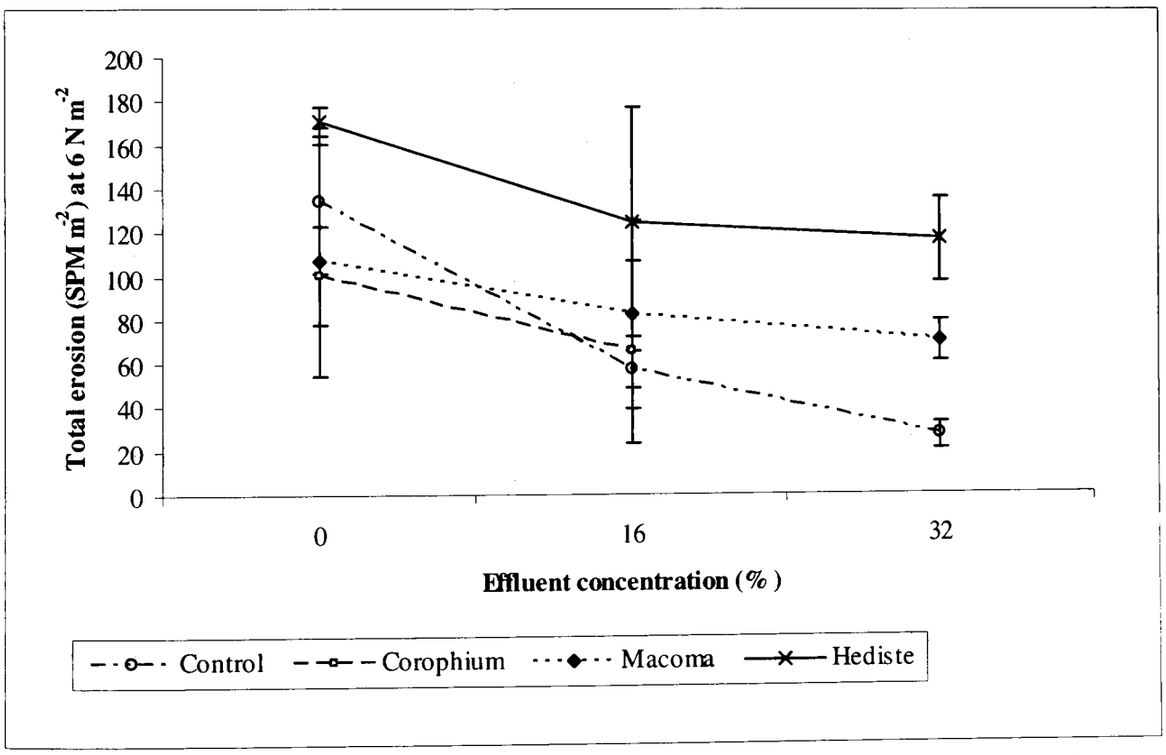


Figure 7.19. Total erosion at 6 Nm^{-2} (mean ± SE) for *H. diversicolor, M. balthica, C. volutator* and controls at 0, 16 and 32% effluent concentration.

Erosion rates were erratic and no obvious patterns between species or concentrations could be seen. These figures have therefore been excluded from the text but can be found in Appendix 12. However, there was an indication that the peak erosion rate occurred at lower shear stress values for the 0% effluent concentration than for the 16 and 32% concentrations.

7.4.4. Shear vane measurements.

As would be expected given the reduction in water content, sediment shear strength, as defined using a shear vane, increased with depth. The July data for Saltend (Figure 7.20) show that this increase in shear strength was greatest at the upper shore sites (S50, S25 and S0 m) and that this difference becomes more pronounced with increasing depth. In general, shear strength at the low shore sites (S200 - S100 m) was significantly ($p<0.05$) lower than that at all other sites at all depths below the top 2-4 cm. Shear strength at the S25 and S0 m sites was consistently higher than at all other sites, at all depths ($p<0.05$). The same pattern was found at Paull with the greatest increase in sediment stability with respect to depth being at the upper shore sites (P50 and P25 m). However, no statistical difference in sediment shear strength between sites was found until a depth of 16 cm was reached. In general, sediment shear strength at the Paull sites was greater ($P<0.05$) than that at the S200, S150 and S100 m sites and, at depths of 30 and 50 cm, was also greater than that at the upper shore Saltend sites.

The March data (Figure 7.21) also showed the same trend of increasing sediment shear strength with depth with statistically significant differences between sites being found at all depths ($p<0.05$). To a depth of 18 cm, the highest values were recorded from the Paull site, after which values for this site were lower than at all other sites. Sediment shear strength at the S200 m site was consistently lower than that at the other Saltend sites and there was an apparent trend of increasing shear strength with increasing shore height.

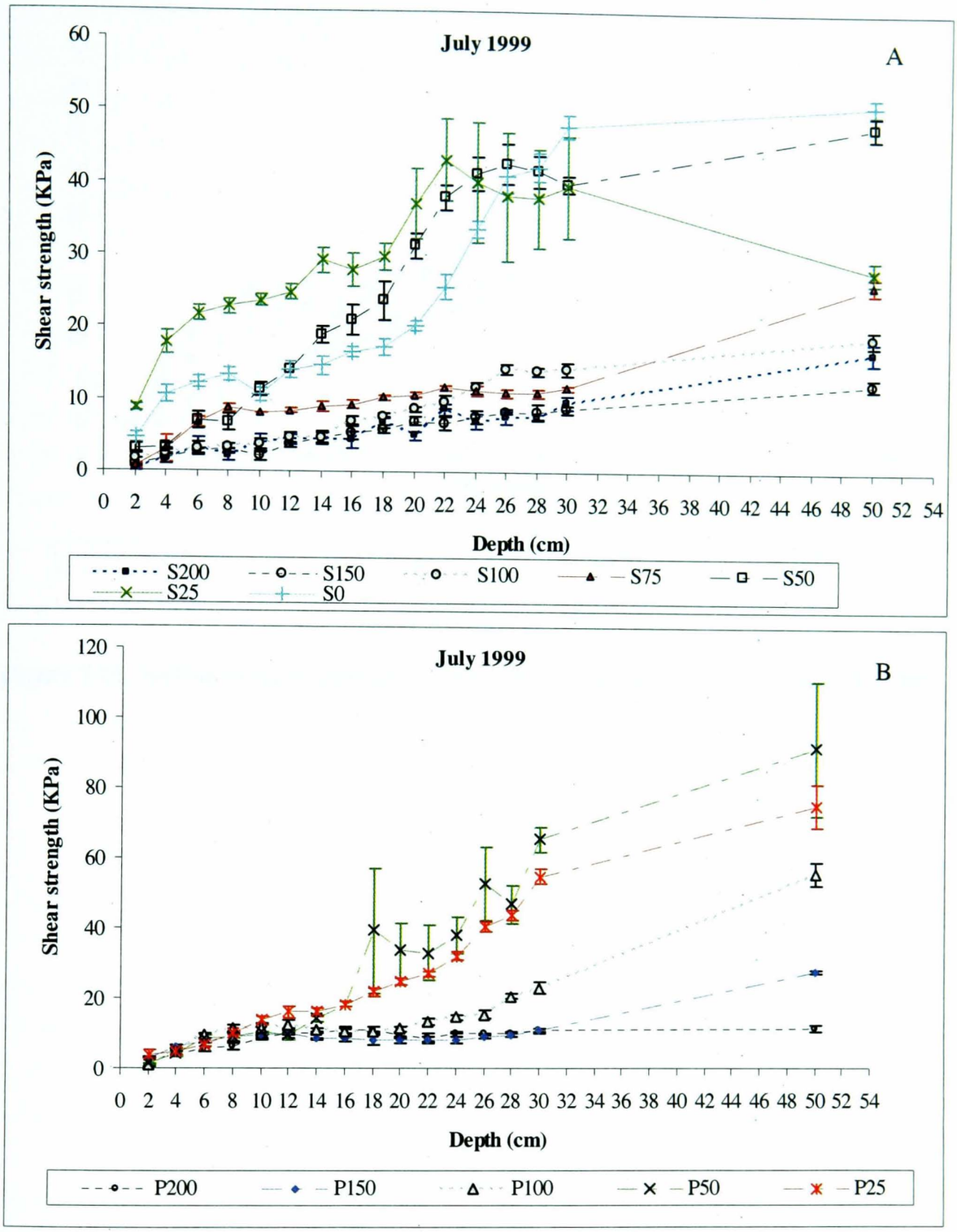


Figure 7.20. Sediment shear strength (mean ± SE) at Saltend (A) and Paull (B) in July 1999.

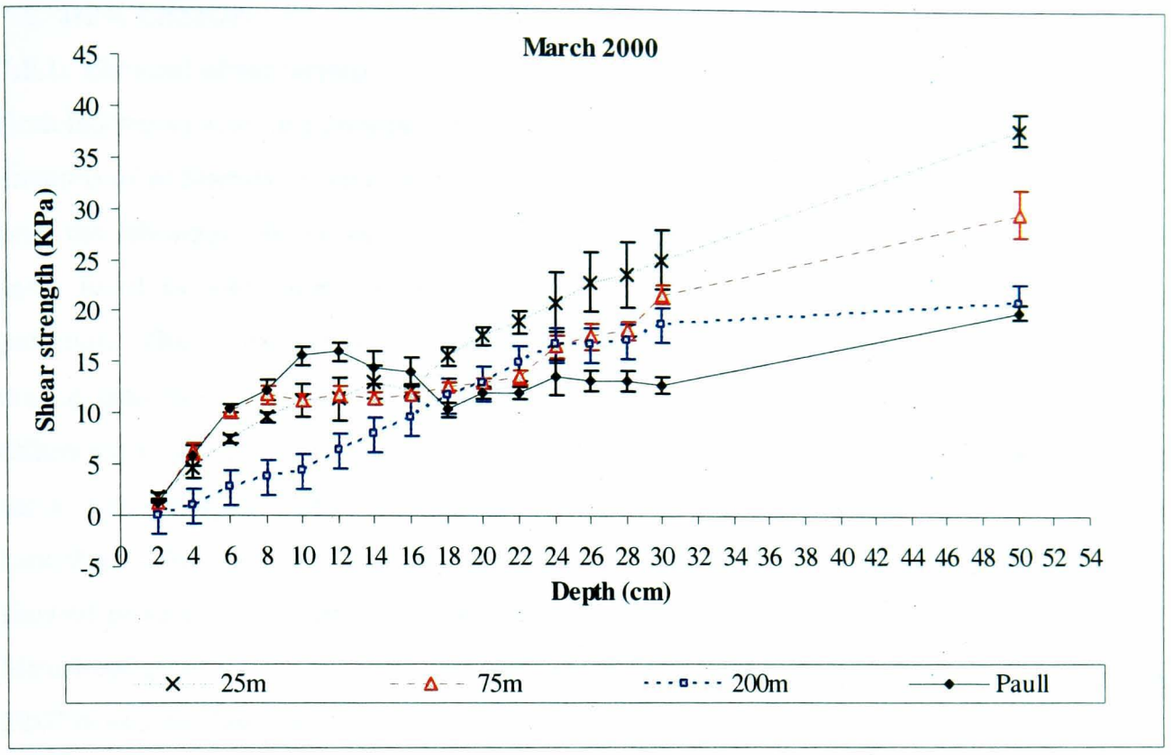


Figure 7.21. Sediment shear strength (mean ± SE) at the four key sites in March 2000.

7.5. DISCUSSION

7.5.1. General observations

Both laboratory and field studies have indicated differences in the erodibility (in terms of total erosion) of sediments at different sites on the Paull and Saltend mudflats. Moreover, use of both the laboratory flume and the CSM (deployed in the field) produced data showing the same trend of increasing erosion potential with increasing distance from the source of pollution. That is, the sediments at the S200 m and the Paull sites appeared to be more easily eroded than those at the more polluted S25 m and S75 m sites. The fact that there was no difference in the erodibility of the sediments at the P25 m and P150 m (i.e. upper and mid shore sites) suggests that the erosion potential of these sediments was attributable to something other than shore height alone. Examination of erosion rates (using the CSM data) showed erosion to commence at higher shear stress values at the S25 m and S75 m sites. Maximum erosion rate at these sites also occurred at higher shear stress values than at the S200 m and the Paull sites.

Further laboratory studies examining the influence of both species and effluent concentration on sediment erosion revealed that sediments containing animals were more easily eroded than those without at all effluent concentrations and that increasing effluent concentration caused a reduction in erosion. Critical shear stress values for sediments containing *Hediste diversicolor* were consistently lower than those for sediments containing *Corophium volutator* or *Macoma balthica*. The opposite was demonstrated by the total erosion values with the maximum amount of material being eroded from sediments containing *H. diversicolor* and minimum erosion occurring in the control sediments (no animals). Whilst differences were found between species, only *H. diversicolor* appeared to have a statistically significant impact on sediment stability. However, animal densities were chosen to represent typical field densities at Paull (i.e. unpolluted conditions) and therefore the number of *H. diversicolor* placed in each core was considerably larger (45) than the number of *M. balthica* (5) or *C. volutator* (30). Had the number of individuals used been equal between species, it is likely that a greater impact of *M. balthica* and *C. volutator* would have been noted. However, the aim of the present study was to assess the impact of pollution induced community changes at Paull and Saltend on sediment erosion potential. That is, to assess the impacts of the species present within these mudflats and the effects of pollution-induced changes to behaviour or removal of the species on the sediment properties. It was therefore considered appropriate to simulate field densities of the main species present. It is also accepted that the process of sieving and re-settlement of the sediment will have affected its erosion properties.

It was also demonstrated that, in the absence of any macrofaunal influence, the effluent had potential to stabilise the sediments. Attempts were made to further validate this by comparing the erodibility of aerobic and anaerobic sediments using the laboratory flume although no differences were found between samples. However, Montague (1984) and Mehta (1989) highlighted the difficulties associated with attempting to create and maintain anoxic conditions in laboratory sediments (particularly in the surface layers). Whilst attempts were made to defaunate the sediments with minimum disturbance to the sediment structure (i.e. using boiling water rather than sieving followed by resettlement), it is thought that the process of macrofaunal removal may also, in part, explain why significant results were not obtained.

The shear vane data showed a general trend of increasing sediment shear strength with increasing shore height. This suggests that the erosion potential of the sediment at these sites should show some relationship with shore height. However, whilst the shear vane was effective at measuring the shear strength of sub-surface sediments (e.g. below 5-10 cm), the precision of the measurements made at the surface is questionable. This is because the vane (approximately 5 cm long) needs to be fully inserted into the sediment in order to obtain a precise measurement. In addition, although the precision of the instrument could be increased by using different sized vanes for sediments with different shear strengths, the resolution was not sufficiently high to detect more subtle differences within the unconsolidated surface layers. It is these layers upon which the eroding forces of the CSM or the flume act and it is therefore not surprising that the sediment shear strength data and the erosion data do not follow the same trends.

The physical properties of the sediment (in terms of water and organic content, particle size and bulk density) do not consistently differ between sites suggesting that differences in the erodibility of the different sediments were primarily caused by:

- Differences in the animals communities present and / or
- Differences in the microalgal and carbohydrate content of the sediment;
- Differences in the redox conditions and the resultant impact on the microbial communities;
- Changes to the cohesive and adhesive properties of the sediment caused by the petrochemical nature of the discharge (i.e. the presence of oils).

These factors, their interaction and their overall effect on sediment stability will be discussed in greater detail in Chapter 8. The present section will focus on the performance of the

erosion devices used, the validity of the data generated and the methods used to determine erosion rates and erosion thresholds.

7.5.2. Comparison with other studies.

Erosion thresholds (as critical shear stress values) determined during the present study (using the CSM) ranged from 1.9 N m^{-2} at the S200 m site to 6 N m^{-2} at the S25 m site. Examination of data collected during a range of other studies suggests that whilst there is great variability in erosion threshold values between sites and between studies, these values are within the range of those recorded by other authors who used the CSM. For example, Watts *et al.* (2003) examined the stability of recently accreted sediments (using the CSM) at the Tollesbury managed realignment site following 6 years of regular tidal inundation. Critical shear stress values (τ_{0crit}) ranged from 1.53 – 4.28 N m^{-2} with the lowest values being associated with the lower shore sites and the highest values being associated with areas of saltmarsh or rapidly draining gullies. Yallop *et al.* (1994) recorded a τ_{0crit} value of 1.2 N m^{-2} from intertidal sandy sediments on the island of Texel in the Wadden Sea whilst Underwood & Paterson (1993a) recorded values of 1-2.9 N m^{-2} from intertidal muddy sediments in the Severn estuary. Tolhurst *et al.* (2000a) recorded erosion threshold values of 0.19-2.3 N m^{-2} from sediments in the Sylt-Rømø Bight.

With regard to the flume data, erosion thresholds have been determined in several ways making comparison between studies difficult, a problem which is discussed in section 7.5.3.2. For example, Madsen *et al.* (1993) and Grant & Daborn (1994) both recorded the erosion threshold as critical shear velocity (u_{*crit}), using a linear, recirculating laboratory flume with values being 2.8 and 2.1 cm s^{-1}, respectively. These values correspond to shear velocities (u_*) calculated from velocity profiles for flume pump settings 5-8 (current velocity 16-27 cm s^{-1}) in the present study (Chapter 6). Bed shear stress values for current velocities within this range were between 0.4 and 0.9 N m^{-2}. Widdows *et al.* (1998c) used an annular, field based, flume and recorded critical erosion velocities of between 21.8 (upper shore area) and 30.8 cm s^{-1} from sediments on the Skeffling mudflat Humber estuary) and Widdows *et al.* (2000) recorded critical erosion velocities of 14-40 cm s^{-1} from the same area. In the present study, critical erosion velocities (determined using regression analysis) ranged from 8-16 cm s^{-1} (S200 m and S 75 m, respectively) for material transported as suspended particulate matter and 18-41 cm s^{-1} (P150 m and S75 m respectively) for material transported as bedload. Bed shear stress values for current velocities within this range are estimated (using Figure 6.7) to be 0.3-0.6 Nm^{-2} for material transported as suspended particulate matter and 0.67-1.5 Nm^{-2} for material transported as bedload. Again, these values are comparable to those recorded in other studies and it can therefore be assumed that the performance of the flume used in the

present study was satisfactory. For example, Austen *et al.* (1999) studied the erosional properties of sediments in the tidal area between the islands of Sylt and Rømø (northern Wadden Sea), using the EROMES device (Schünemann & Kühl, 1991) and reported critical shear stress values of 0.16-3 N m^{-2}. Riethmüller *et al.* (2000) reported similar values (using the EROMES device) of 0-2.5 N m^{-2} from intertidal areas of the Wadden Sea. Ruddy *et al.* (1998) recorded critical shear stress values of 1.4 - 2 N m^{-2} at Skeffling, using a laboratory based annular flume (miniflume). de Dekere (2003) reported erosion threshold values of 0.1-0.6 N m^{-2}, depending upon the time of year, from intertidal areas in the Ems-Dollard estuary using the ISEF (In-Situ Erosion Flume) device (Houwing & Van Rijn, 1998).

Maximum erosion rates calculated (using the laboratory flume) during the present study ranged from 0.4 g m^{-2} s^{-1} (S25 m) to 1.1 g m^{-2} s^{-1} (S75 and S200 m) for sediment eroded as SPM and 0.2 g m^{-2} s^{-1} (P150 m) to 1.05 g m^{-2} s^{-1} (S200 m) for sediment eroded as bedload. *In-situ* studies using the CSM gave maximum erosion rates of 0.02 g m^{-2} s^{-1} (S25 m and S75 m) to 0.05 g m^{-2} s^{-1} (P25 m). Again, these values are within the range of those recorded by other authors. For example, Widdows *et al* (1998c) and Widdows *et al.* (2000) reported erosion rates of 0.049-1 g m^{-2} s^{-1} and 0-0.3 g m^{-2} s^{-1} (respectively) for sediments at Skeffling, subjected to a maximum current velocity of 50 cm s^{-1}, using the *in-situ* annular flume. These values are consistent with those recorded by Amos *et al.* (1998) again, from Skeffling, using the Sea Carousel (Amos *et al.,* 1992a) (maximum current velocity of 70 cm s^{-1}). Tolhurst *et al.* (2000a; 2000b) reported erosion rates of 0-0.15 g m^{-2} s^{-1} for sediments in the Sylt-Rømø Bight. Comparison with other studies indicated that the flume used in the present study gave erosion rates an order of magnitude higher than those given by other devices. However, both SPM concentration and erosion rate were corrected to account for the total volume of water in the flume. It is not clear whether this was done by other authors and this could possibly explain the comparatively high erosion rates calculated in the present study.

In-situ studies carried out by Widdows *et al.* (1998a) at Skeffling showed that following each incremental increase in current velocity, above the erosion threshold, there was a rapid increase in sediment resuspension with maximum erosion rates occurring within one or two minutes of the stepwise increase in current velocity. At lower current velocities, (<37 cm s^{-1}), the suspended sediment concentration reached a quasi-steady state within 5 minutes of the change in current velocity. This was described as Type I erosion. At higher velocities, there was not sufficient time to reach a steady state and erosion rates were variable but continuous. This was described as Type II erosion. Examination of the data in the present study suggests that Type I erosion occurs at all sites but that the degree of Type II (mass) erosion may be higher at the less polluted sites (S200 m and the Paull sites). This is indicated by the higher

bedload erosion rates and the greater total mass of sediment eroded. The increased shear strength at depth (as indicated by use of the shear vane) also suggests that Type II (or bedload) erosion may be lower at the more polluted sites.

7.5.3. Sources of error.

7.5.3.1. Sampling and experimental techniques.

Whilst the results of both the flume and the CSM studies were comparable with those of other studies, there are a number of factors associated with the design of the devices and the way in which they are used which can influence these results. It should, however, be noted that these problems are associated with all studies and not just the present one.

Firstly, using a laboratory based flume necessarily involves the removal of sediment samples from the field and their transport to the laboratory and subsequent transfer to the flume. Tolhurst *et al.* (2000b) examined the differences between the erosion thresholds of sediments tested by a number of different devices. Comparison between the results generated using an 8 m linear recirculating laboratory flume (Armfield Ltd.) and those using the *in-situ* Sea Carousel device showed the laboratory flume to consistently give considerably higher erosion thresholds for sediments taken from the Skeffling mudflats (Humber estuary). Differences in critical shear stress values between laboratory and field measurements arose principally as a result of physical disturbance during sampling and transportation (vibration, compaction and water loss) and ongoing or changes in biological activity. Black & Paterson (1997) also highlighted the differences between laboratory and field measurements and found that following collection and transportation of the samples, visual examination showed large oval shaped depressions in the sediment surface, approximately 3 mm deep. This indicated that settlement had occurred, possibly due to vibrations during transportation. Vibration could cause dewatering and compaction although in some cases, burrow collapse and fluidisation could occur (Tolhurst *et al.*, 2000b). Prolonged exposure to the air could also result in an increase in EPS production by diatoms and infaunal organisms, to prevent desiccation (Tolhurst *et al.*, 2000b) and bioturbation by infaunal organisms may also be reduced (Widdows *et al.*, 1998), thus leading to increased stability of laboratory samples in comparison with field sediments.

However, Tolhurst *et al.* (2000b) transported sediment samples between Skeffling, on the Humber estuary, and St Andrews, in eastern Scotland, and sediment samples would have been exposed to the air for a considerable length of time (several hours). In the present study, samples only had to be transported a few miles from the field to the laboratory and were generally placed in water within 4-5 hours of collection. Tidal exposure of sediments from

mid and upper shore sites would be expected to last for this length of time. Therefore, whilst stabilisation as a result of vibration and subsequent compaction and dewatering and changes in biological activity would be expected to some degree, the effects of transportation were probably not as great as those observed by Tolhurst *et al.*(2000b). Furthermore, a study comparing the laboratory based EROMES device (Schünemann & Kühl, 1991) with the field based CSM, where transportation time and disturbance during transport were minimal, Tolhurst *et al.*(2000b) found only slight differences in the results generated by the two devices.

During the present study, the erosion thresholds of sediments measured in the laboratory flume were lower than those of sediments measured in the field (based on estimated values of critical shear stress for the flume samples). In this case, the effects of sample storage and introduction to the flume need to be considered. Whilst attempts were made to carry out erosion measurements as quickly as possible following collection of the samples, the storage of samples for up to 72 hours was unavoidable due to the fact that only one sample could be placed in the flume at any one time, the time taken for the samples to settle in the flume and the time taken to complete each erosion run. According to Tolhurst *et al.* (2000b), the increased resting time can lead to increased compaction, increased microalgal growth and increased EPS production, all leading to the increased stability of the stored samples. Here it is argued that the effects of the disturbance associated with the transfer of the samples to the flume used in the present study may override these effects leading to decreased stabilisation. In addition, the samples were stored in water. Whist attempts were made to simulate tidal inundation and exposure during low water, drying of the surface sediments is unlikely to have taken place to the same degree in an aquarium (at a constant temperature of 10°C) as it might have done on the tidal flats exposed to higher temperatures, wind and direct sunlight. Tolhurst *et al* (2000a) also found that critical shear stress values generally increase as the size of the test section decreases since surface features such as ripples or faecal mounds can easily (and subjectively) be avoided when devices with small test sections are used.

Tolhurst *et al.* (2000a) demonstrated that erosion thresholds were highly affected by the size of the incremental increases in current velocity over the duration of erosion run. Small scale and large numbers of shear stress steps mean that SPM values will increase gradually and could make determination of the erosion threshold difficult. That is, the point at which erosion increases significantly will be more difficult to define from a smooth, gradual curve than from one where the change in gradient of the slope is more obvious. In contrast, large steps, such as those used in the flume could result in missing the actual erosion threshold and the calculated threshold could end up being greater than the actual threshold. Due to the

design of the flume used in the present study and the available equipment, the large increases in current velocity between pump settings were unavoidable. It was therefore considered more appropriate to examine trends in erosion patterns rather than attempt to calculate precise erosion threshold values (although an indication of these values was given). Tolhurst *et al.* (2000a) also indicated that extended measurements at each shear stress can result in weakening of the bed resulting in lower critical shear stress values. Furthermore, the length of time of exposure to water may reduce the erosion threshold. It was concluded that the behaviour of the sediment may be dependant upon the history of the shear stress applied to it.

Spatial variation of sediment surface features should also be considered as an important influence over sediment stability since features such as ripples, burrows and faecal mounds may increase turbulence at the bed so that the erosion threshold of such sediments may be lower than that of a sediment with a smooth, flat surface (Tolhurst *et al.*, 2000a). The effect of the test section area of the erosion device on erosion thresholds has already been mentioned and it should be pointed out that the biased selection of field sites with smooth surfaces is easier when using a device with a small test section. During the present study, attempts were made to avoid this bias by choosing sampling sites at random although the small test section of the CSM meant that it was difficult to account for surface heterogeneity. Whilst the success of the CSM in terms of its ability to demonstrate the stabilising effects of diatom mats and their associated mucillage secretions has been proven by various authors (e.g. Underwood & Paterson, 1993a; 1993b; Yallop *et al.*, 1994; Underwood & Smith, 1998; Austen *et al.*, 1999; Blanchard *et al.*, 2000; Tolhurst, 2000; deDekere, 2003), it is suggested that a larger device may be more appropriate for studying the effects of animal activity on sediment properties (e.g., the annular flume used by Widdows *et al.*, 1998a; 1998b). Despite this, the CSM proved successful in highlighting differences between the erosion characteristics of the sediments tested during the present study with the results showing the same trends as those generated by the flume.

Paterson *et al.* (2000) found that rain during periods of low tide could enhance sediment erodibility and that the filling of erosion devices may have a similar effect. Measurements by Tolhurst *et al.* (2000a) showed that filling of the CSM test chamber could cause sediment resuspension equivalent to 0.03 Kg m^{-2}. This measurable increase could be set as the erosion threshold although the shear stresses causing it would be unknown. This initial resuspension due to the filling of the device may also influence subsequent erosion. During the present study, light transmission was recorded before applying any shear stress. Where values of below 90% were recorded, the experiment was rejected in order to minimise the effects of filling. Similarly, recirculating flumes have the disadvantage that the presence of suspended

sediment at concentrations of more than 10 g l^{-1} can cause drag reduction which reduces friction at the bed and can result in higher critical erosion thresholds (Gust, 1976; Best & Leeder, 1993).

7.5.3.2. Data analysis.

The two main variables used to describe the erosion properties of sediments are the critical shear stress (τ_{0crit}) and the erosion rate (ε). A number of devices are available for measuring erosion, both in situ and in the laboratory although there are variations between factors such as the area over which erosion takes place (i.e., the test section), the way in which erosion is induced and recorded and the way in which erosion thresholds are defined (Tolhurst *et al.*, 2000a). Furthermore, the attributes of the device used and the method used to determine the critical erosion threshold are often not stated. Consequently, comparison between data collected using different devices is often difficult and standardisation between devices is therefore necessary. The most common method of determining erosion is to measure the SPM (suspended particulate matter), either directly by extraction and filtration of water samples, or indirectly by sensors (nephelometers, optical backscatter). Both the area over which erosion occurs and the volume of water in the system can affect SPM values and SPM therefore needs to be normalised with respect to area and volume and should be expressed as total accumulated mass (e.g., Widdows *et al.*, 1998a; 1998b). In addition, as already stated, the degree of erosion increases as the length of time for which a sediment is exposed to an eroding fluid increases. Calculation of a standardised erosion rate (ε_{st}) as change in SPMn/change in time removes the effects of time (Tolhurst *et al.*, 2000a).

A major problem associated with determining the critical erosion threshold of cohesive sediments is the fact that the objective determination of what actually constitutes erosion is difficult. Miller *et al.* (1972) defined the erosion threshold as the fluid stress just less than that required to initiate grain motion. In non-cohesive sediments, Yalin (1972) and Heizelmann & Wallisch (1991) stated that the simultaneous motion of 10 or more grains represented erosion although Tolhurst *et al.* (2000a) pointed out that this is not appropriate for cohesive sediments since the grains are often too small to see. In cohesive sediments, erosion is normally determined by the accumulation of suspended particulate matter in the eroding fluid (Tolhurst, *et al.*, 2000a) and according to Wainwright (1990) erosion can be defined as the point at which there is a statistically significant increase in SPM above background levels.

However, most natural cohesive sediments have an unstable surface layer of loose flocculent material which is easily resuspended by low shear stresses. Therefore, some authors believe that there is no true erosion threshold for cohesive sediments (Lavelle & Mofjeld, 1987, in

Tolhurst *et al.*, 2000a) and that cohesive sediments may have a number of erosion thresholds depending upon the type of erosion occurring (e.g. surface or type I erosion or mass (type II) erosion). These authors suggested that the erosion properties of the sediment may vary with depth, as a result of differences in compaction and biological activity leading to variations in the erosion rate over time. Determining the erosion threshold becomes much harder when the bed fails in steps as there are a number of different thresholds, any of which could be considered to be the critical erosion threshold. Tolhurst *et al.* (2000a) described the erosion rate profiles of the CSM as confusing since erosion rates can fluctuate widely, often giving negative values indicative of deposition. This was indeed observed both during field and laboratory measurements made in the present study. This is explained by the fact that there is a pause between each pulse of shear stress, allowing resettlement and subsequent resuspension of material between pulses. Since resuspended sediment would settle over the entire bed of the test section, not all of it would be resupended on the next pulse, resulting in lower and lower SPM concentrations until no further erosion occurred. As was observed during the flume experiments, erosion does not necessarily occur as an increase in suspended particulate matter but may occur as flocs or lumps which remain close to the bed. It is possible that erosion occurring in this manner may be missed until the flocs have become sufficiently broken up and the particles dispersed to be detected by devices used for measuring SPM.

Tolhurst *et al.* (2000a) suggested that natural variation and fluctuation in SPM require that some sort of critical erosion threshold be used to determine the onset of erosion. Using the CSM, Paterson *et al.* (2000) defined the critical shear stress as that which caused a 10% reduction in light transmission across the test chamber although due to fluctuations in light transmission readings, defining the point at which such a change in light transmission actually occurs can be subjective. Similarly, Tolhurst *et al.* (2000b) defined the critical erosion shear stress as the point at which light transmission dropped below 90% (using the CSM). Statistical methods to determine erosion thresholds have involved the use of linear regression analysis. For example, Tolhurst *et al* (2000a) plotted separate linear regression lines along a single erosion profile, choosing the points at which the slopes of two or more lines changed as the cut off point for separating the data. The point at which the two lines cross was then defined as the critical shear stress value. However, fitting of the regression lines is carried out by visual examination of the data and is therefore, to a certain degree, subjective. Tolhurst *et al.* (2000a) demonstrated that, depending on which data points were included in the analysis, the critical shear stress for a particular data set could vary between approximately 0.8 and 1.5 Nm^{-2}. Including or excluding a single point was found to significantly change the slope of the

regression line, resulting in a different erosion threshold value. These authors therefore suggested setting a standardised erosion threshold of 0.001Kg m^{-2}.

Sutherland *et al.* (1998) also used linear regression analysis and defined values of critical shear velocity (u_{*crit}) as the point at which the line of best fit intercepted the x-axis. It was argued that this approach was reproducible, statistically valid and uses the entire data set to define the erosion threshold. It was noted that SPM curves were often made up of two (or more in the case of the present study) linear parts, divided by an inflection point and it was suggested that this represented distinct layers in the sediment with differing erosion rates. In several cases, the first data point was found to lie below the regression intercept, determined as the erosion threshold. Sutherland *et al.* (1998) attributed this to 'noise' within the data set which could possibly be explained by the resuspension of a recently deposited layer of loose, flocculent material not associated with the sea bed. Such a layer could consist of very recently deposited material following a period of high tide or biogenic material such as newly deposited faecal pellets or material resuspended and resettled following burrow irrigation by organisms such as *C. volutator* (e.g., Amos, 1992b; Grant & Daborn, 1994).

In the present study, erosion was considered to occur at the point at which a sustained increase in SPM occurred in order to remove some of the subjectivity associated with defining the erosion threshold. Both regression analysis and the point at which a 10% decrease in light transmission (using the CSM) were used to determine the erosion threshold and, in the majority of cases, there was reasonable agreement between the two methods. However, Tolhurst *et al.* (2000a) pointed out that this ignores small unsustained pulses in erosion which may be important on a mudflat wide scale. The criteria for determining the erosion threshold could have implications for estimating the amount of material eroded from a mudflat and may have a significant effect on the understanding of mass transport processes. However, as already stated, Tolhurst *et al.* (2000a) found that filling of the CSM chamber could lead to resuspension equivalent to 0.03 Kg m^{-2} and setting an erosion threshold below this (i.e. 0.001 kg m^{-2}) was considered inappropriate.

Finally, it should be noted that normalising the SPM data to area allows comparison between devices but assumes uniform erosion over the entire test section. This is unlikely to be the case due to the presence of biological features (faecal mounds, tubes, casts and burrows), microbial biofilms, and small scale variations in topography. This problem of oversimplification is likely to increase with increasing area of the test section. Tolhurst *et al.* (2000a) and Riethmüller *et al* (2000) found that standardisation of SPM values and erosion

rates highlighted differences between the devices used to measure erosion with some devices giving values an order of magnitude greater than others.

7.6. SUMMARY AND CONCLUSIONS

- The use of both the laboratory flume and the CSM (deployed in the field) produced data showing the same trend of increasing erosion potential (in terms of total erosion) with increasing distance from the source of pollution. This is thought to be related to the impact of the effluent on both the sediment properties and the faunal communities.
- Laboratory studies using the CSM indicated that increasing effluent concentration could lead to an increase in the erosion threshold of sediments with and without macrofauna. Where macrofauna were present, *H. diversicolor* was found to have the biggest impact in terms of reducing the erosion threshold.
- The erosion thresholds recorded were comparable to those recorded a number of other authors working both in the Humber estuary and at a number of sites throughout the Wadden Sea.
- It is suggested that whilst pollution of this type can increase sediment erosion thresholds and lead to a reduction in the total amount of sediment eroded, the way in which sediments erode may also be of importance. There is an indication that there may be more than one erosion threshold for each of the sediments tested and that Type II erosion may occur to a greater degree in the less polluted sediments.

CHAPTER 8

GENERAL DISCUSSION

8.1. COMMUNITY CHANGES ALONG A POLLUTION GRADIENT

The macrofaunal communities found on the mudflats at Paull and Saltend were considered to be typical of estuarine habitats with low species diversity but high abundance (Chapter 4). The communities at the unpolluted end of the gradient were dominated by the typical middle estuary species *Hediste diversicolor* with moderate abundances of *Corophium volutator*, *Streblospio shrubsolii*, *Manayunkia aestuarina*, oligochaetes and nematodes and low numbers of *Macoma balthica* also being present. The macrofaunal communities along this pollution gradient showed an almost typical response to organic pollution, as described by Pearson & Rosenberg (1978) with a reduction in the number of species and community biomass and a marked increase in animal abundance with increasing proximity to the discharge. In addition, there was also decrease in mean and maximum individual length and biomass at the most polluted sites although examination of individual species showed the oligochaetes (the dominant group) at the most polluted site (S25 m) to be considerably larger than those in less polluted areas (S200 m and P150 m). This suggests that these organisms are not only tolerant of this particular effluent but are actually benefiting from its discharge. Microalgal biomass, and the associated mucous (measured as total and colloidal carbohydrate) secretions, were also found to be enhanced at the most polluted sites (Chapter 2), possibly as a result of nutrient enrichment due to lower dilution of the effluent, and / or reduced grazing pressure from surface deposit feeding species.

Analysis of the physico-chemical properties of the sediment (Chapter 2) revealed no spatial or temporal differences in particle size although comparison with other studies suggests that the sediments in this area are becoming increasingly fine. Water content, bulk density and organic content were variable between sites and these parameters were therefore not considered to be directly responsible for the differences in community structure between sites. Eh was considered to be the most important factor influencing macrofaunal distribution and seasonal changes in this parameter were reflected by changes in the community structure. That is, during the summer months when higher temperatures led to a greater degree of anoxia in the sediments, some of the less pollution tolerant species were found to disappear (either through death or active migration) away from the S75 m site whilst these species would reappear at this site during the cooler months, as redox conditions improved, increasing the

similarity between the communities at the S75 and S200 m sites. Toxicological studies (Chapter 3) also indicated the low toxicity of the effluent but that there were some lethal toxic effects as a result of exposure to the sediment. Again, redox measurements carried out during these tests indicated a high degree of anoxia in the most polluted sediments. However, metals concentration data (C. Nikitik, University of Hull. Pers. comm.) showed a decline in copper and zinc concentrations with increasing distance from the discharge and the effects of this on the faunal community should not be discounted. Similarly, it is likely that there may be several other persistent compounds present in the sediment (e.g. PCBs), the concentrations of which were not determined.

The pollution-induced changes in macrofaunal community structure led to a reduction in bioturbation potential with increasing distance from the discharge, as demonstrated using inert tracers and a resin casting technique (Chapter 5). Furthermore, laboratory studies indicated a reduction in the bioturbation potential of *Hediste diversicolor*, *Macoma balthica* and *Corophium volutator* with increasing effluent concentration. Functional analysis of the community showed a distinct difference in the diversity of the feeding and sediment modification groups present at polluted and non-polluted sites with polluted sites being dominated by non-selective, sub-surface deposit feeding species which have a biodiffusive effect on the sediment. In contrast, the communities at the less polluted sites were composed of selective and non-selective surface and subsurface feeding detritivores, omnivores and carnivores with biodiffusers, regenerators and conveyor belt species all being present.

Both field (using the CSM) and laboratory (using a linear recirculating flume) based erosion studies showed an increase in erosion potential, both as reduced critical shear stress and increased total erosion, of the sediments with increasing distance from the source of pollution. This final chapter aims to provide a possible explanation of why a polluting discharge of this nature, together with the associated structural and functional changes to the faunal communities within its vicinity, could lead to changes in sediment transport potential. Correlation and multiple regression analysis are used to highlight the relationships between the various physical and biological features of the sediment and erosion thresholds with the findings being compared to those of other studies. A brief discussion of the wider environmental implications of pollution induced changes to both faunal communities and sediment transport processes is then given.

8.2. IMPICATIONS FOR SEDIMENT TRANSPORT

8.2.1. Relationships between biological and environmental variables and sediment erosion threshold.

Pearson correlations were carried out (following testing of the data for normality using the Shapiro-Wilks test (n<50)). The analysis was carried out using the data from the CSM experiments since estimations of erosion thresholds using this device were considered to be more precise than those derived from the flume experiments. Statistically significant positive relationships were found between the critical shear stress (τ_{0crit}) and chlorophyll-*a* and oligochaete biomass suggesting that an increase in these parameters would lead to increased sediment stability (Table 8.1). In contrast, increasing redox potential (Eh at 4 cm) and increasing density of *Hediste diversicolor* and *Corophium volutator* will lead to a reduction in the erosion threshold of the sediment. Significant positive correlations were found between total erosion and redox potential and *H. diversicolor* and *C. volutator* abundance. That is, *H. diversicolor* and *C. volutator,* together with increasingly oxidised sediments, appear to enhance erosion. Negative correlations were found between chlorophyll-*a* concentration, sediment carbohydrate (as colloidal-saline and colloidal-EDTA extracts) and oligochaete biomass and total erosion suggesting that as these three parameters increase, the total amount of sediment eroded decreases.

Stepwise multiple regression analysis was attempted in order to determine which combinations of these variables best explain the erosion characteristics of the sediments taken from different sites along the pollution gradient. The results of the stepwise regression were verified using backward regression which gives details of the model at each stage before a variable is removed. Zar (1996) stated that the error associated with the backward technique is generally less than that associated with the forward technique since it begins by including all of the variables. Only variables showing a statistically significant correlation with the sediment erosion characteristics (according to Table 8.1) were included. However, multicollinearity (a strong correlation between two of the independent variables) between the variables (as can be seen in Table 8.1) prevented the determination of a reliable model. Where multicollinearity occurs, entering the two variables may increase the coefficient of determination (r^2) but may increase the error of the regression coefficient and therefore, the error of the predictions made by the model (Zar, 1996). Entering both variables into the model would give the impression that neither was significant, or if stepwise linear regression techniques were used, the least important of the two co-related parameters would be down-weighted in importance (Brinkman *et al.*, 2002). Despite this, *H. diversicolor* abundance was always included in the model with the best model containing this variable alone:

$$\tau_{0crit} = -4.46x10^{-4} \text{ x } \textit{Hediste} \text{ A } + 5.261 \qquad r^2 = 0.47, p<0.05$$

Therefore, the abundance of *H. diversicolor* explained 47% of the variation within the data. If this variable vas removed, chlorophyll-*a* concentration became the next best predictor of τ_{0crit}, explaining 45% of the variation. However, inclusion of both of these variables led to a model which was not statistically significant ($r^2 = 0.47$, $p>0.05$). This was expected given the strong relationship between chlorophyll-*a* concentration and the abundance of *H. diversicolor.*

$$\tau_{0crit} = \text{chla x } 4.31x10^{-2} + 0.97 \qquad r^2 = 0.45, p<0.05$$

Stepwise multiple regression using total erosion as the dependent variable, again, highlighted the importance of the abundance of *H. diversicolor* to sediment erosion potential, explaining 62% of the variance.

$$\text{Total erosion} = \textit{Hediste} \text{ A x } 1.04x10^{-2} + 92.4 \qquad r^2 = 0.62, p<0.01$$

The above model was verified using backward multiple which gave the following model, found to explain 89% of the variance suggesting that this combination of factors is better predictor of total erosion than the abundance of *H. diversicolor* alone:

$$\text{Total erosion} = \textit{Hediste} \text{ A x } 1.6x10^{-2} + \text{olig B x} - 0.82 + Eh_4 \text{ x } 0.74 + \text{coll-S x } 1.43 - 65.15$$

$r^2 = 0.89$, $p<0.01$.

However, examination of the collinearity statistics showed redox potential (Eh_4) oligochaete biomass (olig B) and saline extractable colloidal carbohydrate (coll-S) to have a high Variance Inflation Factor (VIF) indicating problems with multicollinearity between these variables and other independent variables included in the model. The low tolerance value suggested that these variables contributed little to the model and were likely to cause problems in the calculation of the regression coefficients. The correlation coefficients also showed these variables to be highly correlated with the other variables in the model. Therefore, it was concluded that the above equations should be treated cautiously.

The relationships between erosion thresholds and the physical environmental variables (particle size, water content and bulk density) were extremely weak and not statistically significant. This lack of relationship between the physical variables and the erosion properties of the sediment was also noted by Widdows *et al.* (1998a) and Paterson *et al.* (2000) who both found animal abundance (particularly *Macoma balthica* and *Cerastoderma edule*) to have a greater impact on sediment erosion potential. In contrast, strong relationships were

found between some of the biological variables and erosion thresholds suggesting that, in the case of the present study, erosion potential is controlled to some degree by biological activity. It should however, be noted that whilst the presence of animals was found to increase erosion potential, exposure to the effluent in the absence of macrofauna was found to reduce erosion potential. Furthermore, redox potential was found to co-vary with both τ_{0crit} (r = -0.6, $p<0.05$) and total erosion (r = 0.71, $p<0.01$) and sediment organic content was found to be significantly correlated with τ_{0crit} (r = 0.61, $p<0.05$). This suggests that erosion potential is largely a function of a combination of biological activity and pollution-induced physico-chemical changes to the sediment properties. De Boer (1981, in de Dekere, 2003) found that the addition of NaOCl and $CuSO_4$ to sediment reduced sediment stability by breaking the bonds between organic matter and the silt-clay matrix. In the present context, these changes could be to increase cohesion and adhesion between the sediment particles, as a result of oily residues in the effluent and / or enhanced microbial populations resulting from the increased organic content and reduced environment. However, as discussed in Chapter 7, the effect of redox potential on sediment erosion potential is not well understood.

Table 8.1. Pearson correlations between environmental variables, organism abundance (A) and biomass (B) and sediment erosion characteristics (*r* values in italics, significant p values given in bold). Water content, bulk density, particle size and the less common/abundant species have been omitted since no correlation was found between these variables and the erosion characteristics. Correlations are based on environmental and community data for July 1999.

		CHLA	Total carb	Coll_ EDTA	Coll-Saline	Organics	Eh 4	Total A	Total B	Olig A	Olig B	Hed A	Hed B	Cor A	$\tau_0$crit
Total carb	*r*	*0.60*													
	Sig.	**0.01**													
Coll_ EDTA	*r*	*0.92*	*0.67*												
	Sig.	**0.00**	**0.00**												
Coll-Saline	*r*	*0.91*	*0.72*	*0.92*											
	Sig.	**0.00**	**0.00**	**0.00**											
Organics	*r*	*0.86*	*0.66*	*0.87*	*0.82*										
	Sig.	**0.00**	**0.02**	**0.00**	**0.00**										
Eh 4	*r*	*-0.93*	*-0.53*	*-0.88*	*-0.90*	*-0.77*									
	Sig.	**0.00**	0.08	**0.00**	**0.00**	**0.00**									
Total A	*r*	*0.81*	*0.57*	*0.83*	*0.86*	*0.81*	*-0.80*								
	Sig.	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**								
Total B	*r*	*-0.12*	*-0.01*	*-0.04*	*-0.08*	*-0.13*	*-0.15*	*-0.10*							
	Sig.	0.63	0.96	0.89	0.76	0.69	0.64	0.67							
Olig A	*r*	*0.81*	*0.59*	*0.79*	*0.91*	*0.77*	*-0.76*	*0.88*	*0.00*						
	Sig.	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.99						
Olig B	*r*	*0.79*	*0.60*	*0.91*	*0.89*	*0.76*	*-0.79*	*0.71*	*0.02*	*0.83*					
	Sig.	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.93	**0.00**					
Hed A	*r*	*-0.87*	*-0.51*	*-0.86*	*-0.91*	*-0.79*	*0.89*	*-0.74*	*0.21*	*-0.80*	*-0.88*				
	Sig.	**0.00**	**0.03**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.40**	**0.00**	**0.00**				
Hed B	*r*	*-0.45*	*-0.28*	*-0.45*	*-0.50*	*-0.51*	*0.33*	*-0.42*	*0.83*	*-0.41*	*-0.49*	*0.64*			
	Sig.	**0.06**	0.25	0.06	**0.03**	0.09	0.29	0.07	**0.00**	0.09	**0.03**	**0.00**			
Cor A	*r*	*-0.74*	*-0.33*	*-0.70*	*-0.72*	*-0.61*	*0.93*	*-0.68*	*-0.34*	*-0.61*	*-0.63*	*0.61*	*-0.05*		
	Sig.	**0.00**	0.22	**0.00**	**0.00**	**0.04**	**0.00**	**0.01**	0.22	**0.02**	**0.01**	**0.02**	0.87		
$\tau_0$crit	*r*	*0.67*	*0.31*	*0.48*	*0.59*	*0.61*	*-0.60*	*0.27*	*0.19*	*0.53*	*0.61*	*-0.69*	*-0.19*	*-0.62*	
	Sig.	**0.02**	0.36	0.13	0.06	**0.05**	**0.05**	0.41	0.58	0.10	**0.05**	**0.02**	0.57	**0.04**	
Total eros.	*r*	*-0.75*	*-0.42*	*-0.60*	*-0.70*	*-0.53*	*0.71*	*-0.32*	*-0.14*	*-0.59*	*-0.73*	*0.79*	*0.31*	*0.65*	*-0.90*
	Sig.	**0.01**	0.20	**0.05**	**0.02**	0.09	**0.01**	0.33	0.68	0.06	**0.01**	**0.00**	0.36	**0.03**	**0.00**

8.2.2. Impact of animal activity

The stability of intertidal cohesive sediments is influenced by a large number of interacting physical and biological parameters including particle size and density, water content, organic content, depositional history, exposure to air and the composition of the biological community (Burt *et al.*, 1997, in Paterson *et al.*, 2000; Black & Paterson, 1998; Widdows *et al.*, 1998a; Whitehouse *et al.*, 2000). In many intertidal areas, biological activity can be the primary control on sediment stability (Kornman & de Dekere, 1998; Widdows *et al*, 1998a; Paterson, 1989; Tolhurst *et al.*, 1999, in Paterson *et al.*, 2000). The way in which animal activity can impact upon the sediment properties and erosion potential was described in Chapter 7 where it was highlighted that burrowing, feeding and locomotion by benthic organisms could either increase sediment transport or enhance sediment deposition and stabilisation. Briefly, the four broad modes of organism effect on the sediment properties (as defined by Nowell *et al.*, 1981) include (1) alteration of surface roughness, (2) alteration of particle exposure to the flow (e.g., through producing tracks, faecal mounds, burrows and feeding pits), (3) alteration of particle momentum (e.g., the forceful ejection of faecal and pseudo-faecal material into the water column) and (4) alteration of the adhesive and cohesive properties of the sediment particles (through the production of mucous or the consumption of mucous coated particles). It has repeatedly been found that the effects of the activity of a single organism may be simultaneously stabilising and destabilising (Jumars & Nowell, 1984a; Snelgrove & Butman, 1994; Widdows *et al.*, 1998a; 1998c) and that most infaunal estuarine species are capable of changing their mode of feeding and bioturbation so that their activity can be classed as neither consistently stabilising or consistently destabilising (Jumars & Nowell, 1984a; 1984b; Snelgrove & Butman).

The impact of bivalves on sediment transport potential is well documented. Davis (1993) stated that sediment resuspension was dependent upon shear stress at the sediment-water interface and/or by the type, abundance and reworking time of benthic organisms. In the absence of physical shear, the bivalves *Yoldia limatula* and *Macoma tenta* were both found to cause sediment resuspension through the ejection of faecal and pseudofaecal material and individuals of *Y. limatula* were found to be capable of resuspending 20 mg min^{-1}. Bender & Davis (1984) found that individuals of *Y. limatula* could expel quantities of faecal and pseudofaecal material between 10 and 200 times their body weight each day and estimated that a 14 mm long individual could resuspend 440 g of sediment (dry) each year. Davis (1993) stated that bioresuspension could occur independently of shear but that a low level of flow would be required to keep the material in suspension and lead to sediment transport. Intertidal benthic organisms may be exposed to extremely low flows or, during low tide, standing water, during which time resettlement of this resuspended material may occur.

However, it is unlikely that such conditions would remain for a sufficient length of time for this resettled material to consolidate. Therefore, as the flow increased, it is likely that this layer of loose, resettled material may be resuspended and transported in addition to that being ejected by the bivalves. Davis (1993) also found motile deposit feeding species to increase sediment resuspension with the bivalve *Nucula annulata* doubling resuspension at low current velocities. Secondary resuspension through destabilisation at the sediment-water interface, as a result of animal movement through the sediment, is also likely (Bender & Davis, 1984). Nowell *et al.* (1981) found tracks produced on the sediment surface by the motile bivalve *Transenella tantilla* to reduce the critical erosion velocity by 20% when the tracks covered just 10% of the sediment surface. Given the findings of Bender & Davis (1984) and Davis (1993), it is suggested that deposit feeding bivalve species can significantly impact upon sediment transport potential.

During *in-situ* flume studies carried out at Skeffling (Humber estuary) Widdows *et al.* (1998a; 1998b) found a significant positive correlation between the density of *Macoma balthica* and maximum suspended particulate matter concentration at 30 cm s^{-1} (r = 0.93, $p<0.01$) and a negative correlation between *M. balthica* density and the critical erosion velocity (r = -0.96, $p<0.01$). A mudflat density of 1000 *M. balthica* m^{-2} increased sediment erodibility 4-fold and penetrometer measurements demonstrated a significant reduction in sediment shear strength to be associated with this species. Widdows *et al.* (2000) also demonstrated the negative correlation between critical erosion velocity and *M. balthica* density and found temporal changes in sediment stability to coincide with temporal changes in *M. balthica* density. The occurrence of a cold winter led to an increase in the abundance of *M. balthica*, an observation also made by Beukema & Dekker (2003), which caused an increase in sediment erosion potential.

M. balthica densities recorded by Widdows *et al.* (1998a; 1998b) ranged from 100-1575 individuals m^{-2} whereas a maximum of 91 individuals m^{-2} was recorded during the present study (P150 m). This density is consistent with that reported from Paull by Widdows *et al.* (2000) who considered it low and associated it with relatively stable sediments (in comparison to Skeffling where *M. balthica* was abundant) at this site during Spring 1996. This suggests that, in the context of the present study, the densities of *M. balthica* present at Paull may not be sufficiently high to have a major impact on sediment stability, although it should not be assumed that this species has no impact at all. In Chapter 5, the bioturbation potential of *H. diversicolor* and *M. balthica* was found to be similar when similar numbers of organisms were used in each experiment. However, the field density of *H. diversicolor* at all

of the sites studied was much greater than that of *M. balthica* and the greater potential impact of this species on erosion potential (at field densities) was demonstrated in Chapter 7.

The mobile suspension feeding bivalve *Cerastoderma edule* was also found to increase sediment erosion potential but in contrast, it was classed as an important species in terms of biodeposition, capable of depositing up to 6.61 g m^{-2} h^{-1} (Widdows *et al.*, 1998a). It was suggested that erosion caused by this species was the result of the resuspension of recently deposited material, together with its burrowing activity. Ahn (1993) also highlighted the importance of suspension feeding bivalves in biodeposition and biostabilisation. The antarctic species *Laternula elliptica* was found to cause the deposition of 0.26 – 2.17 mg (dry).g $(wet)^{-1}$ d^{-1} with deposition increasing with increasing animal size.

The effects of surface deposit feeding species such as *Corophium volutator* and *Hediste diversicolor* are more complicated and contrasting results have been obtained from various studies. The results of the present study indicate that both species may have a destabilising effect on the sediment (Chapter 7), reducing the critical erosion threshold and increasing the total mass of sediment eroded relative to control sediments containing no macrofauna. Furthermore, *H. diversicolor* was found to be particularly important in terms of its bioturbation potential (Chapter 5). However, Grant & Daborn (1994) found that the effects of *Corophium* activity varied seasonally in relation to the population structure and food availability. During periods of recruitment and high densities of juveniles, sediment erosion rates were found to be negatively correlated with the abundance of *C. volutator*, possibly due to the small body size of the animals, shallow burrowing depth and mucous production. Meadows & Tufail (1986), Meadows *et al.* (1990) and Mouritsen *et al.* (1998) also demonstrated how mucous secretions by *H. diversicolor* and *C. volutator* could lead to increased sediment shear strength.

Over a period of three days, Meadows & Tait (1989) found sediment permeability to decrease with increasing densities of *C. volutator* but to increase as the density of *H. diversicolor* increased. *H. diversicolor* increased sediment permeability to a greater extent than *C. volutator* decreased it. Low densities (1000 individuals m^{-2}) of *H. diversicolor* increased permeability by 37%, field densities (3000 individuals m^{-2}) by 67% and high densities (9000 individuals m^{-2}) by 123%. In contrast, low densities (2500 individuals m^{-2}) of *C. volutator* had no effect on permeability whilst field (7500 individuals m^{-2}) and high (22,500 individuals m^{-2}) densities led to a decrease of 37 and 58%, respectively. These findings were explained by differences in the size, shape and depth of the burrows constructed by these two species and by possible differences in the permeability of the burrow linings. The 'U' shaped

burrows constructed by *C. volutator* would prevent the passage of water beyond the 2-5 cm burrowing depth of this species. In contrast, the burrows of *H. diversicolor* are vertical and considerably deeper.

Both species were associated with increased sediment shear strength and decreased water content with the effect of the two species occurring together being additive (Meadows & Tait, 1989). Neither species was found to increase shear strength at low densities but for field and high densities, *C. volutator* increased shear strength by 50 and 180%, respectively whilst *H. diversicolor* caused increases of 39 (field density) and 80% (high density) (Meadows & Tait, 1989). Widdows *et al.* (1998a) suggested that *H. diversicolor* may act as a sediment stabiliser at Skeffling (Humber estuary) where densities of 25-300 m^{-2} were recorded. Comparable densities were recorded from this site during the present study although given the findings of Meadows & Tait (1989), it is considered unlikely that the stabilising activity of this species is of great importance (in comparison to the activities of other, more abundant species) at Skeffling. *C. volutator* reduced the water content to 88 and 79% of that of the control sediment (no animals) at field and high densities whereas *H. diversicolor* reduced the water content to 80 (field density) and 69% (high density) (Meadows & Tait, 1989).

In contrast, de Dekere *et al.* (2000) found that increasing density of *C. volutator* could lead to increased concentrations of suspended particulate matter under conditions of low flow as a result of feeding, burrow irrigation and diatom predation. de Dekere (2003) also found the sediment surface roughness to increase in the presence of *C. volutator* and suggested that this may also enhance erosion. Grant & Daborn (1994) and Gerdol & Hughes (1994) also found the grazing activity of *C. volutator* to reduce sediment stability. In addition, Grant & Daborn (1994) suggested that scour and subsequent resuspension occurred around the burrows causing the tops of the tubes to protrude above the sediment surface. These tubes eventually became eroded as flocs and transported as bedload material. In addition, plumes of faecal and pseudofaecal material, ejected into the water column due to burrow irrigation, were simultaneously transported as suspended particulate matter. Amos *et al.* (1992b) carried out experiments using the using the Sea Carousel and also found the faecal pellets of *C. volutator* to be an important component of initial surface erosion.

Whilst the results of a number of studies on the effect of *C. volutator* on sediment properties are contrasting, de Dekere (2003) concluded that at densities of >15,000 individuals m^{-2}, the net effect of *C. volutator* was destabilising. At lower densities, the erosion threshold was not affected. de Dekere *et al.* (2000), Gerdol & Hughes (1994a) and Daborn *et al.* (1993) all

found *C. volutator* to decrease the erosion threshold of the sediment and increase both bedload and suspended sediment transport.

Densities of *C. volutator* recorded during the present study ranged from 275-4289 at the P150 m site, 275-3524 at the S200 m site and 0-275 individuals m^{-2} at the S75 m site. The findings of Meadows & Tait (1989) suggest that these densities may not be high enough to have any significant impact on sediment permeability, water content or sediment shear strength although Meadows & Tait (1989) did not indicate the minimum density required to affect these parameters. Grant & Daborn (1994) found *C. volutator* to have both stabilising and destabilising effects on the sediment but field samples in their study contained over 13,000 individuals m^{-2}.

As has already been stated, Meadows & Tait (1989) and Widdows *et al.* (1998a) suggested sediment stabilisation by *H. diversicolor.* On the other hand, Underwood and Paterson (1993a) demonstrated sediment destabilisation in *H. diversicolor* and this species was described, by Winston & Anderson (1971) as one of the most important bioturbating organisms in the Great Bay estuarine system, New Hampshire. Smith *et al.* (1996) suggested that, like *C. volutator, H. diversicolor* may also reduce sediment stability as a result of diatom predation. These findings are consistent with those of the present study. The polychaete *Nephtys incisa* was found to break up the sediment matix, increase porosity and, as a result, increase sediment resuspension 3 to 5 fold and the polychaete *Pectinaria gouldi* was found to cause sediment resuspension through the ejection of faecal and pseudofaecal material (Davis, 1993).

de Dekere *et al* (2001) treated sediment plots (*in-situ*) with the insecticide Vydate which is know to be effective in the removal of macrofauna without killing bacteria and microalgae or altering the physico-chemical properties of the sediment. Macrofaunal and meiofaunal densities decreased by 48 and 34%, respectively, leading to an increase in the critical shear stress of over 300%. A decrease in water content was noted, following the application of the insecticide, and was attributed to the disappearance of a large proportion of the infaunal organisms. Furthermore, there was no significant change in microalgal biomass and it was concluded that the net effect of infauna such as *H. diversicolor, M. balthica, S. shrubsolii* and oligochaetes on muddy sediments was destabilising.

Densities of *H. diversicolor* recorded during the present study ranged from 275-2368 S25 m site, 3909-8425 at the S75 m site, 6057-12,298 at the S200 m site and 4543-7709 individuals m^{-2} at the P150 m site. With reference to the findings of Meadows & Tait (1989), these

abundances suggest that at the S75 m, S200 m and P150 m sites, an increase in permeability by this species would be likely. In addition, particularly at the S200 m site, there is potential for increased sediment shear strength and a reduction in water content as *H. diversicolor* densities at these sites reach their maximum. These effects are likely to be most noticeable at the S200 m and the P150 m sites where comparatively high densities of this species are present throughout the year. Whilst *H. diversicolor* may impact upon the sediment properties to certain degree at the S25 m site, it was not present at densities at which Meadows & Tait (1989) reported even slight effects for the majority of the year. The impact of *C. volutator* on the sediment properties and erosion potential in the case of the present study is unclear but given the findings of other authors, its activity is thought to be less important than that of *H. diversicolor.* This statement is supported by the fact that experiments using equal numbers of *H. diversicolor* and *C. volutator* (Chapter 5) showed *H. diversicolor* to have a greater impact in terms of sediment mixing. Furthermore, laboratory based erosion experiments (using the CSM) showed that, at densities representative of those at Paull, *H. diversicolor* had the greatest potential of the two species to enhance erosion.

Eckman *et al.* (1981) found that, at low densities, the tubes of the polychaete *Owenia fusiformis* (e.g. 4% coverage of the bed) could enhance erosion due to scouring around the base of the tubes. In contrast, at higher densities, erosion was reduced and deposition was enhanced due to protection of the bed by the tubes. At Skeffling (Humber estuary), the tubiculous polychaetes *Pygospio elegans* and *Streblospio shrubsolii* were classified as stabilising organisms by Widdows *et al.* (1998a; 1998b) in areas where they reached high densities. However, at Paull (where these organisms were found in their highest densities during the present study), dense tube mats do not generally occur and it is unlikely that any significant stabilisation (relative to the effects of other species present) would be caused due to the presence of these worms. Densities ranged from 165-2973 individuals m^{-2}, with densities being less than 2000 individuals m^{-2} for most of the year and Allen (2000a) reported a maximum density of 3904 *S. shrubsolii* m^{-2} although densities were generally below 1500 m^{-2}. Widdows *et al.* (1998a) recorded a maximum density of 6000 individuals m^{-2} although, at most mid-lower shore sites, densities were comparable to the maximum density recorded in the present study. Furthermore, Eckman *et al.* (1981) stated that in addition to density, this effect was dependent on tube roughness, geometry, the height : width ratio and the presence of microbial films. Given that the tubes of these species are extremely small, it is also unlikely that isolated tubes would significantly enhance erosion.

Austen *et al.* (1999) found high densities of *Hydrobia ulvae* to cause sediment destabilisation, leading to increased erosion and a reduction in critical shear stress. This was attributed to the

fact that this species feeds on benthic diatoms, therefore reducing microalgal biomass and reducing biostabilisation by microalgae (as discussed in section 8.2.3). Negative correlations were found between microalgal biomass and the density of *H. ulvae*). In addition, *H. ulvae* deposit a large quantity of faecal pellets on the sediment surface which can result in increased sediment transport due to the sediment losing its some of its cohesive properties when it becomes aggregated as faecal pellets (Nowell *et al.*, 1981; Taghon *et al.*, 1984). Blanchard *et al.* (1997) also found that high ($10x10^4$ individuals m^{-2}) densities of *H. ulvae* could increase the faecal pellet content of the sediment by up to 87%. This, again, together with grazing activity and burrowing within the surface layers of the sediment led to enhanced erosion. Whilst densities of this species at Skeffling (particularly at the lowest shore site sampled during the present study) may be sufficiently high to enhance sediment erosion, it is not thought to be of great importance at Paull since it was only recorded once, in very low abundances, throughout the whole sampling period. This species was absent from all sites at Saltend.

Flume studies by Rhoads *et al.* (1978, in Meadows & Tait, 1989) showed that the capitellid polychaete *Heteromastus filiformis* could increase the critical erosion velocity of surface sediments transported as bedload (after 5-10 days of exposure) by 80% and that of sediments transported in suspension by 100%. These head down deposit feeders deposit faecal pellets at the sediment surface in a similar manner to oligochaetes (Clough & Lopez, 1993). Due to the abundant population of oligochaetes at the S25 and S75 m sites, a high density of faecal pellets at the sediment surface would be expected. The enhanced erosion potential of the faecal pellets of *C. volutator* and *H. ulvae* (Amos *et al.*, 1992b; Blanchard *et al.*, 1997; Austen *et al.*, 1999) has already been mentioned and it would be reasonable to assume that a high rate of faecal pellet distribution by oligochaetes might also lead to increased erosion of the surface sediment layers relative to unpelletised sediments.

Nowell *et al.*(1981), demonstrated that, depending upon size and shape, the presence of faecal pellets could either increase or reduce critical erosion thresholds in comparison to unpelletised sediments. However, Nowell *et al.* (1981) stated that pelletisation of a sediment would be more likely to lead to increased erosion potential than the presence of faecal mounds and coils. Taghon *et al.* (1984) also showed that the resuspension, rate and mode of transport and distance transported of faecal pellets produced by the polychaete *Amphicteis scaphobranchiata* was related to age. Recently deposited pellets were transported for greater distances as bedload than old (>6 hours) pellets which quickly became disaggregated and transported as suspended particulate matter. This was attributed to the degradation of adhesive mucous associated with the particle over time. Taghon *et al.* (1994) also suggested

that smaller, and possibly more easily eroded, pellets may be shielded from the flow by larger pellets, thus preventing their resuspension. It was therefore concluded that the relationship between pellet production and sediment transport was not simple and was subject to change according to size, age and associated biofilms. In the present study, laboratory based flume studies indicated little difference between the erosion threshold (both as suspended particulate matter and bedload) between sediment taken from site along the pollution gradient. However, there were marked differences in the total amount of sediment eroded with the greatest degree of erosion being recorded from the non-polluted sites (S200 m and P150 m). These results suggest that once the easily resuspended faecal pellets present on the surface of the most polluted sediments are eroded (Type I erosion), the erosion rate declined whereas erosion at the non-polluted sites continued as Type I and /or Type II erosion. This pattern was not reflected during field studies using the CSM. However, it should be noted that laboratory samples were maintained in a dimly lit room at 10°C and whilst attempts were made to simulate tidal inundation, it is unlikely that drying of these sediments or diatom growth, migration and mucous production, would have occurred to the same extent as in the field.

8.2.3. Impact of microalgae

In the present study, statistically significant correlations were found between chlorophyll-*a* concentration and both critical shear stress (τ_{0crit}) (r = 0.67, $p<0.05$) and total erosion (r = -0.75, $p<0.01$). This suggests a stabilising effect of the enhanced microalgal biomass found to be present at the S25 m site. However, there was no relationship between τ_{0crit} and sediment carbohydrate concentration (as total or saline / EDTA extractable). Total erosion was significantly negatively correlated with both saline (r = -0.7, $p<0.05$) and EDTA (r = -0.6, $p=0.05$) extractable carbohydrate. It is possible that the stabilising effects of the enhanced microalgal biomass found at the most polluted sites are complicated by bioturbation and the deposition of faecal pellets on the sediment surface by the large numbers of oligochaetes present at these sites.

The impact of microalgal populations on the erosion characteristics of cohesive sediments has also been studied extensively. Holland *et al.* (1974), Paterson (1989), Paterson *et al.* (1990), Underwood & Paterson (1993a), Sutherland *et al.* (1998a; 1998b); Austen *et al.* (1999), Paterson *et al.* (2000) all found correlations between chlorophyll-*a* concentration and sediment erosion thresholds with the greatest sediment stability being associated with dense populations of epipelic diatoms. Austen *et al.* (1999) found an extremely strong correlation between chlorophyll-*a* concentration and the sediment erosion threshold (r = 0.99, $p<0.01$) and found the highest erosion thresholds to be associated with sediments with the highest water content and lowest bulk density (Danish Wadden Sea), an observation which was also

made by Williamson & Ockenden (1996). The stabilising effect of the diatoms was found to be greater than the expected destabilising effect of the high water content and Austen *et al.* (1999) therefore considered microalgal biomass to be one of the most important factors controlling the erosion shear stress in the uppermost layers of muddy sediments.

Sediment stabilisation associated with high microalgal biomass arises from the fact that, due to their migratory behaviour within the top few mm of the sediment, diatoms secrete mucous (Blanchard *et al.* 2000; Decho, 2000). The mechanisms by which this mucous is secreted are described in Chapter 2 and in detail by Edgar & Pickett-Heaps (1984) and Hoagland *et al.* (1993). Reithmüller *et al.* (2000) stated that dense diatom populations secreting large amounts of mucous can lead to the formation of smooth gelatinous mats on the sediment surface. This reduces the surface roughness of the sediment which, together with increased adhesion and cohesion between the sediment particles (Decho, 2000), has the overall effect of biostabilisation. The EPS (extracellular polymeric substance) component of this mucous (i.e., the high molecular weight mucous secretions which are composed of polysaccharides) comprises approximately 25% of the material secreted (Underwood *et al.*,1995) and is thought to play the most significant role in sediment stabilisation (Hoagland *et al.*, 1993; Decho, 1990; Decho, 2000).

Whilst a number of authors have reported strong, positive correlations between chlorophyll-*a* concentration and sediment stability, Decho (2000) stated that the presence of a diatom mat is not always linked to increased sediment stability. This was thought to be due to differences in the species composition and the physiological status of the diatoms and the methods used to quantify sediment stability. Furthermore, many authors have reported positive correlations between EPS and colloidal carbohydrate concentration and erosion thresholds (e.g., Underwood & Paterson, 1993a; Amos *et al.*, 1998: Sutherland *et al.* 1998a; 1998b; Blanchard *et al.*, 2000) although, this is not always reported (Tolhurst *et al.*, 2003). Decho (2000) suggested that diatoms secrete several types of EPS, some of which are involved in motility and others which are secreted as secondary metabolites. It is likely that the composition and cohesive properties are specific to each kind of EPS and that the EPS secreted may differ between diatom species. Blanchard *et al.* (2000) also stated that colloidal carbohydrates were readily dissolved in water and that the relationship between EPS and chlorophyll-*a* is therefore expected to be dynamic. Tolhurst *et al.* (2003) suggested that the carbohydrate extracted (using current methods of EPS determination) does not accurately measure the binding capacity of the carbohydrates present in the sediment. Tolhurst *et al.* (2003) also noted that increased chlorophyll concentration in the surface sediments preceded increased stability whereas total and colloidal carbohydrate concentrations increased afterwards. It was

suggested that the diatom cells may armour the sediment which, together with reduced water content, due to emersion, resulted in higher erosion thresholds. In the present study, the relationship between sediment erosion potential (particularly critical shear stress) and carbohydrate concentration were found to be weak in comparison with the relationship between erosion potential and chlorophyll-*a* concentration. Given the findings of Tolhurst *et al.* (2003), this might be expected.

In the present study, sediment stability at the most polluted sites appears to be largely controlled by oligochaete biomass, microalgal biomass and possibly sediment carbohydrate concentration. As the level of pollution declined, structural and functional changes in the community between polluted and non-polluted sites appear to have caused an increase in erosion potential together with a change in the way in which the sediments erode. *H. diversicolor*, together with decreasing microalgal biomass and carbohydrate concentration, appears to become the most important influence over erosion potential. Whilst the net effect of macrofauna may be destabilising (de Dekere *et al.* 2001), erosion potential in the present context appears to be complicated by pollution induced changes to the sediment structure, as indicated by the relationship between erosion potential and redox potential. This was also demonstrated during laboratory studies using the CSM in the absence of macrofauna (Chapter 7). de Dekere *et al.* (2001) suggested that infaunal density alone was an inadequate descriptor of sediment stability and Christie *et al.* (2000) stated that the development of an estuarine mudflat is both complex and difficult to predict because of the multiple relationships between the physical and biological properties of the sediment.

In abiotic sediments, water content and bulk density are the primary controls over sediment stability (Williamson & Ockenden, 1996; Amos *et al*, 1998; Riethmüller *et al*, 1998). Whilst no relationship between sediment erosion potential and water content or bulk density was found, the potential effect of these parameters should not be ignored. Widdows *et al.* (2000) found reduced immersion time to lead to a marked decrease in sediment erodibility in upper shore areas. This was attributed to dehydration of the sediments but also to the reduced bioturbation activity of *M. balthica* during periods of emersion. However, spatial and temporal variability in the stability of the Humber mudflats was found to be consistent with major changes in the biota present rather than with changes in the physical properties of the sediment. Similarly, Tolhurst *et al.* (2000) found the wettest sediments to be the most stable and attributed this to the stabilising action of diatom biofilms.

8.3. WIDER ENVIRONMENTAL IMPLICATIONS

Chapter 1 highlighted the increasingly important role of intertidal mudflats in coastal protection. The effects of pollution, as demonstrated in the present study, led to the redistribution of the various functional groups of organisms. This, in combination with the direct effects of the pollutant on the sediment properties, appears to have led to increased stabilisation of the polluted sediments. Furthermore, a distinct reduction in the depth and complexity of the burrow structures at the more polluted sites was noted and a reduction in bioturbation induced by increasing effluent concentration led to a reduction in the surface roughness of the sediment. Both of these features could play an important role in wave attenuation since the increased frictional forces exerted by a rough bed will be more effective at retarding the flow and, during the flood tide, water may drain into complex burrow structures, further retarding the flow. In contrast, a smooth bed which would not exert such a high frictional force would be less effective at slowing down the flow (Wells & Coleman, 1981, in Dyer, 1998; CPSL, 2001). During an incoming tide or in the event of a storm, this may result in the waves energy being directed at upper shore areas, perhaps at the edge of saltmarshes or at the base of hard sea defences. This may then limit the protection offered by these defences. This is not considered to be an issue at Saltend since the effects of the discharge are extremely localised and are only present in the upper shore part of the mudflat where current speeds would be at a minimum. If an area lower down the shore were impacted, both wave attenuation and the supply of sediment to the upper shore may be reduced, as suggested by Widdows *et al.* (2000). The findings of the present study may therefore be of relevance to environmental impact assessment and to the design and optimal location for a discharge.

Widdows *et al.* (2000) found the intertidal distribution of *M. balthica* to be related to temperature, the highest densities being recorded following a cold winter, and suggested that the future abundance of this species could be adversely affected in the long term by climate change. Given the importance of *M. balthica* as a bioturbator, it was suggested that any change in the distribution and density of biota with important functional roles as 'stabilisers' and 'destabilisers' may alter the balance between the physical and biological processes involved in sediment erosion and deposition. For example, current and wave induced erosion over the lower mudflats may lead to increased accretion in the upper shore areas (Wells & Coleman, 1981, in Dyer, 1998). Therefore, the removal of destabilising macrofauna (and possible subsequent stabilisation of the sediment) from lower and mid shore areas may lead to reduced accretion on the upper shore (Wells & Coleman, 1981, in Dyer, 1998; Widdows *et al.*, 2000; CPSL, 2001). Similarly, the removal of macrofauna may lead to an increase in lower shore microalgal density (due to reduced grazing) and increase sediment stability. Widdows

et al. (2000) indicated that this increased sediment stability will allow more wave energy to reach the upper shore and this may affect the ability of natural sea defences to cope with the predicted sea level rise. Dalrymple & Liu (1978, in Murray *et al.*, 2002) also stated that changes in the elasticity of sediment, resulting from biological activity, will also affect the ability of a sediment to attenuate wave energy.

Finally, the importance of bioturbation on nutrient and contaminant fluxes was highlighted in Chapter 5 and Lee *et al.* (1981) highlighted the importance of the role of macrofauna in the degradation of hydrocarbons. The greater bioturbation potential of the communities present at the Paull and S200 m sites (Chapter 5) suggests greater potential for the removal of contaminants from the sediments at these sites. Gilbert *et al.* (1994), Gilbert *et al.* (1996), Schaffner *et al.* (1994) and Schaffner *et al.* (1997) all found the presence of macrofauna to increase the rate of removal of hydrocarbons from the sediment. This was the result of burrow irrigation together with aeration of the sediment, thus increasing the rate of decomposition, and the transportation of pollutants to the surface allowing photooxidation and evaporation. In contrast, Rasmussen *et al.* (1998) and Petersen *et al.* (1998) found *Arenicola marina* to increase the rate of incorporation of cadmium into the sediment and Petersen *et al.* (1998) found *H. diversicolor* and *C. volutator* to have a similar effect. This was explained by the adsorption of cadmium to metal oxides (magnesium and iron) present in the mucous linings of the burrows of these species. However, it should be noted that these experiments were carried out using clean sediments exposed to contaminated water.

Due to improvements in environmental legislation and tighter controls on effluent quality, it is more likely that organisms in a field situation would be exposed to a greater degree of sediment contamination than water contamination. This is because sediments act as a sink for contaminants following many years of toxic waste disposal in the past. Whilst surface sediments may be comparatively uncontaminated, deeper layers may still contain high concentrations of toxic compounds which could potentially be released during activities such as dredging. Lee *et al* (1981) suggested that whilst the abundance of polychaetes may initially be reduced upon exposure to hydrocarbons, species such as *Arenicola marina* and *Hediste diversicolor* can quickly re-establish themselves following a pollution incident, despite the continued presence of sediment bound contaminants. The effects of bioturbation are therefore of importance and it is suggested that the greater the burrowing depth and mixing potential of an organism, the greater the degree of contaminant removal will be.

Several studies have also indicated that the activity of burrowing macrofauna can enhance nutrient fluxes in and out of the sediment (e.g., Davey & Watson, 1995, Mortimer *et al.*, 1999)

and Rhoads (1974) stated that head down deposit feeding species could reintroduce deep, buried nutrients into the water column or the surface sediments. This can influence the distribution and growth of microorganisms (Hylleberg, 1975, Mortimer *et al.*, 1999) which, as mentioned in the previous section, has implications for sediment stability. However, Davey & Watson (1995) suggested that, in the Tamar estuary, populations of *H. diversicolor* could account for ammonium fluxes to the water column between 10 and 20 times greater than the measured inputs from riverine and sewage sources. Therefore, in estuaries with low flushing times, the activity of burrowing macrofauna may have implications for sewage discharge.

8.4. CONCLUSIONS

It is clear that the relationship between faunal community structure, sediment properties, pollution and their combined effect on sediment stability is complex. However, the study has highlighted the link between the physical, chemical and biological properties of the sediment and the way in which changes to one component can directly affect another component. The overall findings of the study can be summarised in the conceptual model given in Figure 8.1.

The discharge of the effluent from BP (**Figure 8.1, box A**) has had a clear impact on the faunal communities at Saltend in that it has led to reduced species diversity, increased abundance and decreased individual size and biomass. Coupled with the reduction in species diversity was a reduction in the diversity of functional groups present (in terms of their feeding and bioturbation behaviour). The effects of the discharge on benthic community structure were highly localised with maximum impact occurring between 0 and 25-50 m of the outfall. Beyond this, the community progressively recovered with increasing distance to reach maximum diversity at the Paull sites with a transitional community existing between the two extremes. Furthermore, the severity of the impact appeared to be seasonal with maximum effects being noted during the summer when the greatest degree of anoxia was recorded. During this time, the community at the S75 m site (a transitional site) showed a high degree of similarity to the S25 m site whilst, during the cooler months, this site showed a greater degree of similarity to the S200 m site. Toxicological studies showed that this may have been due to a direct link with the effluent toxicity (although this is suspected to be unlikely), accumulation of contaminants in the sediments and indirect effects through the effluents' influence on the sediment properties (i.e. in terms of redox potential). This is supported by the fact that the faunal communities and the sediment redox potential both follow the same seasonal trend.

This pollution-induced alteration of the faunal community (**box B**) has led to a reduction in bioturbation potential (**box B1**), demonstrated both in the field and the laboratory using a variety of techniques. The mode of sediment modification is altered with communities within the immediate vicinity of the discharge being dominated by high numbers of non-selective, sub-surface deposit feeding biodiffusers (**box B1**) in comparison to communities further away which are composed of selective and non-selective surface and subsurface deposit feeders, carnivores, detritivores and omnivores with a mix of conveyor belt species, regenerators and biodiffusers (**box B2**). Whilst the depth of bioturbation increases significantly as the level of pollution declines, it is thought that the rate of bioturbation by the communities close to the outfall may be increased. This is thought to be due to the sheer number of organisms together with the fact that some species are able to increase their rate of burrow irrigation in order to

remove sulphides and other toxins. However, there is no direct evidence for this in the present study and further work is required to confirm this.

In terms of impacts upon the sediment properties, general observations showed that the anoxic sediments at sites close to the discharge were generally black, oily and sticky. That is, their cohesive properties appear to have been enhanced (**box C**) either directly as a result of the oils in the effluent or indirectly through the increased microbial biomass (**box D**) associated with highly anoxic sediments. Sub-surface sediment shear strength increased at the more polluted sites, whilst sub-surface water content was reduced (as demonstrated by the water content profiles) (**box C**). This may either be related to shore height, with upper shore (polluted) sediments being exposed to the air for longer, thus allowing longer periods for consolidation and compaction. However, the impact of animal burrowing should also be considered as an explanation with deeper burrowing organisms increasing the water content and reducing the sediment shear strength at depth at the less polluted sites. During the present study bulk density was only measured in the surface sediments (top 5 cm) and no trends were found between sites. However, the impact of animal activity on bulk density at depth should be considered. Collectively, these changes in sediment properties (**boxes C and D**) could result in a decrease in both Type I (surface) and Type II (sustained erosion at deeper levels) erosion in polluted areas (**box E**). This was demonstrated in defaunated sediments treated with effluent.

Deep bioturbation by large organisms in cleaner sediments (**box B2**) results in the formation of mucous-bound burrow galleries. This may lead to increased Type II erosion (**box E**) as water penetrates the burrows, breaking the sediment off as lumps being transported as bedload, rather than as a suspension of fine particles. In addition, the deposition of faecal pellets, both at the surface and at depth within the sediment, together sediment resuspension due to burrow irrigation and scour due to increased surface roughness, may result in a high rate of Type I erosion (**box E**). In contrast, a high rate of faecal pellet deposition caused by the bioturbatory activity of communities in polluted sediments (**box B1**) may initially lead to a high rate of Type I erosion (**box E**). Once the surface layers are removed, the rate of erosion would be expected to decline until the current velocity became sufficiently high for erosion of the deeper, more consolidated layers to begin. However, in the case of both polluted and unpolluted sediments, the rate of Type I erosion could be reduced by the microbial colonisation of faecal pellets, thus increasing their cohesiveness and resistance to erosion. In the more polluted sediments, the enhanced microalgal biomass with its associated carbohydrate production, could further reduce the rate of Type I erosion (**boxes D and E**).

Whilst there is not sufficient evidence in the present study to predict the way in which pollution induced community change might impact upon the overall erosion potential of an intertidal mudflat, there is a suggestion that the petrochemical effluent examined in the present study has impacted directly upon the sediment properties. In turn, this has impacted upon the macrofaunal communities which, through changes in their bioturbatory activities, have directly influenced the physico chemical and erosional properties of the sediment. In addition to the interaction between the macrofaunal communities and the sediment properties, the discharge (possibly in combination with reduced grazing pressure) has enhanced the microbial communities and the amount of carbohydrate secreted which also has implications for sediment stability.

In summary, the findings of the present study suggest that the petrochemical effluent being discharged onto the Saltend mudflat has modified the sediment properties, macrofaunal community structure and microbial biomass (and the associated mucilage) in such a way that has led to the overall stabilisation of the sediments within the immediate vicinity of this particular discharge.

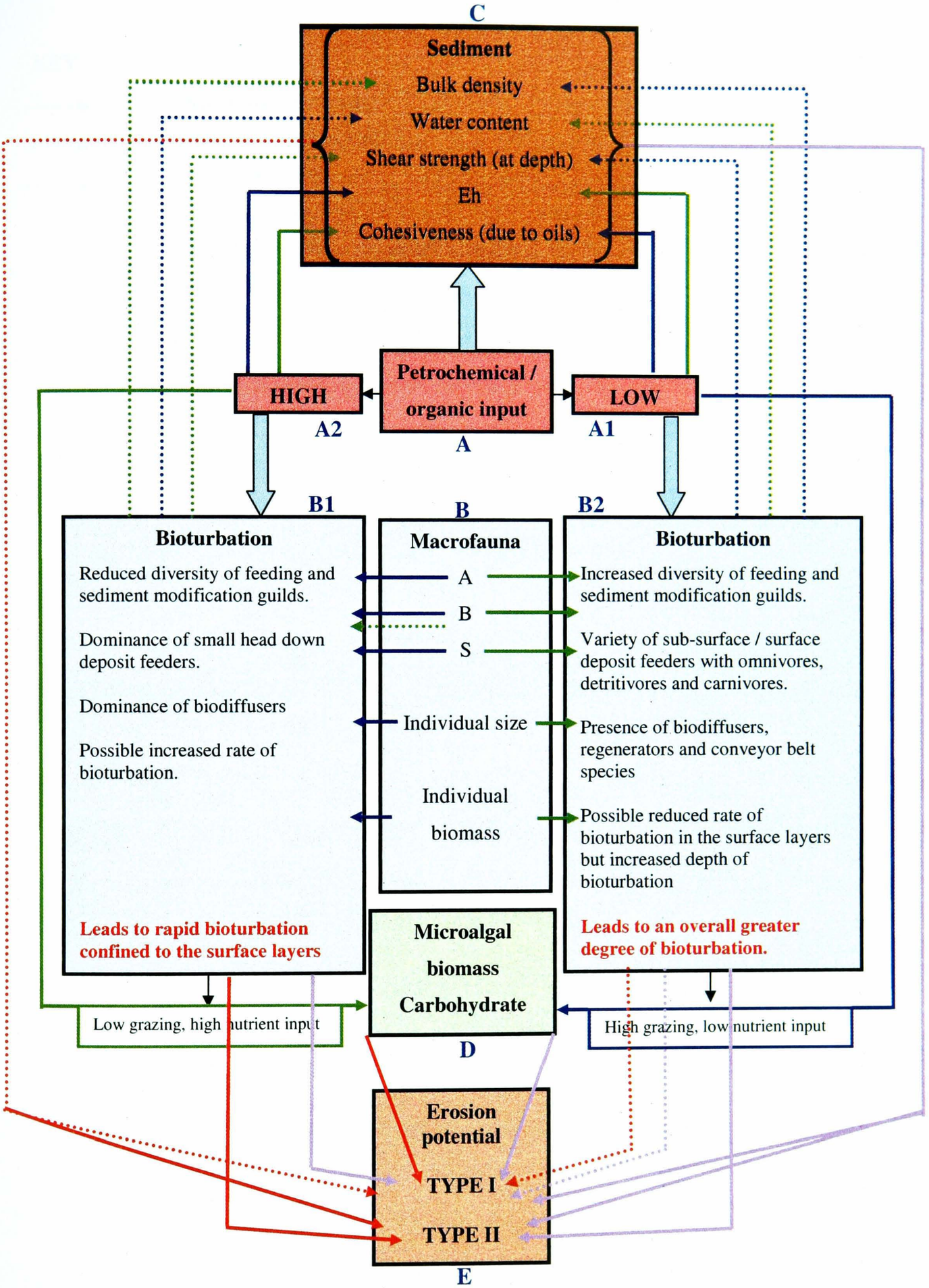


Figure 8.1. Generalised diagram of the interaction between sediment properties, pollution and macrofaunal community structure and their combined effect on erosion potential.

KEY:

Increase in a parameter

Decrease in a parameter

High erosion rate

Low erosion rate

Dotted line indicate an uncertain effect.

8.5. FURTHER STUDY

The present study has provided a detailed but wide ranging insight into the way in which pollution-induced changes to the macrobenthic community structure can impact upon the sediment properties and erosion potential. However, there are certain aspects which need to be considered in greater detail in order to better explain the findings of this study and the overall interaction of animal-sediment relationships and pollution:

- Firstly, due to the difficulties associated with working with such organisms, the importance of oligochaetes, their response to the discharge and their bioturbatory activity has been largely overlooked in the present study. Several assumptions have been made about their potential effects on sediment properties. However, it is now considered necessary *to examine the effects of this type of pollution on oligochaete survival, growth and production. Following this, the effect of pollution on the rate of bioturbation and faecal pellet production should be examined.*

- *The effects of community structure and the associated bioturbatory activity on the physical properties of the sediment (water content profiles, particle size distribution, redox conditions and bulk density)* require further consideration. During the present study, each of these parameters has been measured but no conclusions about the extent to which the various community types or species impact upon these properties could be reached.

- *A more detailed analysis of the size/biomass frequency distribution of the organisms present in relation to pollution.* Following this, a more detailed study of the impact of animal size on bioturbation potential should be carried out since the present study largely concentrated on the effects of individual species, abundance and the structure of the communities present along the pollution gradient.

- *Further development of the field and laboratory techniques* employed in the present study. These were generally successful, particularly in terms of visual representation of differences in bioturbation potential. However, quantification was often difficult and, to a large extent, subjective (e.g., the photographic technique and quantification of burrow volumes from the resin casts). It is suggested that the photographic technique be further developed as a sub-lethal bioassay for the determination of the effects of sediment contamination on infaunal organisms. This could replace assays such as the *Arenicola marina* cast production assay for use with species which do not

produce casts. This would allow toxicity testing of effluents using locally important species exposed to a relevant sediment type, thus removing effects of sub-optimal sediment types on the rate of bioturbation (e.g. *A. marina* does not naturally occur and cannot maintain its burrow in soft, fine grained, waterlogged sediments such as those found at Paull and Saltend).

- *A more thorough examination of the effects of season on community structure, bioturbation and their combined effects on sediment erosion potential* would be of valuable. Furthermore, the findings of the present study relate only to the effluent currently being discharge from BP Chemicals (Saltend) Ltd. Whilst examination of the effects of mixed effluents is of environmental relevance, these studies are site specific and therefore, no assumptions about the effects of 'pollution' in general on bioturbation and erosion potential can be made (although the findings of the present study may be applicable to other areas subject to organic or petrochemical pollution). It would be useful to repeat some of the bioturbation and erosion experiments using a different, perhaps inorganic, pollutant. It is suggested, initially, that for laboratory studies, a more chemically stable compound or effluent be used than that used in the present study. This would allow a more accurate dose-response relationship to be derived.

- *A repeat of the erosion experiments in the field* using a flume such as that used by Widdows *et al.* (1998a; 1998b). Whilst the CSM was useful, it has largely been used to examine the effects of microalgal population on sediment erosion potential. The area over which erosion is measured is extremely small and does not account for the heterogeneity of the sediment surface, caused by burrows, faecal casts, worm tubes and ripples.

- *A more detailed comparison of the erosion potential of aerobic and anaerobic sediments* would allow differentiation between the impact of the organisms and the impact of the effluent (in terms of its influence over Eh) on erosion potential.

- Microalgal biomass and carbohydrate production were found to be enhanced in the most polluted sediments. It would be of value to *examine the rate of microalgal production, and subsequent effect on sediment stability, in relation to effluent concentration.* Furthermore, it would be of value to *examine the effect of the effluent on mucous production by animals.* Mucous production might be expected to increase

due to irritation caused by the effluent. On the other hand, it may decrease as the rate of bioturbation by some of the more sensitive organisms decreases.

- The above work would allow the development of better regression models so that each link in the conceptual model (Figure 8.1) could be quantified. This would lead to the *development of a quantitative predictive mode* which could then be used for environmental management purposes.

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APPENDIX 1. CHARACTERISTICS OF THE BP EFFLUENT (1999-2000) PROVIDED BY THE EVIRONMENT AGENCY (WILLERBY OFFICE).

Determinand	Unit	17-Sep-99	11-Oct-99	04-Nov-99	05-Nov-99	09-Dec-99	09-Feb-00
Ammoniacal nitrogen	mg/l	3.16	3.69	4.76	2.02	1.86	2.89
BOD	mg/l	1459	954	1660	1730	1460	1640
COC	mg/l		3260	2200	2730	2280	2780
Conductivity	uS/cm	1840	2450	1720	1890	1700	1870
Nitrate	mg/l	0.17	1.15	5.86	3.05	0.749	2.98
Nitrite	mg/l	0.11	0.86	0.353	5.43	0.921	5.17
Total oxidised nitrogen	mg/l	0.28	2.01	6.21	8.48	1.67	8.15
Orthophosphate	mg/l	1.58	1.85	1.89	1.12	0.871	1.31
PH	pH units	7.15	6.38	5.84	5.48	5.52	5.1
Total phosphate	mg/l	1.68	2.05	2.05	1.32	1.41	1.62
Suspended solids@105C	mg/l	54	108	30	40	14	44
Arsenic	ug/l	2.88	4.25	7.5	5.19	3.1	5.57
Cadmium	ug/l	1.25	1.47	2.1	2.35	1.31	1.31
Chromium	ug/l	42.1	146	96.5	94.7	43.1	40.1
Copper	ug/l	104	826	197	130	129	176
Iron	ug/l	689	2520	1030	983	1350	1040
Lead	ug/l	4.98	6.16	10.6	3.02	6.12	5.93
Molybdenum	ug/l	437	753	755	716	34.4	774
Nickel	ug/l	56.1	619	75.7	116	98	65.2
Tributyltin	ug/l	0.004	0.004		0.017	0.004	
Triphenyltin	ug/l	0.001	0.001		0.001	0.001	
Zinc	ug/l	313	175	143	129	121	223
1,1,1-Trichloroethane	ug/l	8.5	8.5	1.11	0.46	0.26	0.88
1,2,3-Trichlorobenzene	ug/l	0.125	0.125	0.075		0.075	
1,2,4-Trichlorobenzene	ug/l	0.125	0.125	0.075		0.075	
1,2-Dichloroethane	ug/l	15	15	0.2	0.2	0.2	0.2
1,3,5-Trichlorobenzene	ug/l		0.125	0.075		0.075	
Hexachloro 1,3 Butadiene	ug/l		0.075	0.045		0.075	
Hexachlorobenzene	ug/l	0.05	0.05	0.03		0.05	
PCB congener 028	ug/l	0.1	0.1	0.045		0.1	
PCB congener 052	ug/l	0.125	0.125	0.06		0.125	
PCB congener 101	ug/l	0.025	0.025	0.015		0.025	
PCB congener 118	ug/l	0.1	0.1	0.045		0.1	
PCB congener 138	ug/l	0.05	0.05	0.03		0.05	

Determinand	Unit	17-Sep-99	11-Oct-99	04-Nov-99	05-Nov-99	09-Dec-99	09-Feb-00
PCB congener 153	ug/l	0.075	0.075	0.03		0.075	
PCB congener 180	ug/l	0.05	0.05	0.03		0.05	
Total PCB'S	ug/l	0.525	0.525				
Pentachlorophenol	ug/l	1.2					
Tetrachloroethene	ug/l	7.9	7.9	0.64	0.24	0.13	0.4
Tertachloromethane	ug/l	13	13	0.2	0.2	0.2	0.2
Trichlorobenzene (123, 124, & 135)	ug/l					0.225	
Trichloroethene	ug/l	10	10	0.1	0.1	0.1	0.1
Trichloromethane	ug/l	6.8	6.8	3.8	2.1	0.2	9
Azinphos - Ethyly	ug/l	0.02	0.02	0.05	0.05	0.02	0.02
Azinphos - Methyl	ug/l	0.02	0.02	0.05	0.05	0.02	0.02
Chlorfenvinphos	ug/l	0.02	0.02	0.005	0.01	0.02	0.02
Diazinon	ug/l	0.02	0.02	0.005	0.01	0.02	0.02
Fenitrothion	ug/l	0.02	0.02	0.01	0.01	0.02	0.02
Fenthion	ug/l	0.03	0.03	0.01	0.02	0.03	0.03
Propetamphos	ug/l	0.02	0.02	0.005	0.01	0.02	0.02
Simazine	ug/l	0.06	0.05	0.02	0.04	0.06	0.06
Aldrin	ug/l	0.075	0.075	0.045		0.075	
Atrazine	ug/l	0.06	0.05	0.02	0.04	0.06	0.06
DDE-pp	ug/l	0.05	0.05	0.03		0.05	
DDT-op	ug/l	0.05	0.05	0.03		0.05	
DDT-pp	ug/l	0.05		0.03		0.05	
Total DDT (-op, -pp, DDE-pp, TDE-pp)	ug/l			0.12		0.2	
Dichlorvos	ug/l	0.02	0.02	0.005	0.01	0.02	0.02
Dieldrin	ug/l	0.05	0.05	0.03		0.05	
Endosulphan Alpha	ug/l	0.075	0.075	0.045		0.075	
Endosulphan Beta	ug/l	0.075	0.075	0.045		0.075	
Endrin	ug/l	0.1	0.1	0.03		0.1	
HCH Alpha	ug/l	0.05	0.05	0.03		0.05	
HCH Beta	ug/l	0.1	0.1	0.06		0.1	
HCH Gamma	ug/l	0.05	0.05	0.03		0.05	
Total HCH	ug/l			0.12		0.2	
Isodrin	ug/l	0.05	0.05	0.03		0.05	
Malathion	ug/l	0.02	0.02	0.005	0.01	0.02	0.02
Parathion (ethyl)	ug/l	0.02	0.02	0.005	0.01	0.02	0.02
Parathion (methyl)	ug/l	0.02	0.02	0.005	0.01	0.02	0.02
TDE-pp	ug/l	0.05	0.05	0.03		0.05	
Trifluralin	ug/l	0.125	0.125	0.075		0.125	

APPENDIX 2 A

List of taxa, ranked by abundance (No. m^{-2}) together with their percent contribution to the community for the four key sites.

Paull 150 m (Abundance)

June 1999	A	%	Cum%
Hediste diversicolor	7709.8	48.78	48.78
Oligochaetes	2863.64	18.12	66.9
Manayunkia aestuarina	2478.15	15.68	82.58
Nematodes	1486.89	9.408	91.99
Corophium volutator	715.91	4.53	96.52
Macoma balthica	385.49	2.439	98.95
Spionidae indet.	165.21	1.045	100
Hydrobia ulvae	0	0	100

July 1999	A	%	Cum%
Hediste diversicolor	6608.4	40.34	40.34
Oligochaetes	2891.175	17.65	57.98
Corophium volutator	2409.313	14.71	72.69
Manayunkia aestuarina	2271.638	13.87	86.55
Nematodes	1445.588	8.824	95.38
Spionidae indet.	619.5375	3.782	99.16
Macoma balthica	137.675	0.84	100
Hydrobia ulvae	0	0	100

September 1999	A	%	Cum%
Hediste diversicolor	5025.138	59.84	59.84
Spionidae indet.	1514.425	18.03	77.87
Corophium volutator	1032.563	12.3	90.16
Oligochaetes	550.7	6.557	96.72
Macoma balthica	137.675	1.639	98.36
Nematodes	68.8375	0.82	99.18
Hydrobia ulvae	68.8375	0.82	100
Manayunkia aestuarina	0	0	100

November 1999	A	%	Cum%
Hediste diversicolor	6057.7	39.02	39.02
Corophium volutator	4289.69	27.63	66.65
Spionidae indet.	2973.78	19.16	85.81
Oligochaetes	1376.752	8.869	94.68
Nematodes	495.63	3.193	97.87
Macoma balthica	220.28	1.419	99.29
Manayunkia aestuarina	110.14	0.709	100
Hydrobia ulvae	0	0	100

January 2000	A	%	Cum%
Hediste diversicolor	4543.28	32.8	32.84
Oligochaetes	2753.50	19.9	52.74
Spionidae indet.	1996.29	14.4	67.16
Manayunkia aestuarina	1789.78	12.9	80.10
Corophium volutator	1652.10	11.9	92.04
Nematodes	757.21	5.5	97.51
Macoma balthica	344.19	2.5	100.00
Hydrobia ulvae	0.00	0	100.00

February 2000	A	%	Cum%
Oligochaetes	5562.07	37.27	37.27
Hediste diversicolor	3744.76	25.09	62.36
Spionidae indet.	2092.66	14.02	76.38
Manayunkia aestuarina	1872.38	12.55	88.93
Nematodes	826.05	5.535	94.46
Corophium volutator	605.77	4.059	98.52
Macoma balthica	220.28	1.476	100
Hydrobia ulvae	0	0	100

April 2000	A	%	Cum%
Hediste diversicolor	6553.33	43.12	43.12
Oligochaetes	5672.21	37.32	80.43
Spionidae indet.	1211.54	7.971	88.41
Manayunkia aestuarina	826.05	5.435	93.84
Nematodes	440.56	2.899	96.74
Corophium volutator	275.35	1.812	98.55
Macoma balthica	220.28	1.449	100
Hydrobia ulvae	0	0	100

Saltend 200 m (Abundance)

June 1999	A	%	Cum%
Oligochaetes	10132.88	54.76	54.76
Hediste diversicolor	6388.12	34.52	89.29
Spionidae indet.	881.12	4.762	94.05
Corophium volutator	495.63	2.679	96.73
Nematodes	495.63	2.679	99.4
Manayunkia aestuarina	110.14	0.595	100
Macoma balthica	0	0	100
Hydrobia ulvae	0	0	100

July 1999	A	%	Cum%
Oligochaetes	25791.12	56.31	56.31
Hediste diversicolor	12298.97	26.85	83.17
Nematodes	3120.633	6.814	89.98
Manayunkia aestuarina	2569.933	5.611	95.59
Corophium volutator	1652.1	3.607	99.2
Spionidae indet.	275.35	0.601	99.8
Macoma balthica	91.78333	0.2	100
Hydrobia ulvae	0	0	100

September 1999	A	%	Cum%
Hediste diversicolor	11674.84	42.66	42.66
Oligochaetes	10298.09	37.63	80.28
Corophium volutator	3524.48	12.88	93.16
Manayunkia aestuarina	1266.61	4.628	97.79
Nematodes	330.42	1.207	98.99
Spionidae indet.	165.21	0.604	99.6
Macoma balthica	110.14	0.402	100
Hydrobia ulvae	0	0	100

November 1999	A	%	Cum%
Oligochaetes	19659.99	61.03	61.03
Hediste diversicolor	9196.69	28.55	89.58
Corophium volutator	1266.61	3.932	93.52
Manayunkia aestuarina	1156.47	3.59	97.11
Nematodes	656.84	2.039	99.15
Spionidae indet.	275.35	0.855	100
Macoma balthica	0	0	100
Hydrobia ulvae	0	0	100

January 2000	A	%	Cum%
Hediste diversicolor	6057.7	44.35	44.35
Oligochaetes	5507	40.32	84.68
Nematodes	770.98	5.65	90.32
Manayunkia aestuarina	550.7	4.03	94.35
Spionidae indet.	385.49	2.82	97.18
Corophium volutator	385.49	2.82	100.00
Macoma balthica	0	0.00	100.00
Hydrobia ulvae	0	0.00	100.00

February 2000	A	%	Cum%
Hediste diversicolor	10353.16	44.98	44.98
Oligochaetes	9692.32	42.11	87.08
Nematodes	1982.52	8.612	95.69
Spionidae indet.	440.56	1.914	97.61
Manayunkia aestuarina	275.35	1.196	98.8
Corophium volutator	275.35	1.196	100
Macoma balthica	0	0	100
Hydrobia ulvae	0	0	100

April 2000	A	%	Cum%
Oligochaetes	11564.7	42.94	42.94
Hediste diversicolor	9086.55	33.74	76.69
Corophium volutator	2368.01	8.793	85.48
Manayunkia aestuarina	2202.8	8.18	93.66
Nematodes	1321.68	4.908	98.57
Spionidae indet.	330.42	1.227	99.8
Macoma balthica	55.07	0.204	100
Hydrobia ulvae	0	0	100

Saltend 75 m (Abundance)

June 1999	A	%	Cum%
Oligochaetes	53362.83	80.48	80.48
Hediste diversicolor	8150.36	12.29	92.77
Nematodes	4736.02	7.143	99.92
Spionidae indet.	55.07	0.083	100
Macoma balthica	0	0	100
Manayunkia aestuarina	0	0	100
Corophium volutator	0	0	100
Hydrobia ulvae	0	0	100

July 1999	A	%	Cum%
Oligochaetes	36621.55	65.65	65.65
Nematodes	14813.83	26.55	92.2
Hediste diversicolor	4295.46	7.7	99.9
Manayunkia aestuarina	55.07	0.099	*100*
Macoma balthica	0	0	100
Spionidae indet.	0	0	100
Corophium volutator	0	0	100
Hydrobia ulvae	0	0	100

September 1999	A	%	Cum%
Oligochaetes	25387.27	69.95	69.95
Hediste diversicolor	8425.71	23.22	93.17
Nematodes	1211.54	3.338	96.51
Manayunkia aestuarina	991.26	2.731	99.24
Corophium volutator	275.35	0.759	100
Macoma balthica	0	0	100
Spionidae indet.	0	0	100
Hydrobia ulvae	0	0	100

November 1999	A	%	Cum%
Oligochaetes	9014	53.55	53.55
Hediste diversicolor	6277.98	37.29	90.84
Nematodes	1046.33	6.216	97.06
Manayunkia aestuarina	385.49	2.29	99.35
Corophium volutator	110.14	0.654	100
Macoma balthica	0	0	100
Spionidae indet.	0	0	100
Hydrobia ulvae	0	0	100

January 2000	A	%	Cum%
Oligochaetes	37007.04	89.12	89.12
Hediste diversicolor	4240.39	10.21	99.34
Nematodes	220.28	0.53	99.87
Manayunkia aestuarina	55.07	0.13	100.00
Macoma balthica	0	0.00	100.00
Spionidae indet.	0	0.00	100.00
Corophium volutator	0	0.00	100.00
Hydrobia ulvae	0	0.00	100.00

February 2000	A	%	Cum%
Oligochaetes	66414.42	91.92	91.92
Hediste diversicolor	3909.97	5.412	97.33
Nematodes	1872.38	2.591	99.92
Manayunkia aestuarina	55.07	0.076	100
Macoma balthica	0	0	100
Spionidae indet.	0	0	100
Corophium volutator	0	0	100
Hydrobia ulvae	0	0	100

April 2000	A	%	Cum%
Oligochaetes	60081.37	87.7	87.7
Hediste diversicolor	4680.95	6.833	94.53
Nematodes	3524.48	5.145	99.68
Manayunkia aestuarina	110.14	0.161	99.84
Spionidae indet.	110.14	0.161	100
Macoma balthica	0	0	100
Corophium volutator	0	0	100
Hydrobia ulvae	0	0	100

Saltend 25 m (Abundance)

June 1999	A	%	Cum%
Oligochaetes	123797.4	96.65	96.65
Nematodes	4020.11	3.138	*99.79*
Hediste diversicolor	275.35	0.215	*100*
Macoma balthica	0	0	*100*
Manayunkia aestuarina	0	0	100
Spionidae indet.	0	0	*100*
Corophium volutator	0	0	*100*
Hydrobia ulvae	0	0	100

July 1999	A	%	Cum%
Oligochaetes	75556.04	98.63	98.63
Hediste diversicolor	826.05	1.078	99.71
Nematodes	220.28	0.288	100
Macoma balthica	0	0	100
Manayunkia aestuarina	0	0	100
Spionidae indet.	0	0	100
Corophium volutator	0	0	100
Hydrobia ulvae	0	0	100

September 1999	A	%	Cum%
Oligochaetes	93398.72	98.15	98.15
Hediste diversicolor	1486.89	1.563	99.71
Nematodes	275.35	0.289	100
Macoma balthica	0	0	100
Manayunkia aestuarina	0	0	100
Spionidae indet.	0	0	100
Corophium volutator	0	0	100
Hydrobia ulvae	0	0	100

January 2000	A	%	Cum%
Oligochaetes	18668.73	88.28	88.28
Hediste diversicolor	2368.01	11.2	99.48
Nematodes	110.14	0.521	100
Macoma balthica	*0*	0	100
Manayunkia aestuarina	*0*	0	100
Spionidae indet.	*0*	0	100
Corophium volutator	0	0	100
Hydrobia ulvae	*0*	0	100

February 2000	A	%	Cum%
Oligochaetes	54464.23	96.77	96.77
Hediste diversicolor	1376.75	2.446	99.22
Nematodes	440.56	0.783	100
Macoma balthica	0	0	100
Manayunkia aestuarina	0	0	100
Spionidae indet.	0	0	100
Corophium volutator	0	0	100
Hydrobia ulvae	0	0	100

April 2000	A	%	Cum%
Oligochaetes	72141.7	97.25	97.25
Hediste diversicolor	1817.31	2.45	99.7
Nematodes	220.28	0.297	100
Macoma balthica	0	*0*	100
Manayunkia aestuarina	0	*0*	100
Spionidae indet.	0	*0*	100
Corophium volutator	0	*0*	100
Hydrobia ulvae	0	0	100

APPENDIX 2 B

List of taxa ranked by biomass (g m^{-2}), together with percent contribution to the community, for the four key sites.

Paull 150 m (Biomass)

June 1999	B	%	Cum%
Macoma balthica	76.580	51.73	51.73
Hediste diversicolor	69.482	46.93	98.66
Corophium volutator	1.432	0.967	99.62
Manayunkia aestuarina	0.435	0.294	99.92
Oligochaetes	0.099	0.067	99.98
Nematodes	0.021	0.014	100
Spionidae indet.	0.002	0.001	100
Hydrobia ulvae	0.000	0	100

July 1999	B	%	Cum%
Hediste diversicolor	32.395	83.74	83.74
Macoma balthica	5.004	12.94	96.67
Corophium volutator	0.964	2.491	99.16
Spionidae indet.	0.110	0.285	99.45
Nematodes	0.096	0.249	99.7
Manayunkia aestuarina	0.069	0.178	99.88
Oligochaetes	0.048	0.125	100
Hydrobia ulvae	0.000	0	100

September 1999	B	%	Cum%
Hediste diversicolor	30.181	93.53	93.53
Corophium volutator	0.792	2.455	95.99
Macoma balthica	0.555	1.719	97.71
Hydrobia ulvae	0.511	1.585	99.29
Spionidae indet.	0.199	0.617	99.91
Oligochaetes	0.028	0.087	100
Nematodes	0.001	0.004	100
Manayunkia aestuarina	0.000	0	100

November 1999	B	%	Cum%
Hediste diversicolor	8.197	78.9	78.9
Macoma balthica	1.115	10.73	89.62
Corophium volutator	0.867	8.348	97.97
Spionidae indet.	0.173	1.664	99.64
Oligochaetes	0.038	0.364	100
Manayunkia aestuarina	0.000	0	100
Nematodes	0.000	0	100
Hydrobia ulvae	0.000	0	100

January 2000	B	%	Cum%
Hediste diversicolor	15.499	57.21	57.21
Macoma balthica	10.657	39.33	96.54
Corophium volutator	0.624	2.304	98.84
Oligochaetes	0.139	0.513	99.36
Spionidae indet.	0.114	0.422	99.78
Manayunkia aestuarina	0.043	0.158	99.94
Nematodes	0.017	0.064	100
Hydrobia ulvae	0.000	0	100

February 2000	B	%	Cum%
Macoma balthica	17.589	55.45	55.45
Hediste diversicolor	12.959	40.86	96.31
Spionidae indet.	0.427	1.346	97.65
Oligochaetes	0.361	1.139	98.79
Corophium volutator	0.320	1.009	99.8
Manayunkia aestuarina	0.037	0.118	99.92
Nematodes	0.025	0.08	100
Hydrobia ulvae	0.000	0	100

April 2000	B	%	Cum%
Macoma balthica	75.857	60.64	60.64
Hediste diversicolor	45.713	36.54	97.19
Oligochaetes	2.082	1.665	98.85
Corophium volutator	0.892	0.713	99.56
Manayunkia aestuarina	0.298	0.238	99.8
Spionidae indet.	0.210	0.168	99.97
Nematodes	0.039	0.031	100
Hydrobia ulvae	0.000	0	100

Saltend 200 m (Biomass)

June 1999	B	%	Cum%
Hediste diversicolor	64.625	87.35	87.35
Corophium volutator	7.297	9.862	97.21
Oligochaetes	2.010	2.717	99.93
Spionidae indet.	0.030	0.04	99.97
Manayunkia aestuarina	0.022	0.03	100
Nematodes	0.002	0.003	100
Macoma balthica	0.000	0	100
Hydrobia ulvae	0.000	0	100

July 1999	B	%	Cum%
Hediste diversicolor	114.73	90.65	90.65
Oligochaetes	5.00	3.952	94.6
Macoma balthica	4.78	3.778	98.38
Corophium volutator	1.91	1.508	99.88
Nematodes	0.07	0.058	99.94
Manayunkia aestuarina	0.06	0.044	99.99
Spionidae indet.	0.02	0.015	100
Hydrobia ulvae	0.00	0	100

September 1999	B	%	Cum%
Hediste diversicolor	68.177	70.63	70.63
Macoma balthica	24.947	25.85	96.48
Oligochaetes	2.013	2.085	98.57
Corophium volutator	1.258	1.303	99.87
Manayunkia aestuarina	0.078	0.08	99.95
Spionidae indet.	0.044	0.046	100
Nematodes	0.004	0.004	100
Hydrobia ulvae	0	0	100

November 1999	B	%	Cum%
Hediste diversicolor	17.822	92.29	92.29
Oligochaetes	1.060	5.487	97.78
Corophium volutator	0.336	1.742	99.52
Manayunkia aestuarina	0.074	0.384	99.9
Spionidae indet.	0.015	0.077	99.98
Nematodes	0.004	0.02	100
Macoma balthica	0.000	0	100
Hydrobia ulvae	0.000	0	100

January 2000	B	%	Cum%
Hediste diversicolor	31.591	93.5	93.5
Oligochaetes	1.443	4.272	97.78
Corophium volutator	0.665	1.967	99.74
Spionidae indet.	0.067	0.197	99.94
Nematodes	0.013	0.037	99.98
Manayunkia aestuarina	0.008	0.023	100
Macoma balthica	0.000	0	100
Hydrobia ulvae	0.000	0	100

February 2000	B	%	Cum%
Hediste diversicolor	35.517	90.91	90.91
Oligochaetes	3.319	8.495	99.4
Spionidae indet.	0.105	0.268	99.67
Corophium volutator	0.097	0.248	99.92
Nematodes	0.027	0.069	99.99
Manayunkia aestuarina	0.006	0.014	100
Macoma balthica	0.000	0	100
Hydrobia ulvae	0.000	0	100

April 2000	B	%	Cum%
Hediste diversicolor	91.160	62.2	62.2
Macoma balthica	42.099	28.73	90.93
Oligochaetes	9.414	6.424	97.35
Corophium volutator	3.638	2.482	99.83
Spionidae indet.	0.086	0.059	99.89
Manayunkia aestuarina	0.082	0.056	99.95
Nematodes	0.080	0.054	100
Hydrobia ulvae	0.000	0	100

Saltend 75 m (Biomass)

June 1999	B	%	Cum%
Hediste diversicolor	84.879	91.6	91.6
Oligochaetes	7.710	8.32	99.92
Nematodes	0.074	0.08	100
Spionidae indet.	0.003	0.003	100
Macoma balthica	0.000	0	100
Manayunkia aestuarina	0.000	0	100
Corophium volutator	0.000	0	100
Hydrobia ulvae	0.000	0	100

July 1999	B	%	Cum%
Hediste diversicolor	53.231	88.21	88.21
Oligochaetes	7.071	11.72	99.93
Nematodes	0.039	0.064	99.99
Manayunkia aestuarina	0.006	0.009	100
Macoma balthica	0.000	0	100
Spionidae indet.	0.000	0	100
Corophium volutator	0.000	0	100
Hydrobia ulvae	0.000	0	100

September 1999	B	%	Cum%
Hediste diversicolor	83.948	95.94	95.94
Oligochaetes	3.182	3.637	99.58
Corophium volutator	0.350	0.4	99.98
Nematodes	0.014	0.016	100
Manayunkia aestuarina	0.004	0.004	100
Macoma balthica	0.000	0	100
Spionidae indet.	0.000	0	100
Hydrobia ulvae	0.000	0	100

November 1999	B	%	Cum%
Hediste diversicolor	16.299	96.57	96.57
Oligochaetes	0.513	3.038	99.6
Corophium volutator	0.048	0.287	99.89
Nematodes	0.009	0.055	99.95
Manayunkia aestuarina	0.009	0.055	100
Macoma balthica	0.000	0	100
Spionidae indet.	0.000	0	100
Hydrobia ulvae	0.000	0	100

January 2000	B	%	Cum%
Hediste diversicolor	40.304	90.8	90.8
Oligochaetes	4.074	9.179	99.98
Nematodes	0.007	0.016	100
Manayunkia aestuarina	0.001	0.001	100
Macoma balthica	0.000	0	100
Spionidae indet.	0.000	0	100
Corophium volutator	0.000	0	100
Hydrobia ulvae	0.000	0	100

February 2000	B	%	Cum%
Hediste diversicolor	70.774	79.28	79.28
Oligochaetes	18.470	20.69	99.97
Nematodes	0.029	0.033	100
Manayunkia aestuarina	0.001	0.001	100
Macoma balthica	0.000	0	100
Spionidae indet.	0.000	0	100
Corophium volutator	0.000	0	100
Hydrobia ulvae	0.000	0	100

April 2000	B	%	Cum%
Hediste diversicolor	87.791	78.52	78.52
Oligochaetes	23.967	21.44	99.95
Nematodes	0.052	0.046	100
Spionidae indet.	0.001	1E-03	100
Manayunkia aestuarina	0.001	5E-04	100
Macoma balthica	0.000	0	100
Corophium volutator	0.000	0	100
Hydrobia ulvae	0.000	0	100

Saltend 25 m (Biomass)

June 1999	B	%	Cum%
Oligochaetes	31.70	91.71	91.71
Hediste diversicolor	2.84	8.22	99.93
Nematodes	0.02	0.069	100
Macoma balthica	0.00	0	100
Manayunkia aestuarina	0.00	0	100
Spionidae indet.	0.00	0	100
Corophium volutator	0.00	0	100
Hydrobia ulvae	0.00	0	100

July 1999	B	%	Cum%
Oligochaetes	32.067	59.64	59.64
Hediste diversicolor	21.687	40.34	99.98
Nematodes	0.011	0.02	100
Macoma balthica	0.000	0	100
Manayunkia aestuarina	0.000	0	100
Spionidae indet.	0.000	0	100
Corophium volutator	0.000	0	100
Hydrobia ulvae	0	0	100

September 1999	B	%	Cum%
Oligochaetes	40.150	68.42	68.42
Hediste diversicolor	18.534	31.58	100
Nematodes	0.002	0.003	100
Macoma balthica	0.000	0	100
Manayunkia aestuarina	0.000	0	100
Spionidae indet.	0.000	0	100
Corophium volutator	0.000	0	100
Hydrobia ulvae	0.000	0	100

January 2000	B	%	Cum%
Hediste diversicolor	26.77558	81.58	81.58
Oligochaetes	6.04283	18.41	99.99
Nematodes	0.002754	0.008	100
Macoma balthica	0	0	100
Manayunkia aestuarina	0	0	100
Spionidae indet.	0	0	100
Corophium volutator	0	0	100
Hydrobia ulvae	0	0	100

February 2000	B	%	Cum%
Hediste diversicolor	37.943	53.52	53.52
Oligochaetes	32.946	46.48	100
Nematodes	0.001	0.002	100
Macoma balthica	0.000	0	100
Manayunkia aestuarina	0.000	0	100
Spionidae indet.	0.000	0	100
Corophium volutator	0.000	0	100
Hydrobia ulvae	0.000	0	100

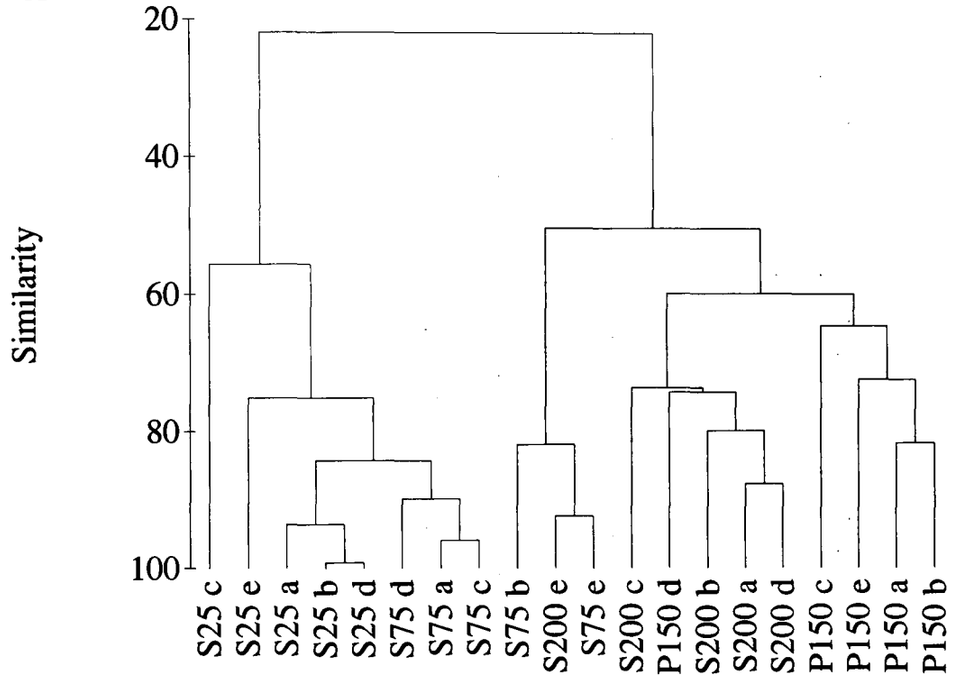
April 2000	B	%	Cum%
Oligochaetes	71.708	61.29	61.29
Hediste diversicolor	45.284	38.71	100
Nematodes	0.004	0.004	100
Macoma balthica	0.000	0	100
Manayunkia aestuarina	0.000	0	100
Spionidae indet.	0.000	0	100
Corophium volutator	0.000	0	100
Hydrobia ulvae	0.000	0	100

APPENDIX 3. Cluster analysis using replicate data (June 1999 - April 2000).

***A* = untransformed; *B* = √√ transformed.**

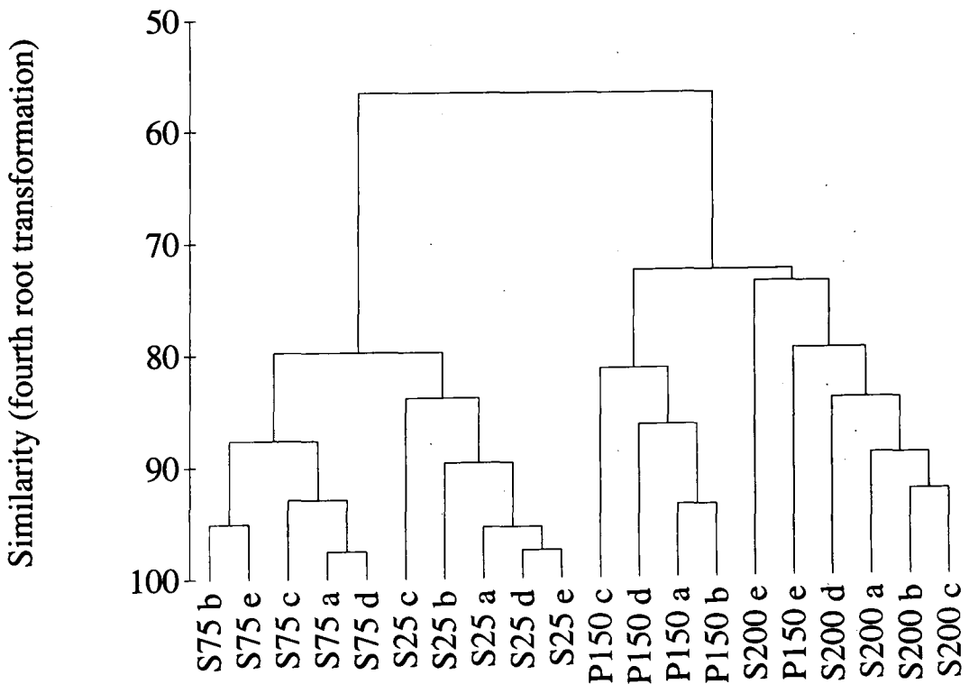
Replicate abundance (June 1999)

A



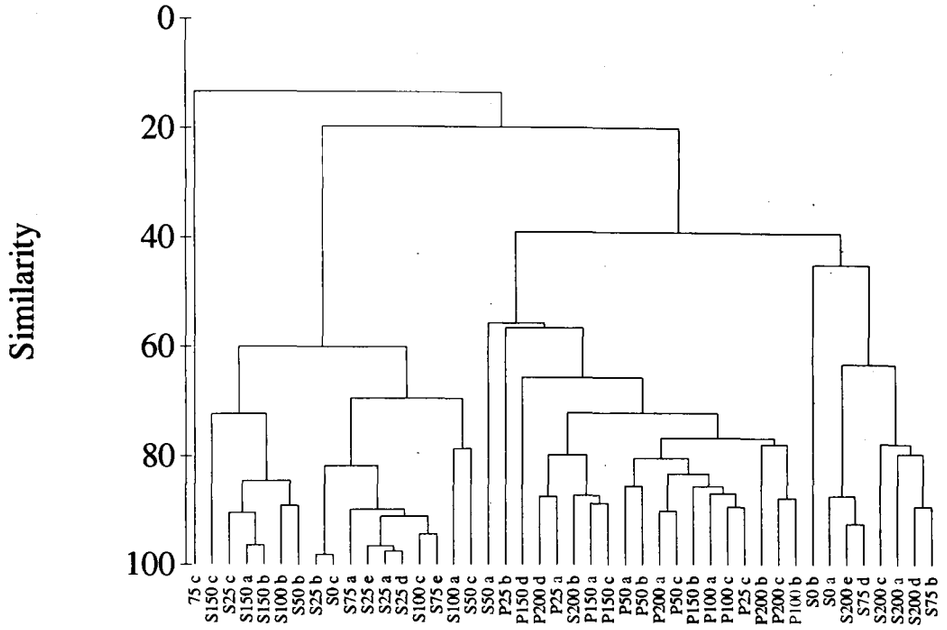
Replicate abundance (June 1999)

B



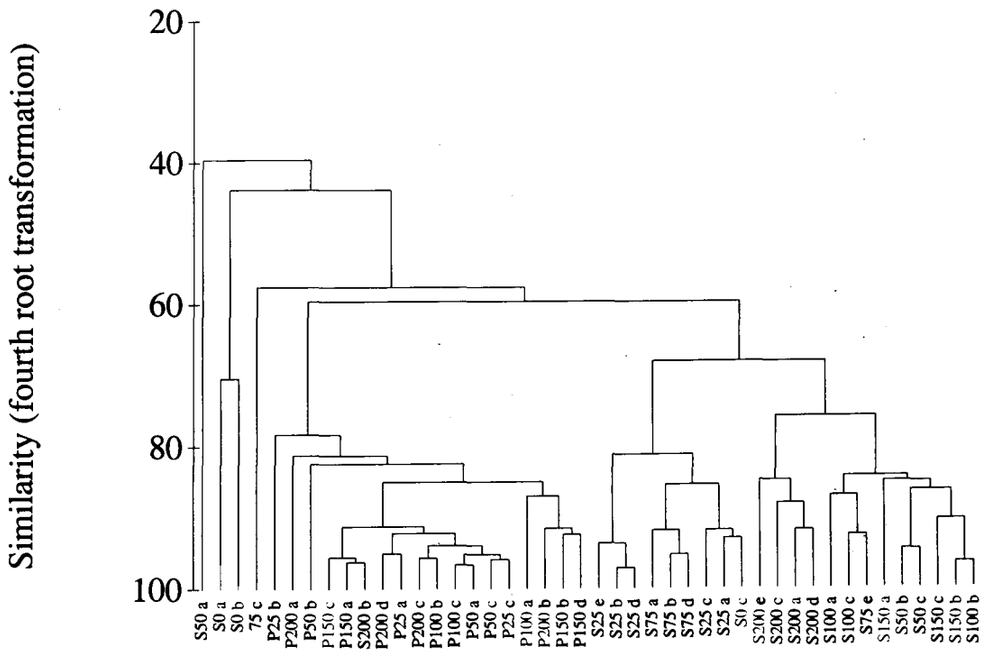
Replicate abundance (July 1999)

A



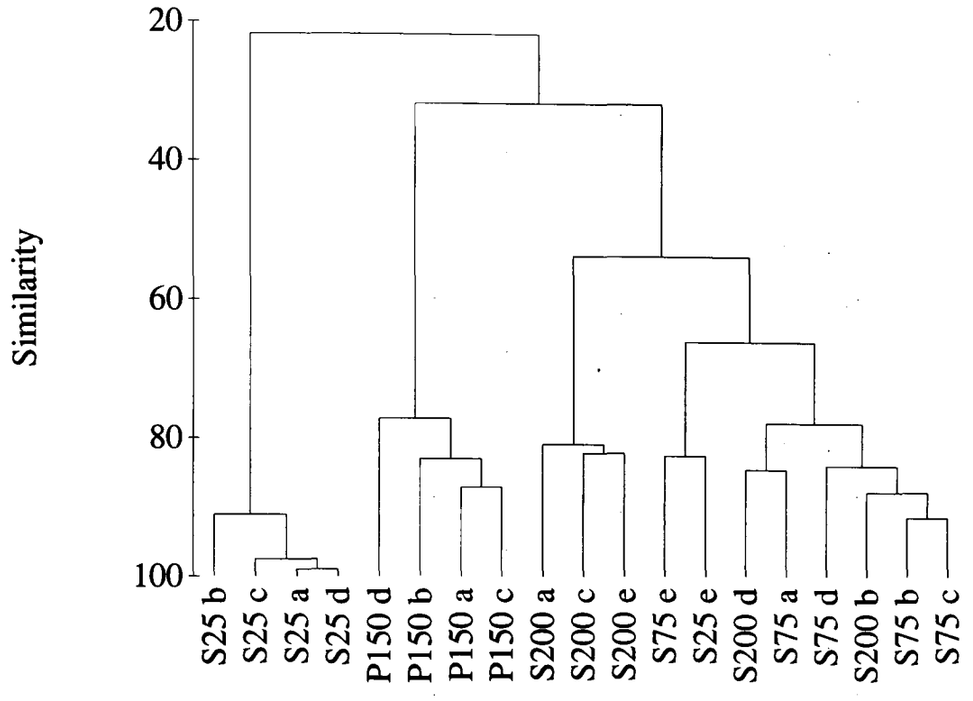
Replicate abundance (July 1999)

B



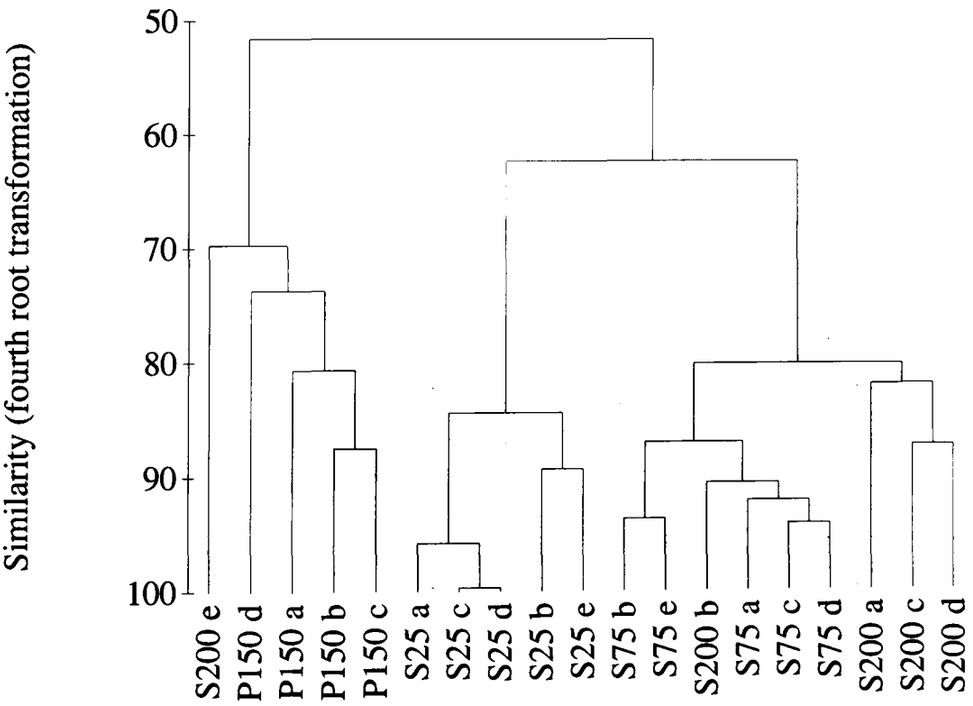
Replicate abundance (September 1999)

A



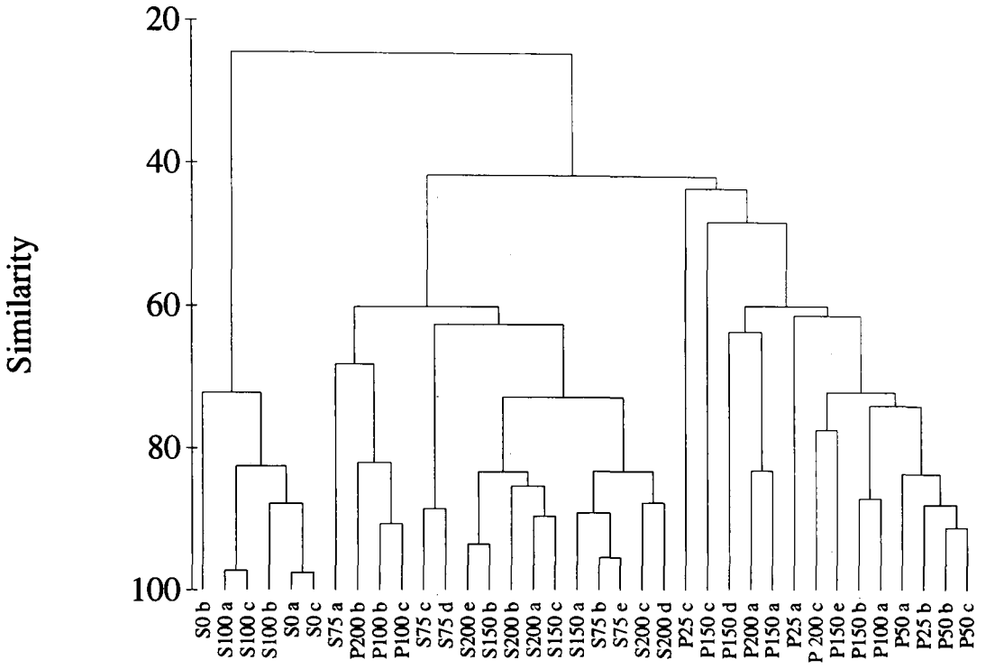
Replicate abundance (September 1999)

B



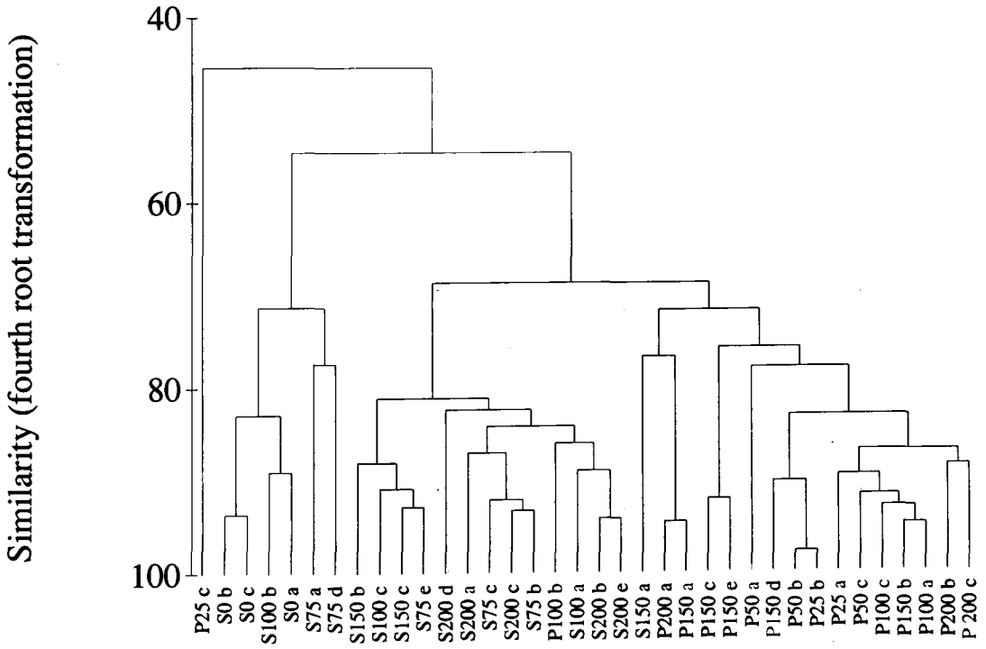
Replicate abundance (November 1999)

A



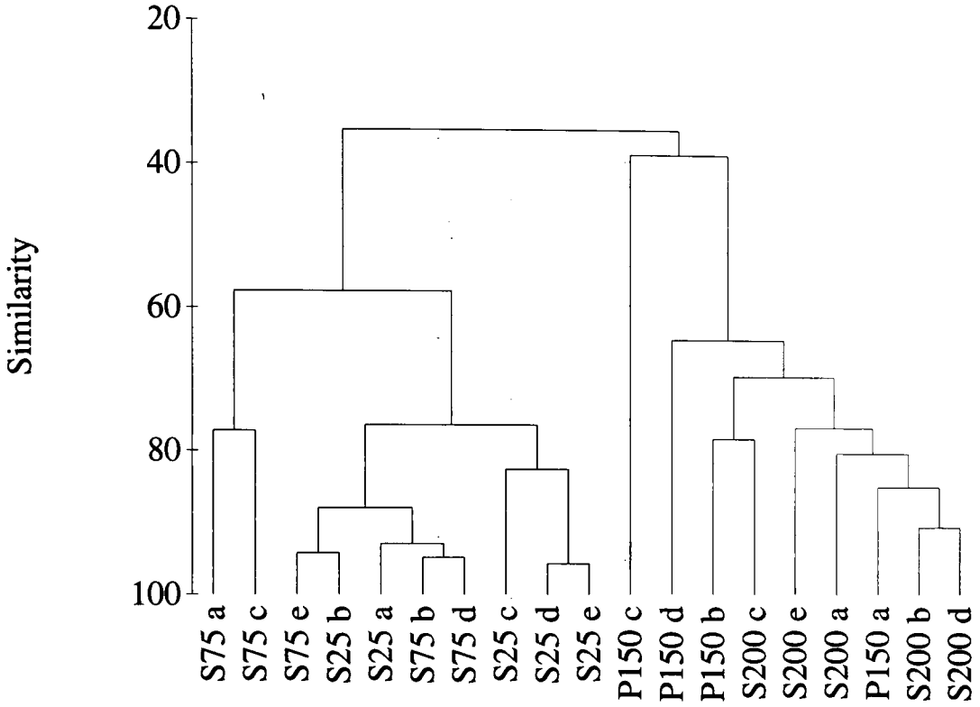
Replicate abundance (November 1999)

B



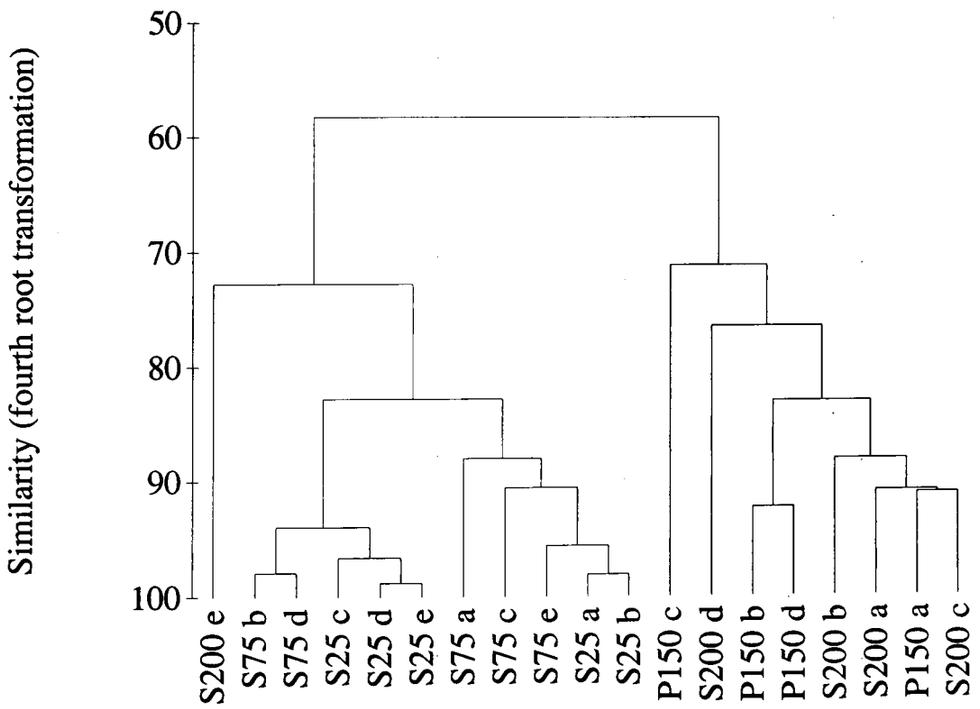
Replicate abundance (January 2000)

A



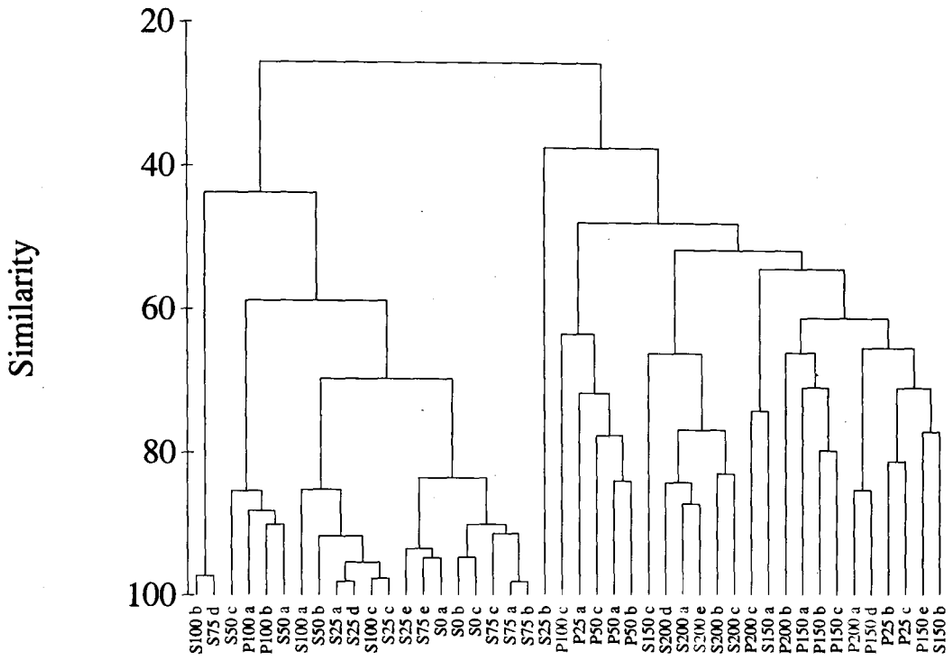
Replicate abundance (January 2000)

B



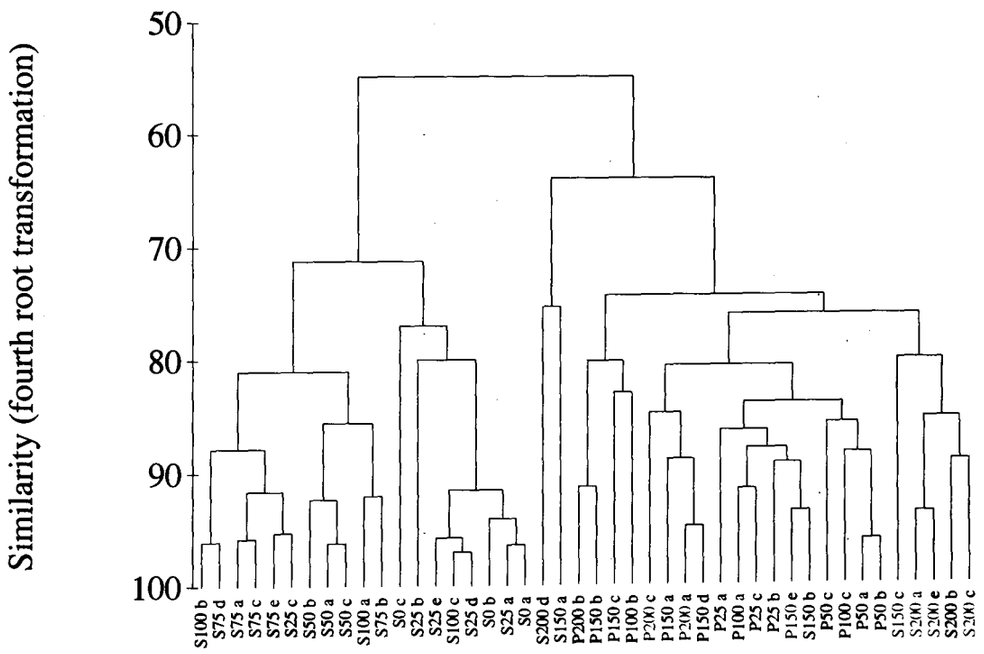
A

Replicate abundance (February 2000)



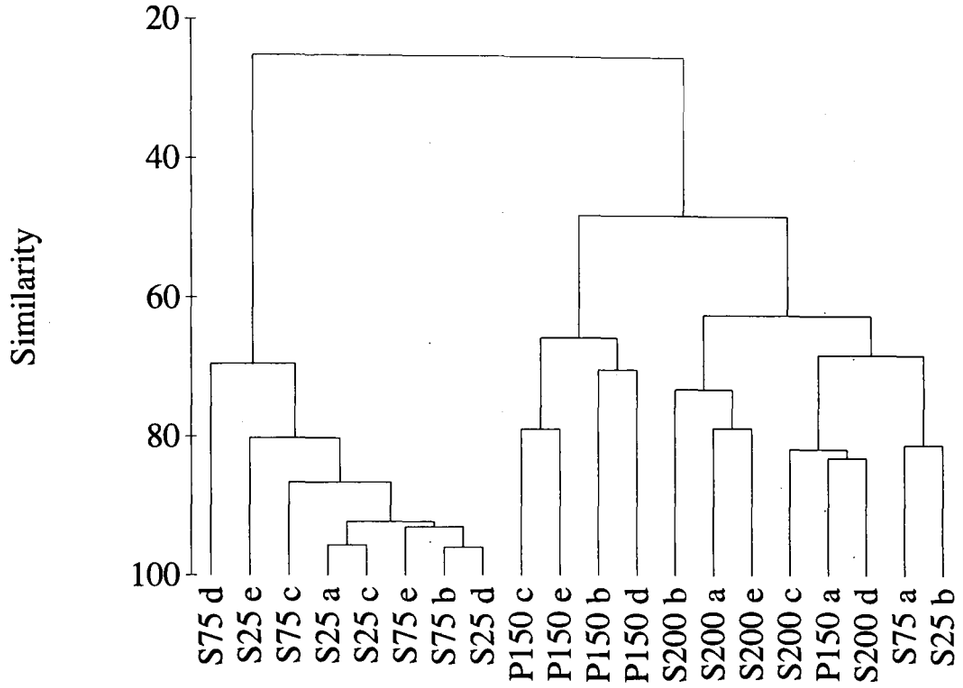
B

Replicate abundance (February 2000)



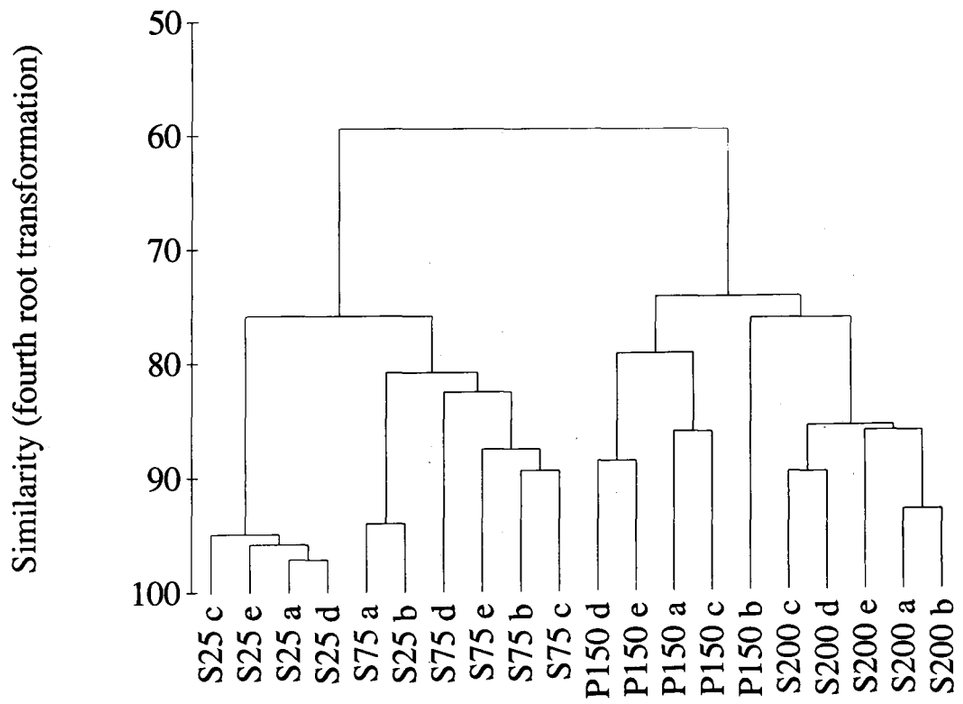
Replicate abundance (April 2000)

A

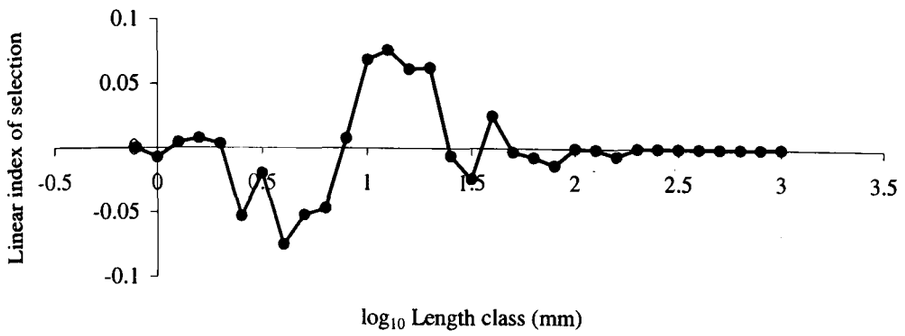


Replicate abundance (April 2000)

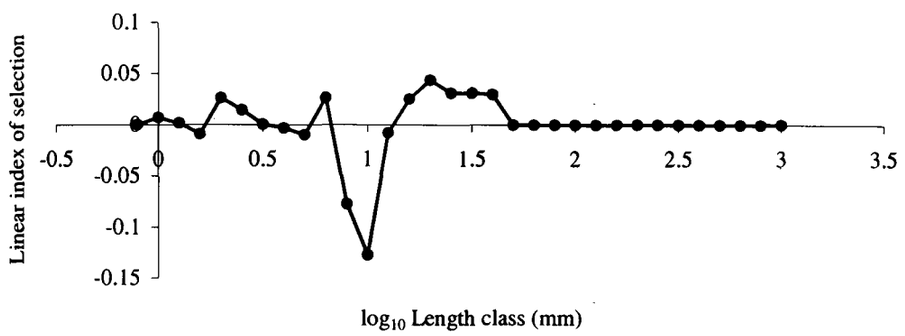
B



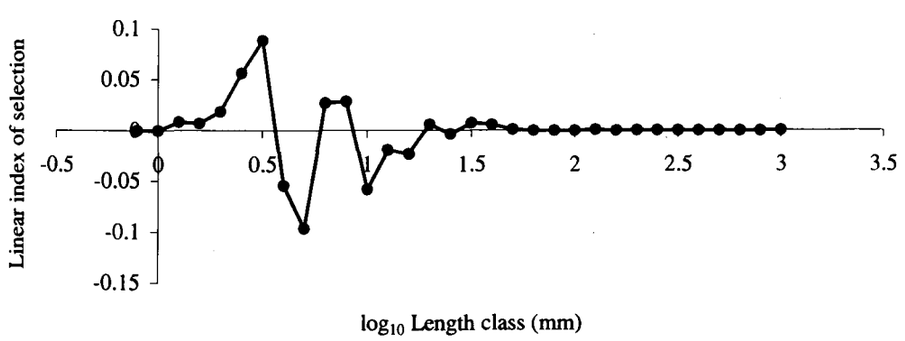
APPENDIX 4A. LINEAR INDEX OF SELECTION (LENGTH)



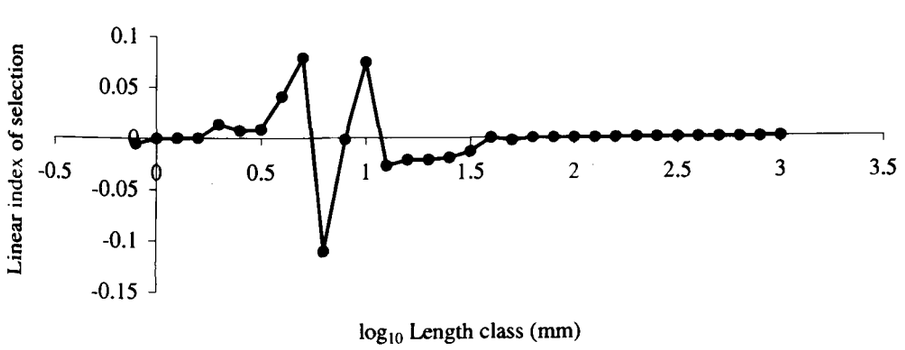
P Jan vs P Jun



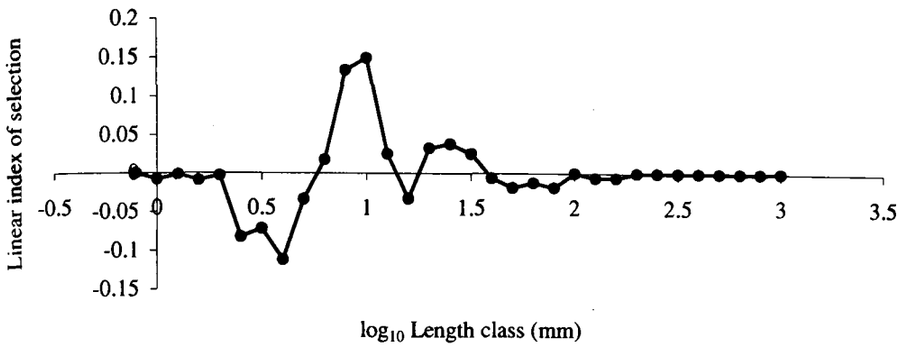
S200 Jan vs S200 Jun



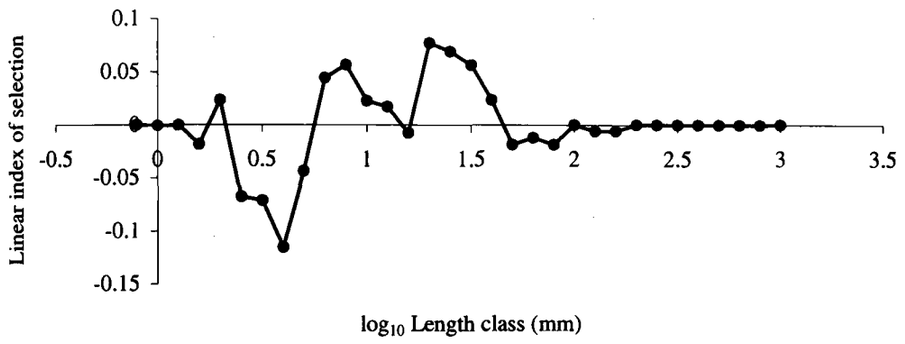
S75 Jan vs S75 Jun



S25 Jan vs S25 Jun



P Jan vs S200 Jan



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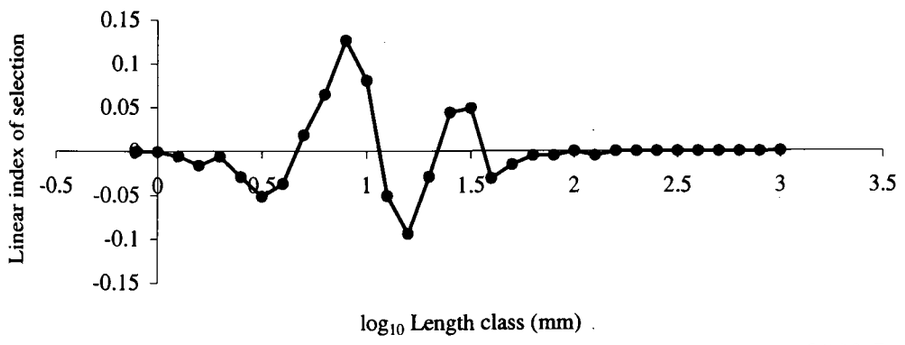
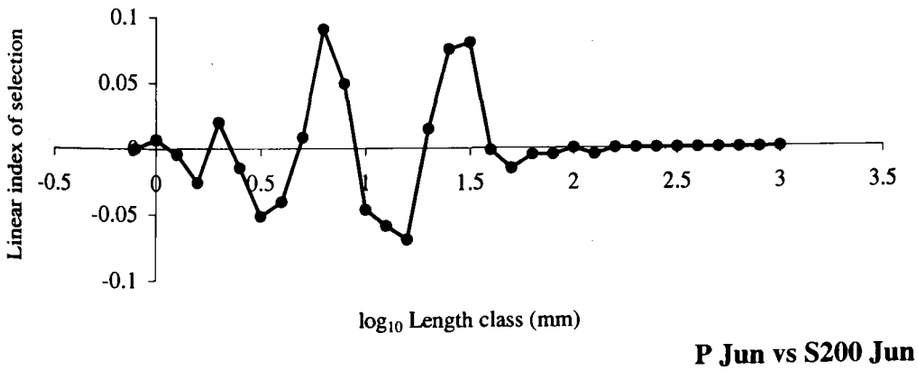


P Jun vs S200 Jan



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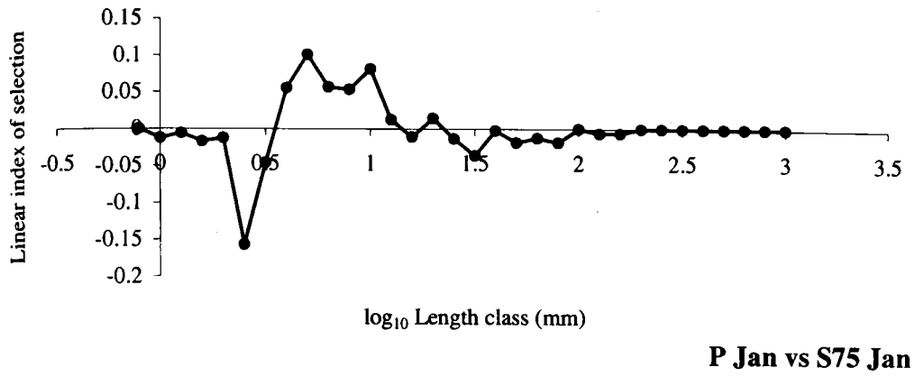
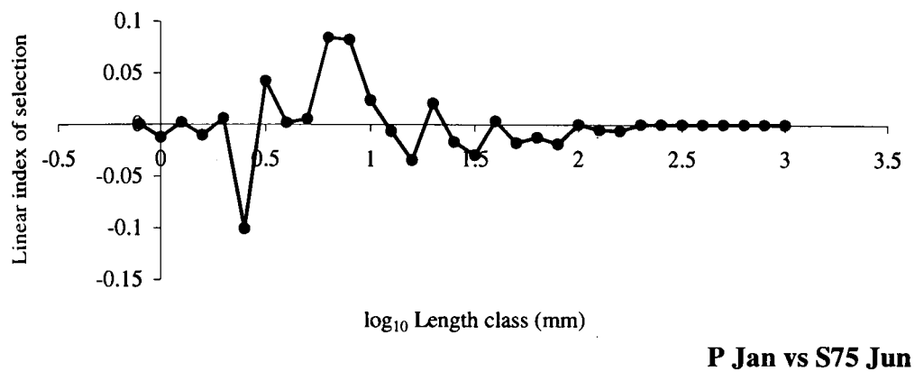
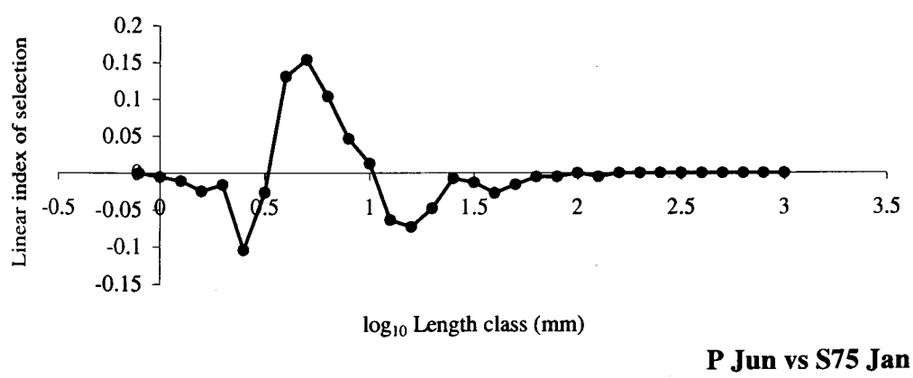


P Jan vs S75 Jan



P Jan vs S75 Jun



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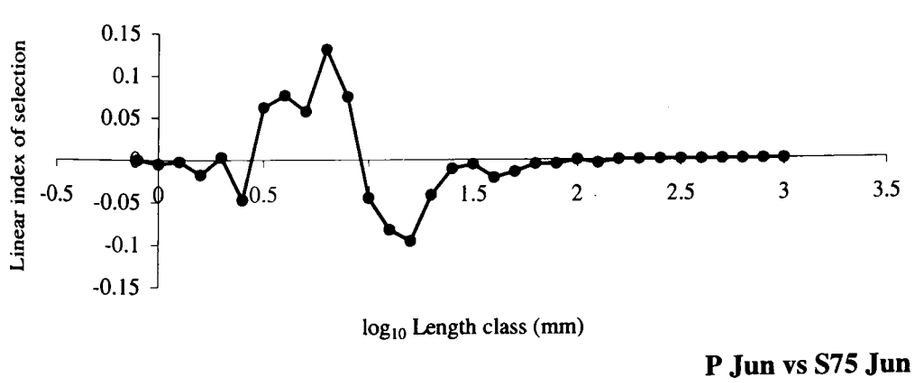
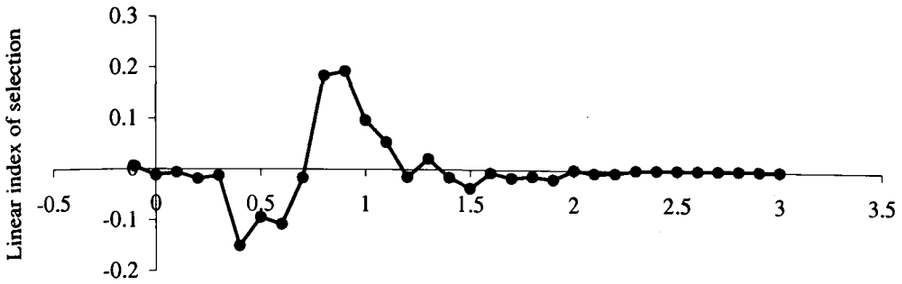


P Jun vs S75 Jun



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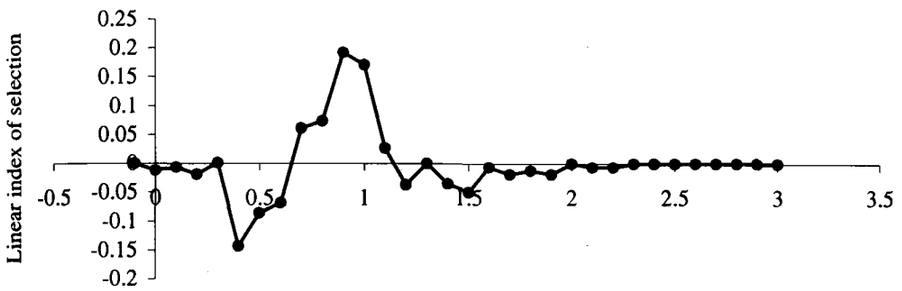
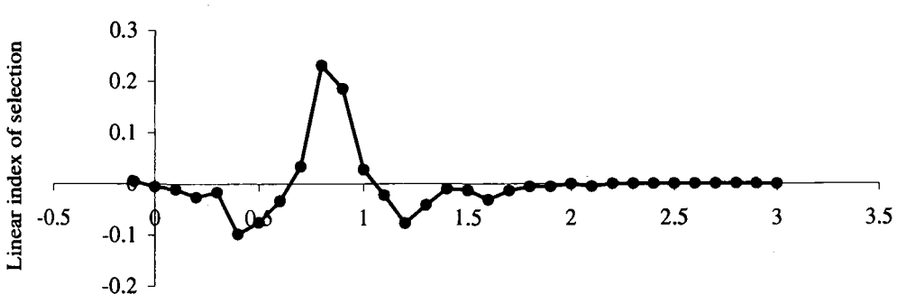
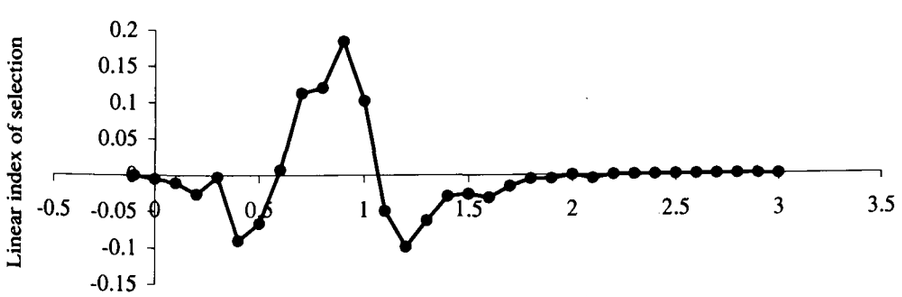


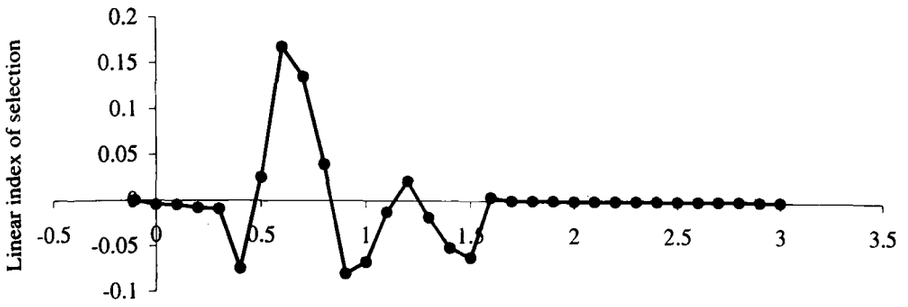
P Jan vs S25 Jun



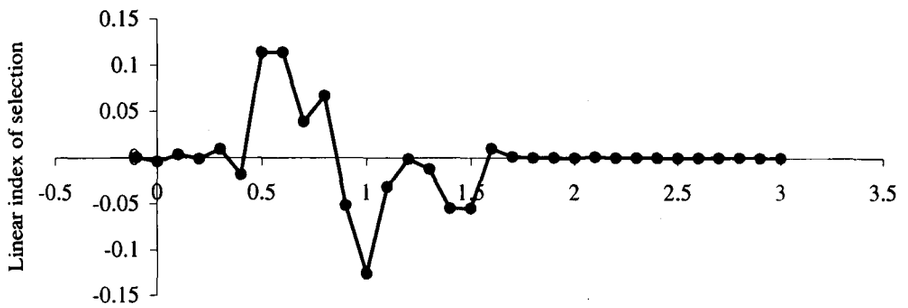
P Jun vs S25 Jan



P Jun vs S25 Jun



S200 Jan vs S75 Jan



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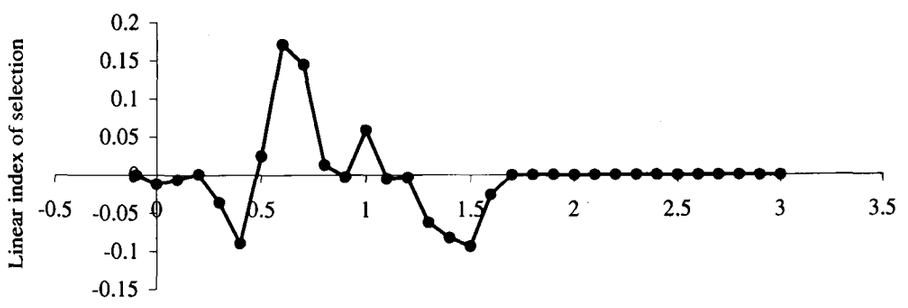

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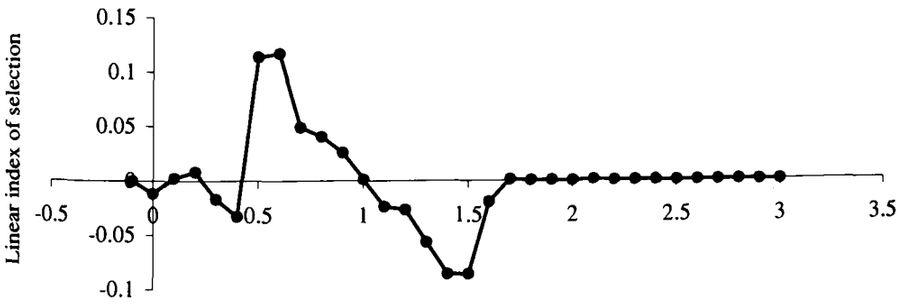

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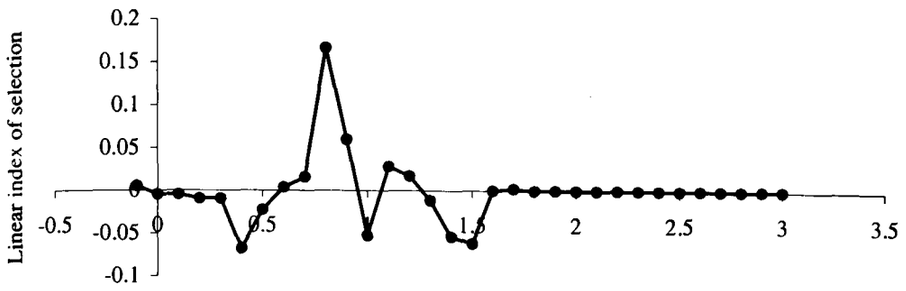

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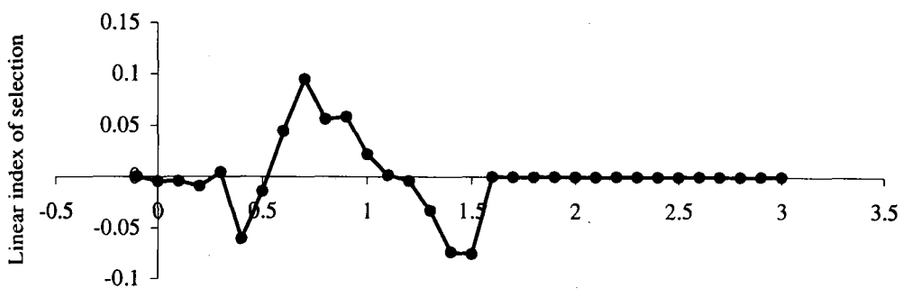

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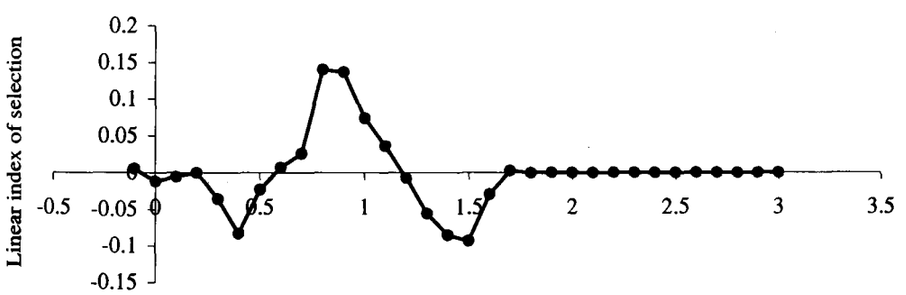

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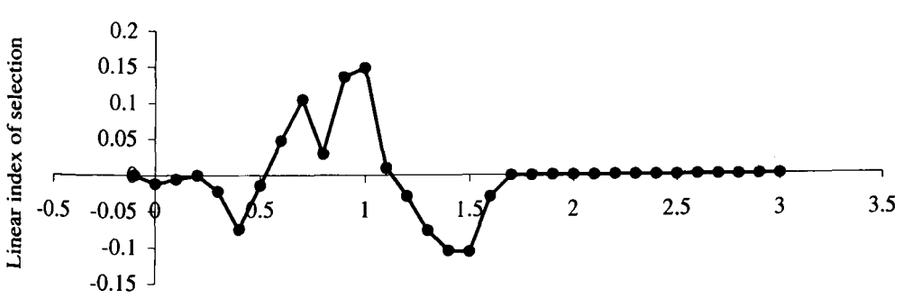
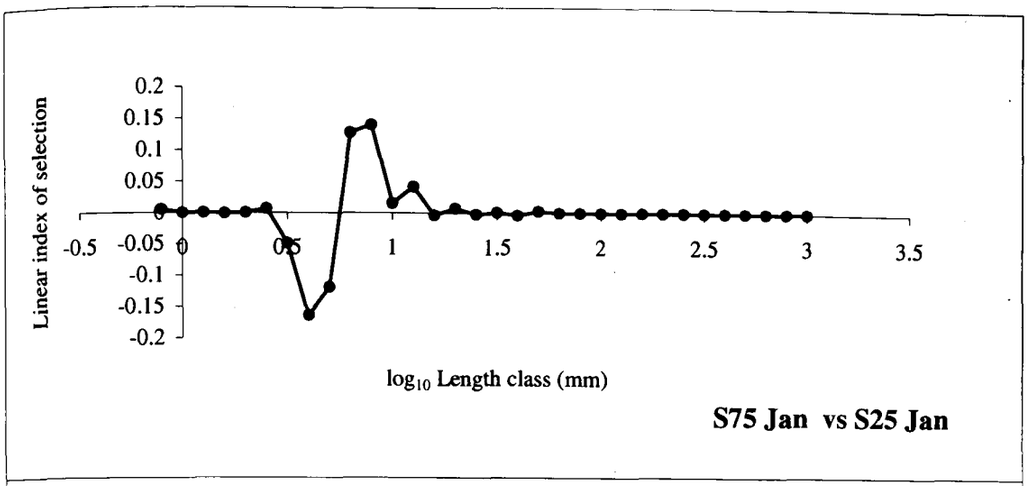
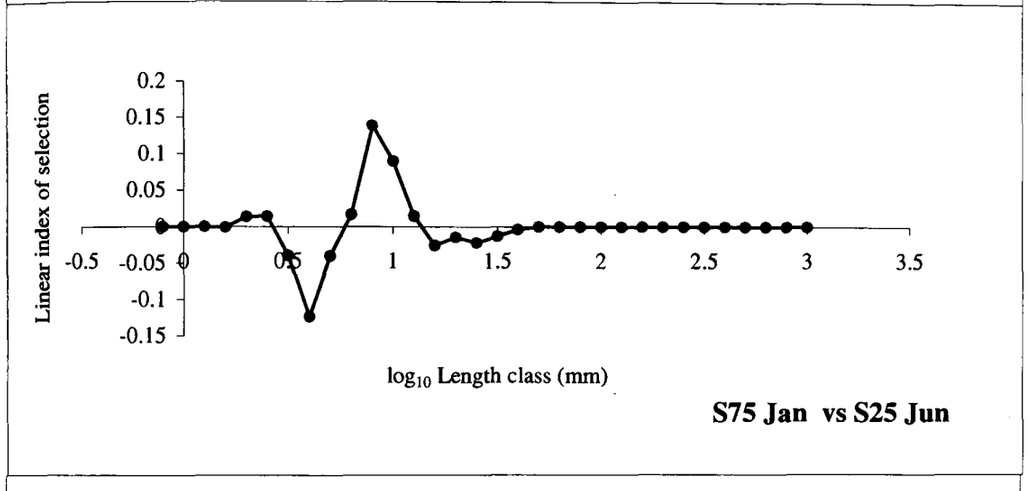


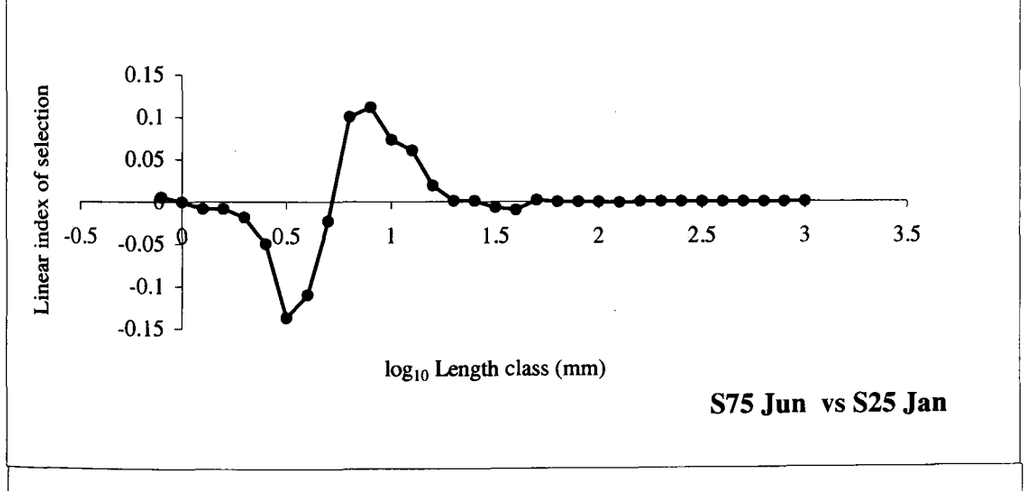
S200 Jun vs S25 Jun



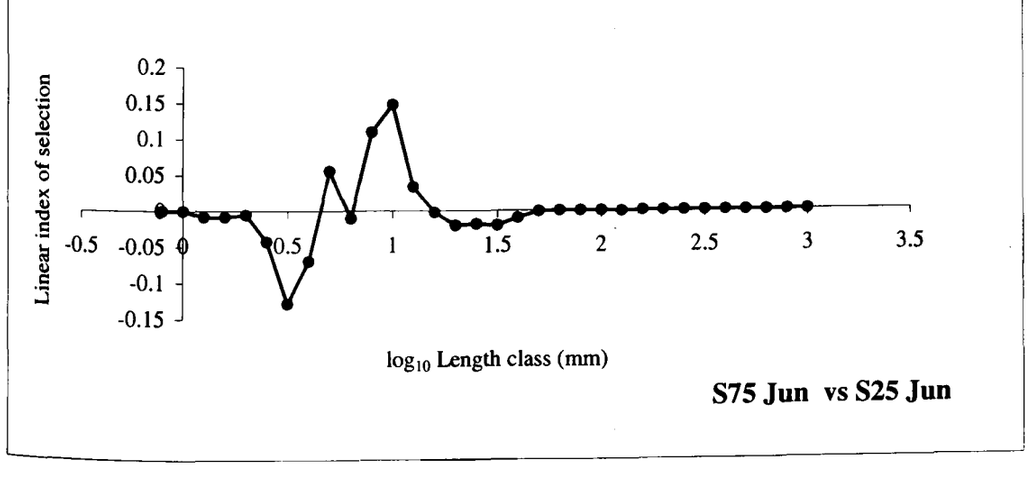
S75 Jan vs S25 Jan



S75 Jan vs S25 Jun

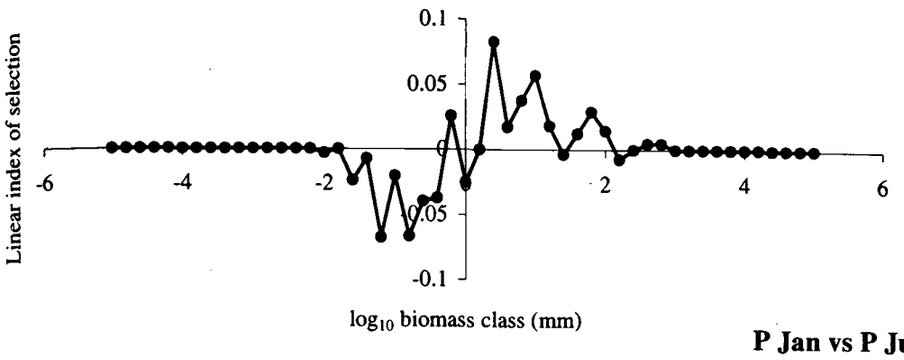


S75 Jun vs S25 Jan

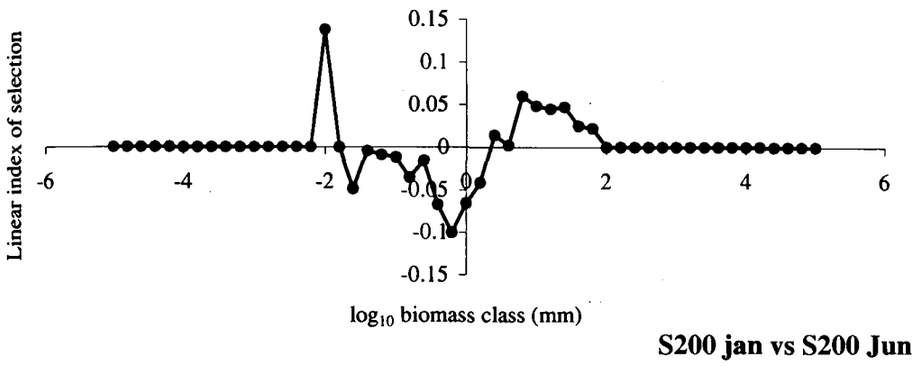


S75 Jun vs S25 Jun

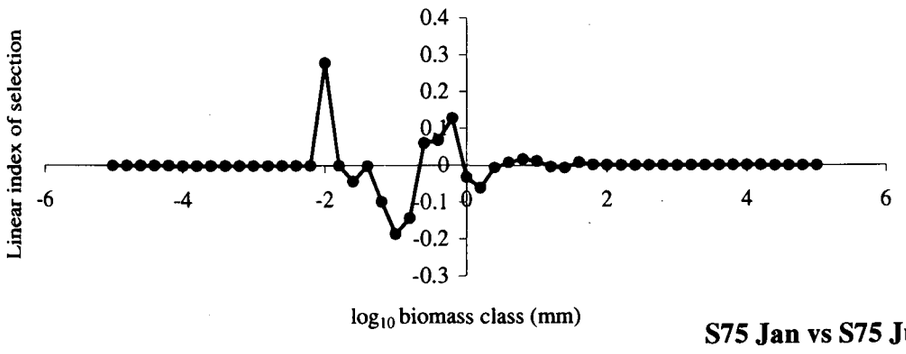
APPENDIX 4B. LINEAR INDEX OF SELECTION (BIOMASS)



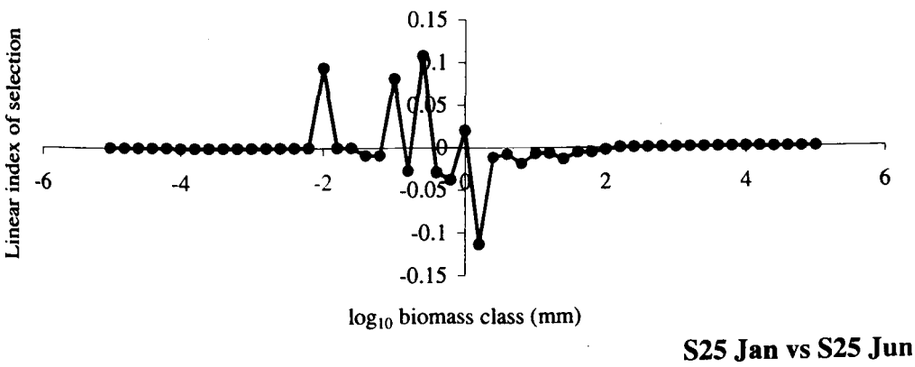
P Jan vs P Jun



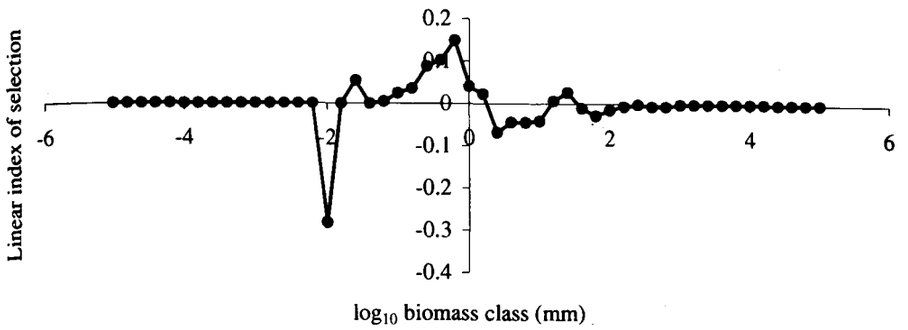
S200 jan vs S200 Jun



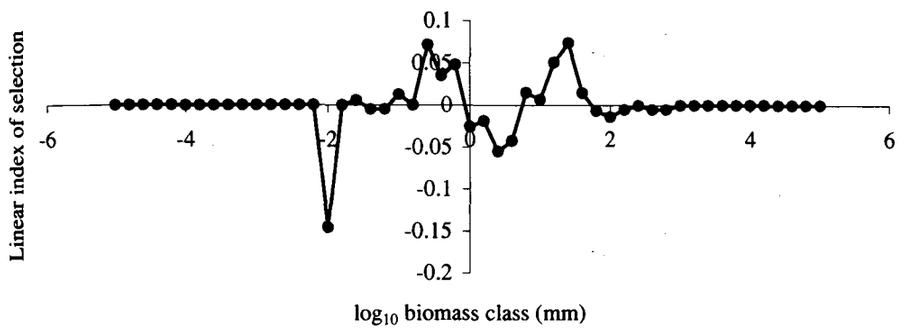
S75 Jan vs S75 Jun



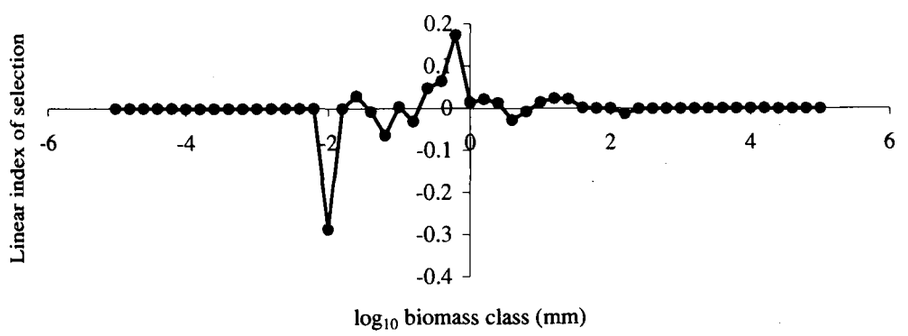
S25 Jan vs S25 Jun



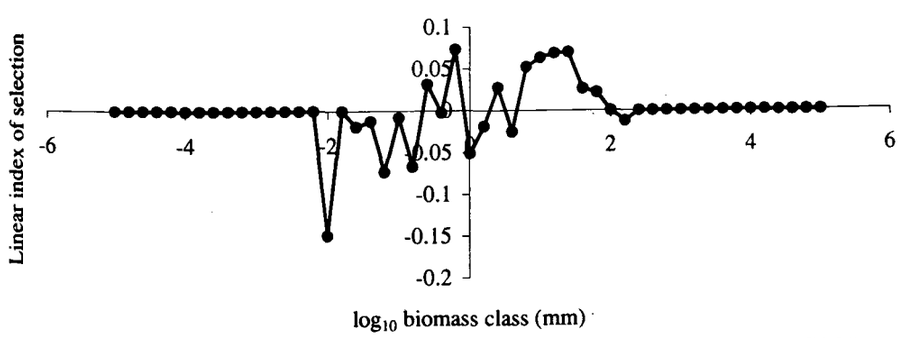
P Jun vs S200 Jan



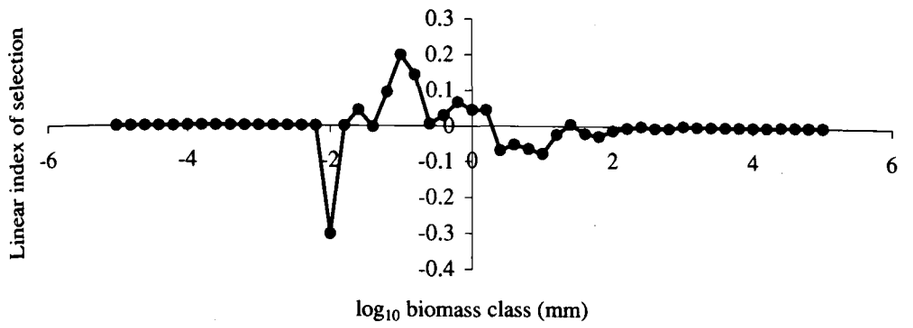
P Jun vs S200 Jun



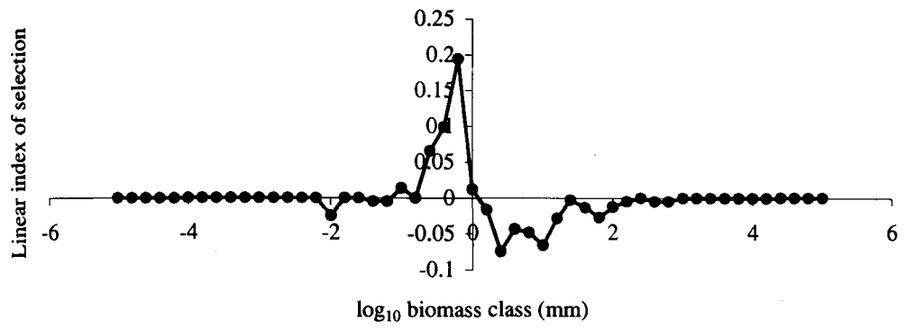
P Jan vs S200 Jan



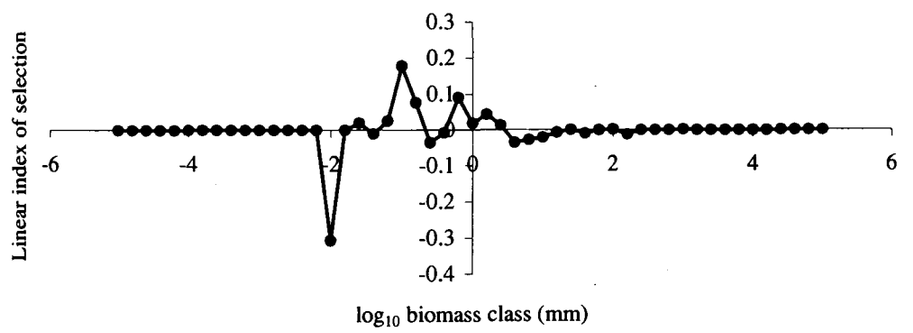
P Jan vs S200 Jun



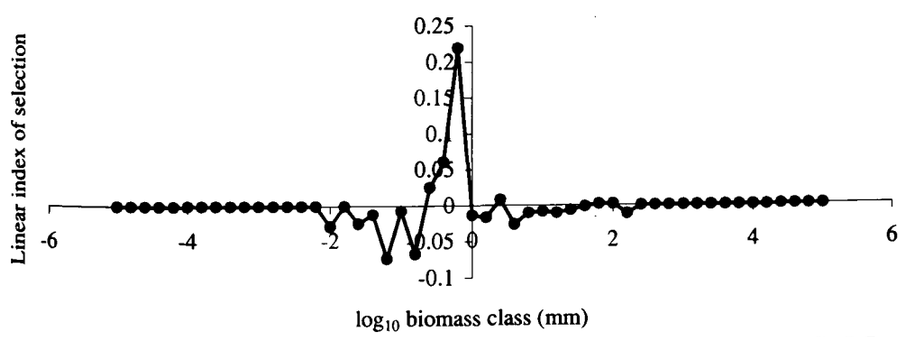
P Jun vs S75 Jan



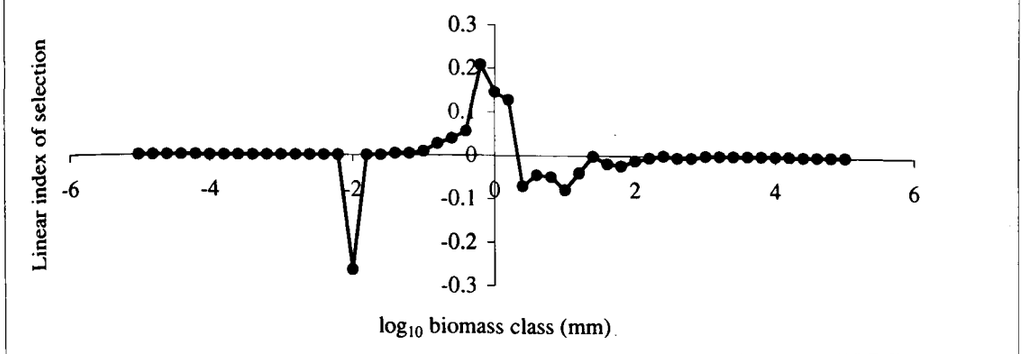
P Jun vs S75 Jun



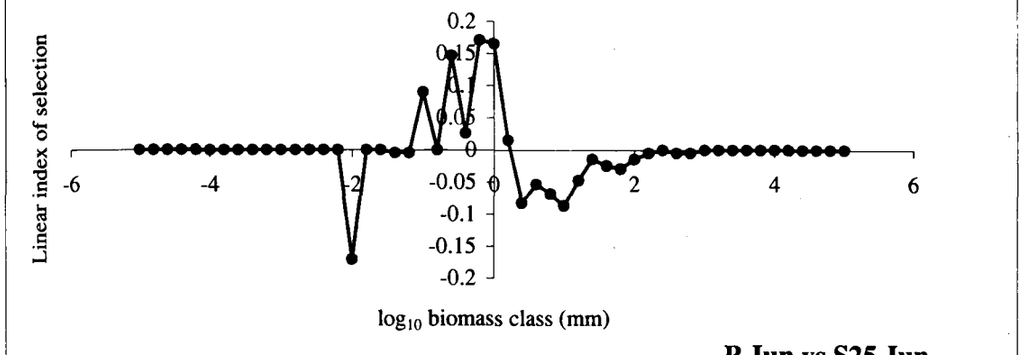
P Jan vs S75 Jan



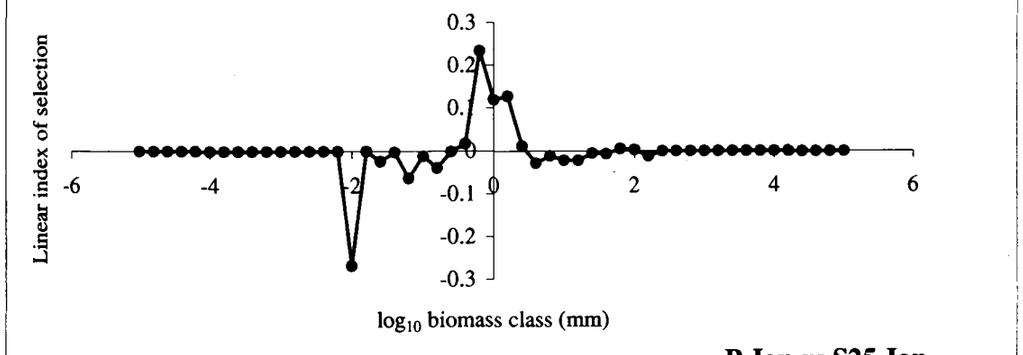
P Jan vs S75 Jun



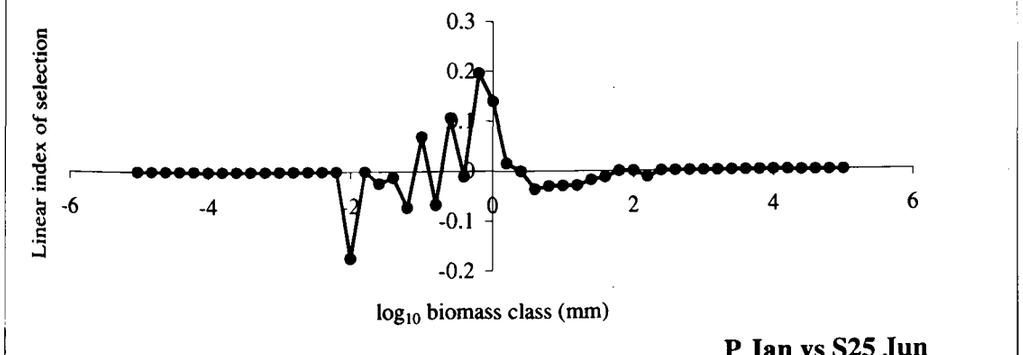
P Jun vs S25 Jan



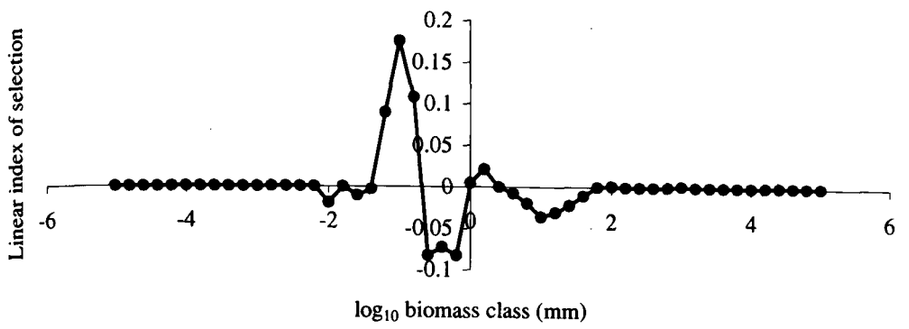
P Jun vs S25 Jun



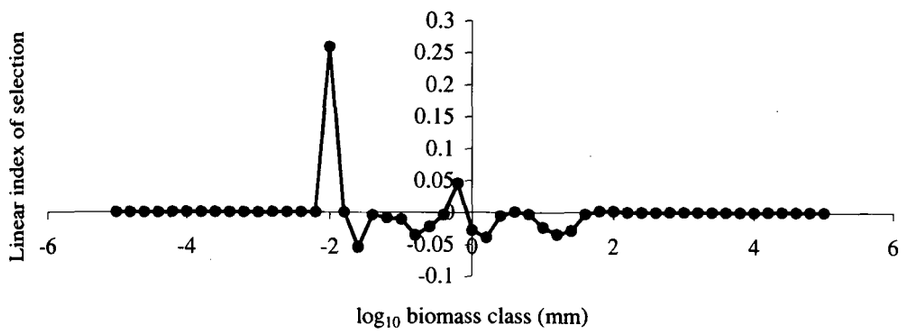
P Jan vs S25 Jan



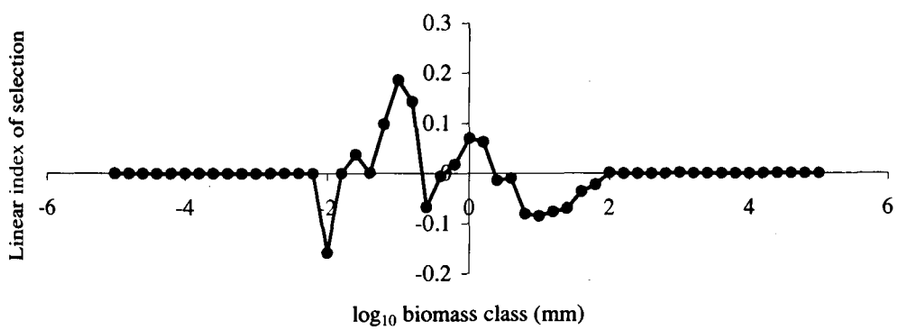
P Jan vs S25 Jun



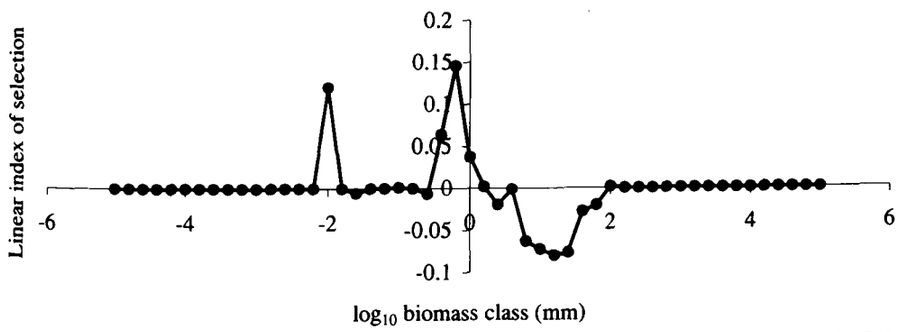
S200 Jan vs S75 Jan



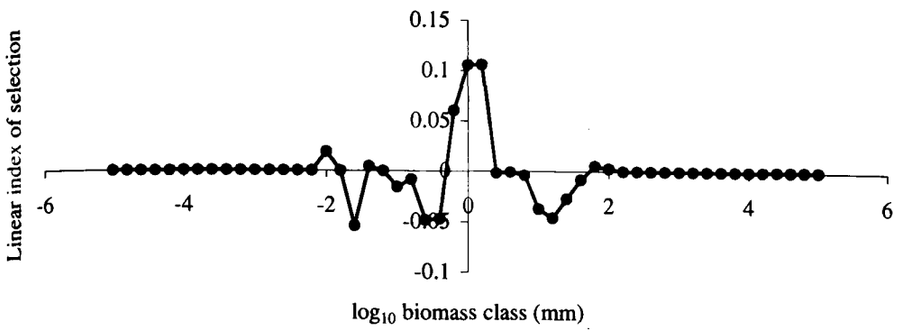
S200 Jan vs S75 Jun



S200 Jun vs S75 Jan



S200 Jun vs S75 Jun



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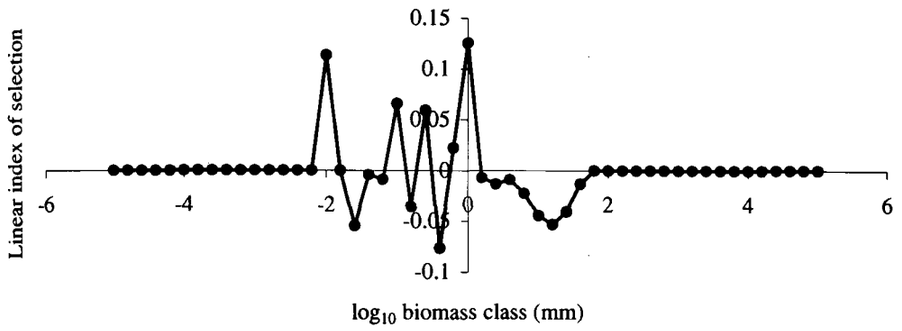
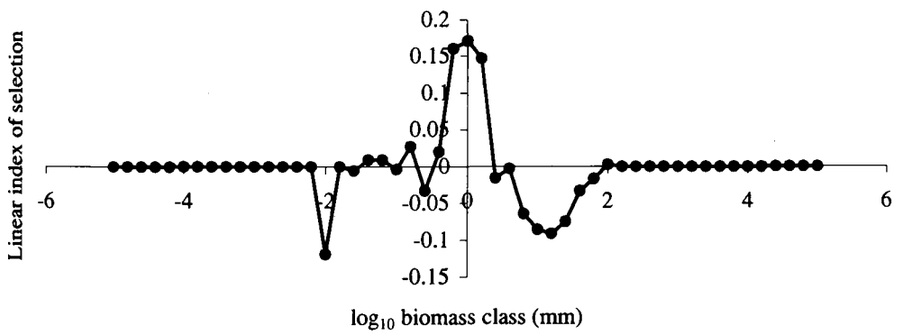


S200 Jan vs S25 Jun



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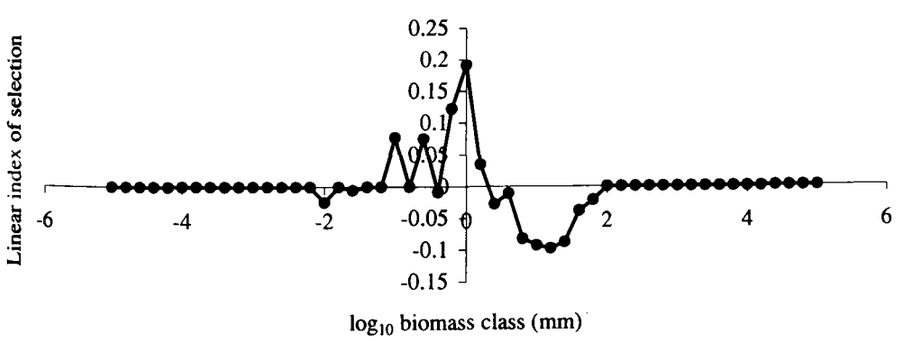
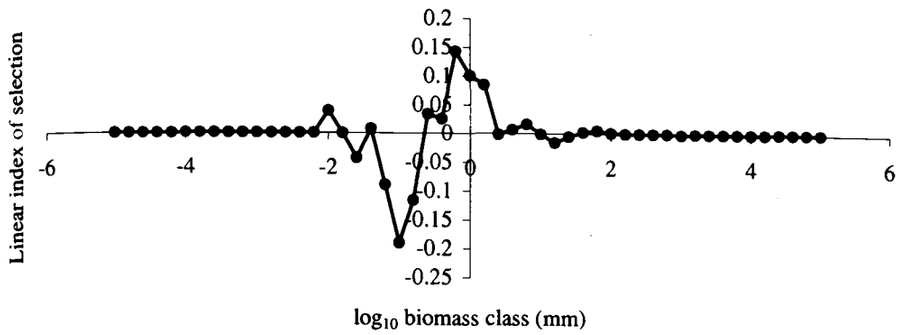
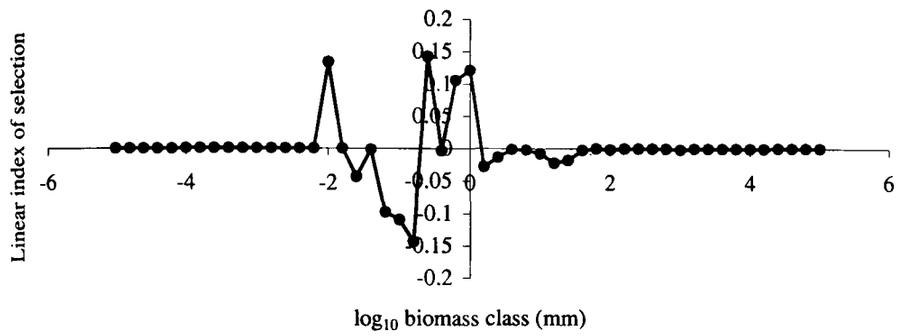


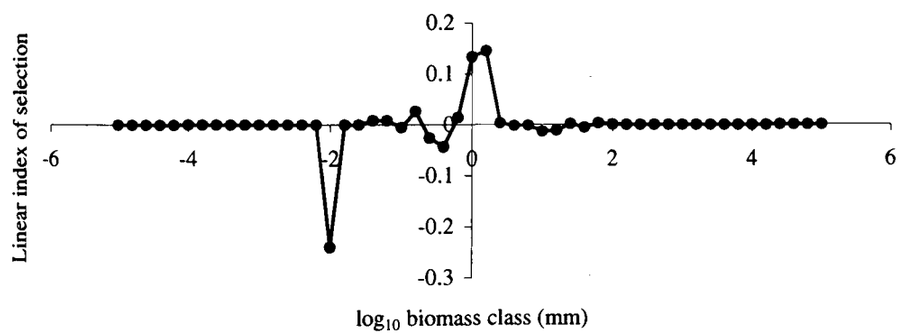
S200 Jun vs S25 Jun



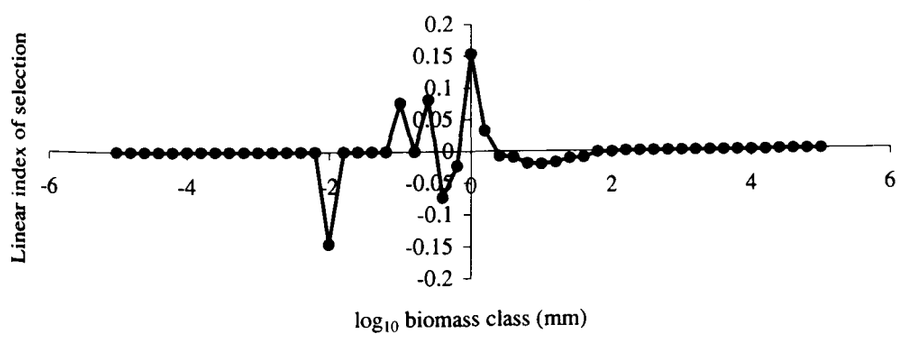
S75 Jan vs S25 Jan



S75 Jan vs S25 Jun



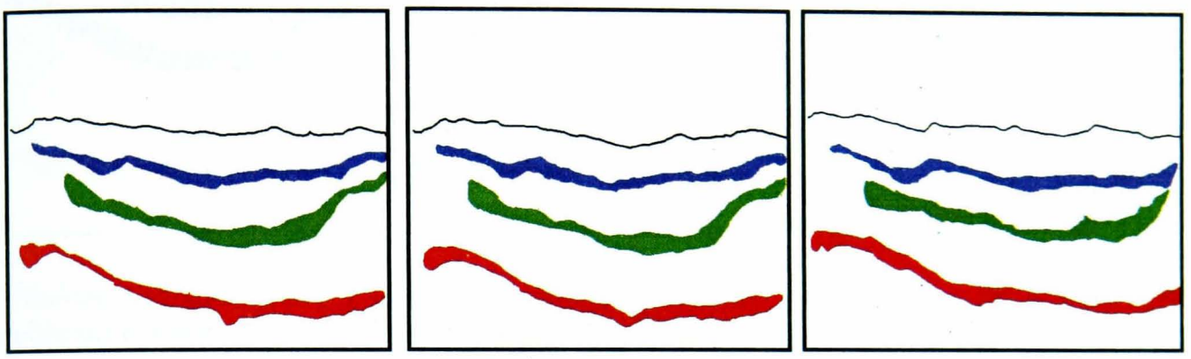
S75 Jun vs S25 Jan



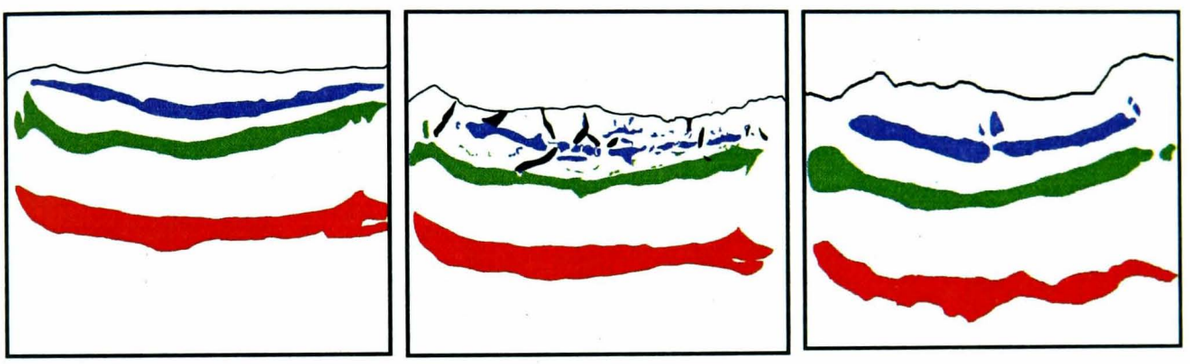
S75 Jun vs S25 Jun

APPENDIX 5.

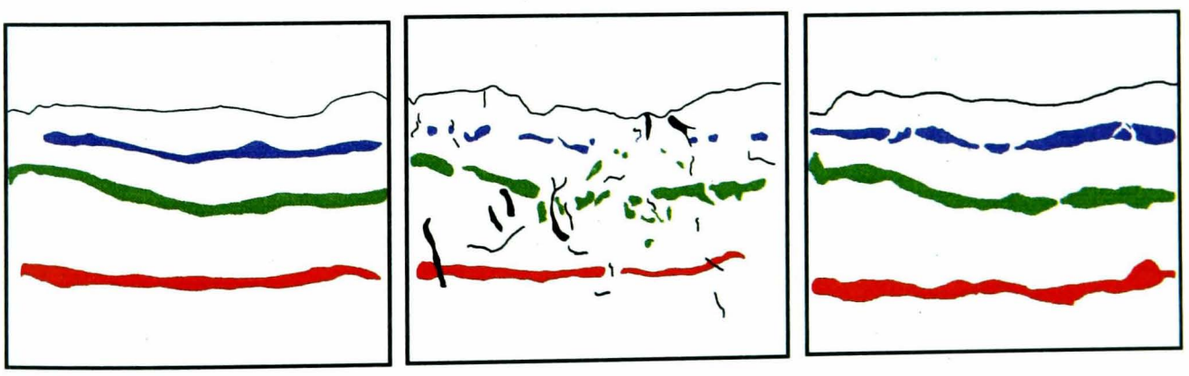
ASSESSMENT OF BIOTURBATION POTENTIAL IN RELATION TO SPECIES AND EFFLUENT CONCENTRATION USING THE THIN SECTION TECHNIQUE.



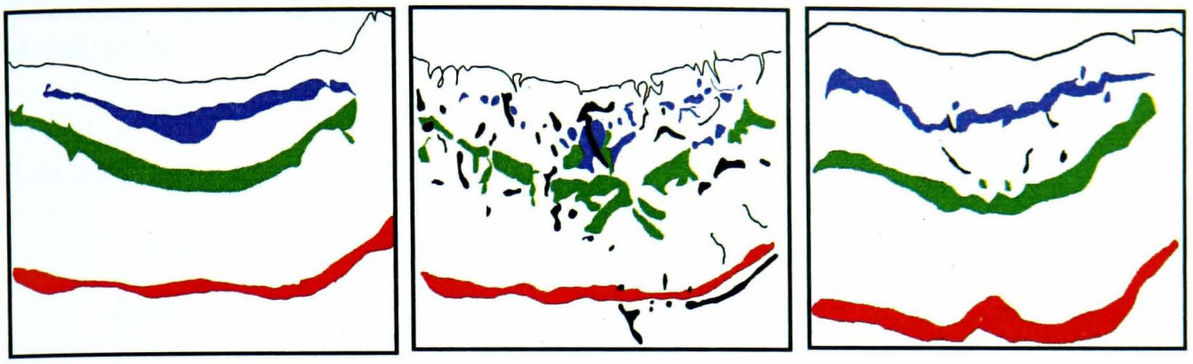
0% controls for day 0 (A) and day 15 (B) and 32% control for day 15 (C)



***Corophium volutator* at 0% effluent concentration for day 0 (A) and day 15 (B) and 32% effluent concentration for day 15. Black areas indicate the presence of burrows.**



***Macoma balthica* at 0% effluent concentration for day 0 (A) and day 15 (B) and 32% effluent concentration for day 15. Black areas indicate the presence of burrows.**



***Hediste diversicolor* at 0% effluent concentration for day 0 (A) and day 15 (B) and 32% effluent concentration for day 15. Black areas indicate the presence of burrows.**

APPENDIX 6. ASSESSMENT OF BIOTURBATION IN RELATION TO TIME, SPECIES AND EFFLUENT CONCENTRATION USING PHOTOGRAPHIC ANALYSIS.

Controls

TIME (DAYS)

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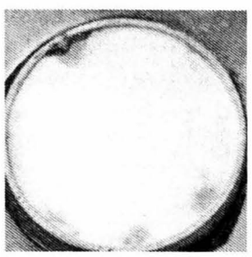
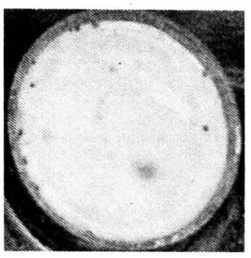
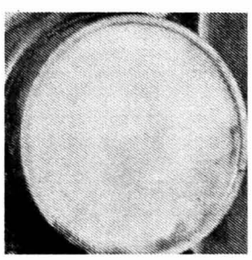
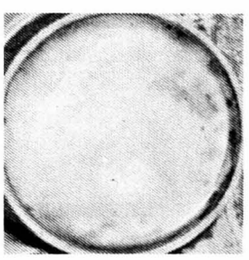
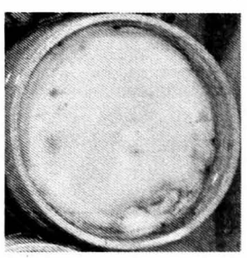
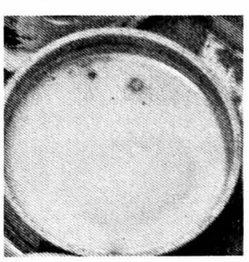
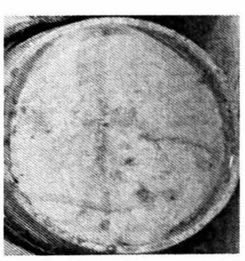
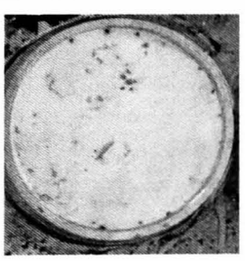
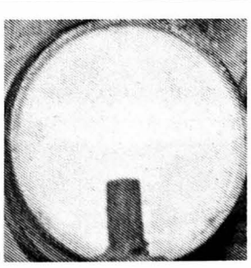
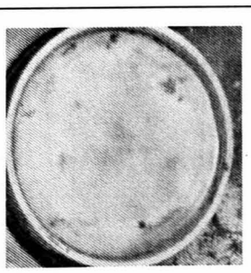
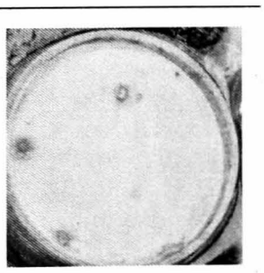
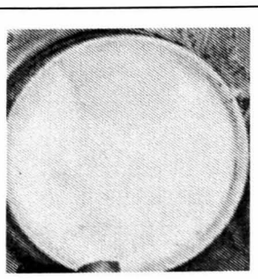
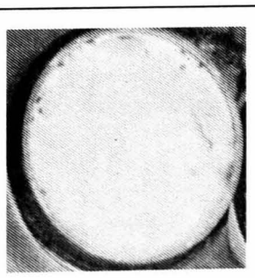
0%				
4%				
8%				
16%				
32%				

Corophium volutator

TIME (DAYS)

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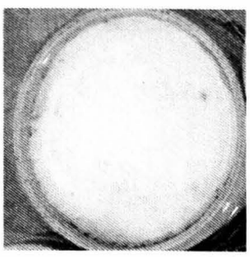
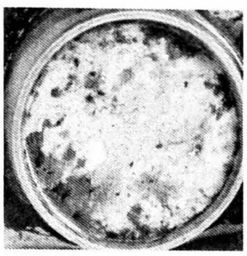
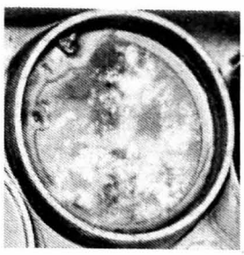
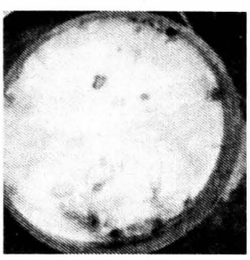
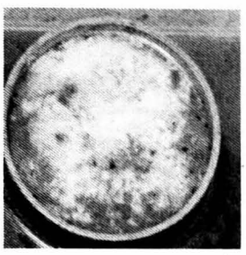
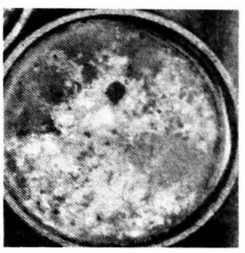
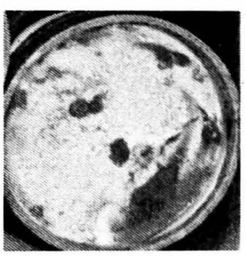
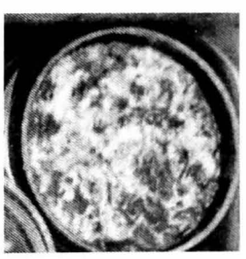
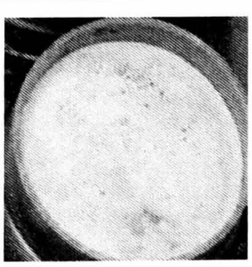
0%				
4%				
8%				
16%				
32%				

Hediste diversicolor

TIME (DAYS)

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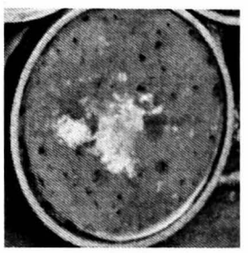
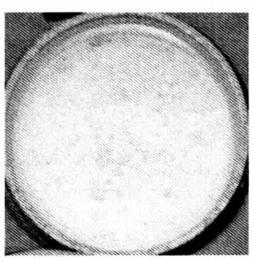
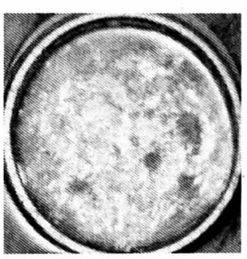
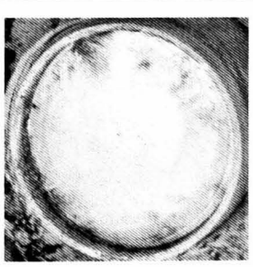
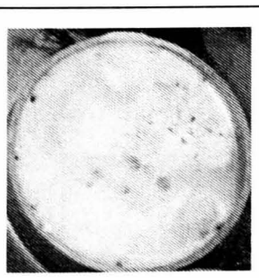
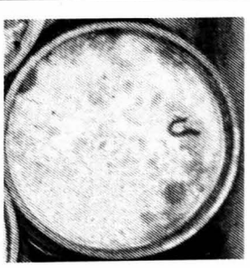
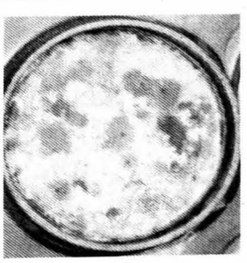
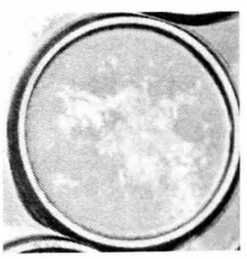
4%				
8%				
16%				
32%				

Macoma balthica

TIME (DAYS)

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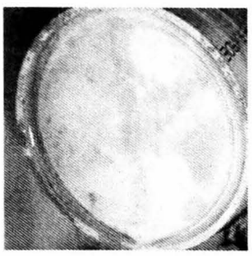
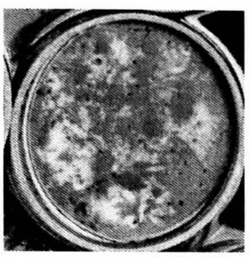
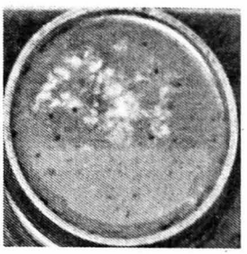
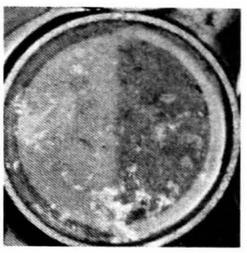
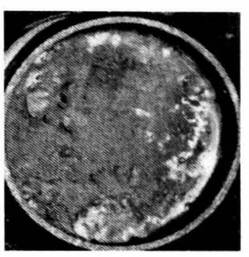
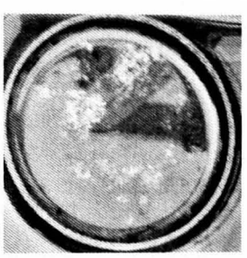
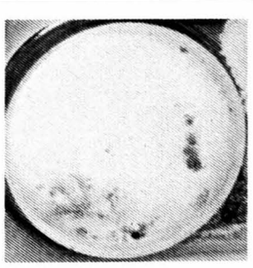
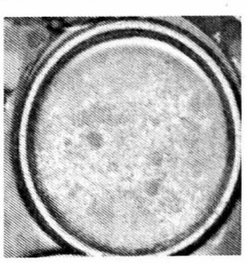
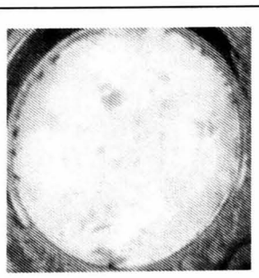
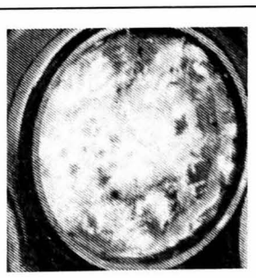
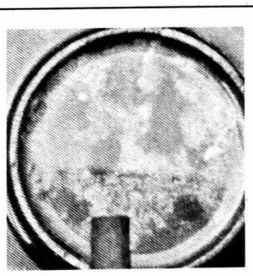
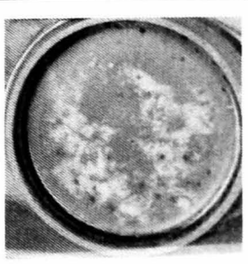
0%				
4%				
8%				
16%				
32%				

Scrobicularia plana

TIME (DAYS)

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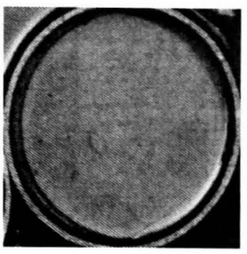
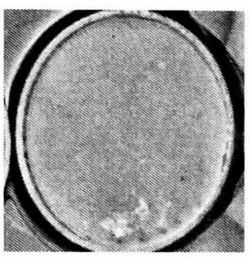
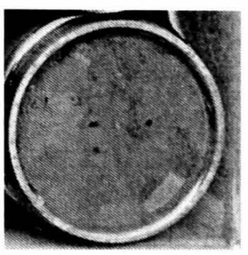
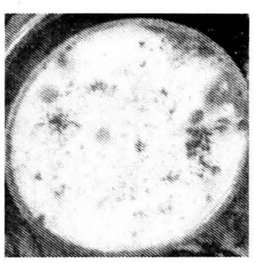
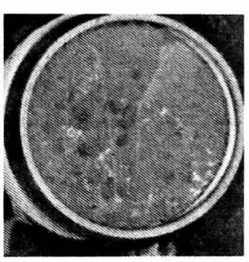
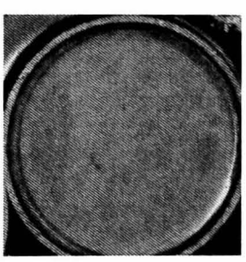
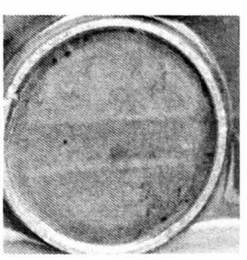
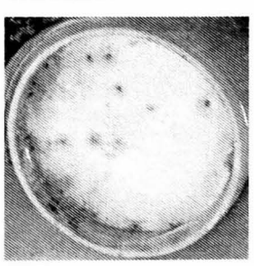
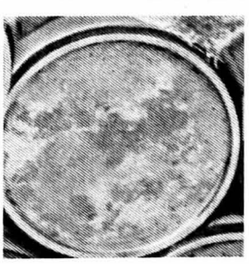
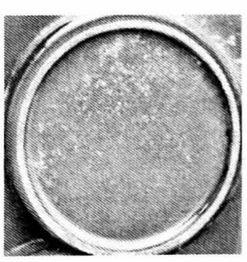
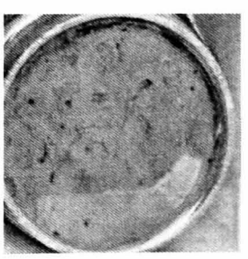
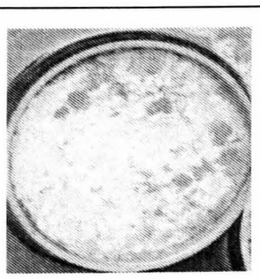
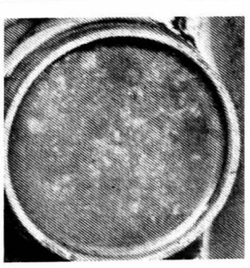
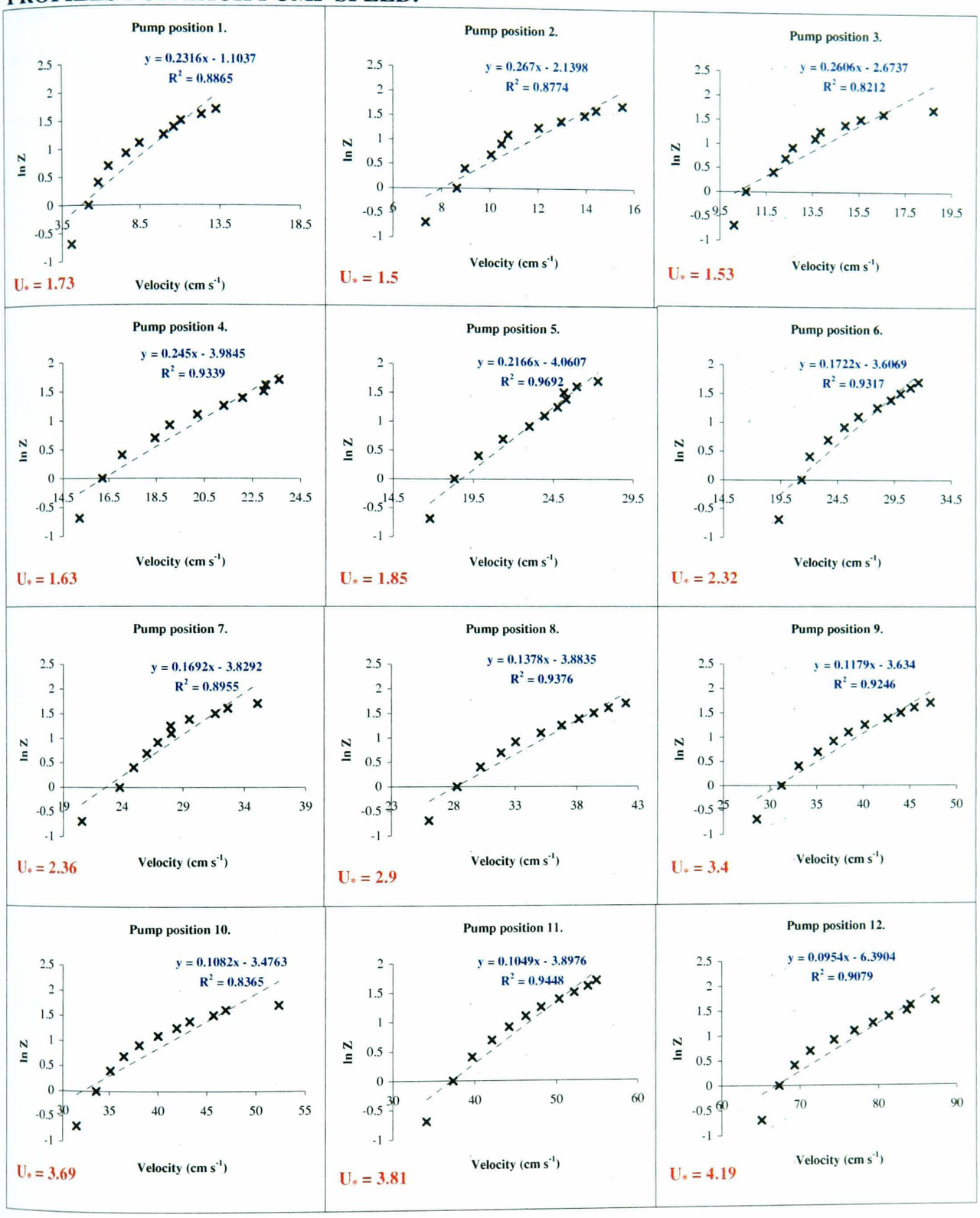
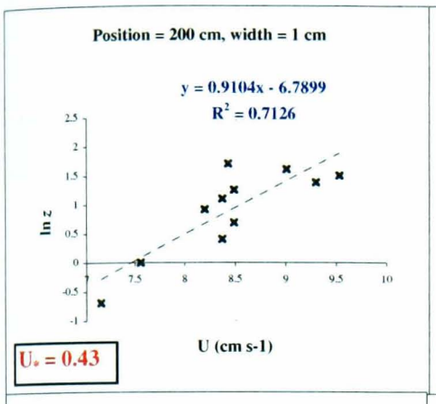
0%				
4%				
8%				
16%				
32%				

APPENDIX 7. PUMP CALIBRATION: CALCULATION OF τ0 FROM VELOCITY PROFILES FOR EACH PUMP SPEED.

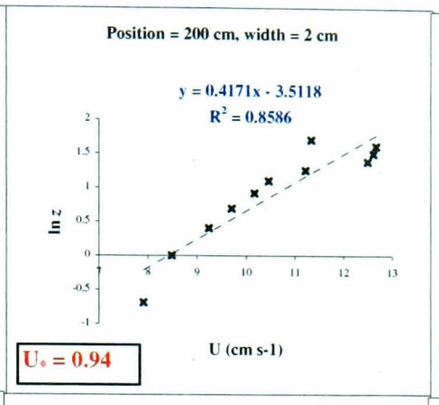


APPENDIX 8. FLOW CHARACTERISATION: CALCULATION OF τ_0 FROM VELOCITY PROFILES AT PUMP SPEED 5 (16.8 CM S^{-1})

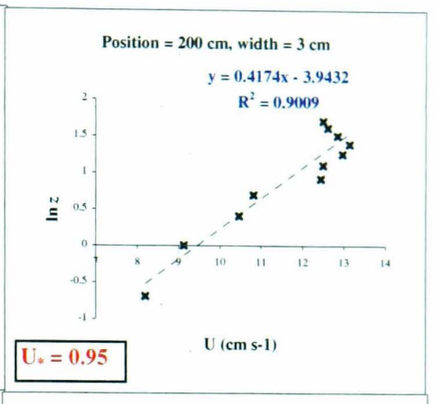
a) Position = 200 cm



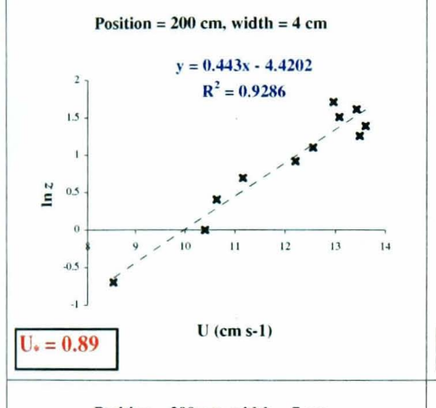
$U_* = 0.43$



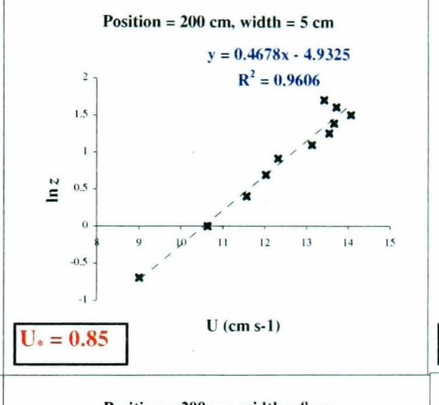
$U_* = 0.94$



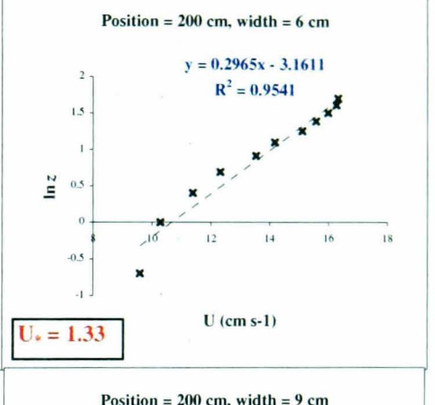
$U_* = 0.95$



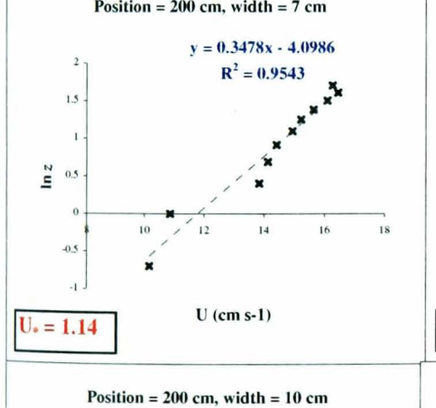
$U_* = 0.89$



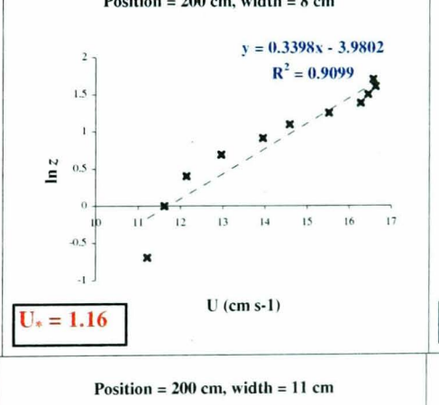
$U_* = 0.85$



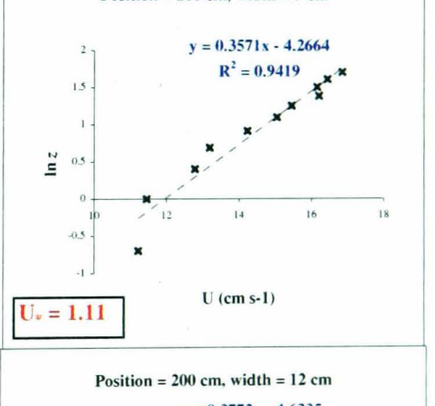
$U_* = 1.33$



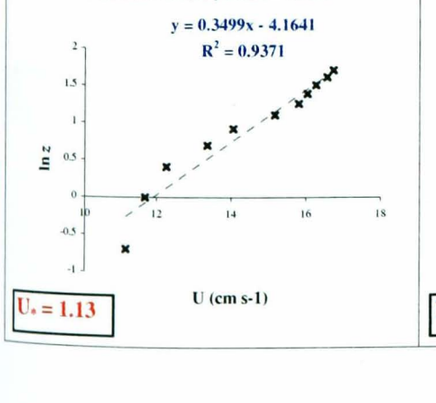
$U_* = 1.14$



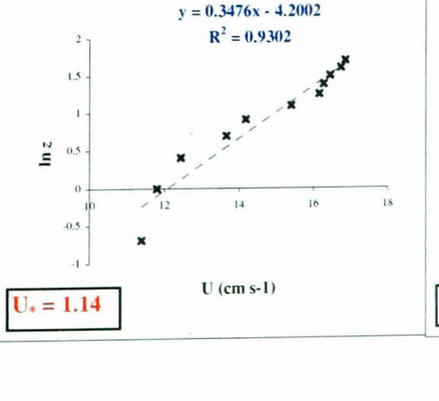
$U_* = 1.16$



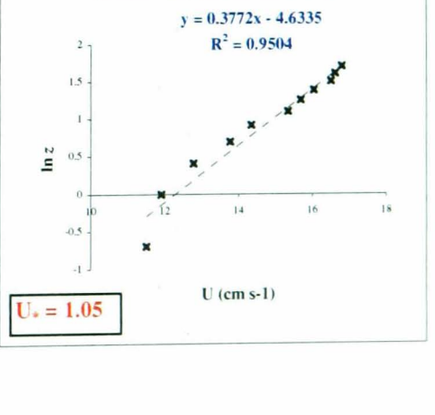
$U_* = 1.11$



$U_* = 1.13$



$U_* = 1.14$

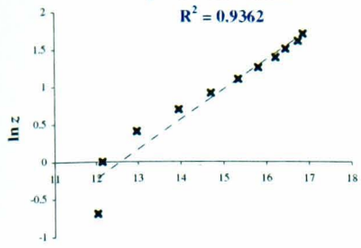


$U_* = 1.05$

Position = 200 cm, width = 13 cm

y = 0.3981x - 5.0081

R^2 = 0.9362

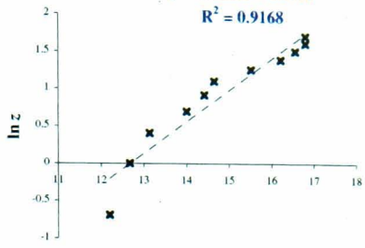


$U_* = 1.13$

Position = 200 cm, width = 14 cm

y = 0.4232x - 5.3711

R^2 = 0.9168

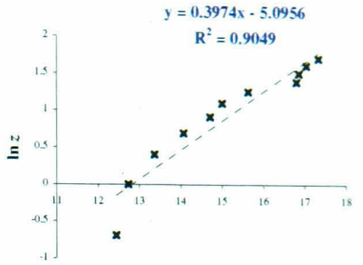


$U_* = 0.93$

Position = 200 cm, width = 15 cm

y = 0.3974x - 5.0956

R^2 = 0.9049

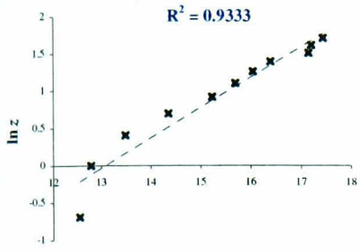


$U_* = 0.99$

Position = 200 cm, width = 16 cm

y = 0.4055x - 5.3063

R^2 = 0.9333

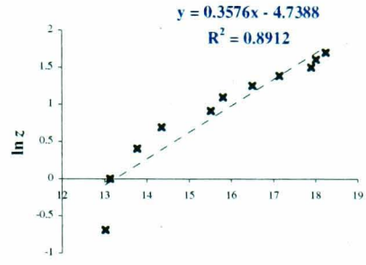


$U_* = 0.97$

Position = 200 cm, width = 17 cm

y = 0.3576x - 4.7388

R^2 = 0.8912

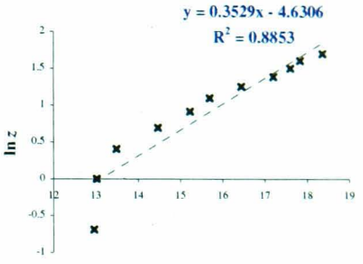


$U_* = 1.1$

Position = 200 cm, width = 18 cm

y = 0.3529x - 4.6306

R^2 = 0.8853

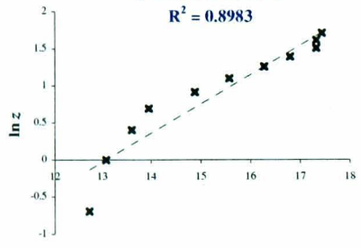


$U_* = 1.12$

Position = 200 cm, width = 19 cm

y = 0.3925x - 5.1305

R^2 = 0.8983

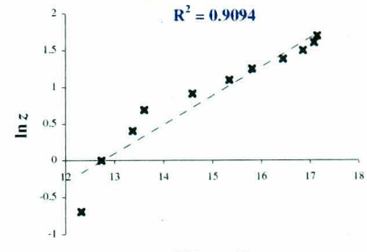


$U_* = 1.0$

Position = 200 cm, width = 20 cm

y = 0.395x - 5.0368

R^2 = 0.9094

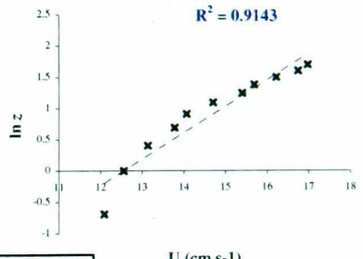


$U_* = 1.0$

Position = 200 cm, width = 21 cm

y = 0.4244x - 5.3263

R^2 = 0.9143

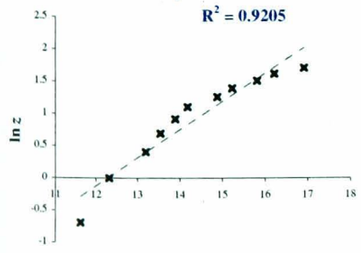


$U_* = 0.93$

Position = 200 cm, width = 22 cm

y = 0.4352x - 5.345

R^2 = 0.9205

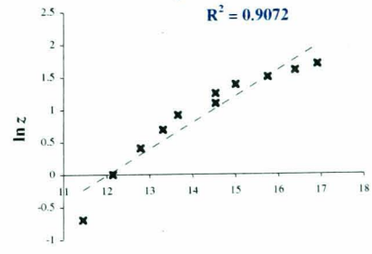


$U_* = 0.91$

Position = 200 cm, width = 23 cm

y = 0.4081x - 4.9062

R^2 = 0.9072

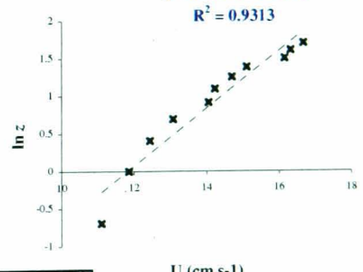


$U_* = 0.96$

Position = 200 cm, width = 24 cm

y = 0.3841x - 4.5402

R^2 = 0.9313

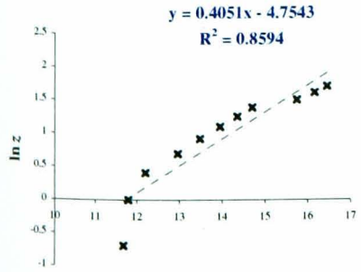


$U_* = 1.03$

Position = 200 cm, width = 25 cm

y = 0.4051x - 4.7543

R^2 = 0.8594

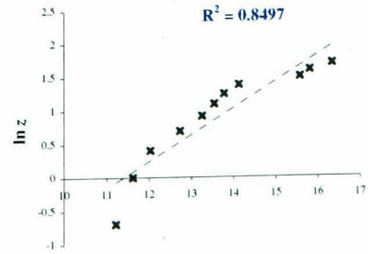


$U_* = 0.97$

Position = 200 cm, width = 26 cm

y = 0.4003x - 4.5607

R^2 = 0.8497

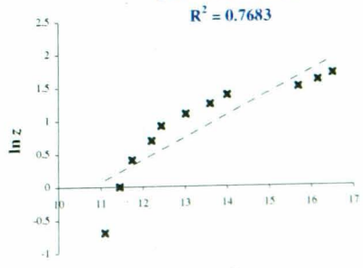


$U_* = 0.98$

Position = 200 cm, width = 27 cm

y = 0.3386x - 3.6551

R^2 = 0.7683

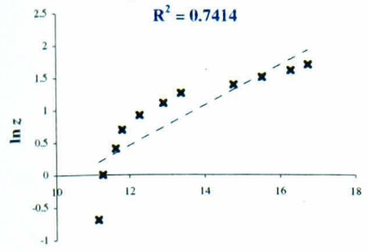


$U_* = 1.16$

Position = 200 cm, width = 28 cm

y = 0.3112x - 3.28

R^2 = 0.7414

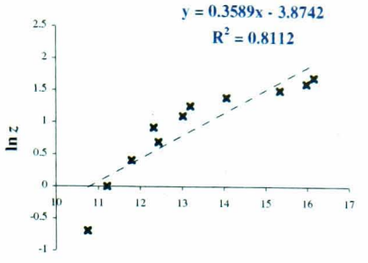


$U_* = 1.26$

Position = 200 cm, width = 29 cm

y = 0.3589x - 3.8742

R^2 = 0.8112

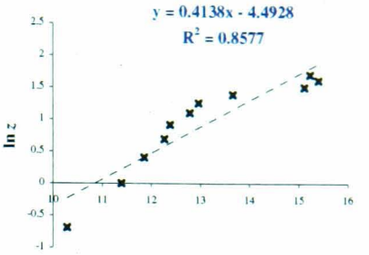


$U_* = 1.09$

Position = 200 cm, width = 30 cm

y = 0.4138x - 4.4928

R^2 = 0.8577

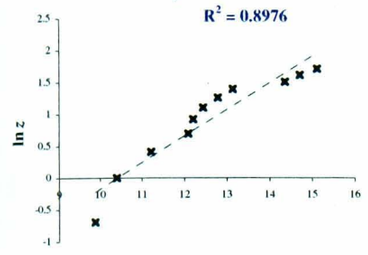


$U_* = 0.95$

Position = 200 cm, width = 31 cm

y = 0.4168x - 4.3432

R^2 = 0.8976

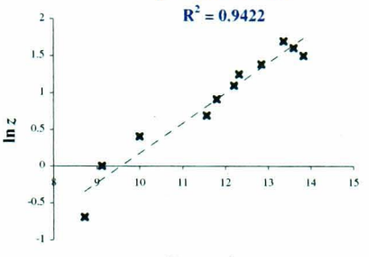


$U_* = 0.94$

Position = 200 cm, width = 32 cm

y = 0.409x - 3.9119

R^2 = 0.9422

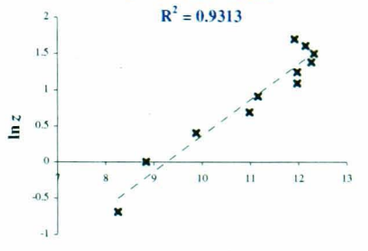


$U_* = 0.96$

Position = 200 cm, width = 33 cm

y = 0.4981x - 4.6132

R^2 = 0.9313

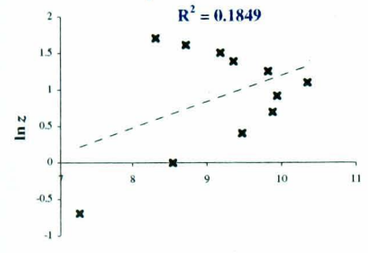


$U_* = 0.8$

Position = 200 cm, width = 34 cm

y = 0.3585x - 2.3887

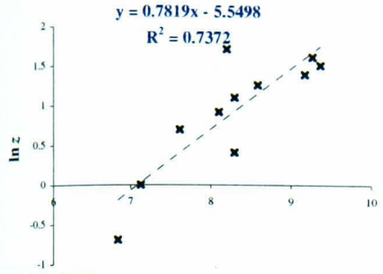
R^2 = 0.1849



$U_* = 1.13$

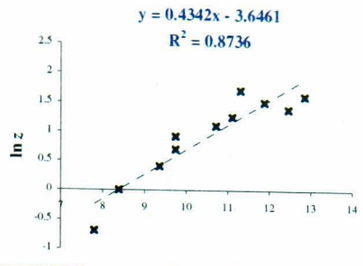
b) Position 210 cm

Position = 210 cm, width = 1 cm



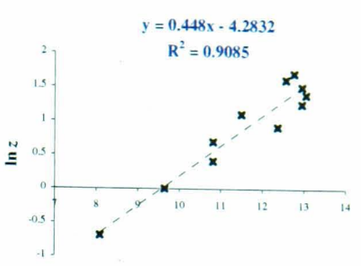
$U_* = 0.51$

Position = 210 cm, width = 2 cm



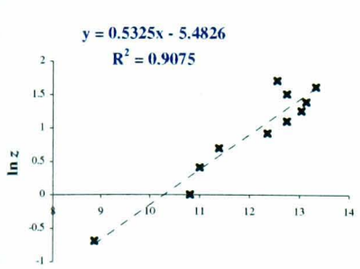
$U_* = 0.92$

Position = 210 cm, width = 3 cm



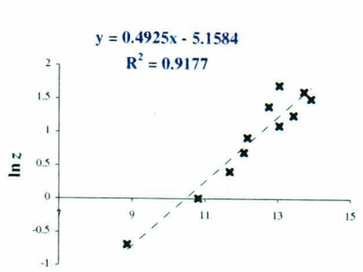
$U_* = 0.89$

Position = 210 cm, width = 4 cm



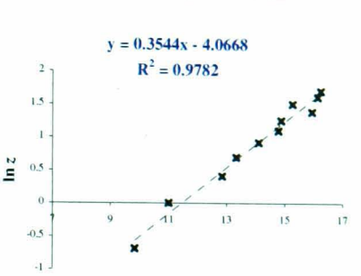
$U_* = 0.75$

Position = 210 cm, width = 5 cm



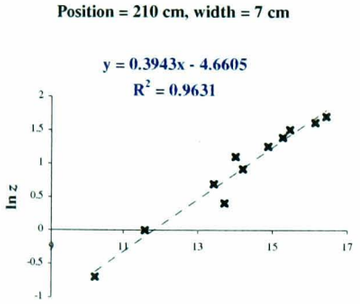
$U_* =$

Position = 210 cm, width = 6 cm



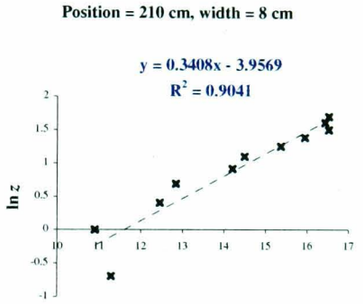
$U_* = 1.13$

Position = 210 cm, width = 7 cm



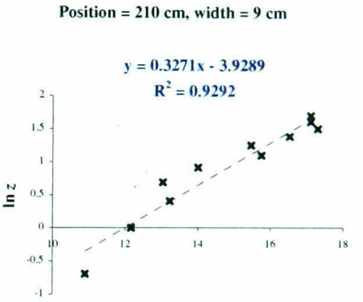
$U_* = 1.01$

Position = 210 cm, width = 8 cm



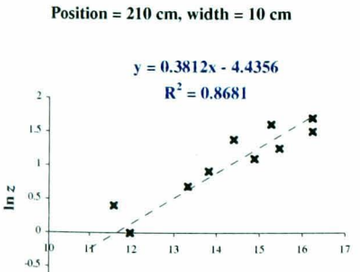
$U_* = 1.17$

Position = 210 cm, width = 9 cm



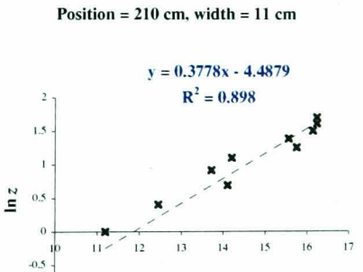
$U_* = 1.22$

Position = 210 cm, width = 10 cm



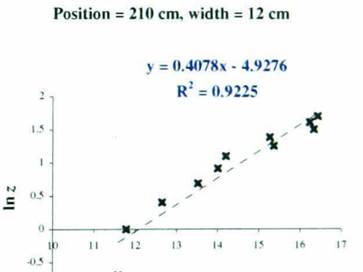
$U_* = 1.05$

Position = 210 cm, width = 11 cm



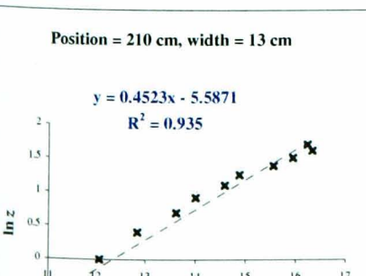
$U_* = 1.06$

Position = 210 cm, width = 12 cm



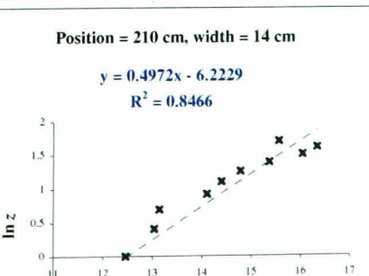
$U_* = 0.98$

Position = 210 cm, width = 13 cm



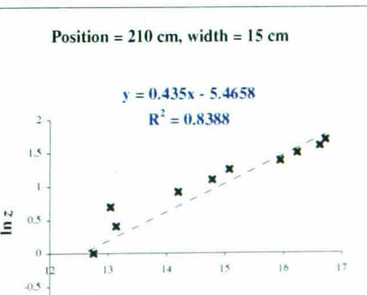
$U_* = 0.88$

Position = 210 cm, width = 14 cm



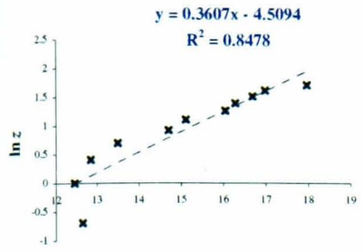
$U_* = 0.8$

Position = 210 cm, width = 15 cm



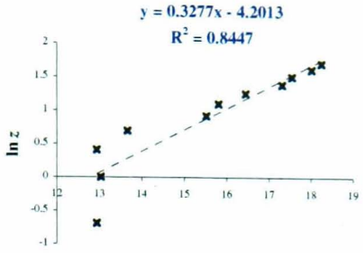
$U_* = 0.92$

Position = 210 cm, width = 16 cm



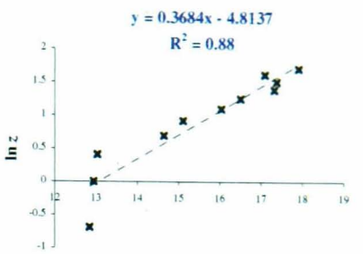
$U_* = 1.11$

Position = 210 cm, width = 17 cm



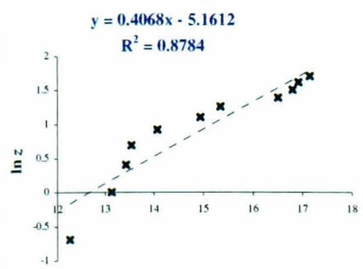
$U_* = 1.22$

Position = 210 cm, width = 18 cm



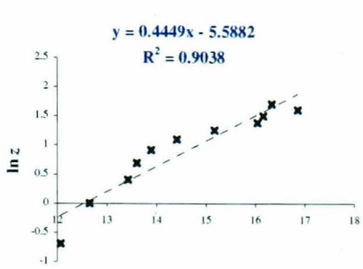
$U_* = 1.09$

Position = 210 cm, width = 19 cm



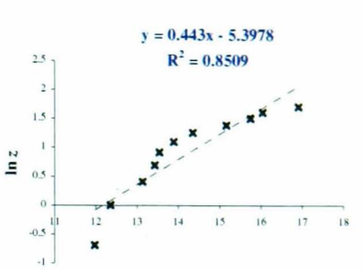
$U_* = 0.98$

Position = 210 cm, width = 20 cm



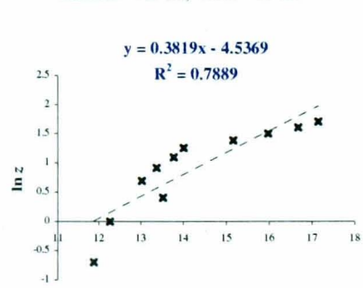
$U_* = 0.89$

Position = 210 cm, width = 21 cm



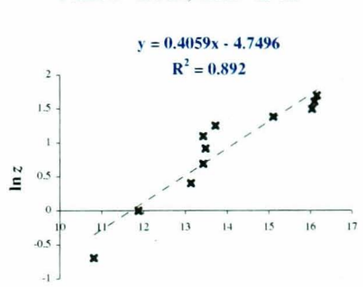
$U_* = 0.9$

Position = 210 cm, width = 22 cm



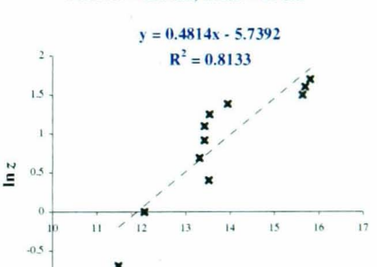
$U_* = 1.05$

Position = 210 cm, width = 23 cm



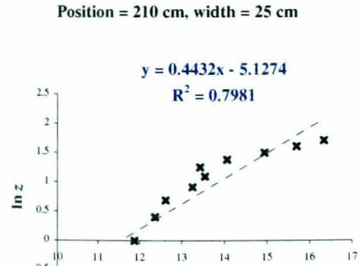
$U_* = 0.98$

Position = 210 cm, width = 24 cm



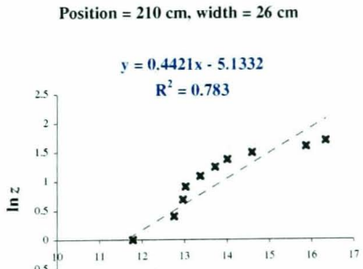
$U_* = 0.83$

Position = 210 cm, width = 25 cm



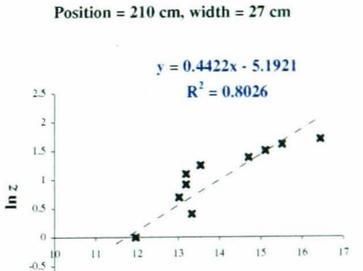
$U_* = 0.9$

Position = 210 cm, width = 26 cm



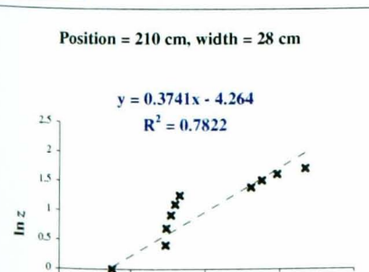
$U_* = 0.89$

Position = 210 cm, width = 27 cm



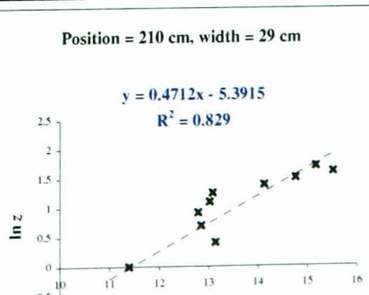
$U_* = 0.89$

Position = 210 cm, width = 28 cm



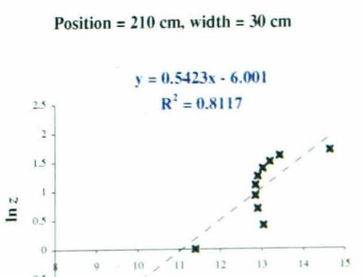
$U_* = 1.05$

Position = 210 cm, width = 29 cm



$U_* = 0.83$

Position = 210 cm, width = 30 cm

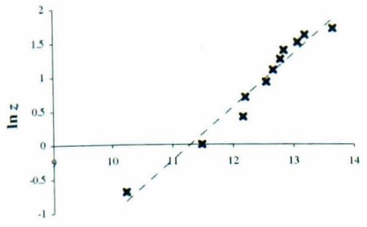


$U_* = 0.73$

Position = 210 cm, width = 31 cm

y = 0.7825x - 8.8384

R^2 = 0.962

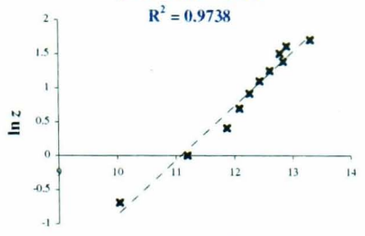


$U_* = 0.5$

Position = 210 cm, width = 32 cm

y = 0.7969x - 8.8356

R^2 = 0.9738

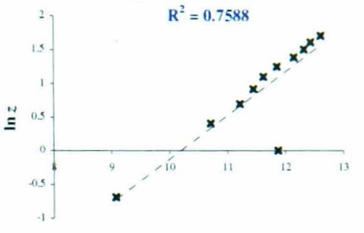


$U_* = 0.5$

Position = 210 cm, width = 33 cm

y = 0.6476x - 6.5977

R^2 = 0.7588

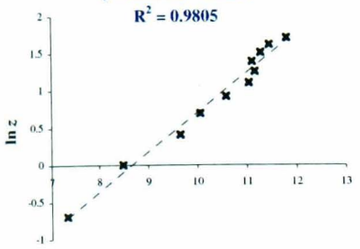


$U_* = 0.61$

Position = 210 cm, width = 1 cm

y = 0.5336x - 4.6286

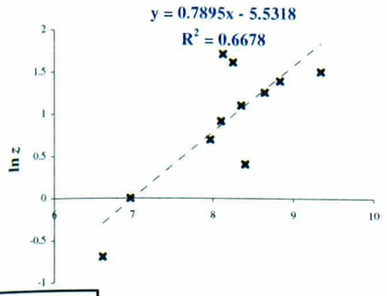
R^2 = 0.9805



$U_* = 0.74$

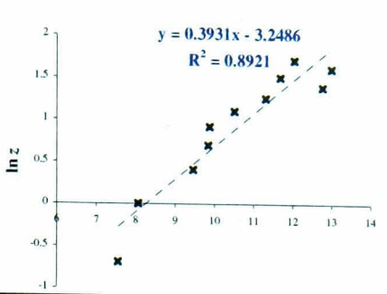
c) Position = 215 cm

Position = 215 cm, width = 1 cm



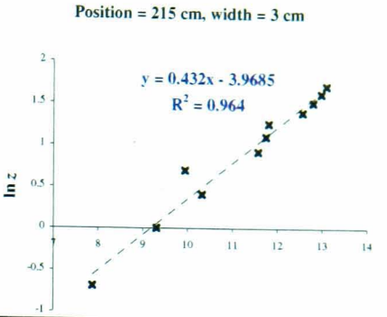
$U_* = 0.51$ U (cm s-1)

Position = 215 cm, width = 2 cm



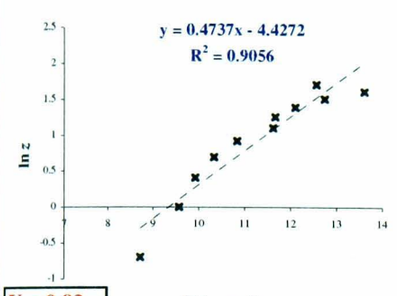
$U_* = 1.0$ U (cm s-1)

Position = 215 cm, width = 3 cm



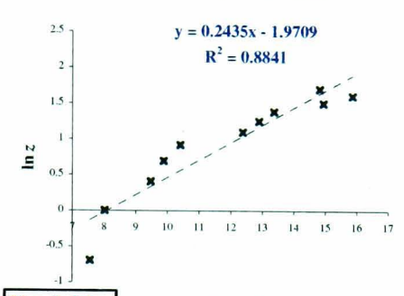
$U_* = 0.91$ U (cm s-1)

Position = 215 cm, width = 4 cm



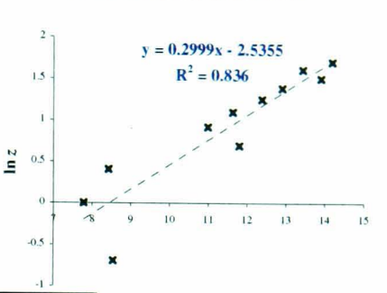
$U_* = 0.83$ U (cm s-1)

Position = 215 cm, width = 5 cm



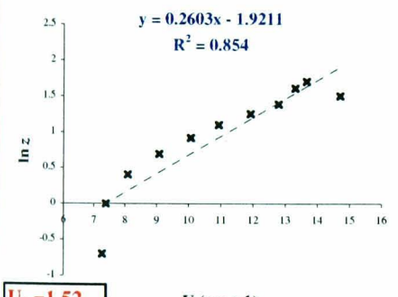
$U_* = 1.61$ U (cm s-1)

Position = 215 cm, width = 6 cm



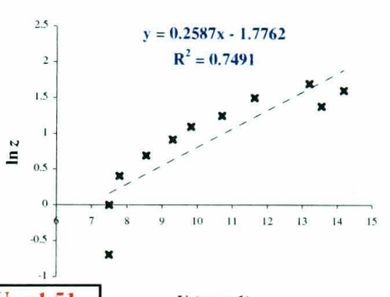
$U_* = 1.32$ U (cm s-1)

Position = 215 cm, width = 7 cm



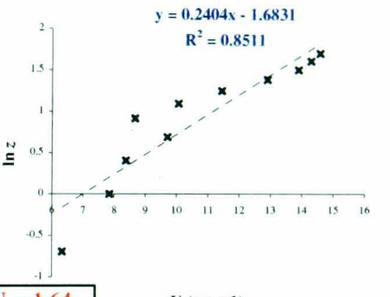
$U_* = 1.52$ U (cm s-1)

Position = 215 cm, width = 8 cm



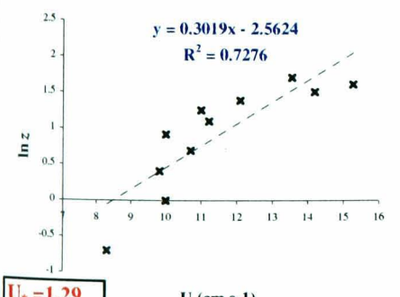
$U_* = 1.51$ U (cm s-1)

Position = 215 cm, width = 9 cm



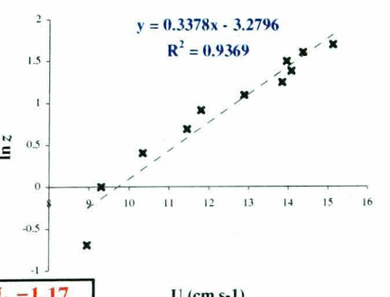
$U_* = 1.64$ U (cm s-1)

Position = 215 cm, width = 10 cm



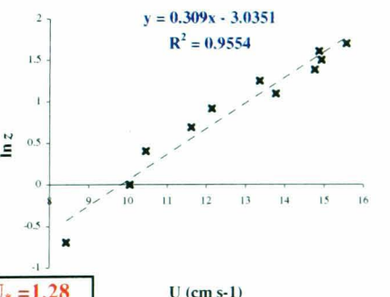
$U_* = 1.29$ U (cm s-1)

Position = 215 cm, width = 11 cm



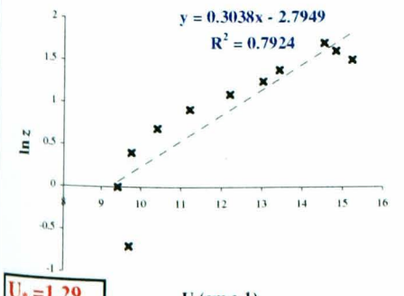
$U_* = 1.17$ U (cm s-1)

Position = 215 cm, width = 12 cm



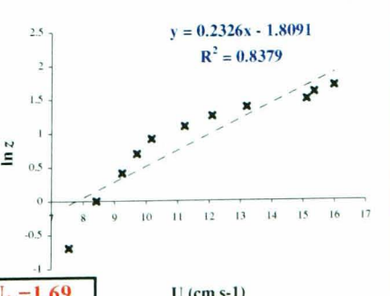
$U_* = 1.28$ U (cm s-1)

Position = 215 cm, width = 13 cm



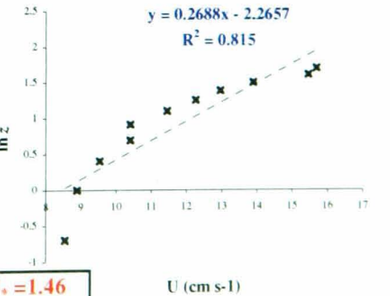
$U_* = 1.29$ U (cm s-1)

Position = 215 cm, width = 14 cm



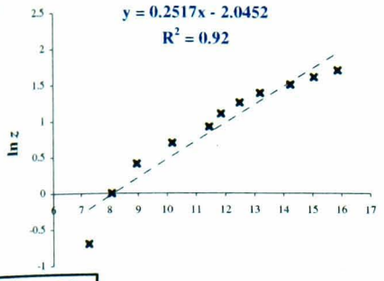
$U_* = 1.69$ U (cm s-1)

Position = 215 cm, width = 15 cm



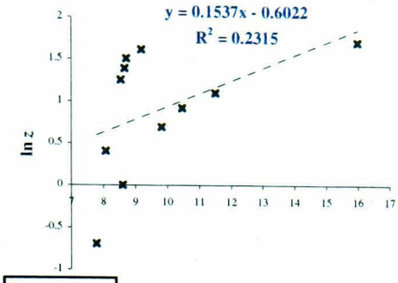
$U_* = 1.46$ U (cm s-1)

Position = 215 cm, width = 16 cm



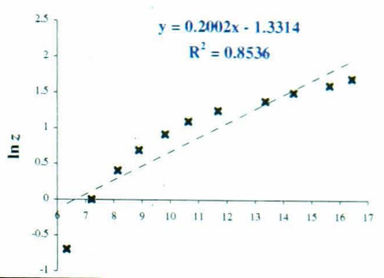
$U_* = 1.56$

Position = 215 cm, width = 17 cm



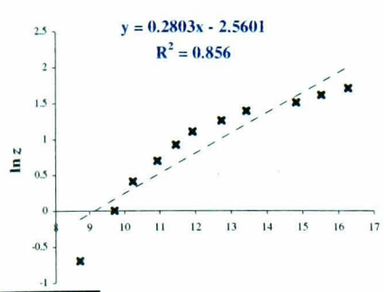
$U_* = 2.6$

Position = 215 cm, width = 18 cm



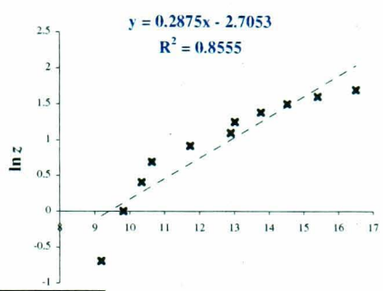
$U_* = 1.96$

Position = 215 cm, width = 19 cm



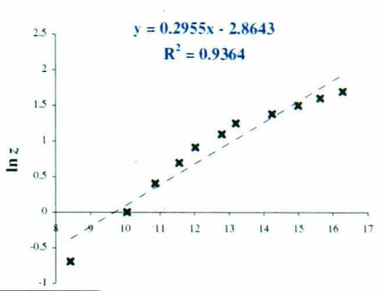
$U_* = 1.4$

Position = 215 cm, width = 20 cm



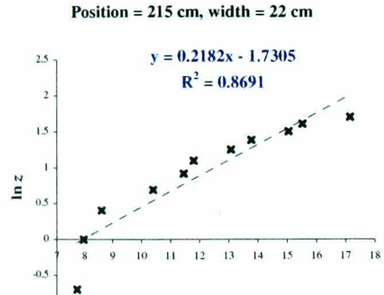
$U_* = 1.37$

Position = 215 cm, width = 21 cm



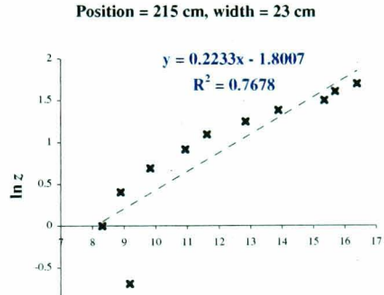
$U_* = 1.33$

Position = 215 cm, width = 22 cm



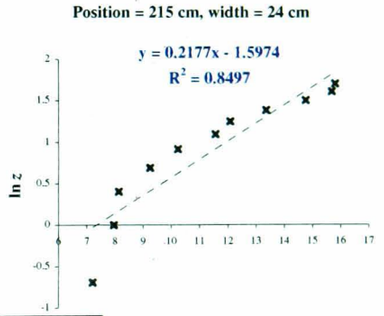
$U_* = 1.81$

Position = 215 cm, width = 23 cm



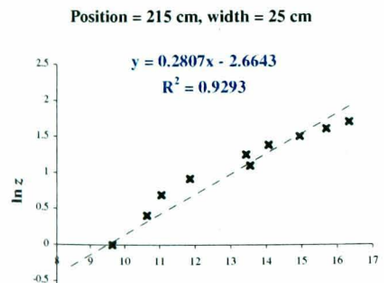
$U_* = 1.76$

Position = 215 cm, width = 24 cm



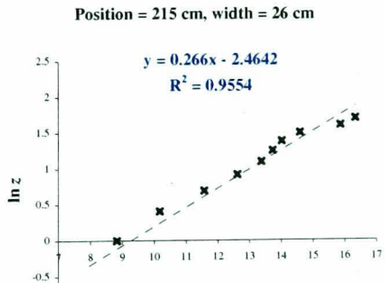
$U_* = 1.8$

Position = 215 cm, width = 25 cm



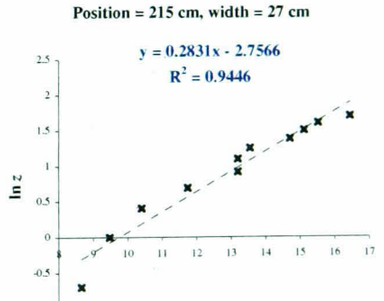
$U_* = 1.4$

Position = 215 cm, width = 26 cm



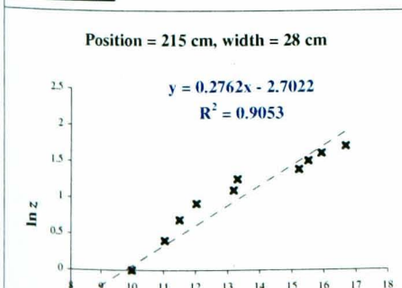
$U_* = 1.48$

Position = 215 cm, width = 27 cm



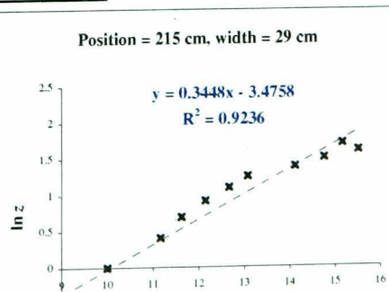
$U_* = 1.39$

Position = 215 cm, width = 28 cm



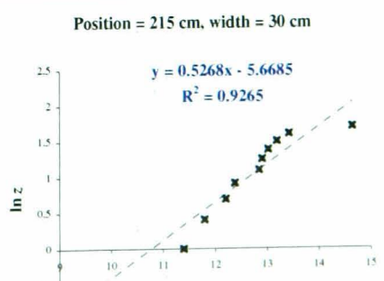
$U_* = 1.43$

Position = 215 cm, width = 29 cm



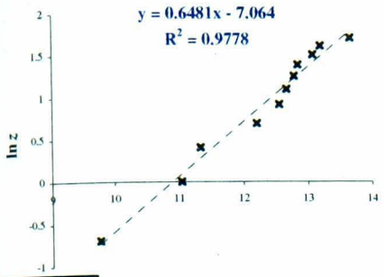
$U_* = 1.14$

Position = 215 cm, width = 30 cm



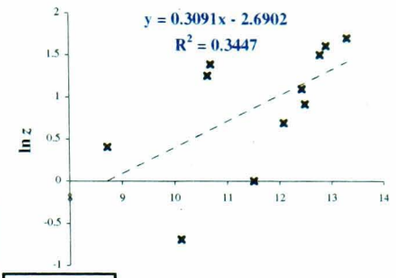
$U_* = 0.75$

Position = 215 cm, width = 31 cm



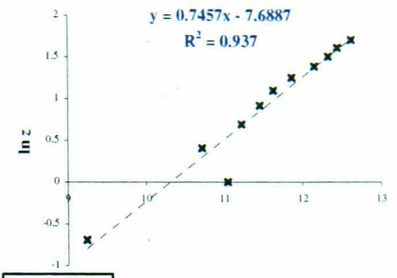
$U_* = 0.61$

Position = 215 cm, width = 32 cm



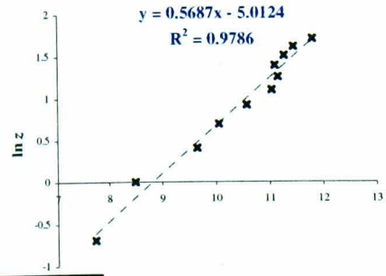
$U_* = 1.27$

Position = 215 cm, width = 33 cm



$U_* = 0.53$

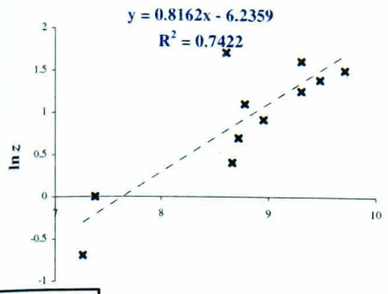
Position = 215 cm, width = 34 cm



$U_* = 0.7$

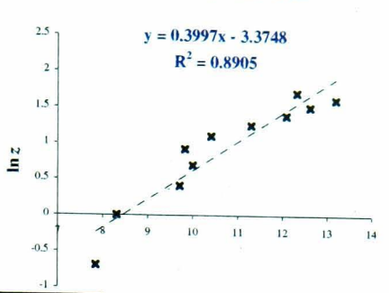
d) Position = 225 cm

Position = 225 cm, width = 1 cm



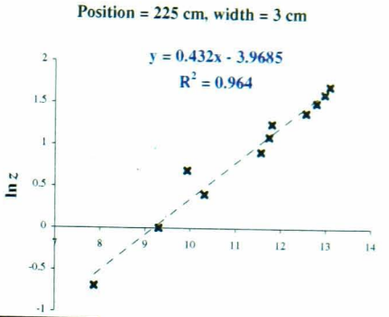
$U_* = 0.48$

Position = 225 cm, width = 2 cm



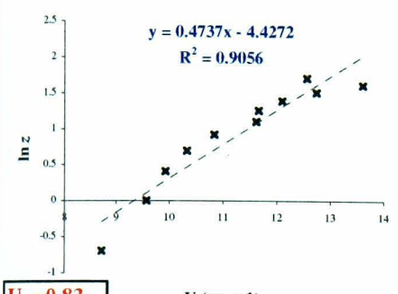
$U_* = 0.98$

Position = 225 cm, width = 3 cm



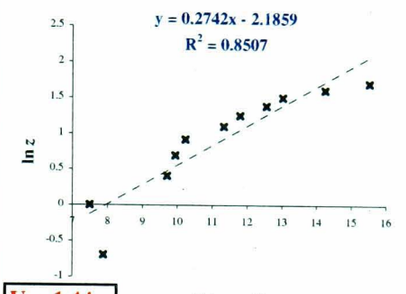
$U_* = 0.91$

Position = 225 cm, width = 4 cm



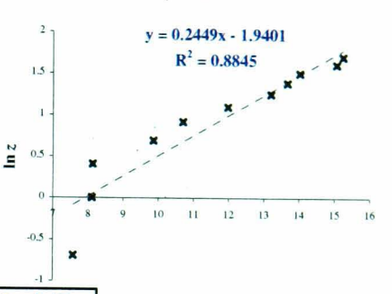
$U_* = 0.83$

Position = 225 cm, width = 5 cm



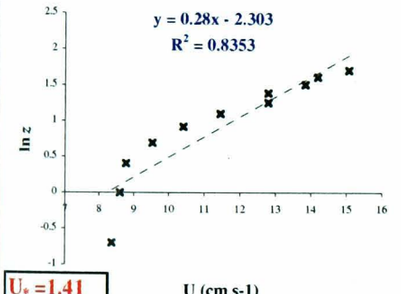
$U_* = 1.44$

Position = 225 cm, width = 6 cm



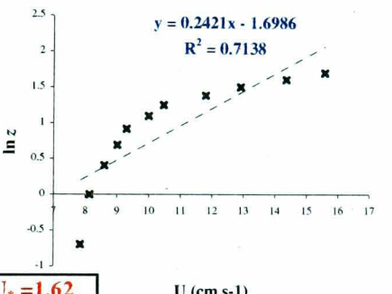
$U_* = 1.61$

Position = 225 cm, width = 7 cm



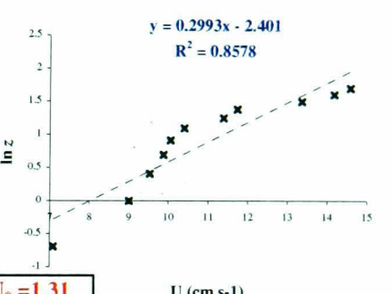
$U_* = 1.41$

Position = 225 cm, width = 8 cm



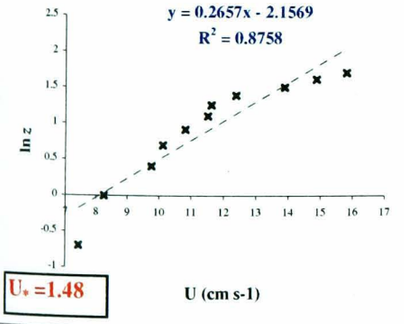
$U_* = 1.62$

Position = 225 cm, width = 9 cm



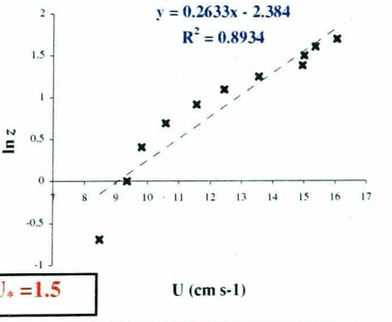
$U_* = 1.31$

Position = 225 cm, width = 10 cm



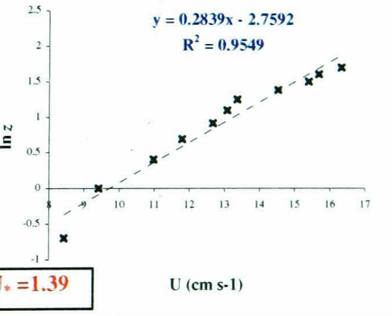
$U_* = 1.48$

Position = 225 cm, width = 11 cm



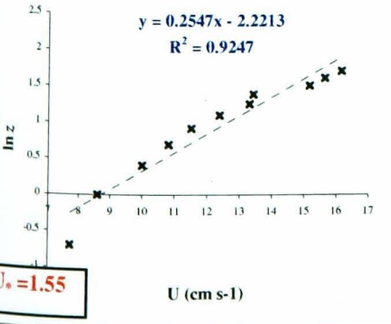
$U_* = 1.5$

Position = 225 cm, width = 12 cm



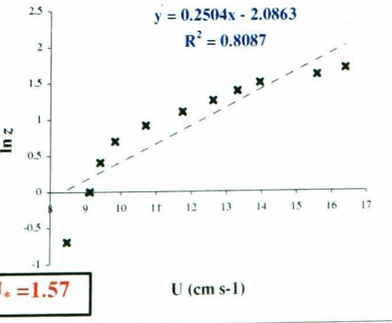
$U_* = 1.39$

Position = 225 cm, width = 13 cm



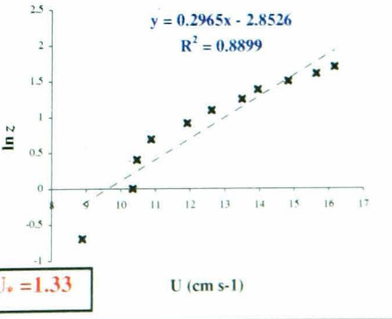
$U_* = 1.55$

Position = 225 cm, width = 14 cm



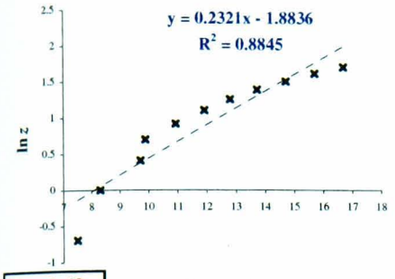
$U_* = 1.57$

Position = 225 cm, width = 15 cm



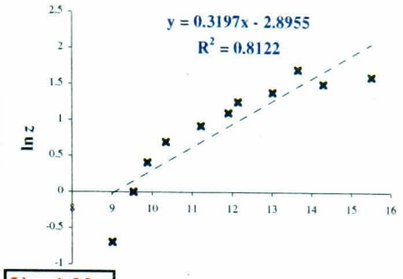
$U_* = 1.33$

Position = 225 cm, width = 16 cm



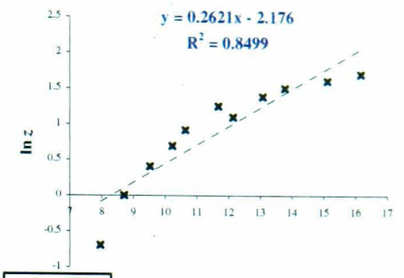
$U_* = 1.69$

Position = 225 cm, width = 17 cm



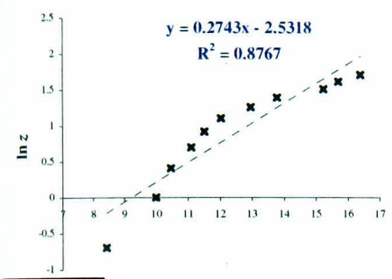
$U_* = 1.22$

Position = 225 cm, width = 18 cm



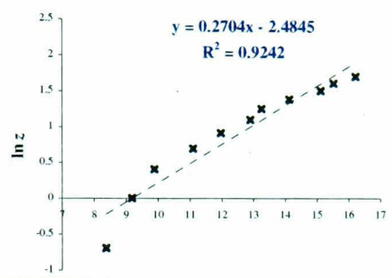
$U_* = 1.5$

Position = 225 cm, width = 19 cm



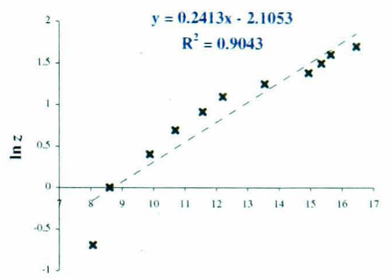
$U_* = 1.43$

Position = 225 cm, width = 20 cm



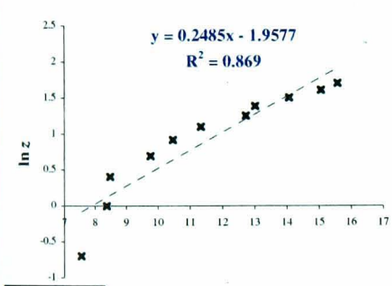
$U_* = 1.46$

Position = 225 cm, width = 21 cm



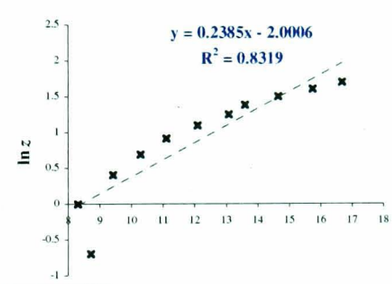
$U_* = 1.63$

Position = 225 cm, width = 22 cm



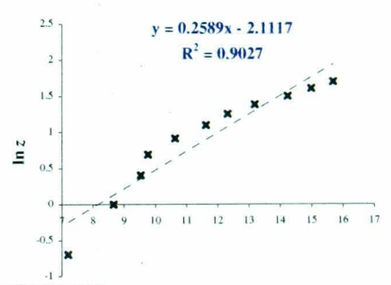
$U_* = 1.58$

Position = 225 cm, width = 23 cm



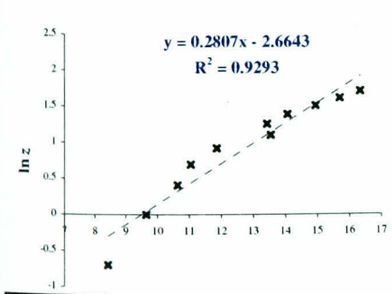
$U_* = 1.65$

Position = 225 cm, width = 24 cm



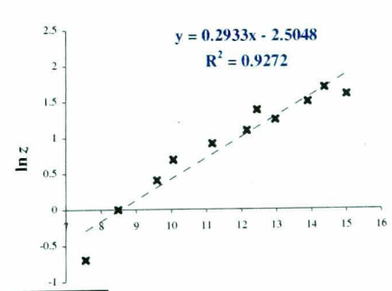
$U_* = 1.52$

Position = 225 cm, width = 25 cm



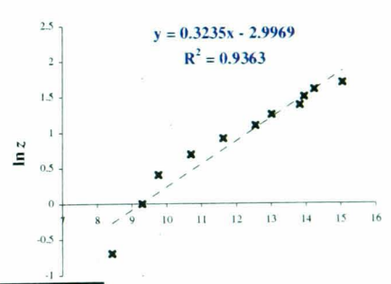
$U_* = 1.4$

Position = 225 cm, width = 26 cm



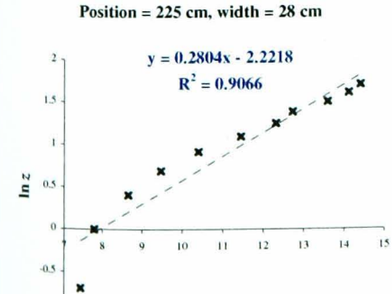
$U_* = 1.34$

Position = 225 cm, width = 27 cm



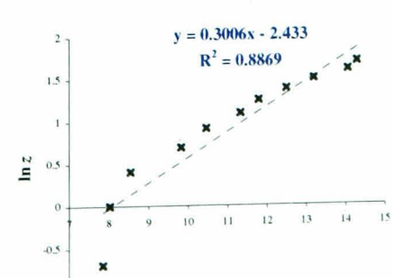
$U_* = 1.22$

Position = 225 cm, width = 28 cm



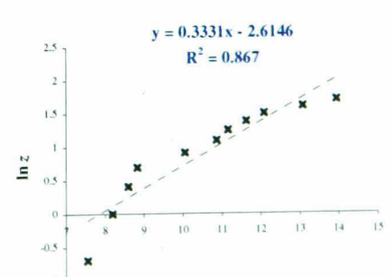
$U_* = 1.4$

Position = 225 cm, width = 29 cm



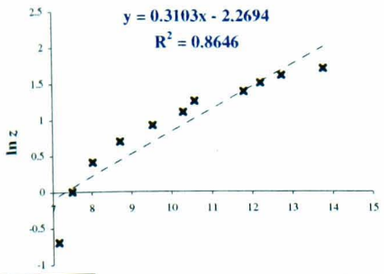
$U_* = 1.31$

Position = 225 cm, width = 30 cm



$U_* = 1.18$

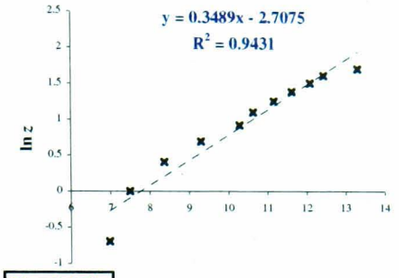
Position = 225 cm, width = 31 cm



$U_* = 1.27$

U (cm s-1)

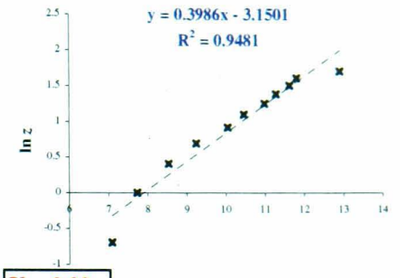
Position = 225 cm, width = 32 cm



$U_* = 1.13$

U (cm s-1)

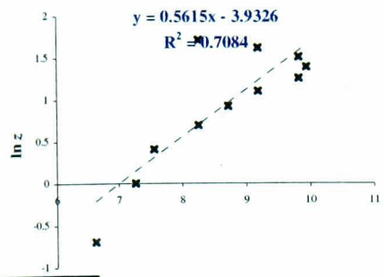
Position = 225 cm, width = 33 cm



$U_* = 0.99$

U (cm s-1)

Position = 225 cm, width = 34 cm

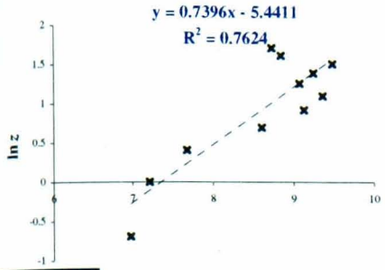


$U_* = 0.71$

U (cm s-1)

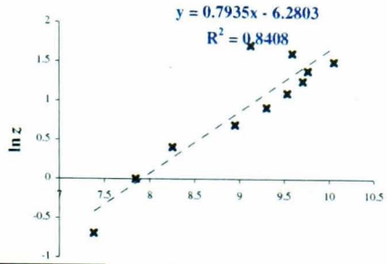
e) Position = 235 cm

Position = 235 cm, width = 1 cm



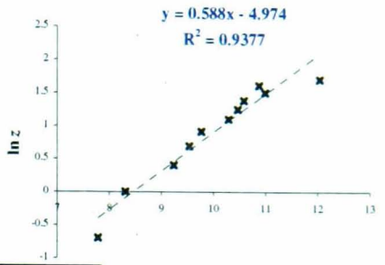
$U_* = 0.54$

Position = 235 cm, width = 2 cm



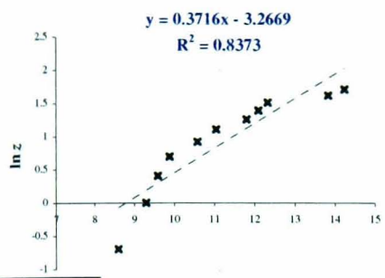
$U_* = 0.5$

Position = 235 cm, width = 3 cm



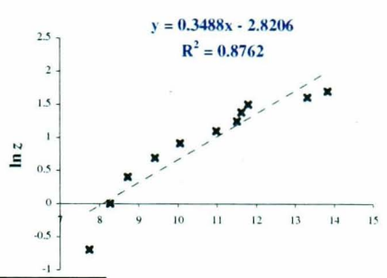
$U_* = 0.68$

Position = 235 cm, width = 4 cm



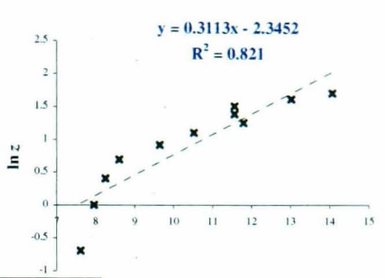
$U_* = 1.06$

Position = 235 cm, width = 5 cm



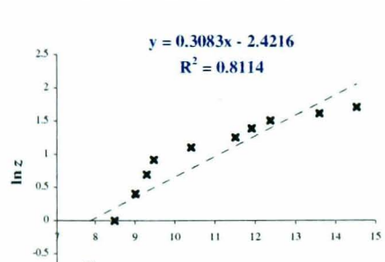
$U_* = 1.13$

Position = 235 cm, width = 6 cm



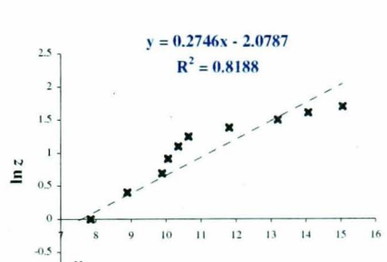
$U_* = 1.26$

Position = 235 cm, width = 7 cm



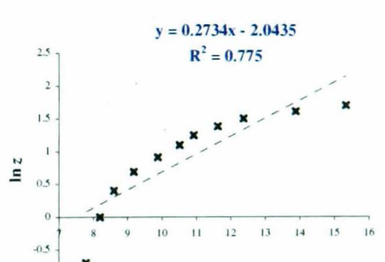
$U_* = 1.27$

Position = 235 cm, width = 8 cm



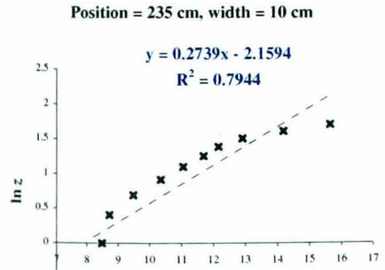
$U_* = 1.43$

Position = 235 cm, width = 9 cm



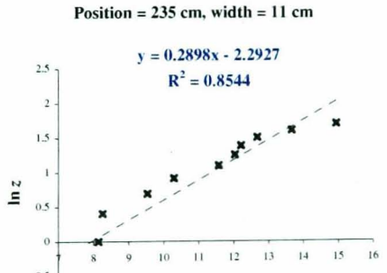
$U_* = 1.44$

Position = 235 cm, width = 10 cm



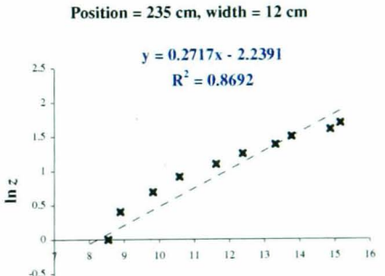
$U_* = 1.44$

Position = 235 cm, width = 11 cm



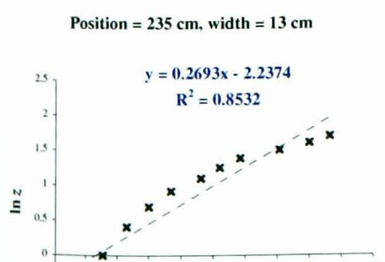
$U_* = 1.36$

Position = 235 cm, width = 12 cm



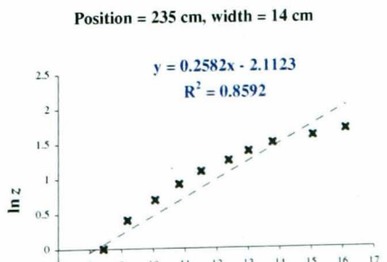
$U_* = 1.45$

Position = 235 cm, width = 13 cm



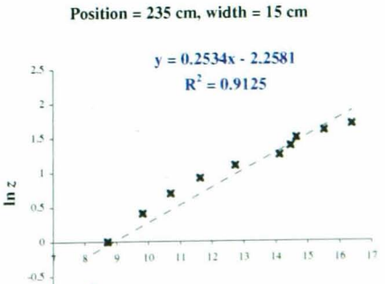
$U_* = 1.46$

Position = 235 cm, width = 14 cm



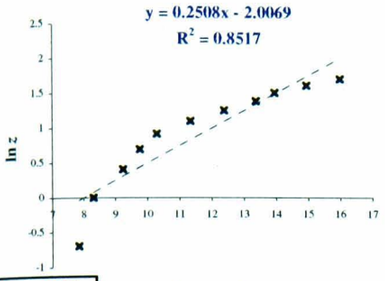
$U_* = 1.52$

Position = 235 cm, width = 15 cm



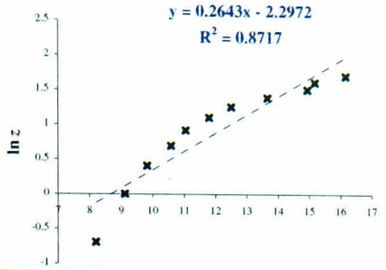
$U_* = 1.56$

Position = 235 cm, width = 16 cm



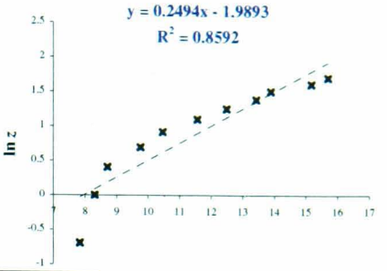
$U_* = 1.57$

Position = 235 cm, width = 17 cm



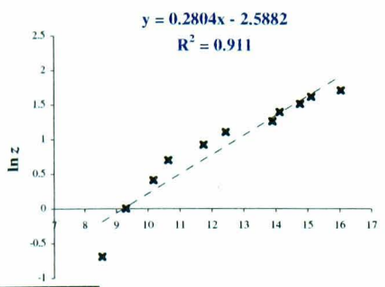
$U_* = 1.49$

Position = 235 cm, width = 18 cm



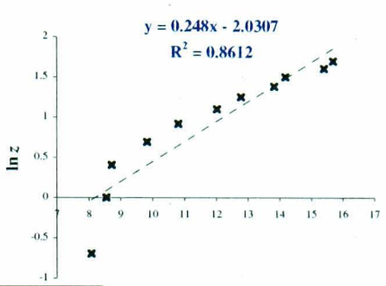
$U_* = 1.58$

Position = 235 cm, width = 19 cm



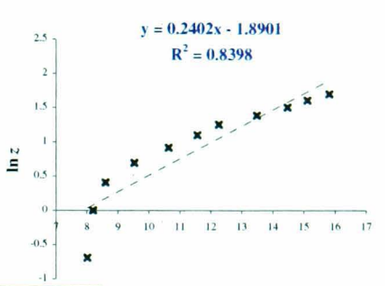
$U_* = 1.41$

Position = 235 cm, width = 20 cm



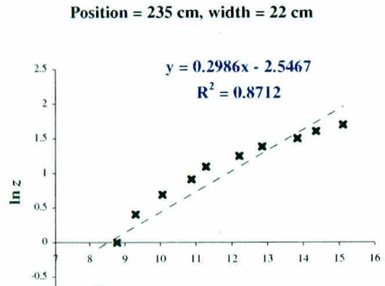
$U_* = 1.58$

Position = 235 cm, width = 21 cm



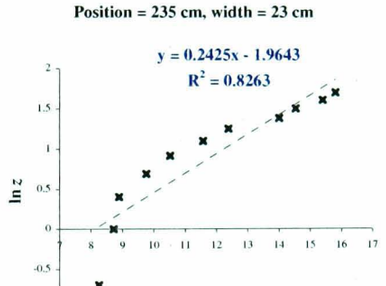
$U_* = 1.64$

Position = 235 cm, width = 22 cm



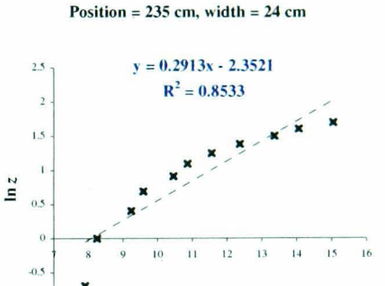
$U_* = 1.32$

Position = 235 cm, width = 23 cm



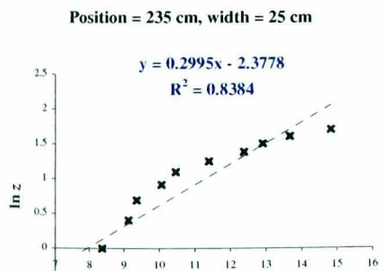
$U_* = 1.62$

Position = 235 cm, width = 24 cm



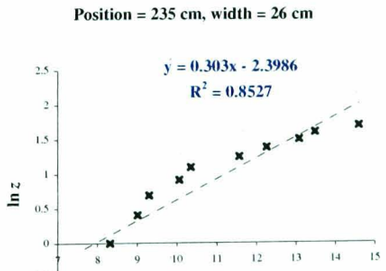
$U_* = 1.35$

Position = 235 cm, width = 25 cm



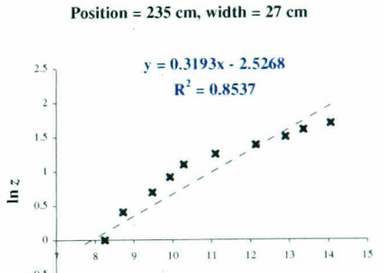
$U_* = 1.31$

Position = 235 cm, width = 26 cm



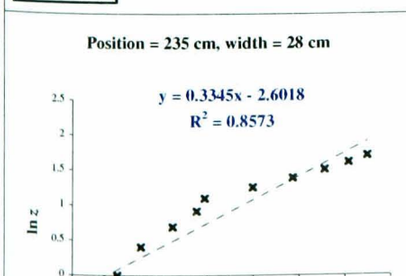
$U_* = 1.3$

Position = 235 cm, width = 27 cm



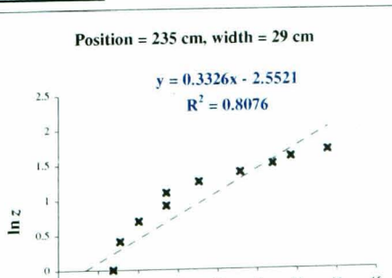
$U_* = 1.23$

Position = 235 cm, width = 28 cm



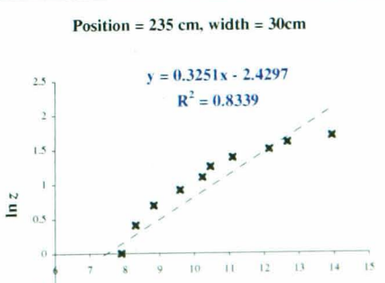
$U_* = 1.18$

Position = 235 cm, width = 29 cm



$U_* = 1.18$

Position = 235 cm, width = 30cm

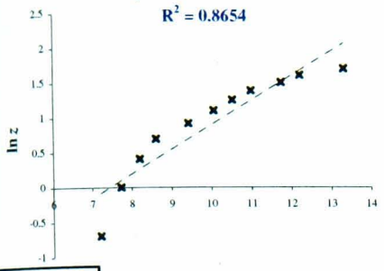


$U_* = 1.21$

Position = 235 cm, width = 31 cm

y = 0.353x - 2.6306

R^2 = 0.8654



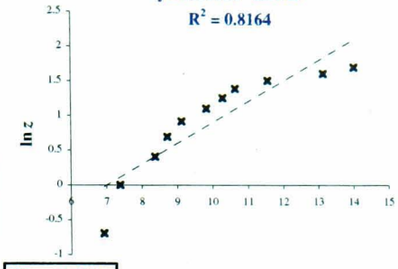
$U_* = 1.12$

U (cm s-1)

Position = 235 cm, width = 32 cm

y = 0.3003x - 2.1045

R^2 = 0.8164



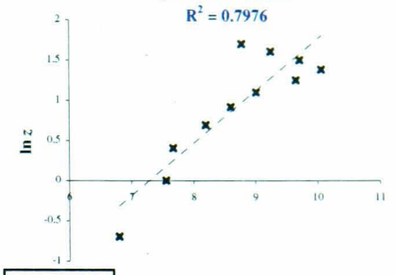
$U_* = 1.31$

U (cm s-1)

Position = 235 cm, width = 33 cm

y = 0.654x - 4.7659

R^2 = 0.7976



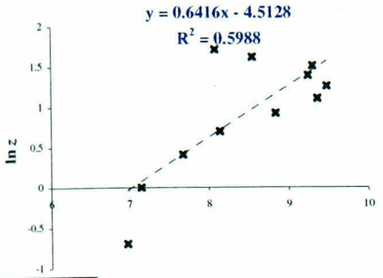
$U_* = 0.61$

U (cm s-1)

Position = 235 cm, width = 34 cm

y = 0.6416x - 4.5128

R^2 = 0.5988



$U_* = 0.62$

U (cm s-1)

APPENDIX 9. STATISTICAL SIGNIFICANCE OF THE CALCULATED VALUES OF u_* AND z_0.

Table 1. u_* and z_0 values for each pump setting

Pump speed	Velocity (cm s^{-1})	Slope	95% CLL	95% CLU	p	U*	ln Z_0	95% CLL	95% CLU	p	Z_0
1	5.3	0.23159928	0.169110912	0.294087649	**<0.01**	1.73	-1.103677701	-1.673105479	-0.534249923	**<0.01**	0.334
2	7.9	0.267045396	0.191785441	0.342305351	**<0.01**	1.5	-2.139815323	-3.016240205	-1.263390442	**<0.01**	0.119
3	10.2	0.260596652	0.168897207	0.352296096	**<0.01**	1.53	-2.673692551	-3.95075261	-1.396632492	**<0.01**	0.070
4	15.2	0.244961455	0.195823687	0.294099223	**<0.01**	1.63	-3.984470853	-4.973494401	-2.995447306	**<0.01**	0.019
5	16.8	0.216627612	0.187529239	0.245725985	**<0.01**	1.85	-4.060671744	-4.733331778	-3.388011709	**<0.01**	0.018
6	19.3	0.172156796	0.137008715	0.207304876	**<0.01**	2.32	-3.606922452	-4.53725082	-2.676594083	**<0.01**	0.028
7	22.5	0.169196295	0.125613691	0.212778899	**<0.01**	2.36	-3.829172799	-5.05907186	-2.599273738	**<0.01**	0.022
8	26	0.137848096	0.111023172	0.164673019	**<0.01**	2.9	-3.883525085	-4.823573303	-2.943476868	**<0.01**	0.021
9	28.6	0.117934296	0.092537175	0.143331418	**<0.01**	3.4	-3.634020294	-4.621008595	-2.647031993	**<0.01**	0.027
10	31.5	0.108166596	0.072102924	0.144230268	**<0.01**	3.69	-3.476326725	-4.950756879	-2.001896571	**<0.01**	0.032
11	34.1	0.104877254	0.085762599	0.123991909	**<0.01**	3.81	-3.897576	-4.780615185	-3.014536815	**<0.01**	0.021
12	65.1	0.095444233	0.072523657	0.118364808	**<0.01**	4.19	-6.390410211	-8.148220983	-4.632599439	**<0.01**	0.002

Table 2. u_* values in the region of the sample with 95% CL and statistical significance (current velocity = 16.8 cm s^{-1}).

	200 cm				210 cm				215 cm				225 cm				235 cm			
Width	Slope	95% CLL	95% CLU	p	Slope	95% CL	95% CLU	p	Slope	95% CL	95% CLU	p	Slope	95% CL	95% CLU	p	Slope	95% CL	95% CLU	p
1	0.91	0.47	1.35	**<0.01**	0.768	0.416	1.121	**<0.01**	0.79	0.37	1.209	**<0.01**	0.816	0.453	1.179	**<0.01**	0.74	0.428	1.051	**<0.01**
2	0.417	0.29	0.54	**<0.01**	0.426	0.297	0.554	**<0.01**	0.393	0.29	0.496	**<0.01**	0.4	0.294	0.505	**<0.01**	0.793	0.533	1.054	**<0.01**
3	0.417	0.31	0.52	**<0.01**	0.444	0.344	0.545	**<0.01**	0.432	0.369	0.495	**<0.01**	0.432	0.369	0.495	**<0.01**	0.588	0.474	0.702	**<0.01**
4	0.443	0.35	0.54	**<0.01**	0.526	0.399	0.653	**<0.01**	0.474	0.358	0.589	**<0.01**	0.474	0.358	0.589	**<0.01**	0.372	0.248	0.495	**<0.01**
5	0.468	0.40	0.54	**<0.01**	0.486	0.377	0.596	**<0.01**	0.243	0.177	0.31	**<0.01**	0.274	0.188	0.361	**<0.01**	0.349	0.25	0.448	**<0.01**
6	0.297	0.25	0.35	**<0.01**	0.35	0.31	0.389	**<0.01**	0.3	0.2	0.4	**<0.01**	0.245	0.178	0.312	**<0.01**	0.311	0.202	0.421	**<0.01**
7	0.348	0.29	0.41	**<0.01**	0.389	0.33	0.448	**<0.01**	0.26	0.179	0.341	**<0.01**	0.28	0.186	0.374	**<0.01**	0.308	0.196	0.42	**<0.01**
8	0.34	0.26	0.42	**<0.01**	0.336	0.253	0.42	**<0.01**	0.259	0.146	0.372	**<0.01**	0.242	0.127	0.358	**<0.01**	0.275	0.177	0.372	**<0.01**
9	0.357	0.29	0.42	**<0.01**	0.323	0.255	0.391	**<0.01**	0.24	0.165	0.316	**<0.01**	0.299	0.207	0.391	**<0.01**	0.273	0.162	0.385	**<0.01**
10	0.35	0.28	0.42	**<0.01**	0.378	0.27	0.486	**<0.01**	0.302	0.163	0.441	**<0.01**	0.266	0.19	0.341	**<0.01**	0.274	0.169	0.379	**<0.01**
11	0.348	0.28	0.42	**<0.01**	0.373	0.277	0.468	**<0.01**	0.338	0.272	0.404	**<0.01**	0.263	0.195	0.332	**<0.01**	0.29	0.2	0.38	**<0.01**
12	0.377	0.31	0.44	**<0.01**	0.402	0.312	0.492	**<0.01**	0.309	0.259	0.359	**<0.01**	0.284	0.237	0.33	**<0.01**	0.272	0.192	0.351	**<0.01**
13	0.398	0.32	0.48	**<0.01**	0.445	0.352	0.538	**<0.01**	0.304	0.187	0.421	**<0.01**	0.255	0.2	0.31	**<0.01**	0.269	0.185	0.353	**<0.01**
14	0.423	0.33	0.52	**<0.01**	0.487	0.324	0.651	**<0.01**	0.233	0.155	0.31	**<0.01**	0.25	0.159	0.342	**<0.01**	0.258	0.179	0.337	**<0.01**
15	0.397	0.30	0.49	**<0.01**	0.428	0.282	0.573	**<0.01**	0.269	0.172	0.365	**<0.01**	0.297	0.218	0.375	**<0.01**	0.253	0.194	0.313	**<0.01**
16	0.406	0.32	0.49	**<0.01**	0.356	0.241	0.47	**<0.01**	0.252	0.196	0.308	**<0.01**	0.232	0.169	0.295	**<0.01**	0.251	0.172	0.33	**<0.01**
17	0.358	0.26	0.45	**<0.01**	0.323	0.216	0.429	**<0.01**	0.154	-0.057	0.365	**0.54**	0.32	0.204	0.436	**<0.01**	0.264	0.188	0.341	**<0.01**
18	0.353	0.26	0.45	**<0.01**	0.364	0.264	0.464	**<0.01**	0.2	0.138	0.263	**<0.01**	0.262	0.179	0.345	**<0.01**	0.249	0.173	0.326	**<0.01**
19	0.393	0.29	0.49	**<0.01**	0.401	0.286	0.516	**<0.01**	0.28	0.194	0.367	**<0.01**	0.274	0.197	0.352	**<0.01**	0.28	0.214	0.347	**<0.01**
20	0.395	0.30	0.49	**<0.01**	0.437	0.323	0.551	**<0.01**	0.288	0.198	0.377	**<0.01**	0.27	0.212	0.329	**<0.01**	0.248	0.173	0.323	**<0.01**
21	0.424	0.33	0.52	**<0.01**	0.436	0.296	0.576	**<0.01**	0.295	0.237	0.354	**<0.01**	0.241	0.182	0.3	**<0.01**	0.24	0.161	0.319	**<0.01**
22	0.435	0.34	0.53	**<0.01**	0.374	0.224	0.525	**<0.01**	0.218	0.154	0.282	**<0.01**	0.248	0.176	0.321	**<0.01**	0.299	0.212	0.385	**<0.01**
23	0.408	0.31	0.51	**<0.01**	0.4	0.29	0.51	**<0.01**	0.223	0.131	0.316	**<0.01**	0.239	0.158	0.319	**<0.01**	0.243	0.159	0.326	**<0.01**

	200 cm				210 cm				215 cm				225 cm				235 cm			
Width	Slope	95% CLL	95% CLU	p	Slope	95% CL	95% CLU	p	Slope	95% CL	95% CLU	p	Slope	95% CL	95% CLU	p	Slope	95% CL	95% CLU	p
24	0.384	0.31	0.46	**<0.01**	0.47	0.3	0.65	**<0.01**	0.218	0.149	0.287	**<0.01**	0.259	0.195	0.323	**<0.01**	0.291	0.2	0.382	**<0.01**
25	0.405	0.28	0.53	**<0.01**	0.435	0.264	0.605	**<0.01**	0.281	0.222	0.339	**<0.01**	0.281	0.222	0.339	**<0.01**	0.3	0.2	0.399	**<0.01**
26	0.4	0.27	0.53	**<0.01**	0.442	0.267	0.618	**<0.01**	0.266	0.223	0.309	**<0.01**	0.293	0.231	0.355	**<0.01**	0.303	0.208	0.398	**<0.01**
27	0.339	0.20	0.48	**<0.01**	0.442	0.277	0.608	**<0.01**	0.283	0.231	0.335	**<0.01**	0.323	0.26	0.387	**<0.01**	0.319	0.22	0.419	**<0.01**
28	0.311	0.17	0.45	**<0.01**	0.374	0.225	0.523	**<0.01**	0.276	0.209	0.344	**<0.01**	0.28	0.213	0.348	**<0.01**	0.335	0.232	0.437	**<0.01**
29	0.359	0.23	0.49	**<0.01**	0.471	0.31	0.633	**<0.01**	0.345	0.27	0.42	**<0.01**	0.301	0.22	0.382	**<0.01**	0.333	0.21	0.455	**<0.01**
30	0.414	0.29	0.54	**<0.01**	0.542	0.345	0.739	**<0.01**	0.527	0.415	0.639	**<0.01**	0.333	0.235	0.431	**<0.01**	0.325	0.216	0.435	**<0.01**
31	0.417	0.31	0.52	**<0.01**	0.783	0.665	0.9	**<0.01**	0.648	0.574	0.722	**<0.01**	0.31	0.218	0.403	**<0.01**	0.353	0.248	0.458	**<0.01**
32	0.409	0.33	0.49	**<0.01**	0.797	0.698	0.896	**<0.01**	0.309	-0.012	0.63	**0.14**	0.349	0.284	0.413	**<0.01**	0.3	0.193	0.408	**<0.01**
33	0.498	0.396	0.6	**<0.01**	0.648	0.372	0.923	**<0.01**	0.746	0.6	0.892	**<0.01**	0.399	0.328	0.469	**<0.01**	0.654	0.406	0.902	**<0.01**
34	0.359	-0.209	0.926	**0.2**	0.534	0.477	0.59	**<0.01**	0.569	0.505	0.632	**<0.01**	0.562	0.29	0.833	**<0.01**	0.642	0.246	1.038	**<0.01**

Table 3. ln Z_0 values in the region of the sample with 95% CL and statistical significance (current velocity = 16.8 cm s^{-1}).

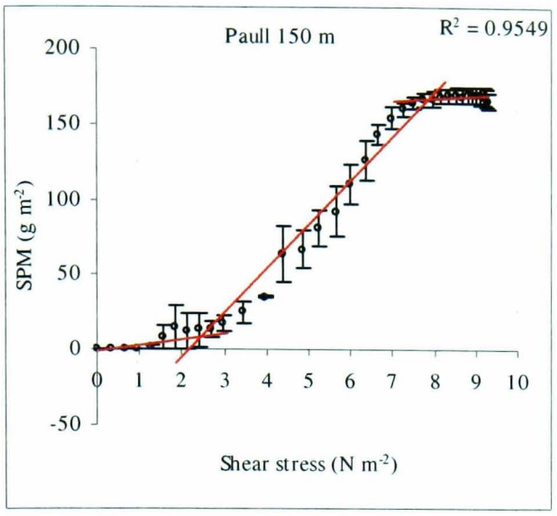
	200 cm				210				215				225				235			
Width	ln Z_0	95% CLL	95% CLU	p	ln Z_0	95% CLL	95% CLU	p	ln Z_0	95% CLL	95% CLU	p	ln Z_0	95% CLL	95% CLU	p	ln Z_0	95% CLL	95% CLU	p
1	-6.79	-10.5	-3.1	**<0.01**	-5.44	-8.37	-2.52	**<0.01**	-5.53	-8.97	-2.1	**<0.01**	-6.24	-9.42	-3.05	**<0.01**	-5.44	-8.12	-2.76	**<0.01**
2	-3.51	-4.88	-2.15	**<0.01**	-3.57	-4.93	-2.2	**<0.01**	-3.25	-4.35	-2.15	**<0.01**	-3.37	-4.52	-2.23	**<0.01**	-6.28	-8.65	-3.91	**<0.01**
3	-3.94	-5.17	-2.72	**<0.01**	-4.25	-5.43	-3.07	**<0.01**	-3.97	-4.68	-3.25	**<0.01**	-3.97	-4.68	-3.25	**<0.01**	-4.97	-6.12	-3.83	**<0.01**
4	-4.42	-5.54	-3.3	**<0.01**	-5.41	-6.94	-3.88	**<0.01**	-4.43	-5.73	-3.12	**<0.01**	-4.43	-5.73	-3.12	**<0.01**	-3.27	-4.67	-1.87	**<0.01**
5	-4.93	-5.83	-4.04	**<0.01**	-5.09	-6.45	-3.73	**<0.01**	-1.97	-2.78	-1.17	**<0.01**	-2.19	-3.18	-1.19	**<0.01**	-2.82	-3.89	-1.75	**<0.01**
6	-3.16	-3.84	-2.48	**<0.01**	-4.01	-4.57	-3.45	**<0.01**	-2.54	-3.7	-1.37	**<0.01**	-1.94	-2.73	-1.15	**<0.01**	-2.35	-3.51	-1.18	**<0.01**
7	-4.1	-4.93	-3.27	**<0.01**	-4.59	-5.43	-3.75	**<0.01**	-1.92	-2.82	-1.02	**<0.01**	-2.3	-3.4	-1.21	**<0.01**	-2.42	-3.65	-1.19	**<0.01**
8	-3.98	-5.15	-2.81	**<0.01**	-3.9	-5.1	-2.7	**<0.01**	-1.78	-2.97	-0.58	**<0.01**	-1.7	-2.97	-0.43	**<0.01**	-2.08	-3.16	-1	**<0.01**
9	-4.27	-5.24	-3.29	**<0.01**	-3.87	-4.89	-2.86	**<0.01**	-1.68	-2.52	-0.84	**<0.01**	-2.4	-3.43	-1.37	**<0.01**	-2.04	-3.27	-0.82	**<0.01**
10	-4.16	-5.16	-3.17	**<0.01**	-4.4	-5.92	-2.87	**<0.01**	-2.56	-4.18	-0.94	**<0.01**	-2.16	-3.04	-1.27	**<0.01**	-2.16	-3.36	-0.96	**<0.01**
11	-4.2	-5.26	-3.14	**<0.01**	-4.42	-5.8	-3.05	**<0.01**	-3.28	-4.11	-2.45	**<0.01**	-2.38	-3.26	-1.51	**<0.01**	-2.29	-3.31	-1.28	**<0.01**
12	-4.63	-5.59	-3.67	**<0.01**	-4.85	-6.14	-3.56	**<0.01**	-3.04	-3.69	-2.38	**<0.01**	-2.76	-3.37	-2.15	**<0.01**	-2.24	-3.18	-1.3	**<0.01**
13	-5.01	-6.18	-3.84	**<0.01**	-5.49	-6.83	-4.15	**<0.01**	-2.79	-4.24	-1.35	**<0.01**	-2.22	-2.91	-1.53	**<0.01**	-2.24	-3.24	-1.24	**<0.01**
14	-5.37	-6.8	-3.94	**<0.01**	-6.09	-8.45	-3.74	**<0.01**	-1.81	-2.73	-0.89	**<0.01**	-2.09	-3.21	-0.97	**<0.01**	-2.11	-3.05	-1.17	**<0.01**
15	-5.1	-6.57	-3.62	**<0.01**	-5.37	-7.5	-3.23	**<0.01**	-2.27	-3.43	-1.11	**<0.01**	-2.85	-3.86	-1.84	**<0.01**	-2.26	-3.01	-1.5	**<0.01**
16	-5.31	-6.56	-4.05	**<0.01**	-4.44	-6.18	-2.71	**<0.01**	-2.05	-2.72	-1.37	**<0.01**	-1.88	-2.66	-1.1	**<0.01**	-2.01	-2.94	-1.07	**<0.01**

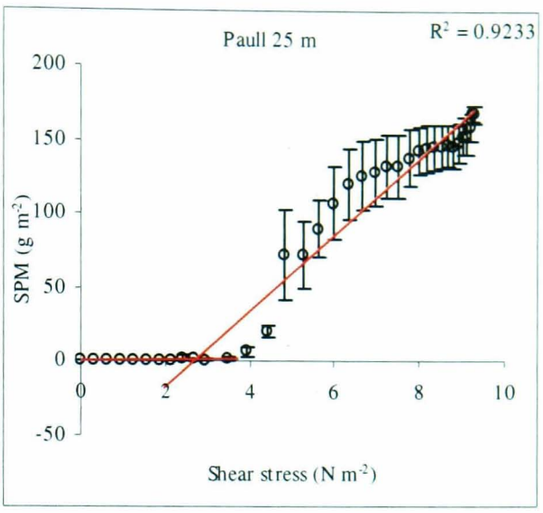
	200 cm				210				215				225				235			
Width	ln Z_0	95% CLL	95% CLU	p	ln Z_0	95% CLL	95% CLU	p	ln Z_0	95% CLL	95% CLU	p	ln Z_0	95% CLL	95% CLU	p	ln Z_0	95% CLL	95% CLU	p
17	-4.74	-6.23	-3.24	**<0.01**	-4.13	-5.8	-2.46	**<0.01**	-0.6	-2.72	1.51	**0.13**	-2.9	-4.29	-1.5	**<0.01**	-2.3	-3.24	-1.35	**<0.01**
18	-4.63	-6.14	-3.12	**<0.01**	-4.76	-6.32	-3.2	**<0.01**	-1.33	-2.06	-0.61	**<0.01**	-2.18	-3.17	-1.18	**<0.01**	-1.99	-2.89	-1.09	**<0.01**
19	-5.13	-6.67	-3.59	**<0.01**	-5.08	-6.8	-3.35	**<0.01**	-2.56	-3.65	-1.47	**<0.01**	-2.53	-3.52	-1.54	**<0.01**	-2.59	-3.43	-1.75	**<0.01**
20	-5.04	-6.46	-3.62	**<0.01**	-5.48	-7.15	-3.81	**<0.01**	-2.71	-3.84	-1.57	**<0.01**	-2.48	-3.23	-1.74	**<0.01**	-2.03	-2.94	-1.12	**<0.01**
21	-5.33	-6.77	-3.88	**<0.01**	-5.31	-7.32	-3.3	**<0.01**	-2.86	-3.62	-2.11	**<0.01**	-2.11	-2.86	-1.35	**<0.01**	-1.89	-2.83	-0.95	**<0.01**
22	-5.34	-6.74	-3.95	**<0.01**	-4.44	-6.6	-2.27	**<0.01**	-1.73	-2.52	-0.94	**<0.01**	-1.96	-2.82	-1.1	**<0.01**	-2.55	-3.56	-1.53	**<0.01**
23	-4.91	-6.32	-3.5	**<0.01**	-4.67	-6.19	-3.14	**<0.01**	-1.8	-2.95	-0.65	**<0.01**	-2	-3.01	-0.99	**<0.01**	-1.96	-2.98	-0.95	**<0.01**
24	-4.54	-5.66	-3.42	**<0.01**	-5.62	-8.07	-3.18	**<0.01**	-1.6	-2.42	-0.78	**<0.01**	-2.11	-2.88	-1.35	**<0.01**	-2.35	-3.39	-1.32	**<0.01**
25	-4.75	-6.49	-3.02	**<0.01**	-5.02	-7.35	-2.69	**<0.01**	-2.66	-3.42	-1.91	**<0.01**	-2.66	-3.42	-1.91	**<0.01**	-2.38	-3.48	-1.27	**<0.01**
26	-4.56	-6.3	-2.82	**<0.01**	-5.13	-7.54	-2.73	**<0.01**	-2.46	-3.02	-1.9	**<0.01**	-2.5	-3.24	-1.77	**<0.01**	-2.4	-3.45	-1.35	**<0.01**
27	-3.66	-5.56	-1.75	**<0.01**	-5.19	-7.48	-2.9	**<0.01**	-2.76	-3.44	-2.08	**<0.01**	-3	-3.78	-2.22	**<0.01**	-2.53	-3.62	-1.44	**<0.01**
28	-3.28	-5.16	-1.4	**<0.01**	-4.26	-6.33	-2.19	**<0.01**	-2.7	-3.6	-1.81	**<0.01**	-2.22	-2.99	-1.45	**<0.01**	-2.6	-3.7	-1.51	**<0.01**
29	-3.87	-5.63	-2.12	**<0.01**	-5.39	-7.56	-3.23	**<0.01**	-3.48	-4.44	-2.52	**<0.01**	-2.43	-3.35	-1.52	**<0.01**	-2.55	-3.84	-1.26	**<0.01**
30	-4.49	-6.16	-2.82	**<0.01**	-6	-8.52	-3.48	**<0.01**	-5.67	-7.07	-4.27	**<0.01**	-2.61	-3.67	-1.56	**<0.01**	-2.43	-3.57	-1.29	**<0.01**
31	-4.34	-5.69	-3	**<0.01**	-8.84	-10.3	-7.38	**<0.01**	-7.06	-7.97	-6.15	**<0.01**	-2.27	-3.24	-1.3	**<0.01**	-2.63	-3.7	-1.56	**<0.01**
32	-3.91	-4.82	-3	**<0.01**	-8.84	-10	-7.63	**<0.01**	-2.69	-6.45	1.07	**0.1**	-2.71	-3.39	-2.03	**<0.01**	-2.1	-3.2	-1.01	**<0.01**
33	-4.61	-5.75	-3.48	**<0.01**	-6.6	-9.8	-3.4	**<0.01**	-7.69	-9.37	-6	**<0.01**	-3.15	-3.87	-2.43	**<0.01**	-4.77	-6.93	-2.6	**<0.01**
34	-2.39	-7.62	2.84	**0.3**	-4.63	-5.22	-4.04	**<0.01**	-5.01	-5.68	-4.35	**<0.01**	-3.93	-6.29	-1.58	**<0.01**	-4.51	-7.87	-1.16	**<0.01**

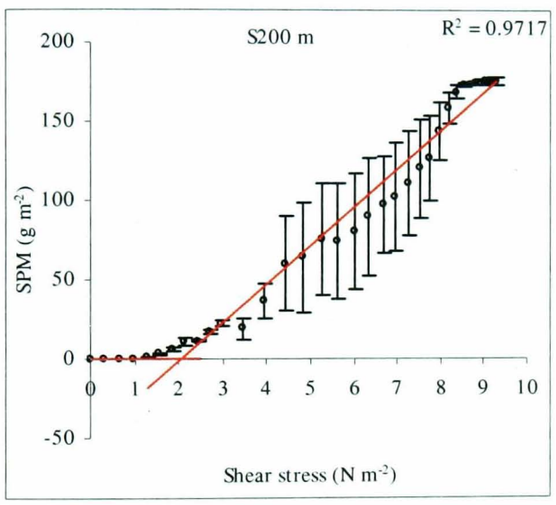
Table 4. Z_0 values in the region of the sample (current velocity = 16.8 cm s^{-1}).

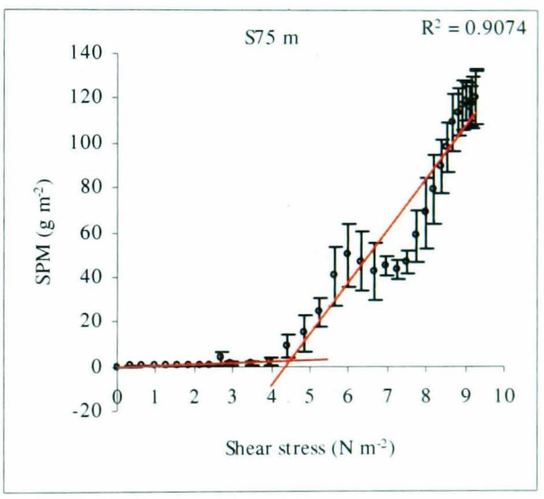
Width	200 cm	210 cm	215 cm	225 cm	235 cm	Width	200 cm	210 cm	215 cm	225 cm	235 cm
1	0.0012	0.0045	0.0041	0.0020	0.0045	18	0.0101	0.0088	0.2665	0.1152	0.1386
2	0.0306	0.0290	0.0397	0.0350	0.0020	19	0.0061	0.0065	0.0786	0.0809	0.0765
3	0.0199	0.0147	0.0194	0.0194	0.0072	2	0.0067	0.0043	0.0681	0.0848	0.1330
4	0.0124	0.0046	0.0123	0.0123	0.0390	21	0.0050	0.0051	0.0581	0.1235	0.1530
5	0.0075	0.0064	0.1412	0.1140	0.0607	22	0.0049	0.0122	0.1793	0.1431	0.0797
6	0.0433	0.0186	0.0806	0.1456	0.0974	23	0.0076	0.0097	0.1672	0.1371	0.1421
7	0.0171	0.0104	0.1484	0.1015	0.0902	24	0.0110	0.0037	0.2046	0.1228	0.0967
8	0.0192	0.0208	0.1713	0.1851	0.1269	25	0.0089	0.0068	0.0709	0.0709	0.0943
9	0.0144	0.0214	0.1879	0.0921	0.1314	26	0.0108	0.0061	0.0865	0.0831	0.0923
10	0.0160	0.0127	0.0785	0.1174	0.1171	27	0.0265	0.0058	0.0647	0.0510	0.0813
11	0.0154	0.0124	0.0385	0.0937	0.1026	28	0.0385	0.0145	0.0683	0.1100	0.0755
12	0.0100	0.0081	0.0491	0.0645	0.1082	29	0.0213	0.0047	0.0317	0.0892	0.0793
13	0.0069	0.0043	0.0623	0.1101	0.1084	30	0.0115	0.0026	0.0036	0.0745	0.0895
14	0.0048	0.0024	0.1658	0.1259	0.1227	31	0.0134	0.0002	0.0009	0.1050	0.0733
15	0.0063	0.0048	0.1054	0.0588	0.1061	32	0.0205	0.0002	0.0691	0.0679	0.1237
16	0.0051	0.0121	0.1312	0.1540	0.1362	33	0.0102	0.0014	0.0005	0.0438	0.0088
17	0.0090	0.0165	0.5498	0.0564	0.1021	34	0.0932	0.0101	0.0069	0.0201	0.0113

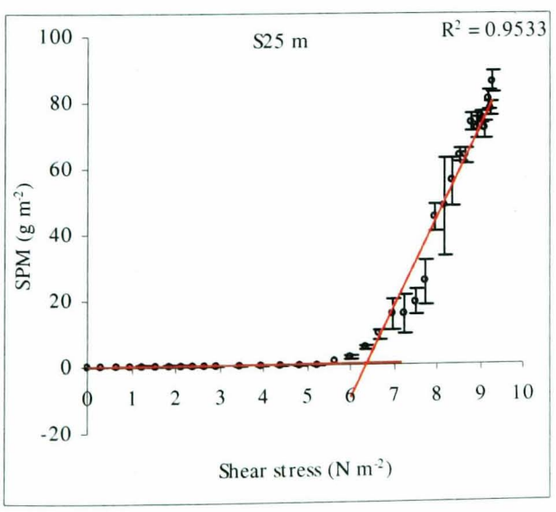
APPENDIX 10. Definition of critical shear stress of Paull and Saltend sediments (CSM field measurements) using regression analysis.



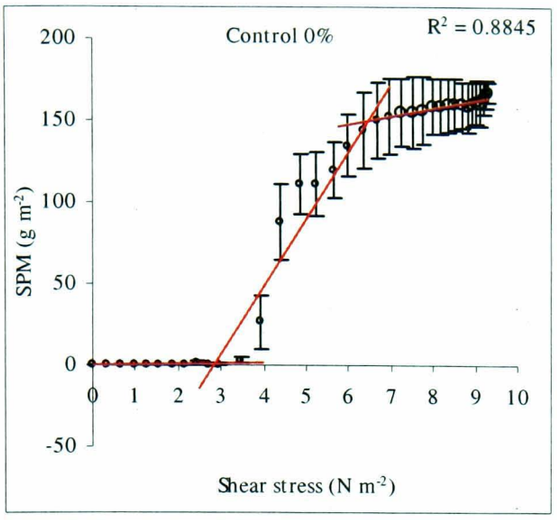


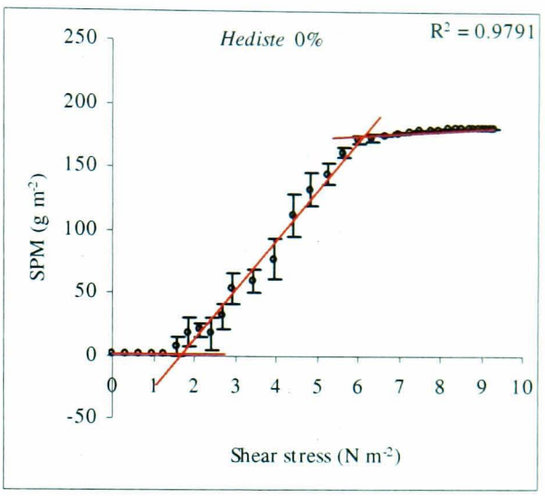


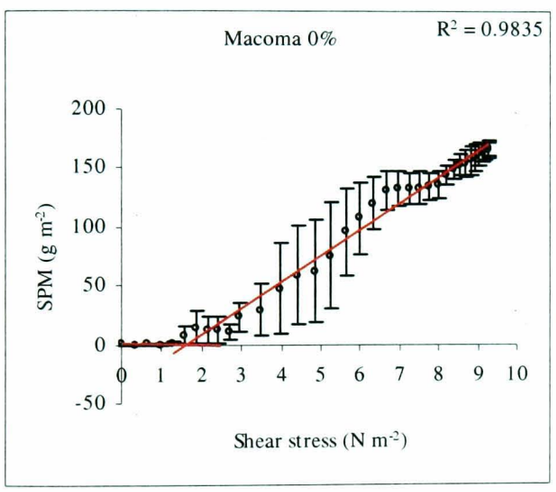


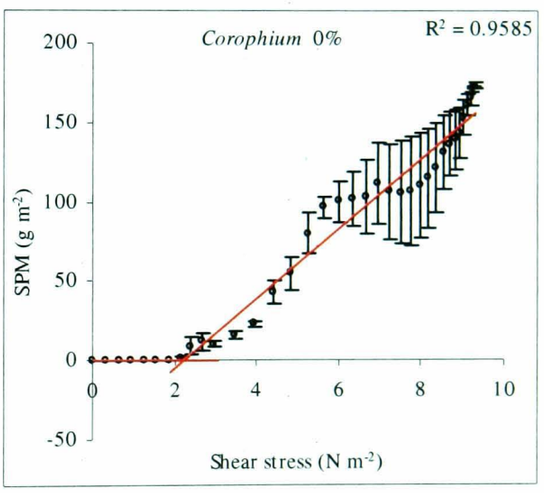


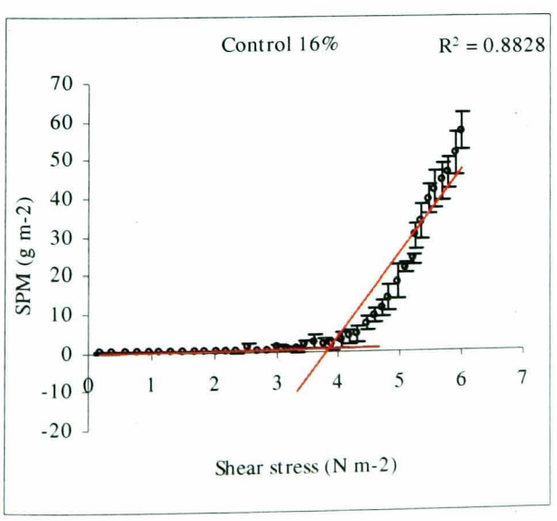
APPENDIX 11. Definition of critical shear stress of sediments exposed to different effluent concentrations (CSM laboratory measurements) using regression analysis.

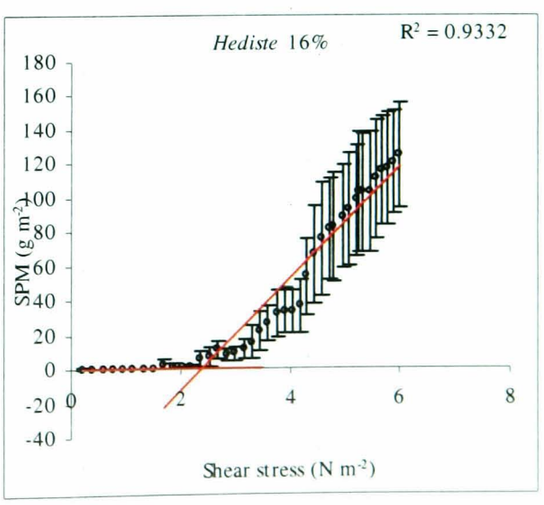


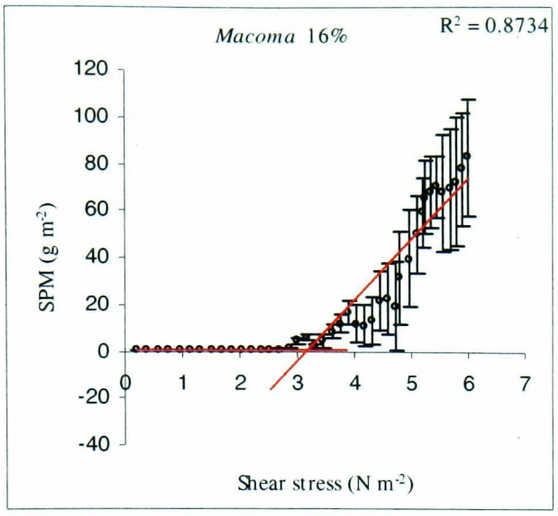


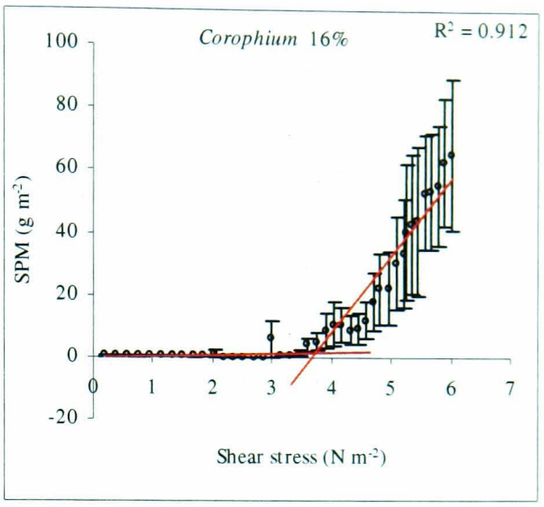


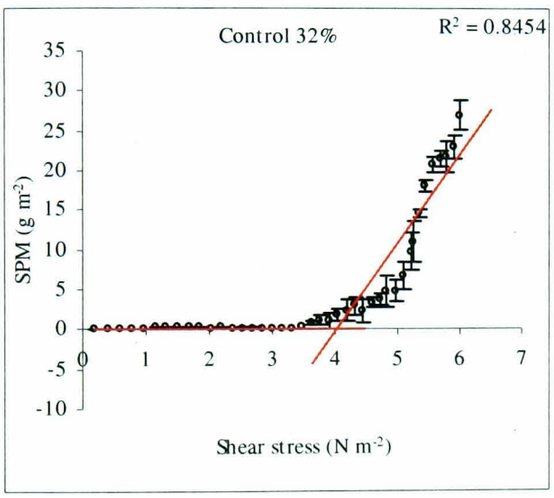


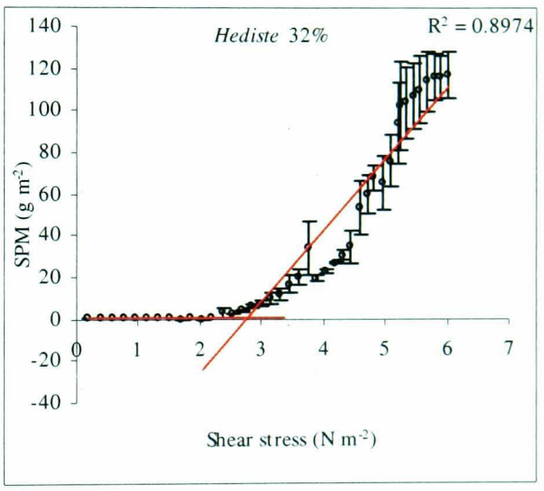


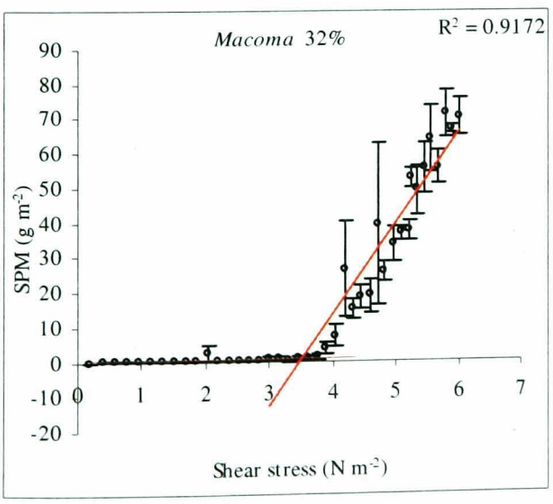



Macoma 16%
$R^2 = 0.8734$
SPM (g m^{-2})
Shear stress (N m^{-2})


Corophium 16%
$R^2 = 0.912$
SPM (g m^{-2})
Shear stress (N m^{-2})


Control 32%
$R^2 = 0.8454$
SPM (g m^{-2})
Shear stress (N m^{-2})


Hediste 32%
$R^2 = 0.8974$
SPM (g m^{-2})
Shear stress (N m^{-2})


Macoma 32%
$R^2 = 0.9172$
SPM (g m^{-2})
Shear stress (N m^{-2})

APPENDIX 12. Erosion rates of sediments exposed to different effluent concentrations (CSM laboratory measurements).