

***INTERACTIONS BETWEEN MARINE BENTHIC INVERTEBRATES
AND SEDIMENTS
IN INTERTIDAL AND DEEP SEA ENVIRONMENTS***

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COPY 1

***INTERACTIONS BETWEEN MARINE BENTHIC INVERTEBRATES
AND SEDIMENTS
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*I do not know what I may appear to the world,
but to myself I seem to have been only like a boy playing on the sea-shore,
and diverting myself in now and then
finding a smoother pebble
or a prettier shell than ordinary
whilst the great ocean of truth lay all undiscovered before me.*

Sir Isaac Newton 1642-1727.

*I dedicate this thesis to my Mother, Father and Brother.
You have each contributed a part to the whole which is me.
Your love and patience have been a strong foundation
through somewhat trying times.*

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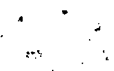
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ABSTRACT

Section I investigated the potential use of *Mytilus edulis* as an environmentally friendly method of protecting intertidal soft sedimentary coastal zones. The section comprises of four main experiments and one set of joint experiments conducted with BSc Honours students. Experiment 1 was a preliminary study of the attachment of *Mytilus edulis* on to sand (particle size $< 500\mu\text{m}$), gravel (particle size range between 4mm and 8mm) and a 1:1 mix of sand and gravel. The results showed that in general mussels on gravel produce more threads than mussels on bare sand. This also means that potentially mussels on sand with underlying gravel will be less likely to be washed away by currents as they will attach to underlying gravel thus anchoring themselves. Experiment 2 developed the findings of experiment 1. The experiment involved allowing *Mytilus edulis* to form clumps on gravel and sand substrates in the laboratory, along with mussels stored in buckets as an un-clumped condition. The mussels from the laboratory were then transported to an intertidal bay, Ardmore Bay in the Clyde Estuary. The results showed that fewer mussels were lost from gravel substrates on the shore than from sand substrates. The effectiveness of using *Mytilus edulis* as a way of protecting intertidal soft sedimentary coastal zones can be determined by the numbers of mussels remaining on top of the sediment. The more mussels covering the sediment surface will mean that less of the sediment's surface is exposed to currents that would cause erosion. The results would indicate that some kind of artificial substrate is required to allow mussels to remain *in situ* on a sandy intertidal coastal zone. Experiment 3 was designed to investigate a novel artificial substrate to allow mussels to be introduced to intertidal soft sedimentary environments. The chosen substrate was plastic mesh with fibres measuring 1mm and 2mm and a square aperture measuring 14mm x 14mm. The experiment was conducted in the laboratory to measure the attachment force of the mussels' byssus threads. The results showed that there was no difference in the attachment strength of mussels on to plastic netting or on to other mussels' shells. This suggests that the attachment onto plastic netting would be as secure as the attachments made in the large

mussel beds present along most coastlines in the intertidal zone. So this type of substrate could potentially be used to introduce mussels onto intertidal soft sedimentary environments in an attempt physically to reduce erosion. Experiment 4 developed the research of experiment 3. The plastic mesh was used in place of the gravel used in experiment 2. The results were similar to those found in experiment 2. Mussels that had been exposed to the plastic mesh substrate prior to being laid on to the field sand were less likely to be washed away by currents. The result shows that the method and the substrate used in this experiment have a potential to be developed into a large-scale method of environmentally friendly method of protecting intertidal soft sedimentary environments. The set of joint field experiments simply develops the work conducted in experiments 1 to 4 and investigates aspects not covered in experiments 1 to 4. Experimental set 1 investigated the attachments on and around isolated boulders within a 30m x 10m transect line at both the upper and lower intertidal on Ardmore Bay. There were three experiments conducted within this set. The experiments showed that in general there were more mussels lost from the lower intertidal site than the upper intertidal site. This highlights the need for a suitable substrate to allow mussels to withstand higher energy environments. Experimental set 2 investigated the behaviour of mussels laid on to sand substrate on the shore. The mussels were laid with no space between mussels, 1cm gap between mussels and pre-clumped. The results of the three experiments in the set showed differences to those of experiment 2 and 3. The results showed that mussels that had been allowed to form clumps in the laboratory lost fewer mussels than those simply placed on to the sand on the shore. Again, this serves to re-emphasise the need for a suitable artificial substrate to allow mussels to remain in high-energy environments.

Section II investigates the affects of an Oxygen minimum zone present off the coast of Oman in the Arabian Sea. The study focuses on the biological mixing, geochemistry and geotechnical properties of the sediments of the area. The parameters measured were Eh, pH, shear strength, biological mixing, water content, carbonate and total organic matter of the sediment samples.

Sediment samples from box cores taken at sites both within and below the OMZ were compared. The samples from within the OMZ showed low redox conditions and high carbonate. The geotechnical properties and biological mixing structures within the OMZ also differed to those outside the OMZ. The differences were related to the level of anoxic conditions and water depth. Samples from within the OMZ showed that Eh, pH and carbonate increased with water depth. The inverse was recorded for sediment water content. Heterogeneity was observed in the sediments of the OMZ just below the surface expressed as two and occasionally three different colours of sediment. The down core trends of Eh, water content and bioturbation decreased as sediment depth increased in OMZ sediment samples. The data for pH showed a slight increase in the top 5cm of sediment from sample sites both within and outside the OMZ. Shear strength in general increased with increasing sediment depth at all sample sites. Correlations at each water depth showed down core sediment trends for the various parameters. Correlations across the water depths showed significant correlations only at deeper water depths. The Eh/pH diagram illustrates a separation of 2 groups, 391m-1008m and 1265m-3396m which fits in well with the predicted range of the OMZ. Cluster analysis showed that the upper and lower sediment depths from separate clusters broke between 4cm to 7.5cm. Shallow water depth samples formed distinctly different clusters to those of deeper water depths. Bottom-water oxygen concentrations taken on board RRS Discovery were also taken into consideration in the correlation analysis.

Section III investigates the potential of marine invertebrate mucus to enhance uptake of radionuclides into sediments. The experiment compared the uptake of Americium (Am^{241}) and Cesium (Cs^{137}) into three experimental substrates. The experimental sediments were natural sand, natural sand with organic matter removed and 1mm glass beads. The results showed that the condition with the natural sand with organic matter removed and mucus added had higher Americium activity than the same sediment without mucus. The experimental substrate of glass beads and mucus showed higher Americium activity after 21 days than the other experimental

sediments. This suggests that mucus is instrumental in the mechanism for radionuclide uptake into sedimentary environments.

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GENERAL INTRODUCTION

This thesis will attempt to investigate three facets of the interaction between marine benthic invertebrates and the sedimentary environments that they inhabit. The sedimentary environments of the continental shelf of both the North Sea and Arabian Sea investigated in this thesis are all terrigenous in origin (Emery 1968). The sediments of these environments can be categorised as either relic or modern (Emery 1968). Relic sediments were formed during the Ice Ages and modern sediments are formed at the present time by erosion and deposition by the action of wind, ice and water.

Emery describes mechanism of the formation of detrital sediments which contribute to the formation of the continental shelves of continents. The mountains of the world's continents are slowly eroded over time by the action of ice freezing and thawing and rainfall (Holmes 1944). The pieces of rock are then transported down rivers being constantly abraded by each other creating ever-smaller pieces (Hingston 1920). Hingston (1920) effectively describes the process of erosion in the following quote. "Heat and cold, rain and river, frost and ice are at work on every side eroding, levelling and sweeping down the mountains. The valleys that are filling and the gorges that are deepening both foretell the same destiny; they predict the building of a plateau of erosion, the lowering of the mountains to the level of the sea." The rivers then flow into the flood plains where some sediment is deposited over time. The river then continues through the flood plain to the coastal zone where large deposits of sediment are accumulated. The fresh water entering the marine environment is less dense and so flows out depositing sediment further out. As the fresh water interacts with the sea water the salt causes flocculation as suspended clay particles adhere to each other and so settle out faster (McLusky 1989). The organic matter introduced by the river discharge is also incorporated into the sediments of the estuary.

The sea itself erodes the coastal zone by the action of tides and waves thus adding to the sediment input of the coastal zone and the continental shelf (Holmes 1944). Typical examples of tidal erosion are the east and south coasts of England, specifically the Norfolk and Kent coastlines.

Periodically sediment is lost from the continental shelves to the abyssal plane via turbidity currents triggered by seismic activity. The turbidity currents flow down channels in the continental shelf which are found mostly where large rivers discharge into the sea (Meadows & Campbell 1988).

All of the marine sedimentary environments support a diverse group of organisms, which live on or within the sediment. The intertidal sedimentary environment of the coastal zone is of particular interest. Not only are these areas of socio-economic importance but they also exhibit a wide range of biodiversity (Beanland 1940, Anderson & Meadows 1969, Beukema 1976, Anderson & Meadows 1978, Reise 1985, Meadows *et al.*, 1998). Intertidal coastal zones contain two of the facets of interaction between sedimentary environments and marine benthic invertebrates that this thesis will focus on.

The first facet of this interaction that I address in my thesis concerns the projected rise in global sea levels in the 21st century that poses a potential threat to intertidal environments. Global warming is the main mechanism by which sea level rise may be induced. Global warming is a natural process of the biosphere however increased levels of carbon dioxide created by man may compound the effects of global warming. The coastal zones of the world are constantly under attack by the action of the sea. This has prompted the intervention by man in many countries around the globe. Man has traditionally used engineering methods to combat the erosion of the coastal zone. Some of the engineering structures take the form of artificial rock or concrete reefs laid offshore to dissipate the force of the tide and waves (US Army corps of engineers 1984 I & II, Harlow 1990, Silvester 1990, Kelletat 1992, DeRond 1996, Simm 1996, Youdeowei & Abam 1997). Engineering protection methods on shore take the form of groins and sea walls. Sea walls prevent the edge of the land being eroded further and protect coastal settlements (US Army corps of engineers 1984 I & II, Harlow 1990). Groins are constructions erected on the sediment of the shore positioned at an angle to onshore currents to prevent sediment transport (Kelletat 1992).

All of the engineering methods reported thus far are heavy engineering methods requiring large-scale constructions. A second type of engineering method used to protect coastal zones involves the use of man-made membranes. These membranes, formed from plastic, have been used

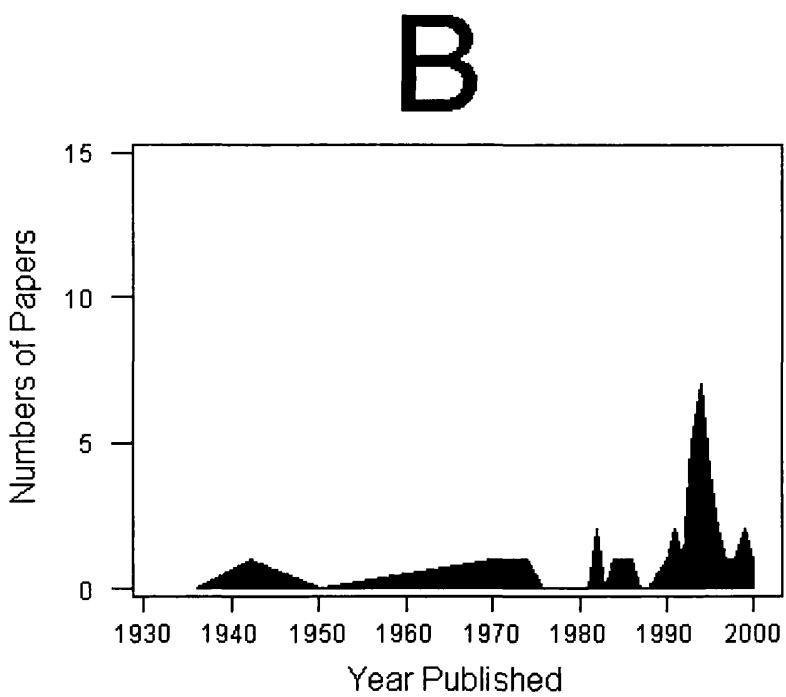
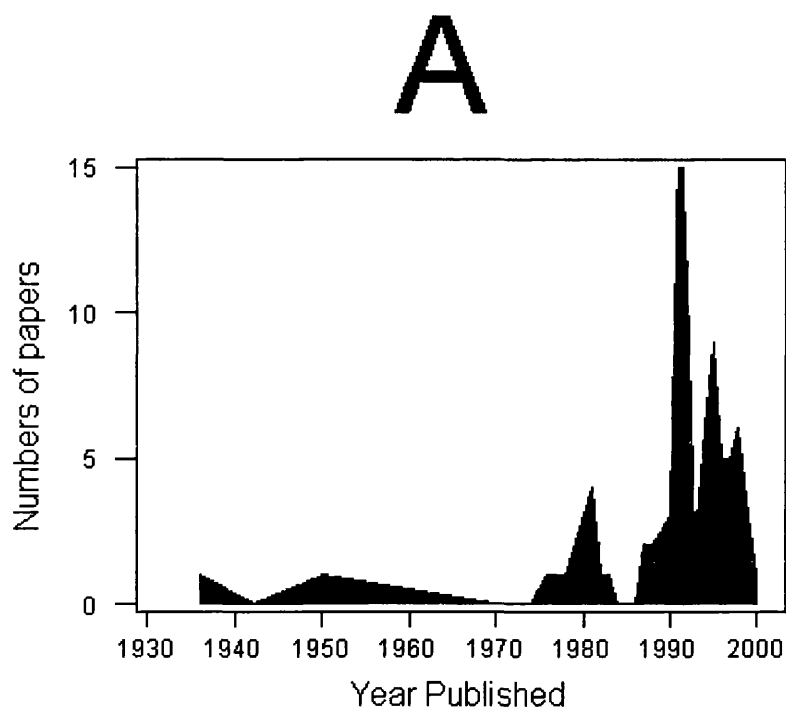
extensively in the terrestrial environment to reinforce and protect soils (Rankilor 1981, Benson & Khire 1994, Fishman & Pal 1994). Membranes have also been developed from plastic membranes and applied to coastal zone protection (Rankilor 1981). These membranes come in various forms of mesh sizes and mesh thickness. The environment to be protected dictates the type of mesh to be used. The membrane is commonly used in conjunction with other materials to provide a strong and long lasting defence mechanism. An example of this would be a heavy armouring material such as large rocks. The membrane would provide a stable base and a filtering mechanism to allow water exchange in the sediment. The hard engineering methods can also be combined with membranes during construction to give permeable separation from the substratum (Rankilor 1981).

Natural methods of coastal protection take many forms plants in particular have been used successfully in some areas to protect coastlines. Some examples of this method can be seen in tropical countries where mangrove swamps are actively cultivated and maintained (Bennett & Reynolds 1993, Weinstock 1994). These mangrove plantations can reduce the wave energy impinging upon the shore. This means that the suspended sediment will no longer be able to be carried by the waves and is deposited amongst the tangled roots of the mangrove trees. There has also been much interest in the microorganisms present in sediment (Anderson & Meadows 1969, Aspras *et al.*, 1971, Pearl 1975, Anderson & Meadows 1978, Muir Wood *et al.*, 1990, Meadows *et al.*, 1994a, Meadows *et al.*, 1994b). The microorganisms such as bacteria, diatoms and blue green algae inhabit the interstitial spaces between the sand grains in sediments. Many of these microorganisms produce mucus. This mucus appears to aid in movement of some groups (diatoms) while providing a means of attachment to surfaces for others (bacteria, blue green algae). The mucus can also act as a weak bonding agent between particles thus increasing the strength of the sediment (Dade *et al.*, 1990). However, despite the research conducted on biological methods of coastal zone protection, little research is focused on the potential of macrobenthic invertebrates for protecting coastal zone environments (Meadows *et al* 1998). In addition, Figure 1 illustrates the clear difference in the activity of research into developing engineering methods compared to the activity of developing environmentally friendly biological methods of coastal zone protection.

Mytilus edulis is an intertidal bivalve mollusc with an extensive range in both northern and southern hemispheres (Suchanek 1978). *M.edulis* is found on both rocky shore environments and soft bottom intertidal environments associated with rock outcrops and isolated boulders. These mussels have also been observed attached to small stones underlying the surface on muddy sand beaches at Ardmore Bay Clyde Estuary and Fairlie Sands Largs (personal observation). *M.edulis* forms attachments to surfaces by means of structures called byssus threads. When in large numbers the aggregates of mussels form a biological armouring surface over the substratum. These aggregations, or mussel beds as they are termed, are a well-known phenomenon, which along with byssus threads have received much attention leading to a number of research investigations (Pujol 1967, Glaus 1968, Van Winkle 1970, Tamarin & Keller 1972, Brown 1975, Allen *et al.*, 1976, Smeathers & Vincent 1979, Price 1980, 1981, 1983, Waite 1983, Young 1983, Witman & Suchanek 1984, Young 1985, Meadows & Shand 1989, Lee *et al.*, 1990, Shand 1991, Dolmer & Svane 1994).

The first section of this thesis attempts to investigate the effectiveness of *Mytilus edulis* as a biological method of coastal zone protection. Biological methods of protecting coastal zones are potentially more flexible and environmentally friendly than engineering methods. When an engineering structure is built, changing it in any way can be very costly. Man-made membranes can also experience degradation in some environments. For example, acid or other inorganic compounds in soils and anoxic sediments can affect plastic membranes (Rankilor 1981). These membranes may also be prone to physical attack by microorganisms (Rankilor 1981). The only course of action here is to replace the membrane, which could mean moving tons of rock. Biological methods of coastal zone protection may also experience attack from chemical and biological compounds, though biological methods can be more resilient.

Figure 1: Plots of numbers of papers published per year for coastal zone protection by engineering methods (A) and biological methods (B).



Biological methods are self-repairing and self-maintaining which will reduce the effect of the attack from compounds and microorganisms.

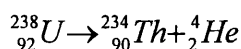
Generally, coastal zone protection methods are implemented on the higher energy area of the shore. It is clear that experimentation is required to reveal any strengths or weaknesses in using mussels to protect coastal zone environments and to this end, various exposure experiments are presented in the first section of the thesis. The number of mussels on different types of substratum remaining from each experiment on the shore after a number of tidal cycles was taken as an indication of the protection provided. If a significant number of mussels were left remaining *in situ* then they will afford physical armouring protection to the sediment beneath them. The mussel aggregations may also encourage sedimentation and will somewhat reduce the force of the waves and currents as they travel further up the shore.

The second facet of interaction that I address in my thesis is concerned with the growing problem of marine pollution in intertidal environments (Kautsky 1988, Goldberg & Bertine 2000). There are many forms of marine pollution with chemical, organic waste and radioactive being the main groups. Within the three main groups, pollutants can be further classified as continuous or episodic. On occasion, there are positive effects arising from pollution. For example, the introduction of sewerage into near shore ecosystems can provide increased nutrients. This however has the detrimental effect of introducing bacteria that may be harmful to many higher organisms by entering the food chain. This illustrates that pollution of any kind must be carefully regulated, monitored and potential threats identified.

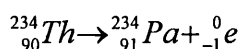
The advent of nuclear technology provided superior weapons which if used as a deterrent could prevent wars, also a vast fuel source to supply the increasing requirements for energy. The major drawback of nuclear technology is the waste that is produced, on a secondary level the accidental release of nuclear radiation is also of concern. Radioactive elements occupy a significant part of the periodic table and as such these elements vary considerably in form and nature of reaction with other elements and surfaces. A number of these elements are naturally occurring though some are

derived through the nuclear decay process. This process is where a nuclear isotope loses energy in the form of radioactive emission. There are three main types of emission, alpha, beta and gamma. Alpha decay involves the radionuclide losing a helium atom thus the atomic mass number is reduced by four and the mass number is reduced by two. Beta decay involves the release of an electron with atomic number zero and mass number minus one. This means that the radionuclide will appear to gain a proton as the mass number increases by one. The equation is balanced out by the electron's mass number of minus one. Gamma decay involves radiation emission with no mass or charge so the radionuclide remains the same.

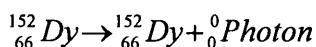
Alpha (α) decay



Beta (β) decay



Gamma (γ) decay



Every time an isotope emits an alpha or beta “particle”, the number of atomic particles is altered for that isotope. This means that in effect the isotope has been transformed into a completely different isotope. This reaction is directly related to the isotope half life, which is the time required for half of the atoms of a sample of the isotope to decay. The longer the half-life of an isotope, the greater the threat to the environment.

Radionuclides enter the sedimentary environment via the sediment water interface. The mechanism by which these radionuclides cross this interface, and the residence times that they remain bound in the sediment, differs between radionuclides. There have been extensive studies of radioactive pollution in the North Sea. The major input of radioactivity into the North Sea comes from the Sellafield Nuclear Reprocessing Plant, Cumbria, UK. Within this input two of the radionuclides

present are Americium ²⁴¹ and Caesium ¹³⁷. These two radionuclides behave very differently with respect to uptake and residence times in sediments. Americium tends to react strongly with particles of organic matter, so is lost from suspension very quickly, and then remains in the sediment for a long time (Kershaw *et al.* 1986). Caesium is less attracted to organic particles and can experience re-solution from the sediment back into the water column above (Evans *et al.* 1983, Santschi *et al.* 1983, Sholkovitz *et al.* 1983, Sholkovitz & Mann 1984).

The amount of organic matter contained in sedimentary environments is controlled by a number of factors. One of the major factors is the presence of benthic invertebrates on or within the sediment. These organisms produce sticky mucus secretions, which are used for various functions a number of these include feeding, ingestion, excretion, burrowing and tube construction. Mucus is a sticky organic substance and so may attract many different types of pollutants. There has been much research conducted on the uptake of heavy metal pollutants by marine invertebrates. This research has identified that areas of the invertebrate organisms bodies associated with mucus experience greater uptake. Specific work conducted by Howell (1982) on marine nematode worms shows the potential of excreted mucus for the uptake of radionuclides. Potentially there is a vast amount of mucus present in marine sediments. If polluting substances come into contact with this mucus within the sediment then the pollutant may be adsorbed onto the mucus. This could pose a potential threat if the pollutants build in concentration over time in the confinement of the sediments. The Organisms within the sediment would experience detrimental effects of the high pollutant concentration, thus interfering with the food chains of many ecosystems. Section III of my thesis attempts to ascertain whether mucus produced by a polychaete *Hediste* (= *Nereis*) *diversicolor* experiences uptake of radionuclides. *Hediste diversicolor* was chosen for the copious amounts of mucus that it secretes. Artificial sediment (glass beads) were compared with natural sediments to determine the role of mucus in the uptake of radionuclides.

The continental slopes of the world's oceans display the third facet of interaction that I address in my thesis between marine benthic invertebrates and sedimentary environments. These areas form an intermediary environment between nutrient rich intertidal environments and low nutrient

abyssal environments. In certain areas of continental slopes with suitable conditions, areas of low oxygen form within the water column and impinge on the continental slope itself. Oxygen minimum zones (OMZ) occur in the eastern tropical Pacific, eastern Atlantic, Indian Ocean and Arabian Sea (Wyrski 1962, Kamykowski & Zentara, Olson et al. 1993).

The Arabian Sea is of particular interest as it is essentially landlocked on three sides. The mixing of the Arabian Sea is directly affected by the monsoons that occurs in the Indian Ocean. The Indian Ocean experiences two very different monsoon currents. The winter monsoonal current appears to be shallow and relatively gentle where as the summer monsoon current is stronger and deeper causing upwelling of bottom water (Currie et al 1973, Wyrski 1973, Bruce 1974,). The upwelling of the deep nutrient rich water will fuel phytoplankton blooms in the surface waters. The phytoplankton in turn will feed many other organisms higher in the food chain. When the nutrients are used up from the surface waters the phytoplankton begin to die off and descend due to lack of sufficient nutrient levels. The decomposition of phytoplankton by bacterial activity in the water column may give rise to a reducing environment thus lowering oxygen levels in the water. This effect may be compounded by the influx of organic matter from the Indus River, which will be decomposed by microorganisms utilising oxygen (Meadows & Meadows 1999).

The organisms inhabiting sediments that the OMZ impinges on may be significantly affected by the low oxygen conditions. The interstitial water present in the sediment may well experience a depletion of oxygen thus making the sediment anaerobic. This would restrict and determine the species and diversity of organisms resident in the area. Organisms living in this environment would have to adapt to lower levels of oxygen by developing highly effective methods of oxygen extraction, have lower metabolisms and be able to use anaerobic metabolic pathways to maximise efficiency (Childress & Seibel 1998). There has been much work conducted on the interactions between benthic invertebrates and sediments of shallow continental slopes to abyssal environments (Rhodes, 1963; Rhodes et al., 1978; Aller, 1982; Anderson & Meadows, 1978; Meadows & Tait, 1989; Meadows & Meadows, 1991; Jones & Jago, 1993; Meadows & Meadows 1994; Meadows et al., 1994b). However, little of this research appears to have been applied to dysaerobic slopes that

may be present in OMZ environments. Section II of my thesis attempts an investigation of the interactions of benthic invertebrates and sediments of the Oman continental slope of the Arabian Sea. Primary indicators of biological activity in the sediment will be the presence of bioturbation and burrow patterns.

SECTION : I

**Investigation of the use of *Mytilus edulis* as an environmentally friendly
method of coastal zone protection**

1.1 INTRODUCTION

1.1.1 Intertidal sediment stabilization

Intertidal sedimentary environments in the coastal zone are of considerable interest, as they exhibit a wide range of biodiversity (Beanland 1940, Anderson & Meadows 1969, Beukema 1976, Anderson & Meadows 1978, Reise 1985, Meadows *et al.*, 1998). The current trend of global warming poses a great potential threat to these environments. The increase of carbon dioxide in the atmosphere will affect the polar ice sheets causing increased melting and raise global sea levels in the 21st century (Hoffman 1984, Jelgersma & Tooley 1992, Shennan 1992, Bird 1996, Raper *et al.*, 1996, Saizar 1997, Olivo 1997). There has therefore been growing interest in coastal zone protection by environmental analyses and effective management (Pilkey 1991). Engineering methods are commonly used to protect these environments. For example, groins, artificial concrete or rock reefs and floating barriers are all employed to dissipate the force of the water before it reaches the shoreline (US Army corps of engineers 1984 a & b, Harlow 1990, Silvester 1990, Kelletat 1992, DeRond 1996, Simm 1996, Youdeowei & Abam 1997). Some engineering methods have been adapted from the terrestrial environment. For example, geomembranes have been routinely used to stabilise slopes on the sides of roadways and in controlling mining subsidence (Kraebel 1936, Bowers 1950). Geomembranes come in many forms however they are mostly constructed from a mesh of plastic fibres as shown in Figure 2. An adaptation of this method in the marine environment would be the use of sand-cement or sand-gravel filled bags and rubber tyre networks, to protect coastal areas (Greene 1978, Youdeowei & Abam 1997). Geomembranes have also been developed to directly dissipate the force of waves breaking on shores (Rankilor 1981). Figure 3 shows the Paraweb synthetic seaweed mat which acts in a similar way to *Laminaria digitata* in dissipating waves before they break on the shore and also caused suspended sand to be deposited.

Figure 2: Scanning electron photomicrograph of a thin permeable membrane demonstrating melded structure and variable pore size. (Courtesy ICI Fibres.) from Rankilor 1981.

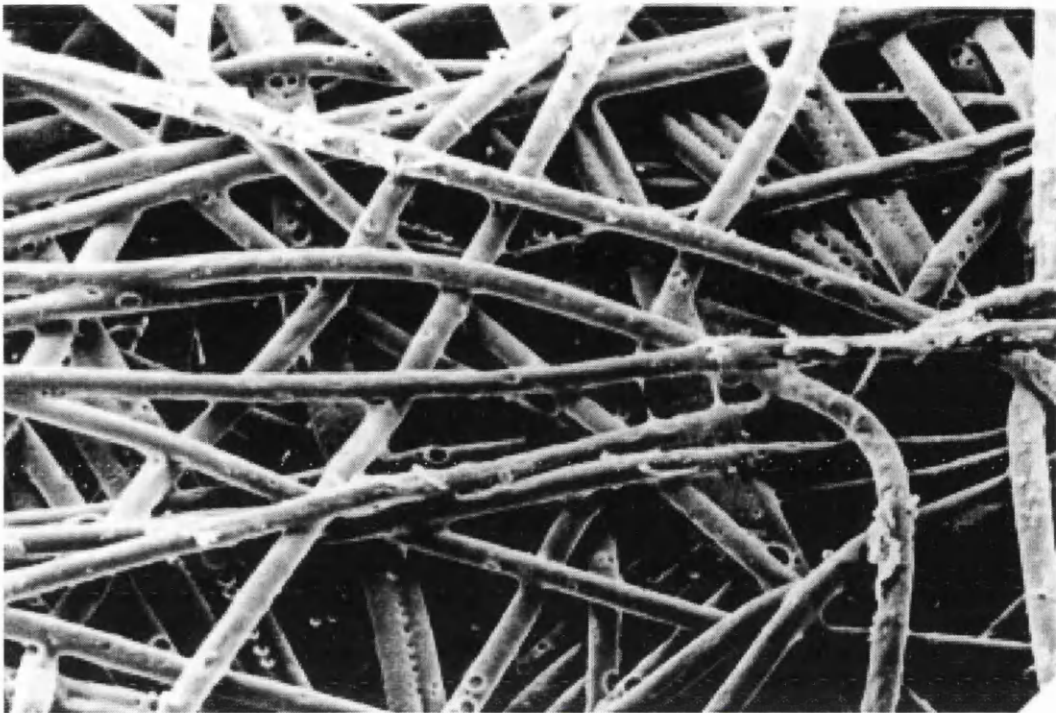
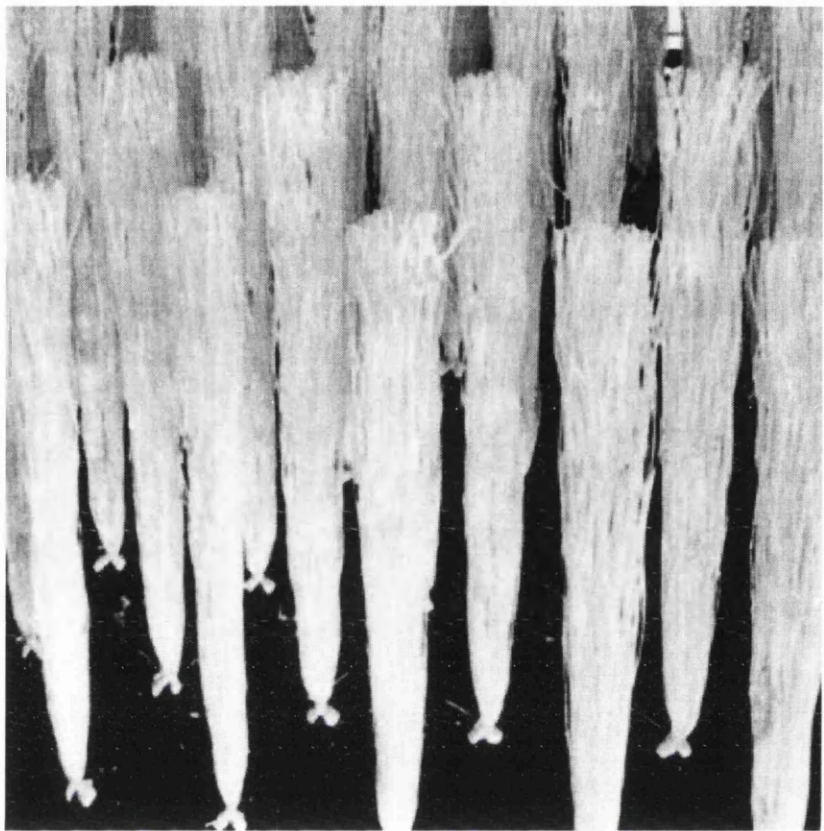


Figure 3: Paraweb synthetic seaweed mat used to dissipate wave forces and cause suspended sediment to settle out and deposit on the sea bed. (Rankilor 1981).



There has recently been a move towards developing more environmentally friendly methods of protecting intertidal coastal zones. The utilisation of biological organisms to protect coastal zones is one such method. These methods concentrate predominantly in tropical countries, due to the great socio-economic pressures placed on coastal zones in these areas. In many areas the cultivation and plantation of mangrove trees is seen as a possible biological method of protection. These plantations have other functions along with protecting the coastal zone. They support local fishing industries as the raised root system provides a naturally safe environment for the young fish fry (Bennett & Reynolds 1993). They are also responsible for providing wood for local and commercial forestry and provide income by being a considerably important tourist attraction (Bennett & Reynolds 1993, Weinstock 1994).

To apply biological methods of coastal zone protection to more temperate areas indigenous organisms need to be considered.

Most organisms in intertidal soft substrate environments are endobenthic, living beneath the sediment surface showing little trace of their existence. Figures 4 and 5 show the mucus meshes produced by microorganisms and microalgae, which bind sediment particles in a similar way to the geomembranes, utilised in engineering sediment strengthening methods. The micro-organisms that inhabit the interstitial spaces between sediment grains produce mucus secretions these have been studied with reference to strengthening sediments (Aspras *et al.*, 1971, Pearl, 1975, Muir Wood *et al.*, 1990, Meadows *et al.*, 1994a, Meadows *et al.*, 1994b). There has also been work conducted on organisms that mechanically strengthen sediments (Holland *et al.*, 1974, Meadows & Tufail 1986, Meadows & Tait 1989, Paterson *et al.* 1990, Meadows *et al.*, 1990, Christian *et al.*, 1991, Jones & Jago 1993, Underwood & Paterson 1993, Grant & Daborn 1994, Shaikh *et al.*, 1998). However, contradictory work by Eckman *et al.*, (1981) shows sediment destabilisation by animal tube constructions.

Figure 4: Sediment particles bound together by the mucus secretions of *Hediste diversicolor*. Scale bar represents 100µm. (Meadows *et al.* 1990).

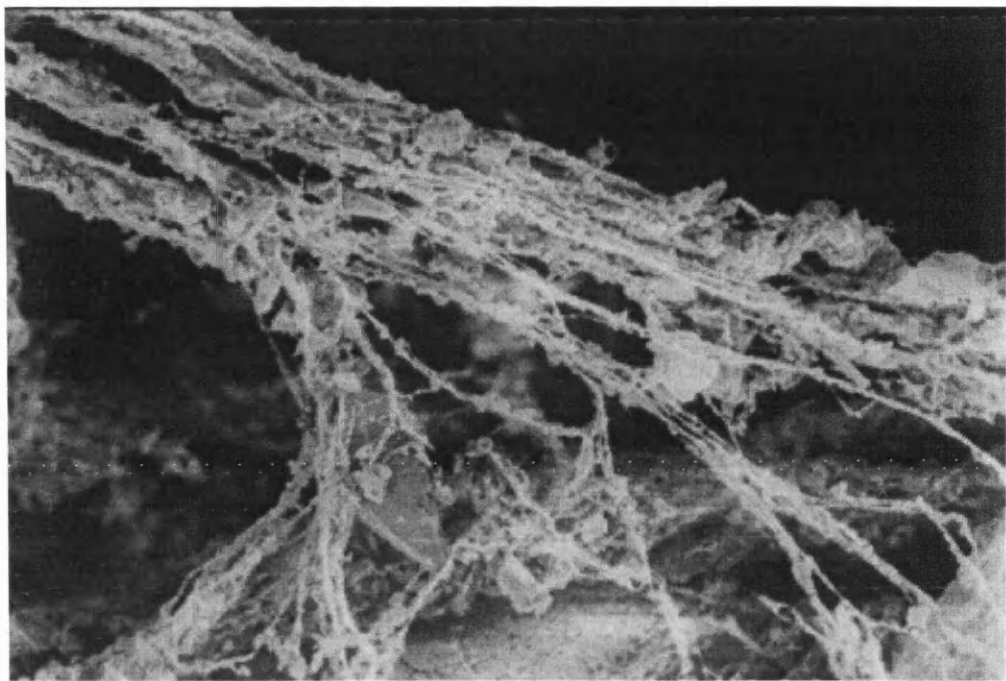
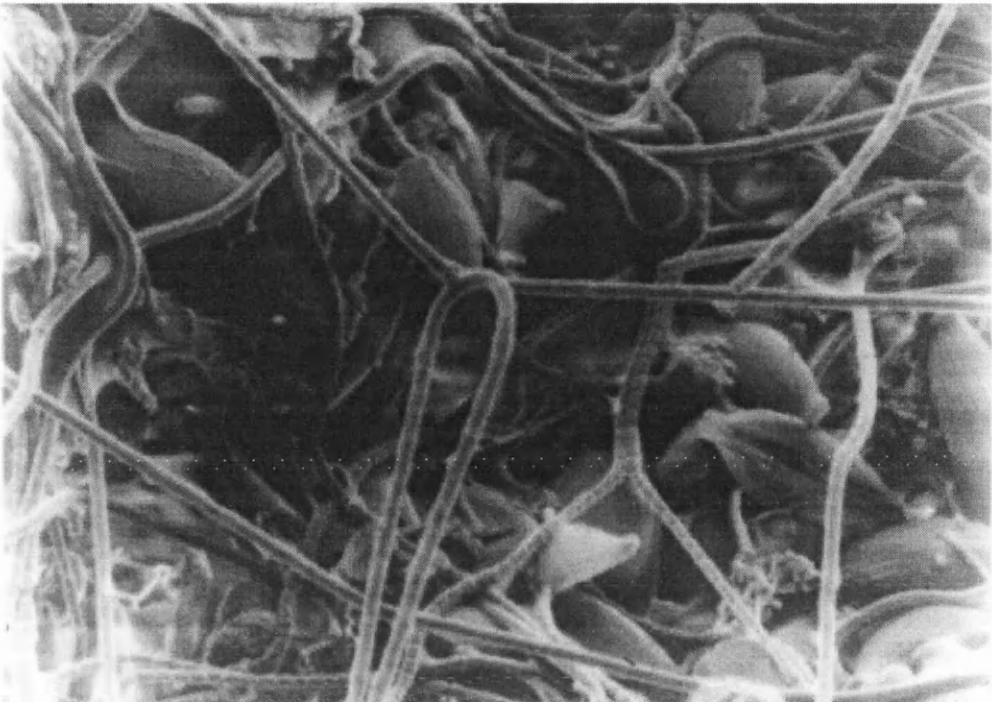


Figure 5: Sediment particles with blue-green algae and diatoms in the sediment interstices.
Scale bar = 50µm; (Tufail 1985)



Other organisms also provide protection to the sediment by physically armouring the sediment from the action of waves and currents. One such organism is the bivalve mollusc *Mytilus edulis*. Mussels produce threadlike secretions to form attachments to surfaces allowing the organism to feed and not be swept away. There have been a number of studies on the production of the threadlike byssus secretions of *Mytilus edulis* (Pujol 1967, Tamarin & Keller 1972, Brown 1975, Price 1983, Young 1985, Lee *et al.*, 1990). The animals attach to the substratum and to their own shells forming a biologically woven-anchored mat. These beds of mussels may act to physically shield sediment from the erosional forces of the sea. The beds, however, can occasionally be destroyed by heavy storms or excessively strong currents. This has resulted in considerable interest in the attachment strength of byssus threads, factors affecting their production and the influence of water currents on their attachment (Glaus 1968, Van Winkle 1970, Allen *et al.*, 1976, Smeathers & Vincent 1979, Price 1980 1981, Waite 1983, Young 1983, Witman & Suchanek 1978, Meadows & Shand 1989, Shand 1991, Dolmer & Svane 1994). However, there appear to be few studies linking mussel attachment on the shore with coastal zone protection (Meadows *et al* 1998).

Mussel beds of *Mytilus edulis* can be found on most coastlines in temperate zones of the northern and southern hemispheres (Suchanek 1978). The research reported in this section was developed as a result of observations made of isolated natural mussel beds measuring 0.5m^2 to 1m^2 in diameter. The mussel beds were observed on an intertidal bay, Ardmore Bay in the Clyde Estuary, Scotland. The opening of the bay faces west into the prevailing winds. There is a clear distinction between the low energy upper intertidal and the high energy lower intertidal. This is indicated firstly by the relatively bare surface sediment of the lower intertidal compared to the sediment surface in the upper intertidal which is interspersed with small boulders. Secondly, when the tide is in, distinctive white capped waves can be seen at the lower intertidal and are not present at the upper intertidal. The general objective of this research was to develop a novel environmentally friendly method of protecting soft intertidal sedimentary environments. A series of laboratory and field experiments was designed to investigate the attachment of *Mytilus edulis* onto various artificial substrata. To protect sediments effectively a significant number of mussels must remain *in situ* to physically

shield the sediment surface from the energy of the waves and currents. The experiments conducted in the field on Ardmore Bay were designed specifically to investigate this.

A significant part of this section has been published in the Geological Society Special Publications (Meadows *et al*, 1998).

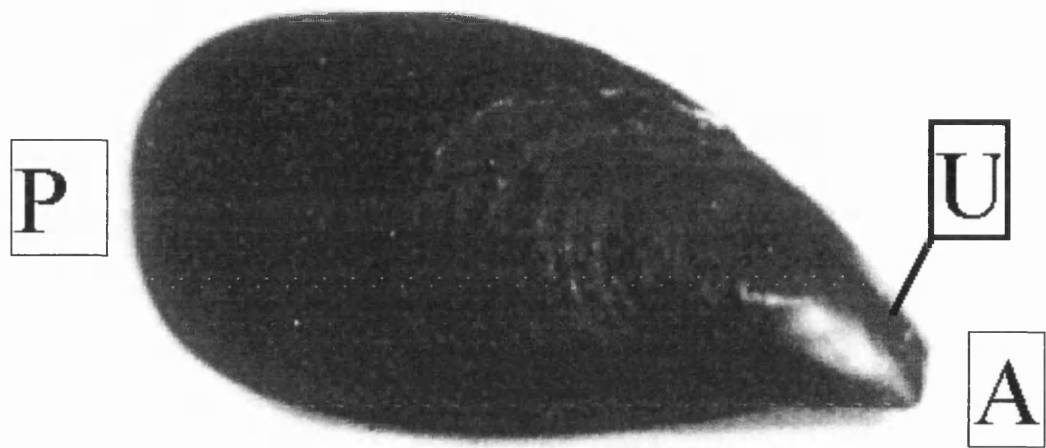
1.1.2 Natural history of *Mytilus edulis*

Mytilus edulis is a semi-sessile benthic invertebrate mollusc, in other words it mainly remains in one position but can move. The range of *Mytilus* is extensive in the Northern and Southern Hemispheres. This distribution is more widespread in the Northern Hemisphere with extremes of temperature being the main controlling factor (Seed 1976).

Mytilus edulis belongs to the family Mytilidae, sub-order Mytilacea of the order Filibranchia, phylum Mollusca. The external morphology consists of a hinged pair of calcareous shells. The shells are hinged by a ligament of elastic protein at the anterior end of the mussel with the umbo or apex located at the extremity of the shells. Figure 6 shows the external morphology from a lateral view of one shell valve indicating orientation and the umbo. The shell is formed and maintained by the mantle layer of the mussel's body. The mantle also provides protection from the potentially abrasive sand particles that on occasion enter the shell cavity without being filtered by the gills. The mantle coats the sand particle with successive layers of calcareous shell material; this eventually becomes spherical in shape forming a pearl. This is also a common phenomenon in other bivalve species such as *Ostrea edule*.

On the shore, *Mytilus* is found in littoral and sub-littoral zones. The species is commonly associated with rocky shore environments though can be found on shingle, mud and sand shores providing there is suitable attachment substrata. On the latter shores mussels are known to develop large beds covering the sediment surface (Field 1922, White 1937, Kuenen 1942, Maas Gesteranus 1942, Verwey 1952, Havinga 1956, Seed 1976). Any organism living in an intertidal environment will experience stresses imposed by that environment. Exposure is a stress factor of any intertidal shoreline. Exposure is essentially the time that organisms are exposed to the air. During this time intertidal organisms cannot feed or respire and must wait until the tide turns and covers the shore again. The period of exposure varies over the year due to the gravitational effect of the moon and sun on the tidal ranges. There are two high tides and two low tides on an intertidal shore per 24-hour period. There are two main types of tides corresponding to

Figure 6: Lateral view of the left valve of *Mytilus edulis* shell indicating orientation and umbo located at the hinge of the shell valves. A = Anterior. P = Posterior. U = Umbo.



the relative positions of the sun and moon. Spring tides occur when both the moon and the sun's gravitational pull act in the same direction. These tides exhibit large amplitude with very high tides and very low tides. Neap tides occur when the sun's gravitational force acts at right angles to the moon's gravitational force. Neap tides are smaller in amplitude as the forces producing them are much weaker. Both spring and neap tides occur twice in the 28-day lunar cycle. During the equinoxes when the sun is directly over the equator in March and September, the amplitude of the spring tides is increased. Figure 7 shows the range of tides over a tidal cycle that will act on intertidal environments. Table 1 shows the abbreviations used for the tidal ranges.

The upper range of *Mytilus edulis* in the intertidal environment is reported to be just above MHWS on gentle sloping shores and can exceed the EHWS on steep exposed Atlantic slopes (Lewis 1964). The lower limit of *Mytilus* is controlled predominantly by predation from crabs, starfish and dogwhelks along with various species of sea birds (Lewis 1964). *Mytilus* is highly adapted to survive the stresses posed by intertidal environments (Bayne *et al.* 1976). This species is also eurythermal being able to tolerate a wide range of temperatures. In summer when the tide is low, the mussels will be exposed to both the air and the heating action of the sun. In winter temperatures may drop below freezing. This means that the water in the body cavity and body of the mussel will also experience freezing. The minimum temperature tolerated by *Mytilus* is reported to be -10°C with less than 64% of the body water frozen as ice beyond this conditions are terminal (Williams 1970). In the summer the clumps and beds that *Mytilus* form may serve some protection from heat and desiccation. The aggregations of mussels will retain some water after the tide recedes and so will raise the local humidity thus affording some protection to the group.

Figure 7: Tidal ranges experienced on an intertidal environment (Meadows & Campbell 1988)

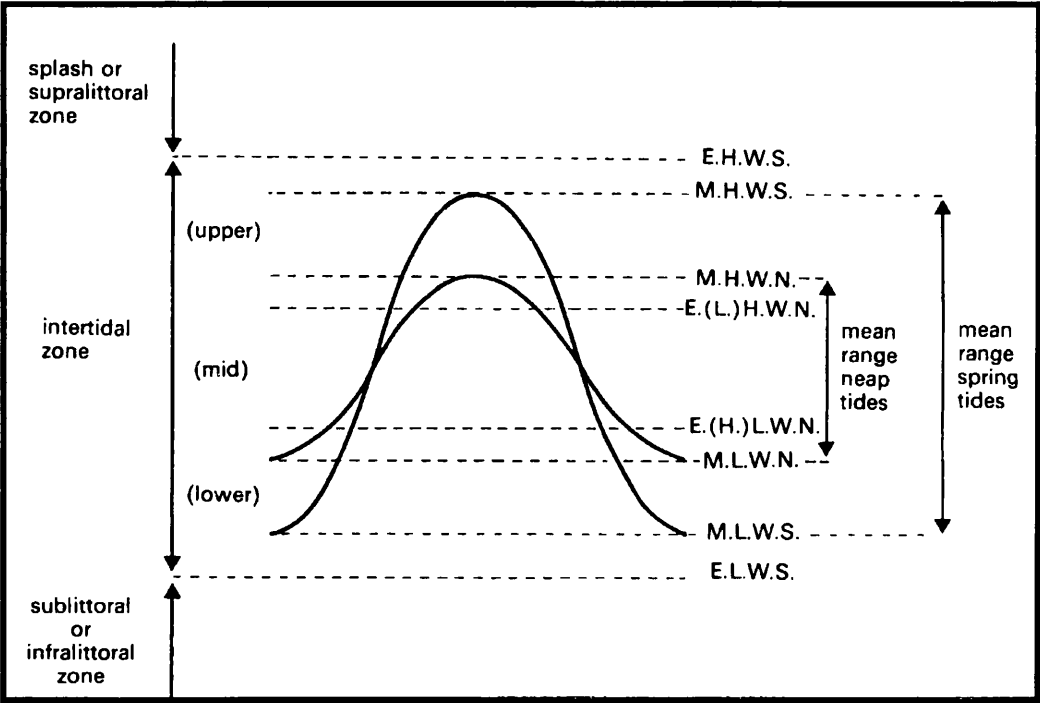


Table 1 Abbreviations used for the tidal ranges

| ABBREVIATION | EXPLANATION |
|--------------|--|
| MHWS | Mean High Water Spring |
| MLWS | Mean Low Water Spring |
| EHWS | Extreme High Water Spring (during equinox) |
| ELWS | Extreme Low Water Spring (during equinox |
| MHWN | Mean high water Neap |
| MLWN | Mean Low Water Neap |
| CD | Chart datum level of zero for all measurements on naval charts |
| | |

Mytilus edulis is a euryhaline species as they thrive in many intertidal environments from fully marine shores to brackish estuaries and salt marshes (Smith 1995a & 1995b, Smith 1956). The extracellular haemolymph and pericardial fluids are known to be isosmotic, with osmotic pressures equalling that of seawater. The mussels close their shell valves during osmotic stress thus affording a short period of protection. If the period of exposure to osmotic stress is great then the mussel will have to open the valves for oxygen and food. When this occurs, the mussel relies on intracellular mechanisms for osmoregulation. These mechanisms involve high concentrations of organic compounds in the cells with a significantly lower concentration in the extracellular fluids.

1.1.2.1 Circulatory system

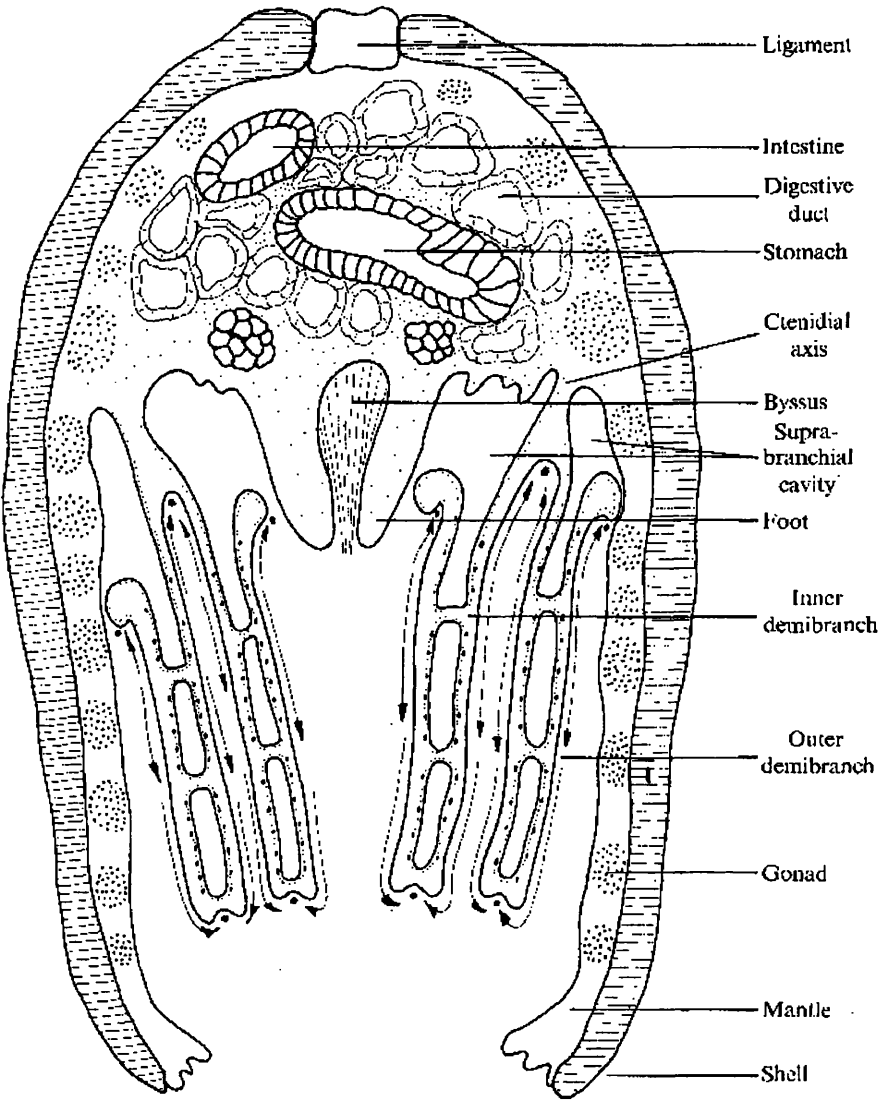
The circulatory system of *Mytilus edulis* is open, with the blood or haemolymph flowing from the heart through the blood vessels to diffuse spaces among the cells known as the haemocoel. The heart is dorsal comprising of three chambers, a median ventricle with two lateral auricles. The heart resides in a thin pericardium membrane situated dorsally. There are two aortae present. These are the dorsal anterior aorta and the posterior ventral aorta. The posterior aorta delivers haemolymph to the posterior of the mantle and the posterior adductor muscle. The anterior aorta delivers haemolymph to the rest of the mussel's body. The posterior aorta has a valve present at its root to prevent haemolymph being driven backwards by the sudden contraction of the foot. The haemolymph is carried back to the heart via a system of veins. There are three main venous sinuses, pallial, pedal and the median ventral sinus. The haemolymph is transported first to the kidneys, then to the gills and finally to the heart. The haemolymph of *Mytilus* has no respiratory pigment present and therefore has a low carrying capacity for oxygen.

1.1.2.2 Gills and feeding mechanism

Mytilus has a pair of gills, which hang in the mantle cavity. Plankton and organic compounds are captured by the gills as water is taken in and filtered through the gills. The water is moved through the gills by the action of the lateral cilia of the gills. The pattern of circulating water inside the body

cavity of *M.edulis* is shown by Figure 8. The gills are formed from two demibranchs on each gill. In turn, each demibranch is comprised of two lamellae, which is the flat surface of the gill formed by the sum of all the filaments on the gill surface. In cross section, this forms a W shape (Figure 8). As water passes over the gills food particles adhere to mucus strings on the lamellae. The particles attached to the mucus are conveyed via the latero-frontal cilia to the frontal cilia. From the frontal cilia, the mucus-bound particles are passed to the marginal groove. Coarse material is passed to the edges of the groove whereas fine particles are directed deep into the groove. The marginal groove conveys the mucus-bound particles to the mouth via ciliated palps. The palps also sort the particles, unsuitable or excess material is passed to rejection tracts, which remove the material. This material is then ejected as pseudofaeces. The particles selected for ingestion by the palps are passed down the oesophagus into the stomach. The mucus string containing the food particles is carried by cilia to a cartilage-like region known as the gastric shield in the stomach. A mucoprotein rod known as the crystalline style protrudes from the style sac into the stomach and rests on the gastric shield. The crystalline style is rotated by cilia located in the style sac. This rotation winds the mucus string around the style, the mechanical action of which breaks up the food particles in the mucus string. The fragments of food are then conveyed by tracts to a sorting caecum attached to the ventral wall of the stomach. Only small particles can enter the caecum. Large material is carried back to the style to be broken down further. Dense sand grains are sorted from the mixture and rejected via the mid gut. The less dense material is eventually passed to the digestive gland. Faecal matter is passed to the anus and is expelled from the rectum. . Particles that are discharged from the mantle cavity without being exposed to the alimentary canal are known as pseudo-faeces. (White 1937).

Figure 8: Cross section through *Mytilus edulis* showing internal structure and flow of feeding currents propelled by cilia represented by arrows (Bayne *et al.*, 1976).



1.1.2.3 Nervous system and sense organs

Mytilus edulis contains three pairs of nerve ganglia. These are the cerebral ganglia, the visceral ganglia and the pedal ganglia. The cerebral ganglia serve the anterior regions of the mussel's body namely, labial palps, anterior adductor, anterior part of the mantle and sense organs. The visceral ganglia serve the gills, heart, and posterior part of the mantle. The pedal ganglia serves the foot and byssal muscles.

There are six different types of sense organ found in *M.edulis*. These are, the eyes, the osphradia, the abdominal sense organs, tactile receptors, the statocysts and a dermal light sense mechanism. The eyes of *M.edulis* are features retained from the larval stage of the mussel. The adults eyes remain unchanged from the larval eyes and as such are very small. The eyes are formed from invaginated epithelium containing a mucoid lens. The osphradia are patches of dark brown pigmented epithelium. This sense organ may be active in chemosensory processes though at present there appears to be no literature on the osphradia of *M.edulis*. The tactile receptors are touch sensitive cells found on the mantle surface. *Mytilus edulis* can be observed retracting when large particles encounter the mantle margin (Bayne et al. 1976). The dermal light sense mechanism is initiated when the ventral border of the mantle is exposed to light stimuli. The statocysts are paired structures located under the epithelium near the pedal ganglia. The physiological function of this pair of sense organs is not reported in the literature. The abdominal sense organs are similar in appearance to the osphradia. Both of these types of sense organs are reported by White 1937 to be water-testing organs.

1.1.2.4 Musculature

The musculature of *M.edulis* is consists of four main groups.

Adductor muscles

There are two adductor muscles. The posterior adductor is generally larger than the anterior adductor. The adductor muscles are used by the mussel to close the shell valves. This action is

countered by the ligament of the shell. Thus, if the mussel is dead the shells gape open, as there is no muscle action to act against the ligament.

Pallial muscles

The pallial muscles are simple in *M. edulis*. There are no siphons present. The main role of the pallial muscle is to contract the mantle.

The foot muscles

The paired retractor pedis muscle is the main muscle associated with the foot. This muscle is located anterior to the byssal muscles. The foot is mostly comprised of longitudinal muscle fibres which are derived from the retractor pedis muscle. The foot has a groove running its length and is terminated in a depression which the mussel uses as a sucker when forming byssal attachments onto suitable substrata. These muscles are all active in retraction and extension of the foot. In locomotion, the foot is used to locate a suitable hard surface. Once located a byssus thread is attached to the surface then the foot pulls on the thread apparently testing strength then sustained pulls move the mussel towards the attachment (Personal observation).

The byssal muscles

The muscles that are associated with the byssal organ consist of a paired anterior byssus retractor muscle and a number of paired posterior byssus retractor muscles. The posterior byssus retractor muscles are located behind the foot with insertion into the shell.

1.1.2.5 Reproduction and recruitment

Normally the sexes in *Mytilus edulis* are separate, however hermaphrodites can occur rarely. The only sign of sexual dimorphism in *M. edulis* is the difference in colour of the reproductive organs, the male being yellow or cream and the female apricot or red. The reproductive system itself consists of branching tubes, which ramify through the visceral mass. The gonad consists of paired gonoducts, five main genital canals on either side of the body and various minor canals terminating in follicles. The ova and spermatozoa are derived from germinal epithelium in the follicles. The

main genital canals converge to form the genital duct. The genital duct runs through the mantle to the posterior on the ventral side of the mussel. The duct opens to the exterior across the ventral side of one of the posterior byssus retractor muscles. The aperture of the genital duct is usually sealed with orange coloured mucus.

The reproductive period can be described as starting with gametogenesis and ending when gametes are released, with a vegetative period in between (Seed 1976). In winter there is a redevelopment of the resting gonad which begins the development of ripe gonads. By spring, the gonads are ripe and ready to spawn. There is some spawning activity here though only minor in quantity. The gonads undergo rapid gametogenesis to regain a ripe condition by summer. Here there is full spawning with complete evacuation of the gonad. The connective tissue around the gonad begins to thicken with deposits of glycogen and fat during autumn. The cycle is completed in winter where redevelopment of the resting gonad begins again. There are various factors controlling this reproductive cycle. These factors can be categorised into exogenous and endogenous factors. The exogenous factors are external factors of the environment. Sea temperature is a major factor in the control of both the time and duration of spawning. There has been a large amount of work done in this area (Young 1945, Chipperfield 1953, Allen 1955, Savage 1956, Moore & Reish 1969). However, the mechanism by which temperature affects reproduction is still in question. The mechanical action of scraping or chipping of the shell of *M. edulis*, along with pulling of the byssus and attack of the adductor muscle all initiate spawning (Field 1922, Young 1942, 1945, 1946). The endogenous factors arise from within the mussel. These factors are generally chemical and act in conjunction with the external environmental stimuli (Seed 1976).

The fertilised egg forms into a ciliated trochophore, which develops into the second larval stage after one day. The second larval stage consists of a veliger form consisting of a pair of shell valves and a velum or ciliated swimming organ. Further stages consist of various veliger forms until the adult form is attained.

The settlement of *M.edulis* larvae is a two-stage process that consists of firstly a primary settlement and then a secondary settlement onto established mussel beds (DeBlock & Geelen 1958). The primary settlement onto filamentous substrata has been well known for some time (Wilson 1886, Johnstone 1898, Maas Geesteranus 1942, Chipperfield 1953, Verwey 1952, 1954). The mussel larvae settle onto numerous primary substrata, then move to and individuals settle on a final substratum, which will usually be a mussel bed (Maas Geesteranus 1942). The surface texture of the mussel bed itself may be a deciding factor in the final settlement (Maas Geesteranus 1942). The primary settlement is thought to be a way of allowing the larvae to attach and grow before joining a bed thus extending the young mussels survival (Thorson 1957).

1.1.2.6 Growth

The rate of growth of *M.edulis* is primarily affected by the availability of food in the surrounding environment (White 1937). Although a number of environmental factors must be taken into account. Temperature can affect growth, the optimum temperature range being between 10°C and 20°C (Coulthard 1929). Reduced salinity is also reported to have a detrimental effect on growth rate (Lubinsky 1958). This low growth rate is compensated by the fact that many predators of *M.edulis* are incapable of existing at lower salinities (Seed 1976). Growth can be measured by one of three ways (Little & Kitching 1996). The first method requires reproduction to be limited to one season in the year. This method appears to be accurate for only two to three years, after this time the annual peaks from size frequency analysis merge due to variations in growth rate. The second method is to physically count growth rings on the mussel shell. These rings are however not always laid down annually therefor they are named disturbance rings.

1.1.2.7 Commercial mussel culture

The focus of this section of the thesis is to investigate the introduction of mussels onto the shore attached to artificial substrata. It is therefore important to discuss commercial mussel culture that grows and cultivates mussels in vast quantities. Cultivation of *M.edulis* is widespread throughout

Europe with different countries generally adopting different methods. There are three main methods: firstly cultivation on poles, secondly cultivation on the seabed and finally cultivation on frames or free floating structures (Mason 1972).

The cultivation of mussels on poles has been used extensively in France for about 700 years (Field 1922). The poles onto which mussel spats settle are known as collector bouchots. These are rows of pine stakes arranged 35 cm apart at the MLWS level of the shore. The growing mussels are then transferred to rearing bouchots, which are 75 cm apart and located nearer to the shore. These bouchots have willow or chestnut branches interwoven horizontally forming a crude mesh surface. The transferred mussels are attached in bags of fine mesh netting designed to rot away with time. The mussels are now exposed for a significant part of the day which prepares them for being closed during transport after harvesting which keeps the mussels fresher. A modification of this method is used in Brittany and Jersey. This new method involves allowing spat to attach to ropes suspended between bouchots. When the mussels begin growing on the ropes the rope is transferred to a clean bouchot and wrapped around it in a spiral fashion. The main advantage of bouchots is that they keep the mussels away from predators. The drawbacks are however that the bouchots are exposed to the air for a significant amount of time, thus limiting feeding and the size of the mussels. The bouchots can also be vulnerable to storms (Mason 1976).

Mussel cultivation on the sea-bed is conducted in Denmark, West Germany and Holland. The spat of *Mytilus* settle onto public ground in the intertidal zone from where they are then dredged with toothless dredges. Farmers are allocated plots in deeper water, which they rent from the government. The mussel beds are thinned out as they grow. This thinning enables the mussels to grow better to a larger more marketable size. The mussels are dredged when they are the size for market; they are then left in an area of the beach to allow silt to be purged. The mussels are then processed in mechanised factories where they are separated, cleaned, graded for size and then packed. The main problem of this method is the exposure to predators such as crabs, seabirds and whelks, which must be controlled.

The cultivation on frames and free-floating structures involves the growth of mussels on suspended ropes. These methods are practised widely around the coasts of the Mediterranean Sea with *Mytilus galloprovincialis*. The ropes are suspended from fixed frames. The ropes are accessible from the top of the frame where the mussels can be pulled up and harvested when the mussels are a suitable size. *M. edulis* has been cultivated in this way for an experiment in Guernsey the results of which were promising. Free floating methods of culture involves the use of modified boats or specially designed rafts. This method is particularly popular in Spain where *Mytilus galloprovincialis* is the species used.

The culture of mussels can experience potential problems from periodic red algae blooms or “red tides”. These algae are filtered and ingested by mussels which if eaten by humans give rise to paralytic shellfish poisoning, (PSP).

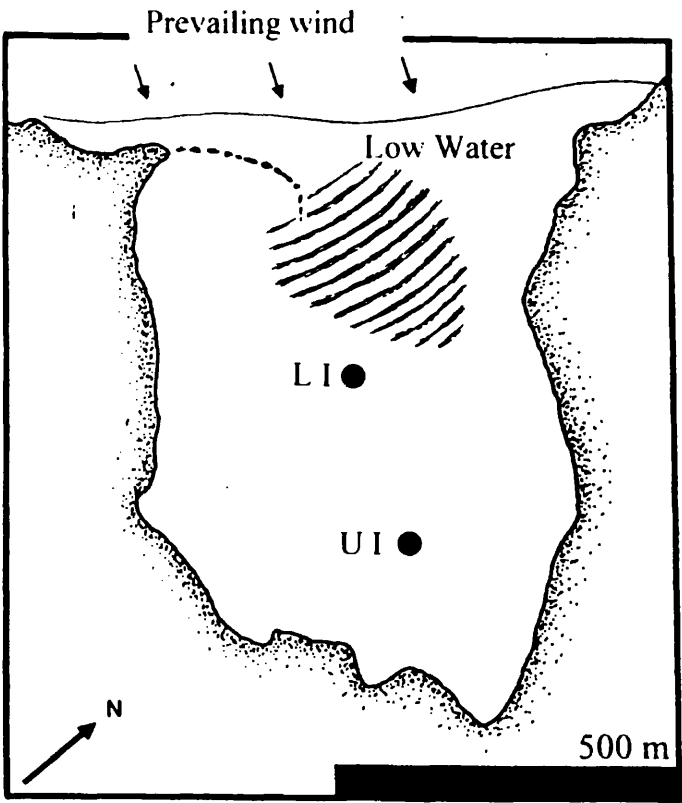
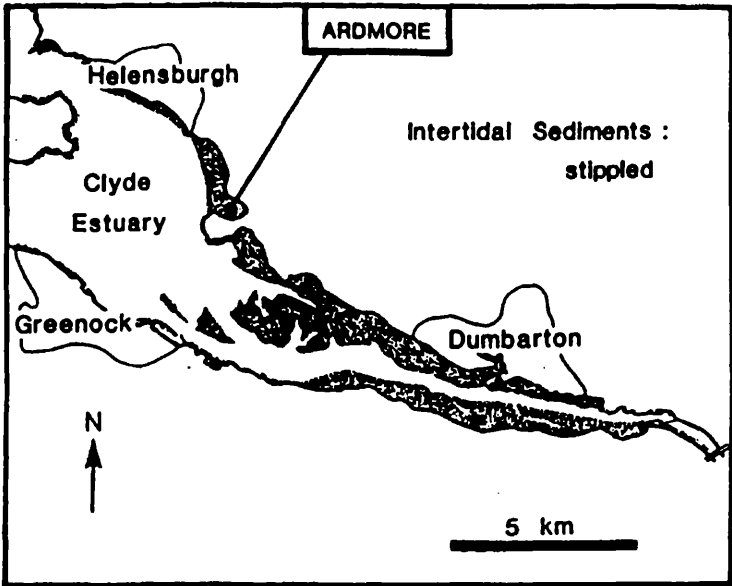
1.2) MATERIALS AND METHOD

This section contains firstly a general method section that applies to all the work conducted in the section. Secondly, there is a detailed description of the experimental procedures conducted. In total there are six experiments reported. The first four experiments conducted by Fraser West are presented with numerical notation. The remaining 2 groups of experiments were conducted jointly with two honours BSc students and are presented with roman numerical notation.

The sediment used in all the experiments in this section, was collected from Ardmore Bay (Figure 9 page) in the Clyde Estuary (Latitude 55° 58' 32" N Longitude 4° 41' 29" W). This intertidal bay was chosen for a number of reasons. Firstly, Ardmore Bay is situated in close proximity to the laboratory thus reducing any stress that may be induced in the transportation of organisms from the bay. Secondly, the bay has been well studied by the members of the Biosedimentology group for many years (Meadows & Tufail 1986, Meadows *et al.*, 1990, Meadows *et al.*, 1998). Finally, the bay is an excellent field laboratory for conducting coastal protection experiments, as it is comprised of a large expanse of fine sand, which will be prone to erosional attack from the marine tidal cycles. There is little physical evidence of erosion present at the edge of the saltmarsh. A conservative estimate for an erosional rate of the saltmarsh is one metre in 15 years (Meadows. Pers. Res. Comm).

The sediment used for all the experiments in section I was chosen as a result of work conducted by Shand (1991). The gravel used in experiments 1 and 2 was collected from the western part of the bay where there is a relatively high-energy beach composed entirely of coarse gravel. The fine sand sediment was retrieved from the mid tidal section of the shore. Only the light brown oxygenated first few cm of sediment was taken using a spade as anoxic sediment may interfere with the experiments.

Figure 9: Location of Ardmore Bay in the Clyde Estuary and diagram indicating the upper intertidal site (UI) along with the lower intertidal site (LI).



The mussels *Mytilus edulis* were collected from the north side of Ardmore bay, where there are large beds extending from an area of rocky shore and isolated boulders. The mussels were selected by size, which was an average of five cm in length.

The seawater used for the experiment was obtained from a storage tank in the Graham Kerr Building (Glasgow University) with a salinity of 36‰. The seawater was originally from the Firth of Clyde collected at Hunterston ore terminal, which was then transported by tanker. Salinity was measured using an Otago optical salinity gauge. Adjustments were made to the salinity each day by adding a volume of 70:30 ratio seawater and freshwater mix. The volume in each experimental dish was corrected to a marked level on the side-wall of the dish.

1.2.1 Sediment preparation

The fine sediment was taken using a spade to remove a layer of 0-3cm depth, which was the light brown aerobic layer. The sediment was wet sieved through a 500µm (0.5mm) British Standards sieve to remove any large organisms and stones giving a particle size of <500µm. This was then washed in fresh sea water 3 times to remove very fine particles thus giving a more uniform particle size distribution.

The gravel was sieved on shore, through an 8mm British Standards sieve then a 4mm British Standards sieve. It was then transported to the laboratory and washed with seawater 3 times.

1.2.2 Animal preparation

In the field, *Mytilus edulis* measuring about 5cm in length were selected. The mussels were gently separated from their attachment by hand and placed in a bucket. They were then transported to the laboratory. In the laboratory, the mussels shells were scraped clean using a blunt knife to remove attached organisms and old byssus threads. This was done so that any new byssus threads attached during the experiments could be detected. The byssus complex was then cut at the opening of the shell valves using scissors. This was done so that the length of byssus threads produced during the

experiment could be recorded. There was a ± 1 cm error in byssus thread measurements as length was recorded from the shell opening to the byssus pad on the attachment surface. This left about 1 cm length from the shell lip to the origin of the thread in the byssal gland in the foot.

Mussels in experiments 2 and 4 that were used for seeding directly into the field were cleaned and separated in the same way as the laboratory animals. These were then stored in seawater in the laboratory.

1.2.3 Plastic netting preparation

Plastic netting used was cut from large lengths supplied by a garden centre.

The plastic netting used was of two different types illustrated in Table 2. There were two different sizes of plastic mesh used in experiment 3 and 4. The first had a square aperture measuring 14mm x 14mm and with a mesh diameter of 2mm. The second mesh had a square aperture measuring 14mm x 14mm with a mesh diameter of 1mm.

The netting was cut into 0.25m square pieces from meter width lengths of netting using scissors.

1.2.4 Statistical analysis of data

The data were statistically analysed using MINITAB Release 11.21 computer software. The methods used were, One-way analysis of variance, and Chi-squared analysis.

Table 2: Table showing the two size grades of plastic netting used in experiments 3 and 4.

| NETTING TYPE | APERTURE | MESH THICKNESS |
|--------------|------------------|----------------|
| A | Square 14 x 14mm | 2mm |
| B | Square 14 x 14mm | 1mm |

1.2.5 EXPERIMENTAL DESIGN

1.2.5.1 Experiment 1: *Mytilus edulis* byssus thread production on fine sand, gravel and on a one to one mix of fine sand and gravel.

The objective of this experiment was to study the attachment of mussels on to soft sedimentary substrata and compare this to relatively hard substrata. The sediments used were fine sand with a particle size of $<500\mu\text{m}$, gravel with a particle size between 4mm and 8mm and a 1:1 mix of these two sediments.

Aim of experiment

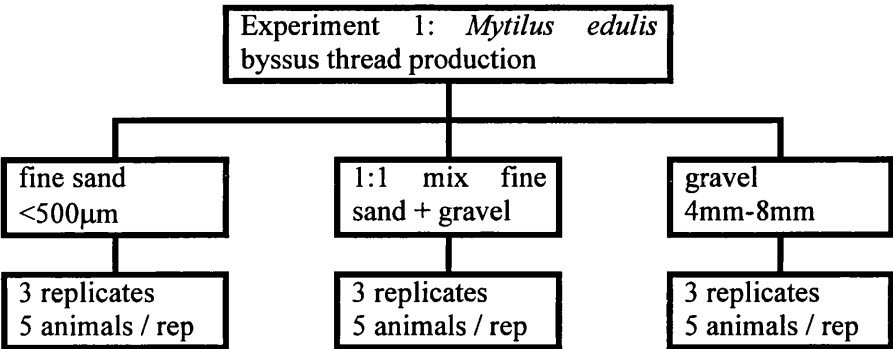
The aim of this experiment was to test the following hypotheses, which are phrased as questions:

- 1.1 Do *Mytilus edulis* attach threads onto particles in all of the three substrata?
- 1.2 Are the numbers of threads produced on gravel, fine sand and a 1:1 mix of gravel and fine sand different?
- 1.3 Are the clump patterns produced by *Mytilus edulis* different on each of the three substrata?

Design and conduct of experiment

The experimental design is shown in Figure 10. The experiment consisted of nine circular containers, three replicates per substrate. The dish dimensions measured 20-21cm diameter and 11cm deep. This size of container was chosen as five mussels could be laid in the centre with areas of free space around the mussels allowing free movement. In total 45 mussels were used in the experiment, five placed in each dish. This number of mussels was chosen as it reflected the numbers of mussels found in many of the isolated groups on the shore.

Figure 10: Experimental design of experiment 1 showing the three different substrata used. Fine sand with a particle size less than 500µm. Gravel with a particle size between 4mm and 8mm. A 1:1 mixture of fine sand and gravel. Three replicate containers per substrate each containing 5 mussels.



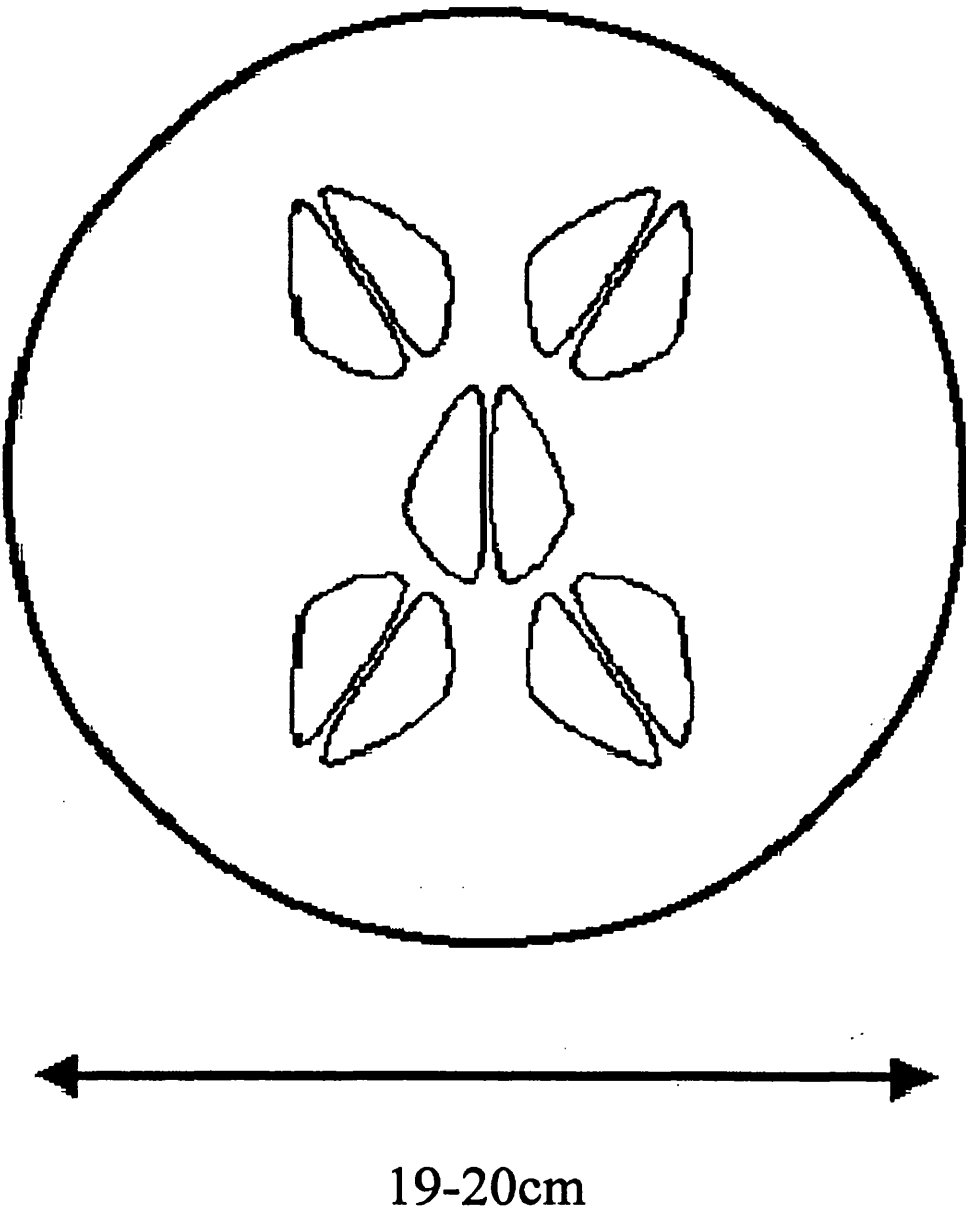
The mussels were laid out in a uniform arrangement in the containers. This allows the pattern of movement to be followed on the three substrata. The initial uniform arrangement is shown in Figure 11.

Details of experiment

In the laboratory, three of the nine containers had 5cm depth of fine sand added. To another three containers 5 cm of a 1:1 ratio mix of fine sand and gravel was added. To the remaining three containers 5 cm of gravel was added. Mussels were then added to all nine containers following the pattern in Figure 11. The containers were then filled with seawater until the mussels were submerged. The containers were then left undisturbed for the five day duration of the experiment. The experiment in the laboratory was subject to natural periods of daylight and room temperature of 18°C.

An observational record was taken over the course of the experiment to monitor the changes in pattern. The experiment was terminated after five days, by adding 1-2ml of Propylene Phenoxitol to the seawater in each dish. This was used to anaesthetise the organisms to prevent the mussels contracting and possibly breaking byssus threads on the addition of Steedman's preservative. The constituents of Steedman's solution are as follows. For 1 litre of concentrated solution, Propylene Phenoxitol 50ml, Propane-1, 2-diol 450ml, Formalin solution 500ml (Steedman 1976). Concentrated Steedman's preservative was added to each dish by removing 125ml water from the dish and then adding 125ml concentrated Steedman's to give a one part in 10 dilution. The position and arrangement of the mussel clumps was then recorded. Steedman's is a harmful substance and as such work was conducted in a well-ventilated part of the laboratory.

Figure 11: Initial pattern arrangement of *Mytilus edulis* in the dishes for all three substrata in experiment 2.



Investigation of clumping pattern behaviour.

Glass sheeting measuring 50cm X 25cm was placed over 2 containers at a time and strong light illuminated the containers. An acetate sheet with a one-millimetre grid was laid onto the glass. Then by viewing from directly above to minimise parallax distortion, the dish and positions of the mussels were traced onto the acetate sheet. The shells and gravel were outlined with black marker, and the byssus threads and pads blue and red respectively for each of the three substrata, fine sand, fine sand and gravel mixed and gravel. These are shown in Figures 27 to 29 pages 83-85. The Steedman's solution was drained from the containers into containers for storage and the dish was then submerged in a tank full of seawater. Sediment was removed from around the clump by a suction hose. In some containers, the mussels had released their entire byssus thread complex. The latter were located and carefully removed for analysis. Great care was taken when removing the mussel clumps from the containers, as the byssus threads were easily broken. The clumps in the containers containing fine sediment were removed by supporting underneath the clump with both hands. The clumps were then transferred into a tray of Steedman's for processing. The removal of mussel clumps in the containers containing gravel and the 1:1 mix required a slightly different procedure due to attached particles and released byssus complexes. The end of the complex released by the mussel was held by forceps and was gently dislodged using a paintbrush. The clump of mussels was dealt with in a similar way; the grains attached to the clump were eased from surrounding grains and the clump was lifted out slowly holding the shells. This, along with the released complex, was transferred to a tray containing Steedman's.

Each mussel was labelled with a Roman numeral from I to V.

Data were noted as follows:

1. The numbers of pads on organisms own shell, on left and right valves.
2. The number of full threads including pads attached to the organism by itself.
3. The number of threads broken from the organism without pad or on the organism's shell with pad and free end.
4. The number of threads attached from one organism's onto another organism,

5. The number of threads attached on the glass side-wall of the dish.
6. The number of threads attached to gravel particles
7. The maximum and minimum length of threads produced per organism.

Each thread was carefully traced from the animal to the gravel particle, then the threads were counted and cut to prevent recounting. This procedure was repeated for the five mussels in each dish. When the mussel had been dealt with, it was cut free from the clump. When measuring the maximum and minimum length of byssus threads, a portion of the thread remained within the shell valves. Measurements were recorded from the distal end of the thread terminated by the pad to the proximal end of the thread protruding from the shell valves. The remainder of the thread inside the shell valves was impossible to measure without opening the organism thus risking breaking the remaining threads still attached. On inspection after all threads had been recorded the shell valves were open and the remainder of the threads was recorded. The estimated error in the byssus length measurements was $\pm 10\text{mm}$.

1.2.5.2 Experiment 2: *Mytilus edulis* laboratory and Field investigation of attachment onto sand and gravel.

Mytilus edulis is known to form beds on soft sediments under field conditions, attaching to large rock surfaces or underlying stones. These attachments may give an extra stability to the integrity of the mussel bed. The objective of this experiment was to investigate the effect of laboratory clumping treatments onto sand and gravel followed by attachment of *Mytilus edulis* under field conditions. The first objective of this experiment was to determine whether *Mytilus edulis* could be used as a biological method of protecting soft sedimentary systems. The second objective was to determine whether pre-formed clumps laid onto sand and gravel in the laboratory, would improve the effectiveness of *Mytilus edulis* as a biological method of protecting soft sedimentary systems.

Aim of experiment

The aim of this experiment was to test the following hypotheses, which are phrased as questions:

1. Is there a difference in numbers of mussels lost and distance transported between the upper and lower intertidal zones?
2. Is there a difference in numbers of mussels lost and the distance transported, for laboratory clumped mussels at the upper intertidal and lower intertidal?
3. Does the introduced gravel substrate affect the numbers of mussels lost and distance transported from the upper and lower intertidal zones?

Design and conduct of experiment

The experiment was conducted in October this would mean that the mussels would be exposed to the beginning of the winter storms thus fully testing their attachment to the substratum. The experiment consisted of six combinations of substratum for six groups of mussels. There were three different densities of mussels used for each group these were single mussels, 5 mussels and 15 mussels. These densities were chosen to span the naturally observed densities of isolated mussel

groups found at Ardmore Bay. There were two replicates of each of these densities. The mussels were collected in the field and transported to the laboratory. The first group of mussels was allowed to form clumps in the laboratory onto gravel and then these were laid onto gravel in the field (Figure 12). The second group of mussels was allowed to form clumps in the laboratory onto gravel and then these were laid onto the field sand. The third group of mussels was allowed to form clumps in the laboratory onto sand and then these were laid onto gravel in the field. The fourth group of mussels was allowed to form clumps in the laboratory onto sand and then these were laid onto the field sand. The fifth group of mussels was laid directly onto the field sand as individual mussels without being clumped. The sixth group of mussels was laid directly onto gravel in the field as individual mussels without being clumped. The two experimental sites on the shore were chosen as they were comparatively different environments. The lower intertidal site was essentially a sandy substratum with no isolated boulders or algae cover. The upper intertidal site was a heterogeneous mixture of sediment, isolated small boulders and algae both on the sediment surface and attached to the boulders. The sedimentary column was also different between the two sites. The sediment of the lower intertidal site being comprised of fine sand with a shell debris layer at about 15 cm depth. The sediment of the upper intertidal site was comprised of fine mud/sand which had patchy deposits of clay distributed randomly over the

upper intertidal zone. In relation to Chart Datum the sites were 2.19 m and 3 m above Chart Datum for the lower intertidal and upper intertidal sites respectively. This was calculated from the mean spring and neap curves for the Standard port of Greenock shown in Figure 13 (Admiralty 2000). Ardmore bay is about 7 miles from the Secondary port of Helensburgh which experiences no time differences in high water and low water when compared to Greenock as shown in Table 3. The lower intertidal site had few isolated boulders in its immediate vicinity. The exposure of Ardmore bay has been rated on an exposure scale for sandy beaches developed by (McLachlan 1980).

Figure 12: Experimental design of experiment 2 showing the six different groups used in the experiment. Mussels in group 1 were laid onto gravel in the laboratory allowed to form clumps then the clumps were transported and laid onto gravel in the field G → G. Mussels in group 2 were laid onto gravel in the laboratory allowed to form clumps then the clumps were transported and laid onto sand in the field S → S. Mussels in group 3 were laid onto sand in the laboratory allowed to form clumps then the clumps were transported and laid onto gravel in the field S → G. Mussels in group 4 were laid onto sand in the laboratory allowed to form clumps then the clumps were transported and laid onto sand in the field S → S. Mussels in group 5 were laid as individual mussels with 1cm spacing between individuals onto gravel in the field G. Mussels in group 5 were laid as individual mussels with 1cm spacing between individuals onto sand in the field S. For each group 2 replicates of individual mussels, groups of 5 mussels and groups of 15 mussels were used. The six groups were laid into three lines of 12 quadrats at both the upper and lower intertidal sites.

| | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 | GROUP 5 | GROUP 6 |
|------------|------------|------------|------------|------------|------------|------------|
| LABORATORY | G | G | S | S | -- | -- |
| | ↓ | ↓ | ↓ | ↓ | | |
| FIELD | G | S | G | S | S | G |

Details of experiment

In the laboratory, 48 circular containers were set up by first adding fine sand of 6cm depth to all of the containers. The containers were grouped into three different sizes for each of the three mussel densities. The containers for fifteen mussels measured 34.5cm internal diameter (ID), the containers for five mussels measured 20 cm ID and single mussel containers measured 12.5 cm ID. To half of each of the three size groups of containers, a 1cm deep circular area of gravel was added to the centre of the container. The area of gravel corresponded to the number of mussels in the dish. The mussels were laid as single animals, groups of five animals or groups of 15 animals. The greater the number of mussels the larger the diameter of the gravel disc. Around the gravel disc, a ring of fine sand of the same depth (1cm) was laid down. The mussels in the laboratory were allowed to form clumps for three days and then placed in polythene bags for transport to the shore. Salinity was checked daily using an optical salinity gauge and adjusted using a 70:30 ratio mix of seawater and freshwater. The experiment in the laboratory was subject to natural periods of daylight and room temperature of 18°C. Care was taken when removing the clumps from gravel and the number of grains attached were noted and left attached. These mussels were either laid onto gravel (G→G or S→G), or onto sand (G→S or S→S).

Figure 13: Mean spring and neap curves for the Standard port of Greenock (Admiralty 2000).

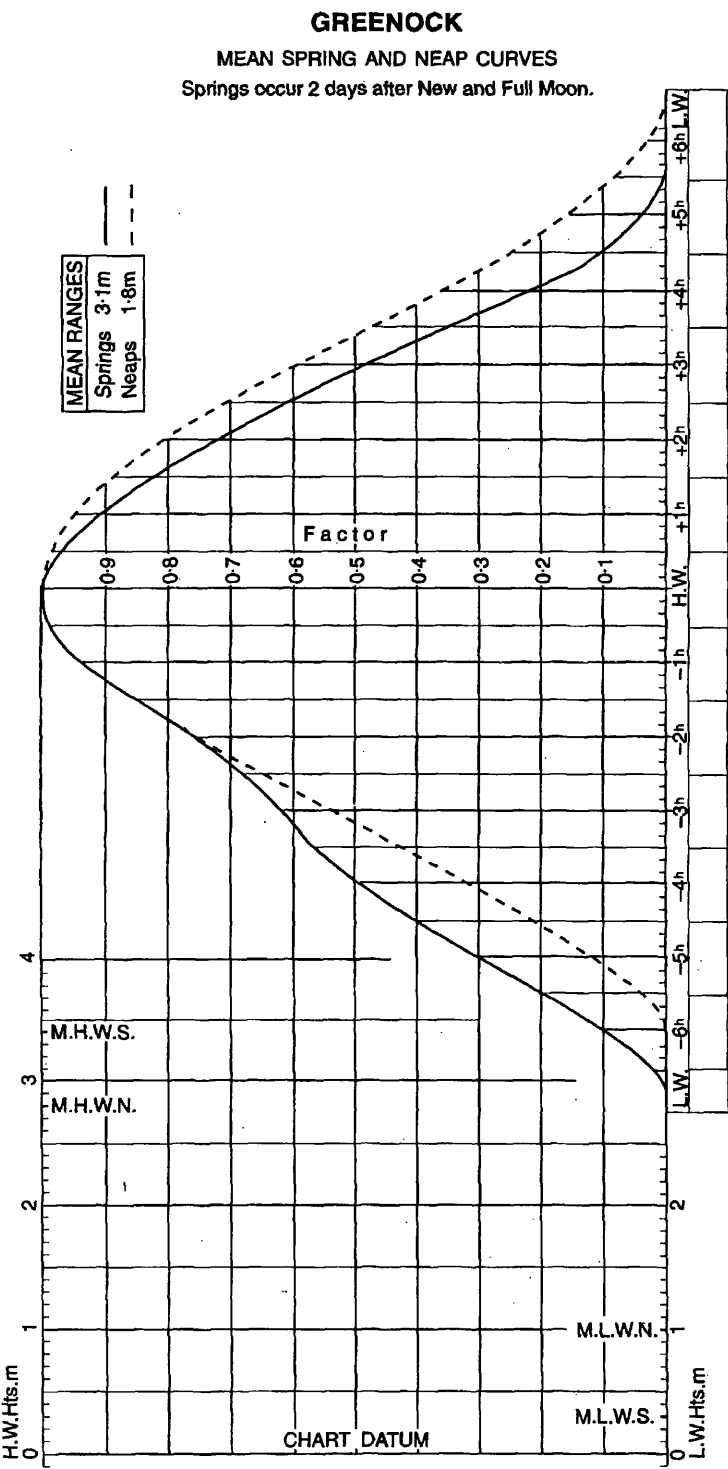
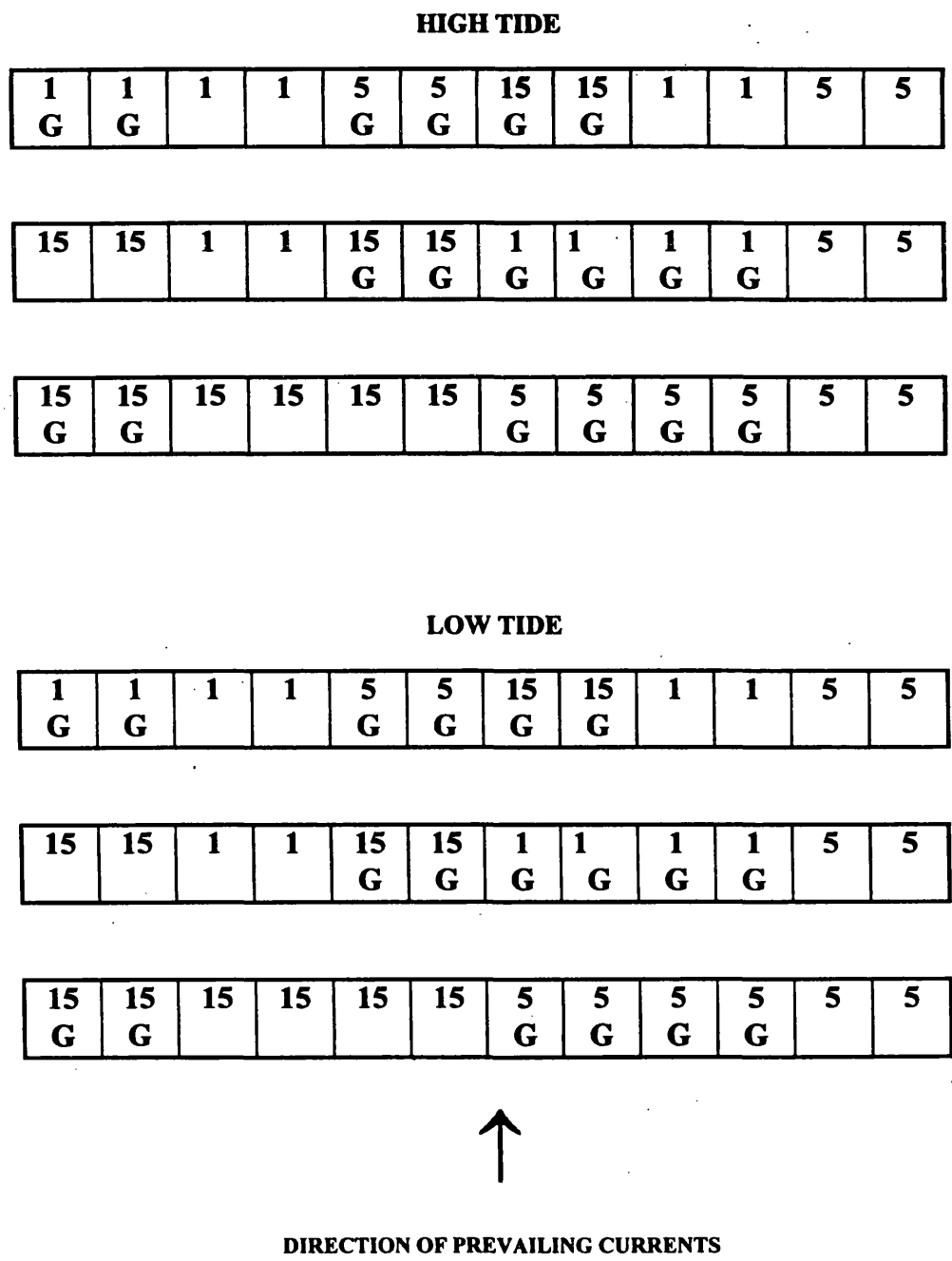


Figure 14: Field layout of mussels in an array of quadrats at both the upper and lower intertidal sites. Gravel G was placed in randomly selected quadrats at both tidal levels. The mussels were laid into the quadrats as single mussels 1, groups of 5 mussels 5 and groups of 15 mussels 15. These mussels were laid randomly into the array the random pattern determined by random number Tables. The direction of the prevailing currents is also shown.

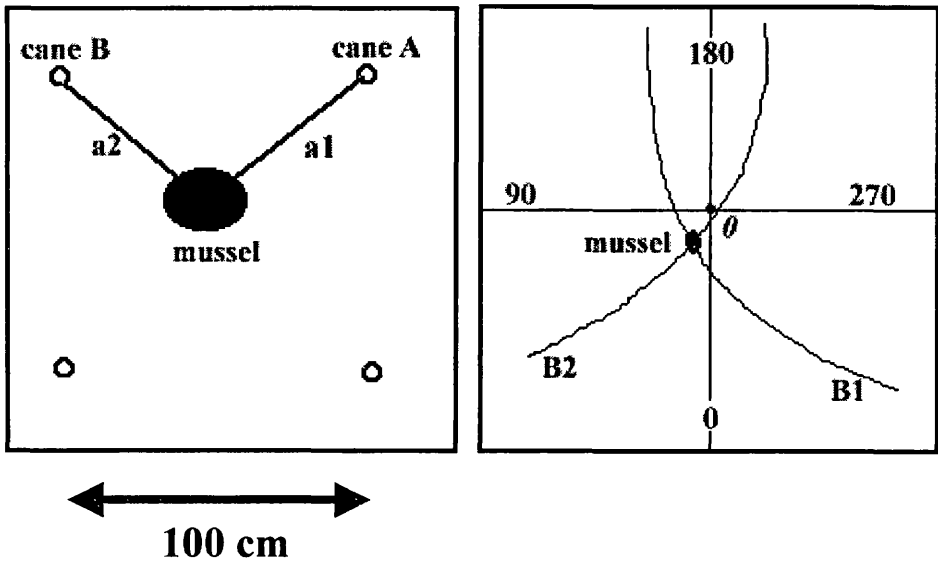


The single animals and clumps of animals were laid onto a matrix of quadrats at the upper intertidal site and at the lower intertidal site (Figure 14). The matrix consisted of three rows of twelve 1m^2 quadrats whose corners were identified by thin bamboo sticks. Gravel was placed in the centre of the selected quadrats (Figure 14), selected by random number Tables (Fisher & Yates 1957). The quadrats to which the laboratory and field seeded mussels were assigned, were determined using random number Tables (Fisher & Yates 1957). Two replicates of each treatment were included. Replicate quadrats were situated next to each other in the matrix. The animals were marked with a small paint coding unique to each quadrat. The paint-coded single animals, clumps of five animals and clumps of 15 animals were placed in the centre of their assigned quadrats. The duration of the field experiment was 18 days, measurements were taken on days 3, 5, 7, 8, 9, 16 and 18. Three measurements were taken for all quadrats at both tidal levels.

1. Numbers of animals inside each quadrat.
2. Numbers of animals outside each quadrat.
3. The displacement distance of these mussels from the origin of each quadrat.

These distance measurements were taken using a 30-metre fibreglass tape. The displacement of mussels was calculated using trigonometry from triangulation measurements taken on the shore. The mussels were associated with their assigned quadrats by their unique paint codes.

Figure 15: Method of recording the position of mussels on the shore in relation to their associated quadrats using triangulation (A). Method of plotting field data of mussel positions onto graph paper in the laboratory (B). The direction of the sea in B is indicated by 0° and the origin or centre of the quadrat is indicated by θ .



The triangulation measurement was taken as follows. Measurements were uniformly taken from the side of the quadrat furthest from the sea. The right hand cane was designated pole A. The left hand cane was designated pole B. Both were identified whilst facing away from the sea (Figure 15A). The first triangulation measure was taken from cane A to the clump or single animal. The second measure was taken from cane B to the single animal or clump. These data were plotted onto one millimetre graph paper in the laboratory using the scale 1m = 10cm (Figure 15B). A quadrat was outlined on a page and the direction of the sea was noted and was given the convention \mathcal{O} . The other four points around the quadrat were then marked 90° , 180° and 270° . The side marked 180° was furthest from the sea and had cane A and B at either end. The scaled triangulation measures were then used to plot the positions of the mussels in relation to the quadrat. The distance measure from cane A was used to form an arc using compasses. This arc would intersect with a similar arc created from cane B. The intersection was circled and annotated with the number of mussels. A line was then drawn from the centre of the quadrat (\mathcal{O}) to the centre of the intersection. This was multiplied by the scale factor to give actual distance on the shore. The angle was measured using a protractor. This was the direction that the mussels had been transported. These data were calculated for all animals at both the upper and lower intertidal sites. At the end of the experiment all mussels were collected and placed in separate bags from each quadrat.

The quadrats with gravel on the sediment were then cored to assess the down core movement of gravel (Figure 14). A 50cm ID plastic core was used to retrieve cores of 30cm depth. The underlying rock inhibited the retrieval of deeper cores. The cores were sectioned on site at intervals of, 0-2cm, 2-5cm, 5-10cm, 10-15cm, 15-20cm, 20-25cm and 25-30cm. Each section was placed into a separate labelled polythene bag for later gravel analysis. Due to constraints of tidal cycles, the coring was completed over a four-day period. In the laboratory each sectioned core was sieved through a 4mm sieve and the weight retained on the sieve for each section of a core was noted to give the down core profile of gravel particles.

Three representative cores collected from the quadrats containing gravel were chosen one from each of the three rows at both tidal levels. The 0-2cm section was used for particle size analysis. This was pre-sieved through a 4mm sieve to remove the introduced gravel. The sample was then

air-dried. When dry, the sample was mixed thoroughly and 100g was taken for analysis. The particle size analysis was undertaken on these using a 0.5 ϕ (Phi) scale sieve stack (44 μ m, 63 μ m, 90 μ m, 125 μ m, 180 μ m, 250 μ m, 355 μ m, 500 μ m, 710 μ m and 1mm) on an octagon sieve shaker. Table 3 shows the half Phi scale and corresponding millimetre scale. The weights retained on each sieve were noted including the catch pan at the base (<44 μ m), these were then entered into a GW BASIC computer program to calculate particle size. (Table of particle size analysis given in results. Table 10 page 104).

Table 3: Table showing the half Phi (ϕ) scale used in the measurement of particle size of sediments. The corresponding scale in millimetres (mm) is also shown.

| Millimetres (mm) | Phi (ϕ) |
|------------------|----------------|
| 1 | 0 |
| 0.71 | 0.5 |
| 0.5 | 1 |
| 0.35 | 1.5 |
| 0.25 | 2 |
| 0.18 | 2.5 |
| 0.125 | 3 |
| 0.090 | 3.5 |
| 0.063 | 4 |
| 0.044 | 4.5 |

1.2.5.3 Experiment 3: *Mytilus edulis* laboratory investigation of attachment to and pull-off measurements from plastic netting.

Mytilus edulis form beds under field conditions on soft substrata and have been found attaching to underlying stones. These attachments may give an extra stability to the integrity of the mussel bed. In this experiment the investigation of a possible artificial seeding surface was conducted.

This experiment is a development of experiment 2. Plastic mesh was used which replaces the gravel in experiment two as the artificial substrate.

The first objective of this experiment was to determine further whether *Mytilus edulis* could be used as a biological method of protecting soft sedimentary systems. The second objective was to determine whether mussels would form clumps onto plastic mesh in the laboratory.

Aim of experiment

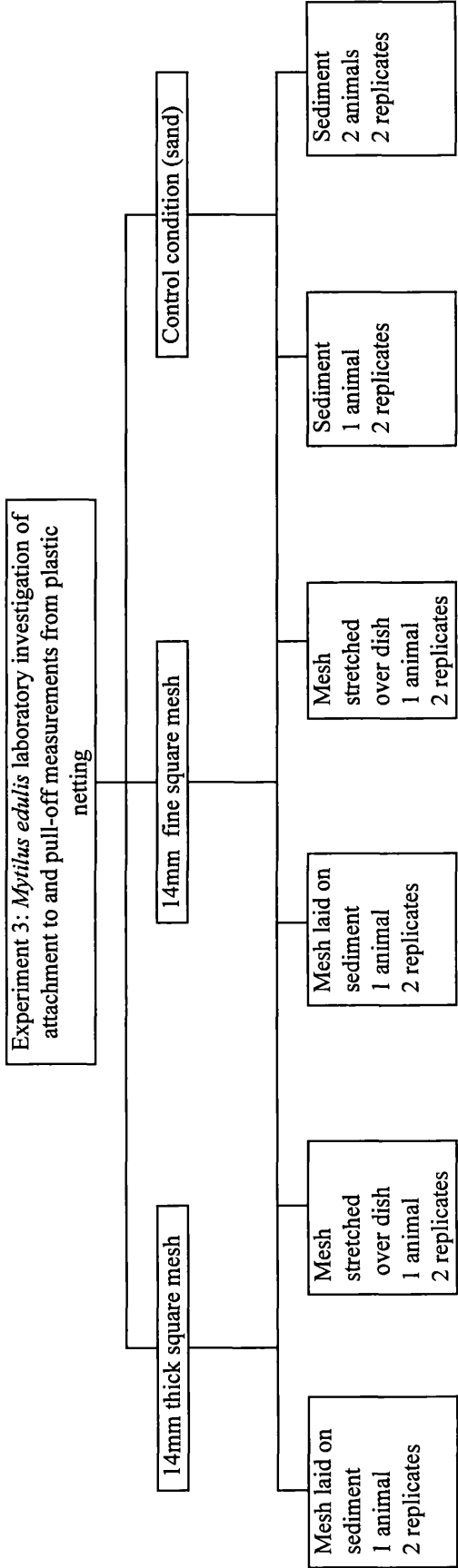
The aim of this experiment was to test the following hypotheses, which are phrased as questions:

1. Will *Mytilus edulis* attach to the plastic netting substrate given?
2. Do the pull-off measurements vary between the two different types of netting?
3. Will the number of threads produced vary between the two different types of netting?

Design and conduct of experiment

The experimental design is shown in Figure 16. The experiment consisted of 12 glass circular containers with a diameter of 20cm. There were six substrata two replicates for each substrata. There were two mesh types, both differed in thickness and aperture size. Mesh type A had an aperture measuring 14mm square with a 2mm-diameter mesh. Mesh type B had an aperture measuring 14mm square with a 1mm-diameter mesh.

Figure 16: Experimental design of experiment 3 showing the three different substrates used. Mesh type A (14mm square aperture mesh with 2mm diameter mesh) was placed over the top of 2 19cm ID circular dishes and laid onto sand inside 2 19cm ID circular dishes. Each dish contained 5cm of sand and 1 mussel was placed into the centre of the mesh in each dish. Mesh type B (14mm square aperture mesh with 1mm diameter mesh) was placed over the top of 2 19cm ID circular dishes and laid onto sand inside 2 19cm ID circular dishes. Each dish contained 5cm of sand and 1 mussel was placed into the centre of the mesh in each dish. There were two control conditions on sand. The first consisted of 2 19cm ID circular dishes each containing 5cm depth of sand with one mussel per dish. The second consisted of 2 19cm ID circular dishes each containing 5cm depth of sand with two mussels per dish.



The containers were prepared in the following way. To six of the containers, fine sediment was added to a depth of about 4cm. The netting to be placed inside the containers was cut using scissors. The netting square was placed over the dish and the scissors were used to cut out the internal circumference. The remaining four netting containers were prepared by placing the netting over the top of the containers and tied down with twine. The corners of these netting squares were cut diagonally to allow the netting to fold over the sides of the dish more easily. One animal was added to each of the netting containers and to one of the sediment containers. To the remaining sediment dish two animals were added. The mussels were placed in the centre of each dish so that the movement about the substrate could be observed.

The experiment was left for seven days. After seven days, the water was drained off carefully. The containers were traced using clear acetate with a 1mm grid, which was placed onto a sheet of glass and positioned, just above the dish as in experiment 1.

The numbers of threads attached to the animal's own shell, threads attached to other animal's shells and numbers of threads attached to netting were recorded for all containers. The force required to break the attachment to the substrata was measured in the following way using a calibrated weighing scale. Calibration involved attaching the weighing scale to standard weights and comparing values measured on a top pan balance. The dish was held down while a clamp was fixed to a mussel. The clamp was attached to the calibrated weighing scale. The scale was pulled up vertically at a constant speed and the resistance mass of the attachment registered on the scale. The measurement of pull-off in kilograms was taken when the byssus threads attached to the substrate broke. The pull-off in kg was converted into force by the equation (force (N) = mass x acceleration). The acceleration of the weighing scale vertically was 0.025m/s^2 . This was repeated for all the containers.

1.2.5.4 Experiment 4: *Mytilus edulis* field investigation of attachment onto netting

The objective of this experiment was to investigate the effect of attachment of *Mytilus edulis* onto netting substrate treatments under field conditions. This was a continuation of the investigation of a possible artificial seeding surface as in experiment 3 for the application in coastal zone protection.

Aim of experiment

The aim of this experiment was to test the following hypotheses phrased as questions:

1. Is there a difference in numbers of mussels lost and distance transported between the upper and lower intertidal zones?
2. Does the introduced plastic mesh substrate affect the numbers of mussels lost and distance transported from the upper and lower intertidal zones?

Design and conduct of experiment

The experiment was conducted in October this would mean that the mussels would be exposed to the beginning of the winter storms thus fully testing their attachment to the substratum. This experiment was conducted at the same time of year as experiment two and as such results from both can be compared. The experimental design is shown in Figure 17. The experiment was a development of experiment 2 page 6 and so most of the basic techniques are the same. The experiment consisted of 24-metre square quadrats arranged in three rows at the upper and lower intertidal sites with twelve replicate pairs in total. There were three different densities of mussels used, single mussels, 5 mussels and 15 mussels. A 14mm square plastic mesh substrate was used in this experiment. The mussels had been collected, cleaned and painted with a unique paint code. In total, there were four groups of mussels used in the experiment (Figure 17). Each group contained 42 mussels in total.

Figure 17: Experimental design of experiment 4 showing the four groups of substrate treatments used in the experiment. Mussels in group 1 were laid onto sand in the laboratory allowed to form clumps and then the clumps were transported and laid onto sand in the field S→S. Mussels in group 2 were laid onto 14mm square plastic mesh in the laboratory allowed to form clumps and then the clumps were transported and laid onto 14mm plastic mesh in the field M→M. Mussels in group 3 were laid as individual mussels with 1cm spacing between mussels onto sand in the field S. Mussels in group 4 were laid as individual mussels with 1cm spacing between mussels onto 14mm square plastic mesh in the field M.

| | GROUP 1 | GROUP 2 | GROUP 5 | GROUP 6 |
|------------|------------|------------|------------|------------|
| LABORATORY | S | M | -- | -- |
| | ↓ | ↓ | | |
| FIELD | S | M | S | M |

There were two replicates each of single mussels, 5 mussels and 15 mussels. Two groups of mussels were allowed to form clumps in the laboratory then these clumps were transported to the field. The first group was laid onto 6cm of sand in six containers to form clumps. These were then transported to the shore and laid onto field sand (S→S). The second group was placed onto plastic mesh suspended over six containers to form clumps and attach to the mesh. These clumps and single mussels attached to mesh were then transported to the shore and laid onto field sand (M→M). Two other groups were laid un-clumped into the field. The first group was laid as individual mussels into six quadrats containing field sand (S). The second group was laid as individual mussels into six quadrats containing plastic mesh substrate (M).

Details of experiment

In the laboratory, the two groups of mussels were clumped by the following method. In total 12 containers were set up. These containers measured 34.5 cm internal diameter. To six of the containers 6cm of fine sand particle size $<500\mu\text{m}$ was added. The remaining six containers had 0.25m^2 plastic mesh placed over empty containers and tied down with twine. The mussels in densities of single, five and 15 mussels were then added one density to each dish with two replicates for each density. This gave two sets of single, five and 15 mussels on sand, with an identical arrangement on the plastic mesh. All the containers were then submerged in seawater. The animals were left to form clumps over a four-day period. The third and fourth groups were kept in buckets over this time; these were the un-clumped conditions.

The mussels were transported to the shore as follows. After four days, the mussels on sand were placed into labelled plastic bags and the mussels laid on netting were placed with the netting flat onto trays. In both cases, care was taken not to disturb any clumps that had formed. The un-clumped mussels were simply transported in the buckets. The mussels were then transported to the shore.

The three rows of eight quadrats were prepared at the upper and lower intertidal as in experiment 2 page six. The array of quadrats was laid out using a 1m^2 metal square. The metal square was laid onto the sand and four bamboo canes were put at each of the corners. The spacing between the quadrats was one metre square. Due to tidal constraint the lower intertidal was set out first. This involved placing 0.25m square pieces of mesh in six of the 24 quadrats. This was used for the un-clumped mesh condition. To six other quadrats, un-clumped mussels were laid onto the field sand. Mussels were laid onto the mesh and sand from the buckets. Care was taken to pull apart any clumps before placing mussels on the mesh and sand. The mussels were laid at a spacing of approximately 1cm between mussels. The mussels that had formed clumps onto mesh in the laboratory were laid into the centre of another six quadrats. All the mesh squares were then secured using a bamboo cane in each corner tied to the mesh with plastic tie wraps. To the remaining 6 quadrats the clumps or single mussels laid onto sand in the laboratory were placed in the centre of each of the six quadrats. The duration of the experiment was 18 days. Over this period measurements identical to those in experiment 2 were taken. As in experiment 2 the trigonometry measurements were plotted onto scaled plots.

1.2.5.5 JOINT FIELD INVESTIGATIONS OF THE POTENTIAL USE OF *MYTILUS EDULIS* AS BIOLOGICAL STABILISERS IN THE INTERTIDAL COASTAL ZONE

The joint fieldwork was conducted at Ardmore Bay. In total two experimental sections are reported. Experiments in Experiment set I were conducted with Suzanne McCulloch and experiments in Experiment set II were conducted with Patricia Whitaker. These were individually written up by the authors and submitted as BSc honours project reports to Glasgow University in 1997/1998.

Both of these experimental sets were developed from the experiments 1 to 4 already reported. These experimental sets were developed from concepts of introducing artificially clumped mussels into the field from Experiment 2 Page 6. Experiments in section 1.3.5.1 were also designed partly from observations made of clumps around boulders from frequent experimental field visits. Experiments in section 1.3.5.2 were designed from the concept behind experiment 2 Page 6, although the research was designed to investigate mussel behaviour on the field sand in more detail.

1.2.4.5.1) Experiment set I: large scale analysis of mussel bed formation at successively greater distances from the rocky shore

Aim of experiment

The aim of this experiment was to test the following hypotheses, which are phrased as questions:

- 1 Do the number of mussel beds and the size of mussel beds vary between the upper and lower intertidal zones?
- 2 Is there a difference between the areas covered by mussels and other organisms on boulders at the upper and lower intertidal zones?
- 2 Is there a difference in the numbers of mussels lost, comparing the upper intertidal and lower intertidal zones?
- 4 Is the orientation of mussel bed formation constrained by tidal forces?

Design and conduct of experiment

The experiment was conducted in November this would mean that the mussels would be exposed to the beginning of the winter storms thus fully testing their attachment to the substratum. The experimental designs of the three experiments are shown in Figures 18 to 20. The experiments were conducted in a 10m X 30m transect set out at both the upper and lower intertidal zones using trigonometry. A length of plastic coated twine had metal rings attached at points of, 5m, 10m and 11.18m. The rings were placed over bamboo canes to form a right-angled triangle I (Figure 21). Rings A and B were flipped over seaward to form a new triangle II. This was repeated flipping ring C to form another triangle III. Rings A and B were flipped over seaward a final time to form a new triangle IV.

Figure 18: Experimental design of experiment A showing the two main groups of mussels used in the experiment. The mussels in group 1 were laid into the 30m x 10m transect as individual mussels. One mussel was laid onto each of the four selected boulders in the transect (B1 - B4). On the boulders, the mussels were laid onto the surface cover, which was covering the greatest area of the boulder surface. For boulder 1 (B1) algae had the greatest cover on the boulders surface area (A). For boulder 2 (B2) mussels had the greatest cover on the boulders surface area (M). For boulder 3 (B3) barnacles had the greatest cover on the boulders surface area (B). For boulder 4 (B4) bare rock had the greatest cover on the boulders surface area (BA). In group 2 there were two mussels laid onto each of the boulders 5 to 8 (B5 to B8). On these boulders, there were two types of cover on the boulders surface area. One mussel was placed on each of the two types of cover. For boulder 5 (B5) algae and mussel had the greatest cover on the boulders surface area (A+M). For boulder 6 (B6) algae and barnacle had the greatest cover on the boulders surface area (A+B). For boulder 7 (B7) algae and bare rock had the greatest cover on the boulders surface area (A+BA). For boulder 8 (B8), mussel and barnacle had the greatest cover on the boulders surface area (M+B).

| | EXPERIMENT A CONDUCTED AT BOTH THE UI AND LI SITES | | | | | | | |
|---|--|----|----|----|-----|-----|------|-----|
| | BOULDER NUMBER | | | | | | | |
| | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 |
| GROUP 1 ONE MUSSEL Type of surface cover of boulder | A | M | B | BA | | | | |
| GROUP 2 TWO MUSSELS Type of surface cover of boulder | | | | | A+M | A+B | A+BA | M+B |

Figure 19: Experimental design of experiment B showing the two groups used in the experiment. In group 1 mussels were laid at 0cm from the boulder at orientations of North (N), South (S), East (E) and West (W). In group 2 mussels were laid at 15cm from the boulder at orientations of North East (NE), South East (SE), South West (SW) and North West (NW).

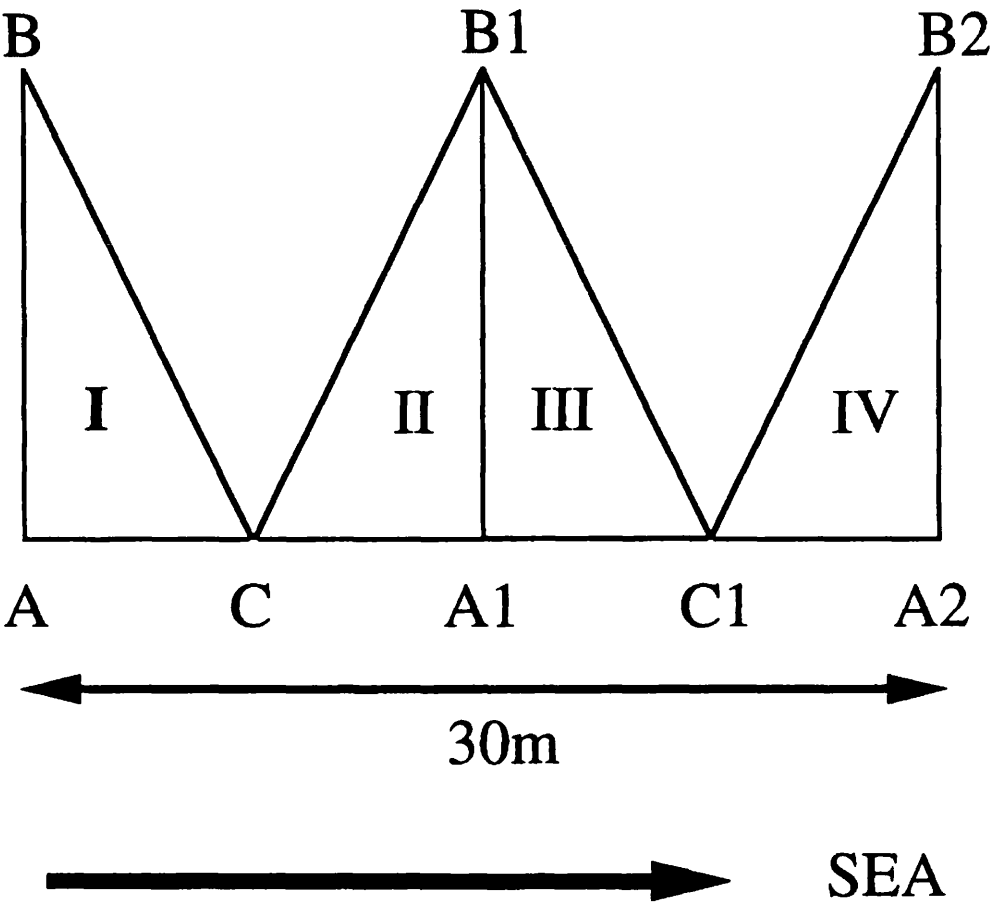
| | EXPERIMENT B CONDUCTED AT BOTH THE UI AND LI SITES | | | | | | | |
|--|--|-----|-----|-----|------|------|------|------|
| | ORIENATATION AROUND THE BOULDER | | | | | | | |
| | N | S | E | W | NE | SE | SW | NW |
| GROUP 1 ONE MUSSEL Distance from boulder | 0cm | 0cm | 0cm | 0cm | | | | |
| GROUP 2 ONE MUSSEL Distance from boulder | | | | | 15cm | 15cm | 15cm | 15cm |

Figure 20: Experimental design of experiment C showing the two groups used. In group 1 1 mussel was laid at 0cm from the boulder at orientations in line with the prevailing current at the front of the boulder (P.C./F) and behind the boulder (P.C./B). At right angles to the prevailing current to the right (P.C. + 90°/R) and to the left of the boulder (P.C. + 90°/L) orientated in the direction of the prevailing current.

| | EXPERIMENT C CONDUCTED AT BOTH THE UI AND LI SITES | | | | | | | |
|--|--|------|-------------|-------------|------|------|-------------|-------------|
| | ORIENATATION AROUND THE BOULDER | | | | | | | |
| | PC/F | PC/B | PC+90/ R | PC+90/ L | PC/F | PC/B | PC+90/ R | PC+90/ L |
| GROUP 1 ONE MUSSEL Distance from boulder | 0cm | 0cm | 0cm | 0cm | | | | |
| GROUP 2 ONE MUSSEL Distance from boulder | | | | | 15cm | 15cm | 15cm | 15cm |

At the upper and the lower intertidal sites 35 boulders were chosen randomly from each transect. The boulder positions were mapped using the canes as reference points. The distances between the boulders were also measured. These data were plotted onto graph paper to give a plan view of each transect. Perimeters of boulders and associated mussel beds were measured using string and a tape measure. The organisms covering each of the selected boulders was also recorded. There were four categories recorded, algae, mussel, barnacle and uncovered rock. Coverage of a particular category was only recorded when it was in contact with the boulder surface. These data were important for Experiment A when mussels were laid onto particular surfaces on the rock. In each of the intertidal sites, eight boulders were chosen for the highest percentage of each of the categories (B1 to B8). There were eight categories 1 for each boulder; A = Algae M = mussel, B = barnacle, BA = bare rock, A+M = algae plus mussel, A+B = algae plus barnacle, A+BA = algae plus bare rock and M+B = mussel plus barnacle. In total 64 mussels were collected from the shore and transported back to the laboratory. In the laboratory the mussels were cleaned and painted with a unique paint code. These were transported to the shore where four mussels were seeded onto each of the eight boulders at both tidal sites. Where a boulder had two types of cover two mussels were laid on each cover. The mussels were left for five days to form attachments. After five days the numbers remaining attached were recorded. There were two further seeding experiments designed to monitor attachment ability of mussels around the base of the boulder. Experiment B consisted of single mussels being laid close to the boulder or associated mussel bed. They were laid at bearings of north, south, east and west. These were interspersed with mussels laid at bearings of NE, SE, SW and NW at a distance of 15cm. The mussels were left for five days after which the numbers remaining were recorded. The initial and final positions of each mussel were also recorded and plotted onto graph paper.

Figure 21: Method used to construct the 30 x 10m quadrat at both the upper and lower intertidal sites used for experiments A, B and C. A length of plastic coated twine was used which had metal rings attached at points of, 5m, 10m and 11.18m. The rings were placed over bamboo canes to form a right-angled triangle I. Rings A and B were flipped over seaward to form a new triangle II. This was repeated flipping ring C to form another triangle III. Rings A and B were flipped over seaward a final time to form a new triangle IV.



Experiment C was similar to experiment B. Two mussels were laid in the direction of the prevailing current (P.C.) and two at right angles to this current (P.C. + 90°). The mussels were again laid with two placed 0cm from the boulder and two at 15cm from the boulder. After five days the number remaining were recorded. The data for the initial and final positions of the mussels were plotted onto graph paper.

1.2.5.5.2 Experimental set II: artificial formation of mussel beds on soft sediment by clumping

Aim of experiment

The aim of this experiment was to test the following hypotheses, which are phrased as questions:

- 1 Will mussels remain in position if laid directly onto soft sediment?
- 2 Will mussels attach to other mussels to form clumps?
- 3 Is there a difference between numbers of mussels lost comparing clumped and non-clumped mussels?

The experiment was split into three main sections. Experiment I was a preliminary study of mussel clumping on the shore. Experiment II was concerned with whether the numbers of mussels affected the clumping and whether they remain in position. Experiment III compared pre-formed clumps and un-clumped mussels for numbers remaining in position. Experiment II was conducted in November and experiment III was conducted in January. Both experiments would be subjected to winter storms which would fully test the mussels attachment to the substratum.

The design of preliminary Experiment I is shown in Figure 22. A total of 30 mussels were collected on the shore. These were then laid into four 0.5m^2 quadrats, four mussels per quadrat at the mid tide zone. There were four different conditions used for each quadrat. The conditions are as follows.

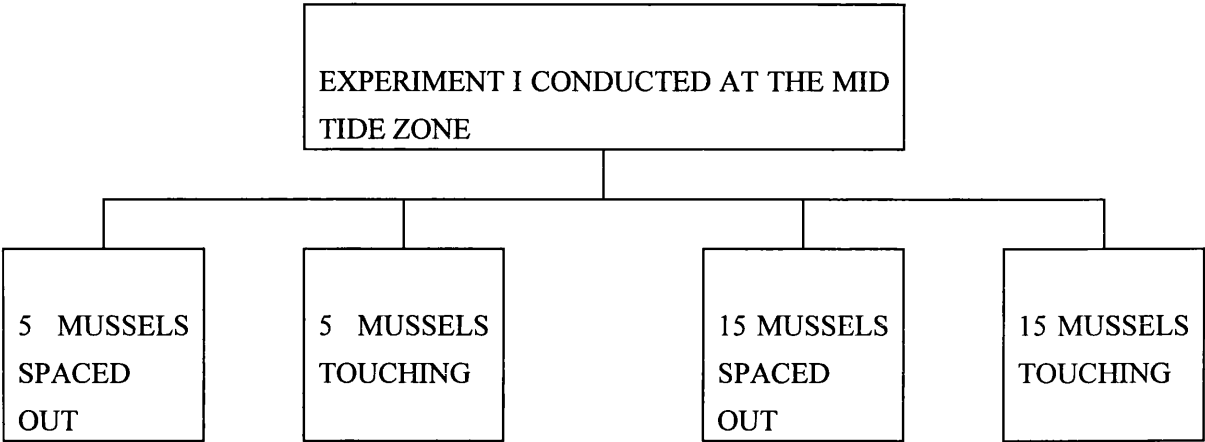
5 mussels spaced out and unattached.

5 mussels touching and unattached.

10 mussels spaced out and unattached.

10 mussels touching and unattached.

Figure 22: Experimental design of experiment I showing the 4 groups used. Group 1 consisted of 5 mussels laid onto the field sand as individual mussels with 1cm spacing between mussels. Group 2 consisted of 5 mussels laid onto the field sand as individual mussels with no space between the mussels. Group 3 consisted of 15 mussels laid onto the field sand as individual mussels with 1cm spacing between mussels. Group 4 consisted of 15 mussels laid onto the field sand as individual mussels with no spacing between the mussels.



Each of the condition terms will now be explained. Spaced out here means mussels are laid down with a 1cm space between individual mussels. Touching simply refers to the mussels being laid down in contact with other mussels in the quadrat. In all cases, the mussels are unattached, they are individual mussels with no byssus attachments with other mussels. The experiment was left for seven days. After this time, the numbers of mussels remaining within each quadrat was recorded. If mussels were missing from a quadrat, a search was initiated on a 360° radius of 3 m from the centre of the quadrat.

Experiment II was a development of the preliminary experiment, experiment 1. The experimental design is shown in Figure 23. This experiment used three different densities of mussel, single mussels, 5 mussels and 15 mussels. In total 220 mussels were collected and transported back to the laboratory. In the laboratory the mussels were cleaned and painted with a unique paint code for identification. The mussels were maintained in seawater overnight then transported to the shore the next day. There were two rows of 1m² quadrats set up at both an upper intertidal and a lower intertidal site with ten quadrats per row and 2m in-between the rows. Mussels were identified by their paint codes and placed in the centre of their designated quadrats. There were two replicates for each of the 5 conditions. These were as follows.

Single mussel

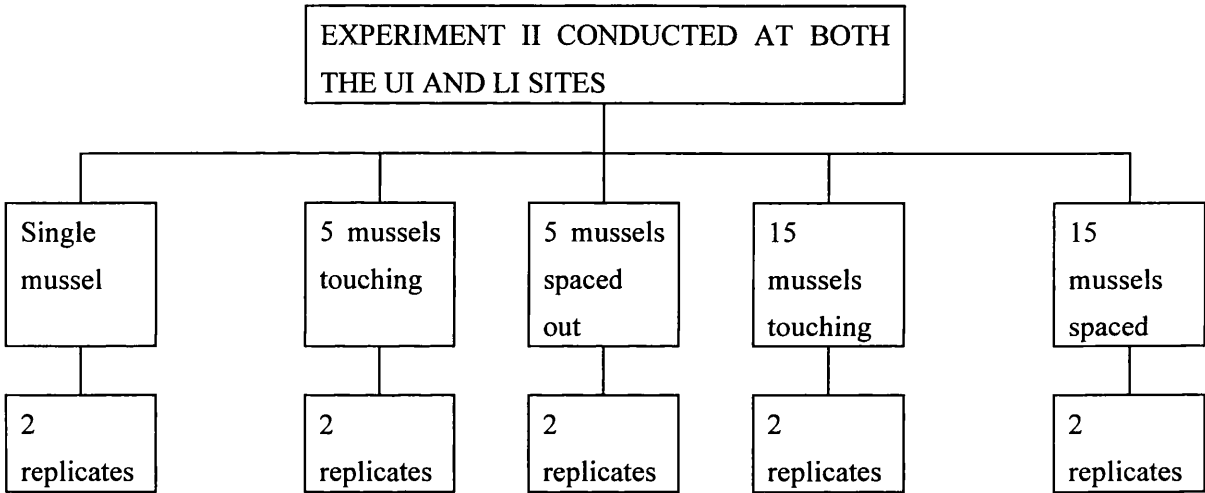
5 mussels touching

5 mussels spaced out

15 mussels touching

15 mussels spaced out

Figure 23: Experimental design of experiment II showing the 5 groups of mussels used. Group 1 consisted of individual mussels laid onto field sand with two replicates at both the upper and lower intertidal. Group 2 consisted of 5 mussels laid onto field sand with no space between the individual mussels with two replicates at the upper and lower intertidal. Group 3 consisted of 5 mussels laid onto field sand with a 1cm space between individual mussels with two replicates at the upper and lower intertidal. Group 4 consisted of 15 mussels laid onto field sand with no space between individual mussels with two replicates at the upper and lower intertidal sites. Group 5 consisted of 15 mussels laid onto field sand having a 1cm space between individual mussels with two replicates at both the upper and lower intertidal.



The placement of the different conditions in the quadrats was randomised using random number Tables. The experiment was left for eight days. After this time numbers of mussels remaining inside and outside each quadrat were recorded. The distance travelled from the centre of the quadrat was calculated using the trigonometry method from experiment 2 section 1.3.2. The data were plotted onto graph paper. The organisms were taken back to the laboratory in labelled bags and numbers of threads attached between mussels in the clumps were recorded.

Experiment III used clumps of attached mussels that were formed in the laboratory. In total 300 mussels were collected for the experiment. The experimental design is shown in Figure 24. The experiment consisted of three main conditions. The first condition was the mussels being laid in the laboratory and the 5 and 15 mussel groups were allowed to form clumps. In the second condition mussels were laid down in the centre of the quadrat with 1cm spaces between the mussels. The third and final condition mussels were laid down in the centre of the quadrat with all the mussels touching. There were three rows of quadrats on the shore two containing five quadrats and a third containing six quadrats. The third row was set up three metres north in line with the seaward row. The allocation of quadrats to each of the conditions, which had two replicates per condition, was achieved using random number Tables. The mussels in the laboratory were left for seven days to form clumps. During this time, the other mussels collected were maintained in seawater. The mussels were placed in labelled polythene bags and transported to the shore. The duration of the experiment was 24 days. Measurements were taken on days six, ten and twenty-four.

Figure 24: Experimental design of experiment III showing the three experimental groups used. Group 1 consisted of mussels laid onto sand in the laboratory allowed to form clumps then the clumps were transported and laid onto the field sand. This was done for individual mussels groups of 5 mussels and groups of 15 mussels with two replicates each. Group 2 consisted of mussels laid onto field sand with a 1cm space between mussels at both the upper intertidal and the lower intertidal. This was done for individual mussels groups of 5 mussels and groups of 15 mussels with two replicates each. Group 3 consisted of mussels laid onto field sand having no space between mussels at both the upper intertidal and the lower intertidal. This was done for individual mussels groups of 5 mussels and groups of 15 mussels with two replicates each.

| EXPERIMENT 3 CONDUCTED AT BOTH THE UI AND LI SITES | | | | | | | | | |
|--|-------------|----|----|-------------|----|----|-------------|----|----|
| | Condition 1 | | | Condition 2 | | | Condition 3 | | |
| Laboratory | 1 | 5 | 15 | - | - | - | - | - | - |
| | ↓ | ↓ | ↓ | | | | | | |
| Field | 1 | 5 | 15 | 1 | 5 | 15 | 1 | 5 | 15 |
| Replicates | X2 | X2 | X2 | X2 | X2 | X2 | X2 | X2 | X2 |

1.3) RESULTS

The results are separated into five sections to correspond to the five experiments described in the materials and methods section. Firstly, a laboratory-based experiment using gravel (experiment 1). Second, a field experiment using gravel (experiment 2). Third, a laboratory based experiment using plastic mesh (experiment 3). Fourth, a field set of experiments using mesh (experiment 4). Finally, joint field investigations (section 1.2.4.5).

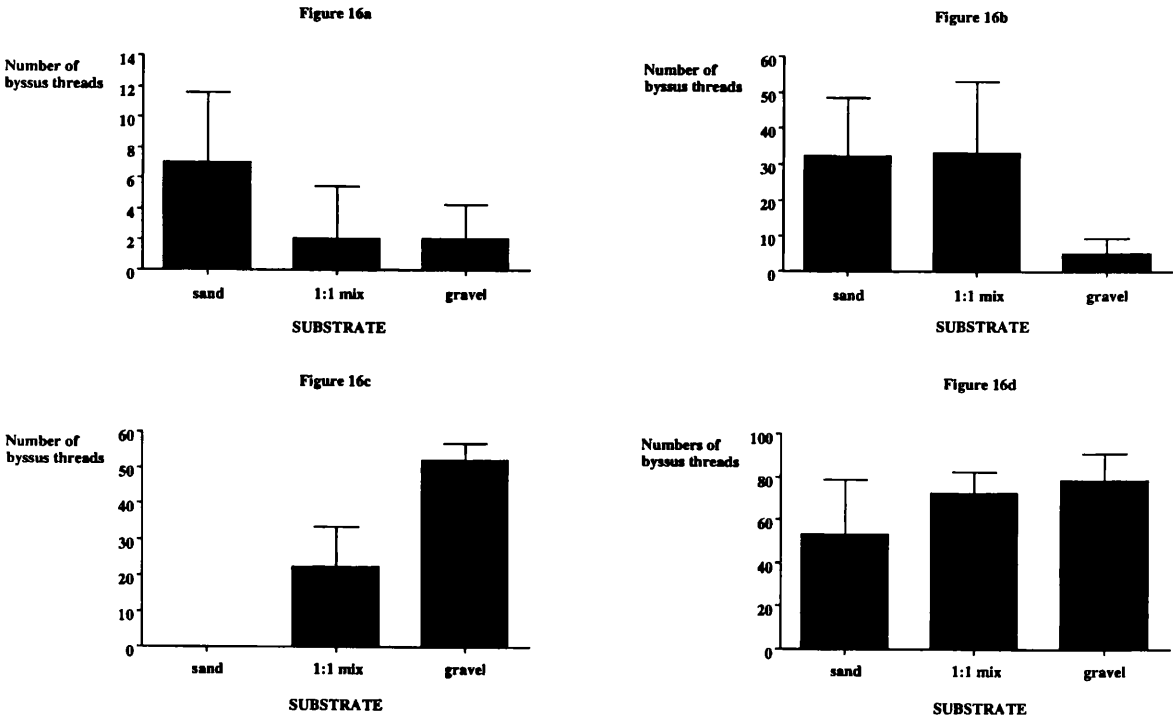
1.3.1) Experiment 1 laboratory investigation of *Mytilus edulis* clumping behaviour on three different substratum, gravel, sand and an equal mix of both

The results of the experiment are shown in Figure 25, and analysed in Table 4. There are highly significant differences in the pattern of thread production by the mussels on sand, gravel and a 1:1 mix of sand and gravel substrata. The following statements are based on the four, one way analysis of variance in Table 4, one of which was statistically significant. The lowest number of threads was produced by mussels on sand, intermediate numbers by mussels on sand + gravel mix and highest numbers on gravel, (Figure 25 D. and Table 4 : $F = 1.68$, d.f. = 2, $0.30 > P > 0.25$ n.s.). Mussels attached the greatest number of threads to their own shells when on sand, fewer on the sand + gravel and least on gravel (Fig. 25 A and Table 4: $F = 2.25$, d.f. = 2, $0.20 > P > 0.15$ n.s.). The numbers of threads attached onto shells of other mussels on sand and on the 1:1 mix of sand and gravel were very similar, on gravel there were very few threads attached to other mussels shells (Fig. 25 B and Table 4: $F = 3.35$, d.f. = 2, $0.15 > P > 0.10$ n.s.). More threads were attached to gravel sized grains on gravel than on 1:1 mix of sand and gravel and no threads were attached to gravel sized particles on sand (Fig. 25 C and Table 4: $F = 39.84$, d.f. = 2, $P < 0.001^{***}$).

Table 4. Laboratory experiment 1 using groups of five *Mytilus edulis*. One way analysis of variance comparing the numbers of byssus threads attached to different surfaces for three types of sediment. S/SG/G = sand/sand plus gravel mix/gravel. d.f. = Degrees of freedom. *F* = variance ratio and *P* = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non-asterisked probabilities are not significant.

| Comparison | Source of variation | d.f. | Sum of squares | Mean squares | <i>F</i> | <i>P</i> |
|-------------------------|---------------------|------|----------------|--------------|----------|-------------|
| Threads to own shell | S/SG/G | 2 | 57.6 | 28.8 | 2.25 | 0.20>P>0.15 |
| | Error | 6 | 76.7 | 12.8 | | |
| | Total | 8 | 134.2 | | | |
| Threads to other shells | S/SG/G | 2 | 1461 | 730 | 3.35 | 0.15>P>0.10 |
| | Error | 6 | 1309 | 218 | | |
| | Total | 8 | 2770 | | | |
| Threads to grains | S/SG/G | 2 | 4028.7 | 2014.3 | 39.84 | P<0.001*** |
| | Error | 6 | 303.3 | 50.6 | | |
| | Total | 8 | 4332.0 | | | |
| Total number of threads | S/SG/G | 2 | 736 | 368 | 1.68 | 0.30>P>0.25 |
| | Error | 6 | 1313 | 219 | | |
| | Total | 8 | 2049 | | | |

Figure 25: Four plots showing the mean numbers of byssus threads produced by *Mytilus edulis* onto different surfaces for three different substrata. The mean number of byssus threads attached onto mussels own shell for 5 mussels with three replicates each laid onto fine sand particle size < 500µm, gravel particle size between 4mm and 8mm and a 1:1 mix of these two sediments (A). The mean number of byssus threads attached from one mussel onto another mussels shell for 5 mussels with three replicates each laid onto fine sand particle size < 500µm, gravel particle size between 4mm and 8mm and a 1:1 mix of these two sediments (B). The mean number of threads attached to gravel sized grains for 5 mussels with three replicates each laid onto fine sand particle size < 500µm, gravel particle size between 4mm and 8mm and a 1:1 mix of these two sediments (C). The mean total number of byssus threads produced for 5 mussels with three replicates each laid onto fine sand particle size < 500µm, gravel particle size between 4mm and 8mm and a 1:1 mix of these two sediments (D).



Investigation of clumping behaviour

The results of the clumping pattern investigation are shown in Table 5 and Figures 26 to 29. The three replicates of mussels laid onto fine sand were found to all form tight clumps inside the initial pattern area (Figure 27). In general, the mussels laid onto the 1:1 mix of fine sand and gravel formed more spread out clumps (Figure 28). Two of the three replicates formed clumps inside the initial pattern area however, one replicate contained one clump inside the initial pattern area and two individual mussels outside. The three replicates of mussels laid onto the gravel substrate showed the greatest variation in pattern (Figure 29). Two of the three replicates contained individual mussels outside the initial pattern area. A Chi-squared analysis was conducted on the numbers of mussels remaining inside the initial pattern area. This revealed no significant difference between the three substrata ($\chi^2 = 0.789$, d.f. = 4, $1.0 > P > 0.9$ n.s.).

Table 5a: Laboratory experiment 1 using groups of 5 *Mytilus edulis*. Data for number of mussels inside the initial mussel pattern area for the three replicates of the three sediments.

| REPLICATES | SUBSTATES | | |
|-------------|-----------|----------------------|--------|
| | FINE SAND | FINE SAND/GRAVEL MIX | GRAVEL |
| REPLICATE 1 | 5 | 3 | 4 |
| REPLICATE 2 | 5 | 5 | 3 |
| REPLICATE 3 | 5 | 5 | 5 |

Table 5b: Laboratory experiment 1 using groups of 5 *Mytilus edulis*. Chi-squared (χ^2) statistical analysis of numbers of mussels inside the initial mussel pattern area for the three replicates from the three different sediments. D.f. = degrees of freedom. *P* = probability. Probabilities given are asterisked significant according to the following system. 0.05>*P*>0.01*, 0.01>*P*>0.001** and *P*<0.001***. All non-asterisked probabilities are not significant.

| Comparison | d.f. | χ^2 | <i>P</i> |
|--|------|----------|--------------------|
| All replicates fine sand/mix/gravel | 4 | 0.789 | 1.0> <i>P</i> >0.9 |

Figure 26: Figure showing the initial pattern arrangement of groups of five mussels added to 9 19cm ID circular dishes in experiment 1. The dashed circle drawn around the mussels indicates the extent of the initial pattern area used to determine movement of mussels over the duration of the experiment.

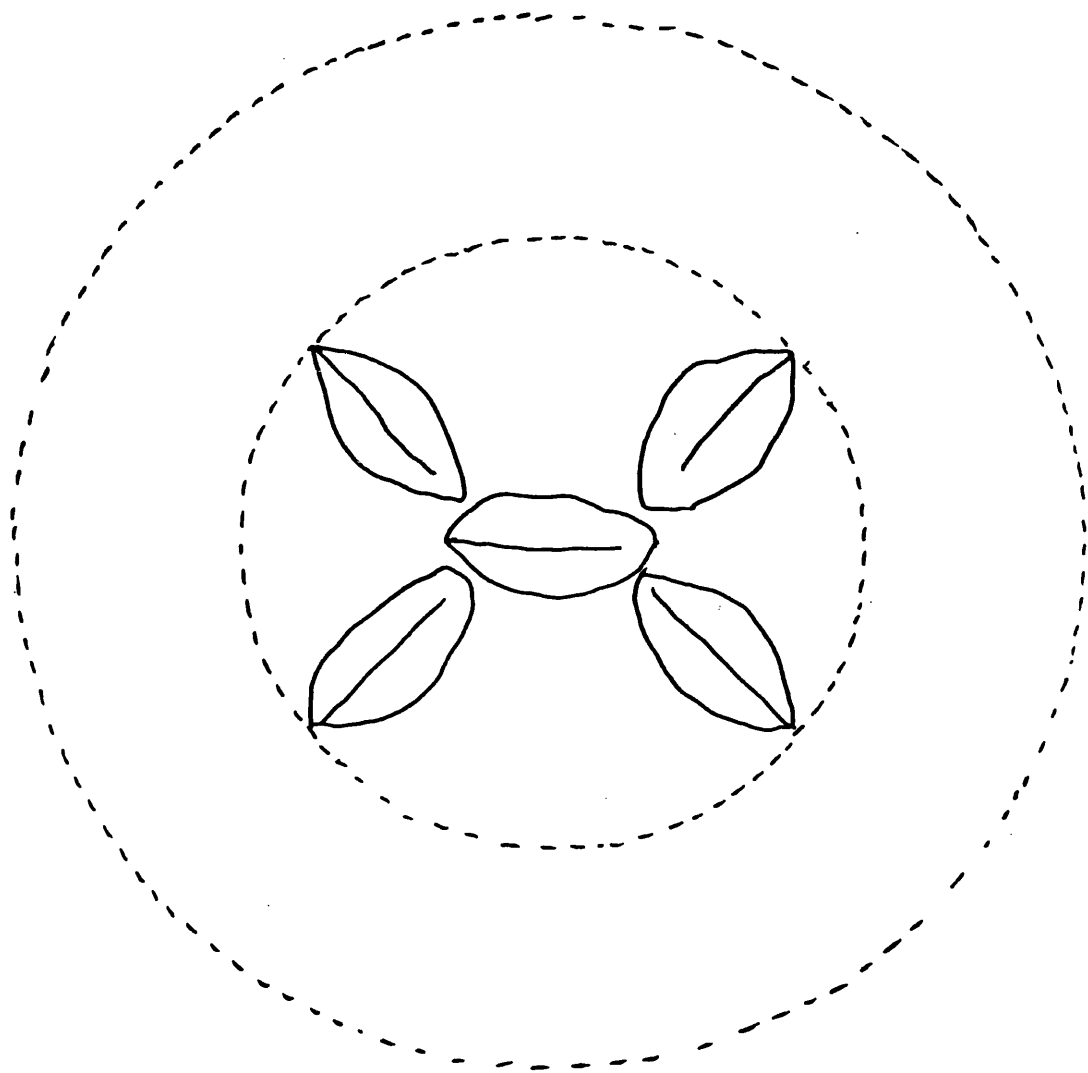


Figure 27: Figure showing the patterns of mussels after five days that had been laid onto fine sand. The three replicates are shown 1, 2, 3 as A, B and C respectively. Mussels are outline by solid line, byssus threads are shown by dashed lines and byssus pads are shown with solid fill.

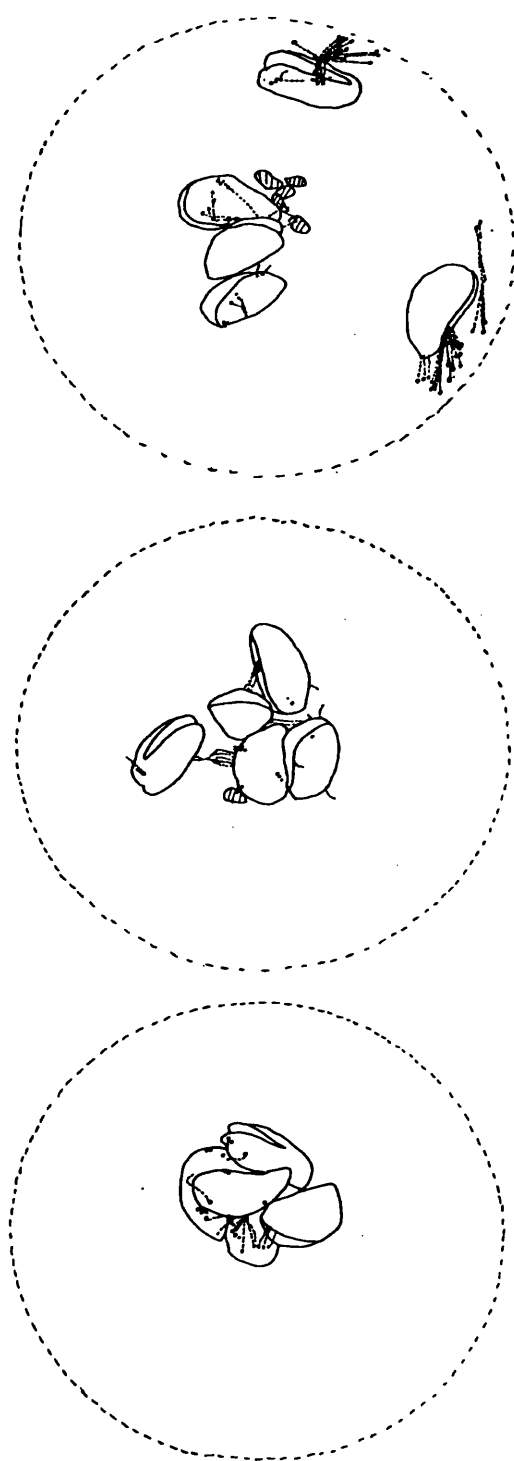


Figure 28: Figure showing the patterns of mussels after five days that had been laid onto the fine sand and gravel 1:1 mix. The three replicates are shown 1, 2, 3 as A, B and C respectively. Mussels are outline by solid line, byssus threads are shown by dashed lines and byssus pads are shown with solid fill.

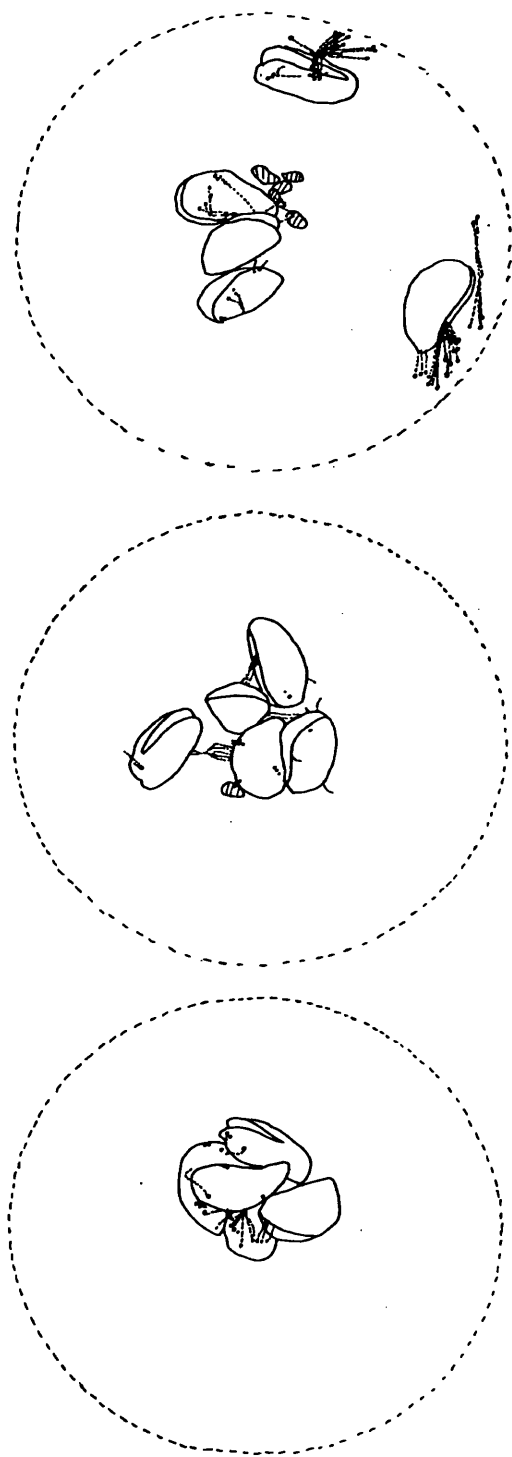
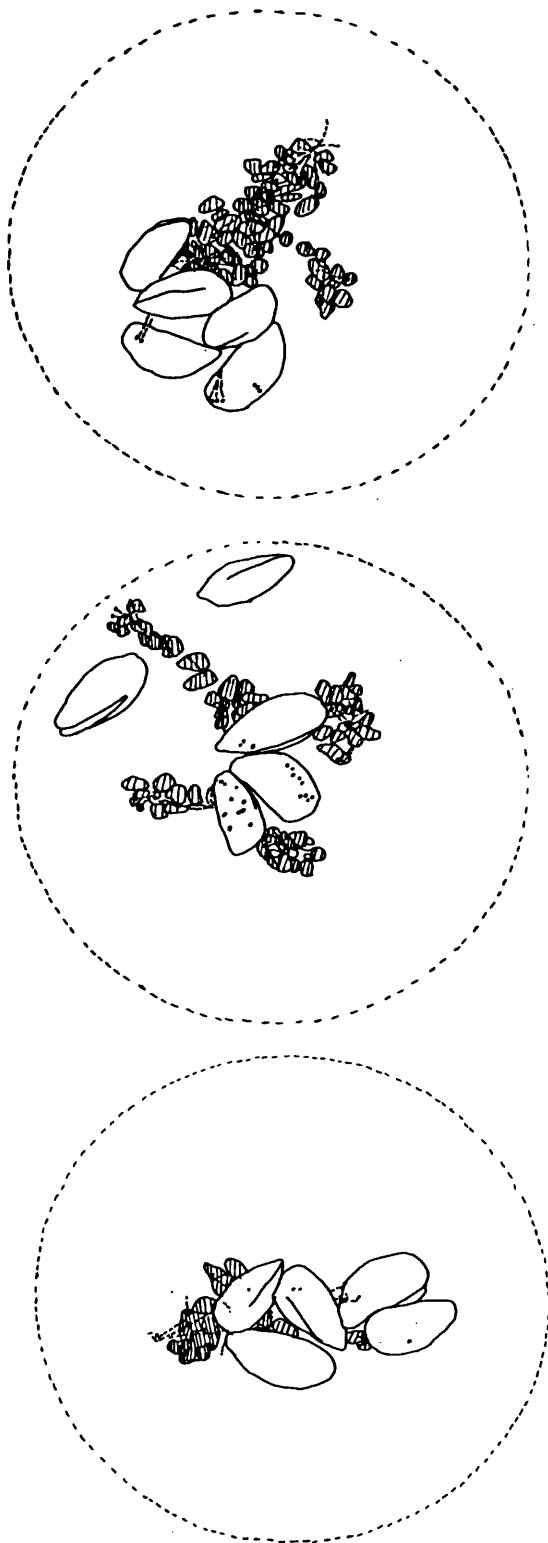


Figure 29: Figure showing the patterns of mussels after five days that had been laid onto gravel. The three replicates are shown 1, 2, 3 as A, B and C respectively. Mussels are outline by solid line, byssus threads are shown by dashed lines and byssus pads are shown with solid fill.



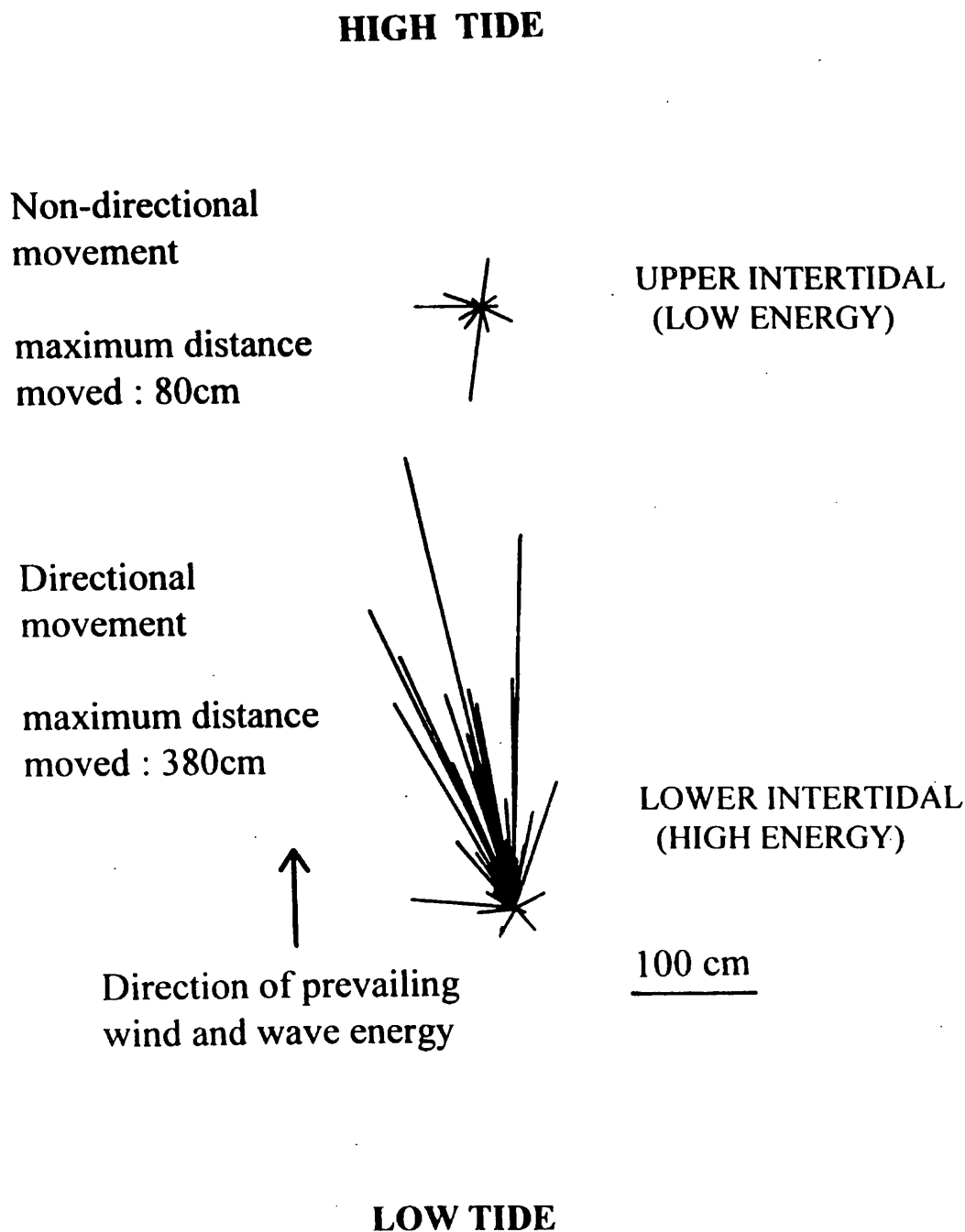
1.3.2) Experiment 2 Intertidal field study of attachment and loss of *Mytilus edulis* to gravel introduced to a sandy shore

The results for the gravel substrate field experiment are shown in the diagram (Figure 30) and Tables 6 to 10.

Transportation of mussels on the shore

In Figure 30 the differences between the low energy upper intertidal and the high energy lower intertidal sites for the distance and direction that mussels were transported on the shore is clearly shown. In the upper intertidal the mussels are transported smaller distances than in the lower intertidal environment (maximum distance: 80cm and 380cm respectively). The pattern of transport is also a clear contrast in both environments. In the upper intertidal environment, there is a distinctly random pattern. In comparison, the lower intertidal environment shows a directional transportation pattern in the direction of the prevailing wind and currents. A series of One-way analyses of variance was conducted on the distances the mussels were transported at the UI and LI sites for day 3 and day 18 of the experiment (Table 6). The comparisons of UI and LI on day 3 and day 18 both showed significant differences. This clearly substantiates the data displayed in Figure 30. The comparison of UI and LI between day 3 and day 18 for each tidal level gave only one significant difference (Table 6 UI_{d3}/UI_{d21}: $F=8.43$, d.f. = 1, $0.01 > P > 0.001^{**}$). This means that for the UI there was a significant difference in the distances that mussels were transported between day 3 and day 18. The converse was true for the LI site, as this showed no significant difference in the distance that mussels were transported between day 3 and day 18 (Table 6 LI_{d3}/LI_{d21}: $F= 0.21$, d.f. = 1, $P > 0.05$ n.s.). A more detailed set of One-way analyses of variance was then conducted for each tidal site on day 3 and then day 18. The comparison analysed the effect of the six substrata (Figure 12, page 47) used in the experiment on distances that mussels were transported on the shore. The comparison of UI on day 18 showed significant differences between the six substrata (Table 6 UI_{d18all}: $F= 4.94$, d.f. = 1 $0.01 > P > 0.001^{**}$). The upper intertidal comparison of the six

Figure 30: Figure showing the movement and direction that mussels were transported on the shore at both the upper and lower intertidal in experiment 2. The upper intertidal low energy site displays a random pattern of transport, the maximum distance that mussels were transported was 80cm. The lower intertidal high-energy site displays a directional pattern of transport in the direction of the prevailing wind and wave energy. The maximum distance that mussels were transported at the lower intertidal was 380cm.



substrata showed no significant difference between the six substrata on day 3 (Table 6 UIId3all: $F=1.30$, d.f. = 1, $P>0.05$ n.s.).

The direction mussels were transported shown in Figure 30 was further analysed using a Chi squared statistical test. The 360° transportation area was split into 45° sections to focus more clearly on statistical differences. The data are analysed statistically in Table 7. In general, there are significant differences in space but not over time. The comparisons over time show that there is no significant difference between the initial measurements on day 3 and the final condition on day 18. This was the case for both the upper intertidal and lower intertidal ($\chi^2 = 6.875$, d.f. = 7, $0.4 > p > 0.5$ n.s. and $\chi^2 = 10.324$, d.f. = 7, $0.1 > p > 0.2$ n.s. respectively). The spatial comparisons showed significant differences between the upper intertidal and the lower intertidal sites. The day three comparison between the upper intertidal site and the lower intertidal site was highly significant ($\chi^2 = 40.565$, d.f. = 7, $p < 0.001^{***}$). The day 18 comparison between the upper intertidal site and the lower intertidal site was also significant ($\chi^2 = 23.091$, d.f. = 7, $0.01 > p > 0.001^{**}$).

Table 6: Experiment 2 *Mytilus edulis* field experiment. One-way analysis of variance conducted on the distances mussels were transported at the upper and lower intertidal sites for day 3 and day18 of the experiment. UI = upper intertidal. LI = lower intertidal. d.f. = degrees of freedom. F = variance ratio. *P* = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non-asterisked probabilities are not significant.

| Comparison | Source of variation | d.f. | Sum of squares | Mean squares | F | <i>P</i> |
|--|-------------------------------|-----------------|---------------------------|---------------|-------|-------------------------|
| UI day 3 Compared to LI day 3 | htd3/ltd3 Error Total | 1 125 126 | 90749 468837 559585 | 90749 3751 | 24.20 | $P < 0.001^{***}$ |
| UI day 18 Compared to LI day18 | htd18/ltd18 Error Total | 1 100 101 | 46059 499298 545357 | 46059 4993 | 9022 | $0.01 > P > 0.001^{**}$ |
| UI day 3 Compared to UI day 18 | htd3/htd18 Error Total | 1 108 109 | 9156 117258 126415 | 9156 1086 | 8.43 | $0.01 > P > 0.001^{**}$ |
| LI day 3 Compared to LI day 18 | ltd3/ltd18 Error Total | 1 117 118 | 1510 850877 852387 | 1510 7272 | 0.21 | $P > 0.05$ |
| UI day 3 all substrata compared | htd3all Error Total | 5 53 58 | 880 7183 8063 | 176 136 | 1.30 | $0.30 > P > 0.20$ |
| LI day 3 all substrata compared | ltd3all Error Total | 5 72 77 | 31359 476877 508235 | 6272 6623 | 0.95 | $0.50 > P > 0.40$ |
| UI day 18 all substrata compared | htd18all Error Total | 5 45 50 | 38691 70504 109195 | 7738 1567 | 4.94 | $0.01 > P > 0.001^{**}$ |
| LI day 18 all substrata compared | ltd18all Error Total | 5 45 50 | 73586 316517 390103 | 14717 7034 | 2.09 | $0.09 > P > 0.08$ |

Table 7: Experiment 2 *Mytilus edulis* field experiment. Chi-squared (χ) statistical analysis of the direction that mussels were transported on the shore at both the upper and lower intertidal for day 3 and day 18. UI = upper intertidal. LI = lower intertidal. D.f. = degrees of freedom. P = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non-asterisked probabilities are not significant.

| Comparison | d.f. | χ^2 | P |
|---------------------------|------|----------|-------------------------|
| Day 3 UI Vs Day 18 UI | 7 | 6.875 | $0.50 > P > 0.40$ |
| Day 3 LI Vs Day 18 LI | 7 | 10.324 | $0.20 > P > 0.10$ |
| Day 3 UI Vs Day 3 LI | 7 | 40.565 | $P < 0.001^{***}$ |
| Day 18 UI Vs Day 18 LI | 7 | 23.091 | $0.01 > P > 0.001^{**}$ |

Loss of seeded mussels on the shore

The loss of mussels after 18 days exposure on the shore is given in Table 8 along with the statistical analysis of the data. Mussels were deemed lost only after a thorough search of a 10-metre diameter area with its centre at the centre of the quadrat. At the upper intertidal site there was a significant difference (Table 8b: $\chi^2 = 18.19$, d.f. = 2, $P < 0.001^{***}$) between numbers lost from quadrats containing 1, 5 and 15 animals. The greatest number being lost from the single animal quadrats (42%), fewer from the clumps of five animals (15%) and least from the clumps of fifteen animals (6%). In the lower intertidal environment there was no significant difference between the three animal densities (Table 8a row 9: 67%, 77%, 84%, Table 8b: $\chi^2 = 3.337$, d.f. = 2, $0.20 > P > 0.10$ n.s.). Comparing animal densities at the upper and lower intertidal sites showed that single animals had no significant difference between tidal levels ($\chi^2 = 1.510$, d.f. = 1, $0.30 > P > 0.20$ n.s.). There was a significant difference between the UI and LI sites for the 5 and 15 animal clumps ($\chi^2 = 45.95$, d.f. = 1, $P < 0.001^{***}$ and $\chi^2 = 220.0$, d.f. = 1, $P < 0.001^{***}$ respectively).

Table 8a: Table showing the numbers of mussels lost from the upper and lower intertidal sites comparing densities of individual mussels, groups of 5 mussels and groups of 15 mussels.

| | Clump size | | |
|-----------------------|---------------|----------|-----------|
| | Single mussel | 5 mussel | 15 mussel |
| Upper intertidal site | | | |
| Number lost | 5 | 9 | 11 |
| Total | 12 | 60 | 180 |
| Percentage lost | 42% | 15% | 6% |
| Lower intertidal site | | | |
| Number lost | 8 | 46 | 151 |
| Total | 12 | 60 | 180 |
| Percentage lost | 67% | 77% | 84% |

Table 8b: Experiment 2 statistical analysis of data by Chi-squared (χ^2). R/L = numbers remaining/numbers lost. d.f. = Degrees of freedom. P = probability. UI = Upper intertidal site. LI = Lower intertidal site. Probabilities given are asterisked significant according to the following system. $0.05>P>0.01^*$, $0.01>P>0.001^{**}$ and $P<0.001^{***}$. All non-asterisked probabilities are not significant.

| Data | Comparison | χ^2 | d.f | P |
|----------------|-------------------|----------|-----|-----------------|
| UI site | R/L versus 1/5/15 | 18.19 | 2 | $P<0.001^{***}$ |
| LI site | R/L versus 1/5/15 | 3.337 | 2 | $0.20>p>0.10$ |
| Single animals | R/L versus UI/LI | 1.510 | 1 | $0.30>p>0.20$ |
| Clumps of 5 | R/L versus UI/LI | 45.95 | 1 | $P<0.001^{***}$ |
| Clumps of 15 | R/L versus UI/LI | 220.0 | 1 | $P<0.001^{***}$ |

Effect of gravel on mussel loss

Table 9 shows and statistically documents the results for mussels that were exposed to a gravel substrate in the laboratory and then seeded into the field. The Chi-squared analysis in Table 9 shows that animals exposed to gravel in the laboratory before seeding into the field lost significantly fewer animals. Table 9a UI: 2% compared to 14% and 13% $2 \times 3 \chi^2 = 8.08$, d.f. = 2, $0.05 > P > 0.01^*$; LI: 6% compared with 31% and 19% $2 \times 3 \chi^2 = 19.381$, d.f. = 2, $P > 0.001^{***}$. The remaining χ^2 analyses also substantiate this conclusion.

Table 9a: Table showing the numbers of mussels lost for the upper and lower intertidal sites comparing the presence or absence of a gravel substrate for individual mussels, groups of 5 mussels and groups of 15 mussels. G→G = mussels laid onto gravel in the laboratory allowed to form clumps then the clumps were transported and laid onto gravel in the field. G→S = mussels laid onto gravel in the laboratory allowed to form clumps then the clumps were transported and laid onto sand in the field. S→G = mussels laid onto sand in the laboratory allowed to form clumps then the clumps were transported and laid onto gravel in the field. S→S = mussels laid onto sand in the laboratory allowed to form clumps then the clumps were transported and laid onto sand in the field. G = mussels laid as individual mussels with no space between the mussels directly into the field onto gravel. S = mussels laid as individual mussels with no space between the mussels directly into the field onto sand.

| | | Gravel treatment | | |
|-----------|------------|------------------|-------------|----------|
| | | G→G and G→S | S→G and S→S | G and S |
| Upper | intertidal | | | |
| site | | | | |
| Remaining | | 82 | 72 | 73 |
| Lost | | 2 (2%) | 12 (14%) | 11 (13%) |
| Total | | 84 | 84 | 84 |
| Lower | intertidal | | | |
| site | | | | |
| Remaining | | 79 | 58 | 68 |
| Lost | | 5 (6%) | 26 (31%) | 11 (19%) |
| Total | | 84 | 84 | 84 |

Table 9b: Table showing the Chi-squared (χ^2) statistical analysis of the numbers of mussels lost from the upper and lower intertidal sites comparing the presence or absence of gravel. R/L = numbers of mussels remaining Vs numbers of mussels lost. d.f. = degrees of freedom. P = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non-asterisked probabilities are not significant.

| Tidal level | comparison | χ^2 | d.f | p |
|-----------------------|--|----------|-----|-------------------------|
| Upper intertidal site | R/L versus G+G and G+S/S+G and S+S/G and S | 8.08 | 2 | $0.05 > P > 0.01^*$ |
| | R/L versus G+G and G+S/S+G and S+S | 7.792 | 1 | $0.01 > P > 0.001^{**}$ |
| | R/L versus G+G and G+S/G and S | 6.753 | 1 | $0.01 > P > 0.001^{**}$ |
| | R/L versus S+G and S+S/G and S | 0.050 | 1 | $0.85 > P > 0.80$ |
| Lower intertidal site | R/L versus G+G and G+S/S+G and S+S/G and S | 19.381 | 2 | $P > 0.001^{***}$ |
| | R/L versus G+G and S+S/S+G and S+S | 17.445 | 1 | $P > 0.001^{***}$ |
| | R/L versus G+G and G+S/G and S | 2.923 | 1 | $0.090 > P > 0.080$ |
| | R/L versus S+G and S+S/G and S | 6.728 | 1 | $0.01 > P > 0.001^{**}$ |

Loss and gain of gravel particles by seeded animals in the field

The loss and gain of gravel particles after 18 days exposure on the shore is documented in Table 10a. A Chi-squared analysis was used to assess any differences between numbers lost from different clump sizes and between the upper and lower intertidal environments. The clumps of animals seeded directly onto gravel in the field picked up greater numbers of gravel particles, of these clumps of 15 picked up the greatest (Table 10a row. 12). Animals at the lower intertidal site picked up significantly fewer particles of gravel than those at the upper intertidal site (Table 10b: one animal $\chi^2 = 33.014$, d.f. = 3, $p < 0.001^{***}$; five animals $\chi^2 = 35.319$, d.f. = 4, $p < 0.001^{***}$ and 15 animals $\chi^2 = 289.775$, d.f. = 5, $p < 0.001^{***}$). The animals seeded onto sand picked up a small number of gravel sized particles. Table 10a rows 4-7 shows the data for the loss of numbers of gravel grains attached to mussels exposed to a gravel substrate in the laboratory then seeded onto gravel (G→G) or sand (G→S) in the field. These showed significant differences in numbers of gravel grains remaining at both the upper and lower intertidal sites. The clumps of fifteen animals showed the greatest number of gravel grains attached, followed by clumps of five, with the least being attached to single animals. This was true for both the upper and the lower intertidal sites. The data were analysed by Chi-squared analysis comparing the numbers of gravel particles before and after field seeding for the three different animal densities (Table 10c). The analysis was applied to the G→G and the S→S data at both the upper and the lower intertidal sites. All four of these Chi-squared analyses were found to be significant. This means that the three different densities of animals lost or gained very different proportions of gravel particles. These are expressed as percentages of gravel particles before and after seeding. In all four cases single animals lose gravel grains in the field (83%, 53%, 47% and 74%). The clumps of five animals showed that three lost gravel and one clump gained gravel (56%, 68%, 65% and 162%). All of the clumps of fifteen animals gained particles (221%, 228%, 117% and 137%).

Table 10a: Table showing the number of gravel particles attached to byssus threads at the beginning and at the end of experiment 2. Data for the upper and lower intertidal sites and the three mussel densities of individual mussels, groups of 5 mussels and groups of 15 mussels. Other notations as in the description for Table 9a.

| | | | UI | | | LI | | |
|------------|--------------|--------|-------|--------|--------|-------|-------|--------|
| | | | 1 | 5 | 15 | 1 | 5 | 15 |
| Lab | Field | | | | | | | |
| G → | G | before | 36 | 50 | 146 | 32 | 66 | 134 |
| | | After | 30 | 81 | 322 | 17 | 37 | 306 |
| | | | (83%) | (162%) | (221%) | (53%) | (56%) | (228%) |
| G → | S | before | 43 | 47 | 163 | 46 | 62 | 114 |
| | | After | 20 | 32 | 191 | 34 | 40 | 156 |
| | | | (47%) | (68%) | (117%) | (74%) | (65%) | (137%) |
| S → | G | before | 0 | 0 | 0 | 0 | 0 | 0 |
| | | After | 11 | 105 | 208 | 5 | 39 | 117 |
| S → | S | before | 0 | 0 | 0 | 0 | 0 | 0 |
| | | After | 0 | 0 | 3 | 0 | 0 | 0 |
| -- | G | before | -- | -- | -- | -- | -- | -- |
| | | After | 30 | 45 | 425 | 0 | 5 | 64 |
| -- | S | before | -- | -- | -- | -- | -- | -- |
| | | after | 0 | 8 | 1 | 0 | 0 | 0 |

Table 10b: Table showing the Chi-squared (χ^2) statistical analysis of numbers of gravel grains attached to mussel byssus threads. 1 mussel Vs UI/LI = the numbers of gravel grains attached to the individual mussels comparing the upper intertidal (UI) and lower intertidal sites (LI). 5 mussels Vs UI/LI = the number of gravel grains attached to the groups of 5 mussels comparing the upper intertidal (UI) and the lower intertidal (LI) sites. 15 mussels Vs UI/LI = the number of gravel grains attached to the groups of 15 mussels comparing the upper intertidal (UI) and the lower intertidal (LI) sites. d.f. = degrees of freedom. P = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non asterisked probabilities are not significant.

| Comparison | χ^2 | d.f. | P |
|---------------------|----------|------|-------------------|
| 1 mussel Vs UI/LI | 33.014 | 3 | $P < 0.001^{***}$ |
| 5 mussels Vs UI/LI | 35.319 | 4 | $P < 0.001^{***}$ |
| 15 mussels Vs UI/LI | 289.775 | 5 | $P < 0.001^{***}$ |

Table 10c: Table showing the Chi-square (χ^2) Statistical analysis of differences in number of gravel particles lost or gained by 1 animal, clumps of 5 animals and clumps of 15 animals (1/5/15 respectively). B/A = number of gravel particles attached to mussels after laboratory clumping prior to field seeding/ number of gravel particles attached to mussels after 18 days exposure to field conditions. d.f. = degrees of freedom and P = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non asterisked probabilities are not significant.

| Comparison | χ^2 | d.f. | p |
|------------------------------|----------|------|-------------------------|
| Upper intertidal site | | | |
| G+G B/A versus 1/5/15 | 14.66 | 2 | $P < 0.001^{***}$ |
| G+S B/A versus 1/5/15 | 13.26 | 2 | $0.01 > p > 0.001^{**}$ |
| Lower intertidal site | | | |
| G+G B/A versus 1/5/15 | 54.88 | 2 | $P < 0.001^{***}$ |
| G+S B/A versus 1/5/15 | 12.94 | 2 | $0.01 > p > 0.001^{**}$ |

Table 10d: Table showing the Chi-square (χ^2) Statistical analysis of differences at the upper and lower intertidal sites for number of gravel particles lost or gained by clumps of 1 animal, 5 animals and 15 animals (1/5/15 respectively). UI = upper intertidal site. LI = Lower intertidal site. B/A = numbers of gravel particles attached to mussels at the end of the prior laboratory clumping/numbers of gravel particles attached to mussels at the end of the 18 day field seeding. G→G = mussels laid onto gravel in the laboratory allowed to form clumps then the clumps were transported and laid onto gravel in the field. G→S = mussels laid onto gravel in the laboratory allowed to form clumps then the clumps were transported and laid onto sand in the field. d.f. = degrees of freedom and P = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non-asterisked probabilities are not significant.

| Data | | Comparison | | χ^2 | d.f. | p |
|------------------|--|------------------------|--|----------|------|-------------------------|
| Single animal UI | | B/A versus G→G and G→S | | 2.552 | 1 | $0.20 > p > 0.10$ |
| Single animal LI | | B/A versus G→G and G→S | | 0.775 | 1 | $0.50 > p > 0.30$ |
| 5 animals UI | | B/A versus G→G and G→S | | 9.017 | 1 | $0.01 > p > 0.001^{**}$ |
| 5 animals LI | | B/A versus G→G and G→S | | 0.237 | 1 | $0.70 > p > 0.50$ |
| 15 animals UI | | B/A versus G→G and G→S | | 18.94 | 1 | $P < 0.001^{***}$ |
| 15 animals LI | | B/A versus G→G and G→S | | 10.19 | 1 | $P < 0.001^{***}$ |

A set of Chi-squared analyses was then employed to analyse the data further. These analyses were designed to test differences in the number of gravel particles lost or gained by (G→G) and (G→S) seeded animals. The Chi-squared was again applied to all animal densities at both the upper and the lower intertidal sites. For the single animals, neither Chi-squared test was found to be significant (Table 10d: UI: $\chi^2 = 2.552$, d.f. = 1, $0.20 > P > 0.10$ n.s. and LI: $\chi^2 = 0.775$, d.f. = 1, $0.50 > P > 0.30$ n.s.). For clumps containing five animals, one Chi-squared was significant (Table 10d UI: $\chi^2 = 9.017$, d.f. = 1, $0.01 > P > 0.001^{**}$). This means that for clumps of five in the upper intertidal seeded onto gravel gain gravel and lose gravel if seeded onto sand (162% and 68%). Both of the 15 animal clumps were highly significant (Table 10d: UI: $\chi^2 = 18.94$, d.f. = 1, $P < 0.001^{***}$, LI: $\chi^2 = 10.19$, d.f. = 1, $P < 0.001^{***}$). So at both the upper and lower intertidal sites clumps of fifteen animals gain more gravel if they are seeded onto gravel than if they are seeded onto sand (UI 221%, 117%; LI 228%, 137%).

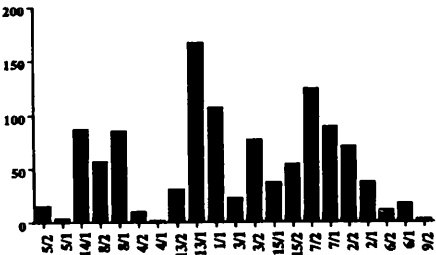
Movements of gravel down the sedimentary column

The results for gravel dispersion down the sedimentary column are shown in Figures 31. The most significant observation from the plots is that there is some natural gravel along with introduced gravel in the sedimentary column. This is shown by quadrats 4, 5 and 6. None of these quadrats had any introduced gravel laid onto them. The only gravel to be introduced here would be the gravel that had attached to the mussels in the laboratory. This means that no relevant conclusions can be made from numbers of gravel grains retrieved from the 5-10cm sediment depth. However, the 0-2 cm sediment depth can be used as an indication of a movement of gravel grains down the sedimentary column. This depth slice will experience mixing due to wave action over a minor scale whereas deeper depths will experience mixing over longer time scales. It is also worth noting that the data re-emphasise the strong contrast between the upper intertidal and the lower intertidal for the data. In general the upper intertidal has considerably more gravel grains at the three sediment depths than the lower intertidal.

Figure 31: Six plots showing the numbers of gravel particles present moving down the sediment column from quadrats containing gravel in experiment two. The three sediment depth ranges used were 0-2cm, 2-5cm and 5-10cm. The x axis shows the quadrat identification number and the replicate number (quadrat number/replicate).

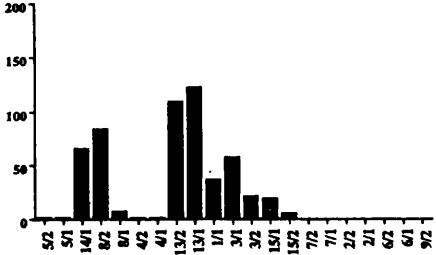
UPPER INTERTIDAL

0-2cm SEDIMENT DEPTH

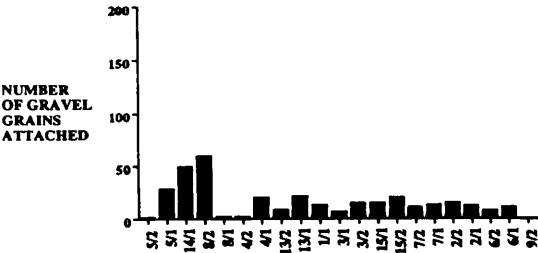


LOWER INTERTIDAL

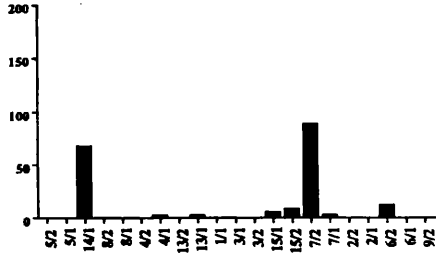
0-2cm SEDIMENT DEPTH



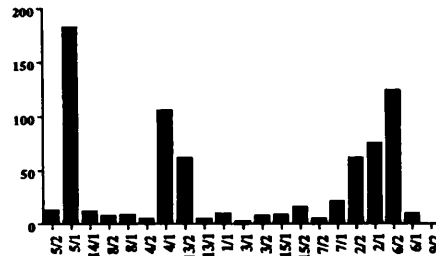
2-5cm SEDIMENT DEPTH



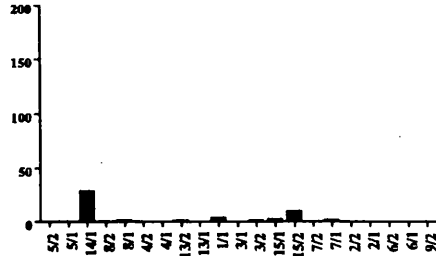
2-5cm SEDIMENT DEPTH



5-10cm SEDIMENT DEPTH



5-10cm SEDIMENT DEPTH



QUADRAT/REPLICATE

QUADRAT/REPLICATE

Particle size analysis

Table 11 shows the results of the particle size analysis on three replicates taken from the upper intertidal site and the lower intertidal site. The results show that in general the surface (0cm to 2 cm) sediment at the upper and lower intertidal have a similar overall size shown by the mean particle diameter. The mean particle diameter simply calculates the average size class of particles measured in phi units (Table 3 page 55) that occurs in a sample of sediment. The samples are also shown to be moderately well sorted at the upper intertidal site and well sorted at the lower intertidal site. Folk (1966) give a grading of sorting, in general the lower the sorting the then the sample is well sorted. A poorly sorted sediment may be predominantly made up from either very small or very large particles. The samples also show a negative skewness. This suggests that there is a significant amount of fine particles present in the samples. The kurtosis describes the amount of a particular particle size class in a distribution. High values of kurtosis (>3) are termed leptokurtic, low values of kurtosis (<3) are termed platykurtic. Leptokurtic sediment samples among the upper and lower intertidal replicate samples will have high peak shaped distributions giving small amounts of the fine and coarse particle size fractions. Platykurtic sediment samples will have flat shaped distribution patterns giving relatively high amounts of both the fine and coarse particle size fractions.

Table 11: Particle size analysis of sediment at the upper and lower intertidal sites of Ardmore Bay. Three replicates per tidal level. UI = upper intertidal. LI = lower intertidal.

| Sample | Mean particle diameter (phi) | Standard deviation (sorting) | Skewness | Kurtosis |
|----------------|------------------------------|------------------------------|----------|----------|
| UI replicate 1 | 2.179 | 0.8741 | -0.3025 | 0.1325 |
| UI replicate 2 | 2.732 | 0.7605 | -0.8717 | 4.306 |
| UI replicate 3 | 2.606 | 0.7639 | -0.5873 | 1.490 |
| LI replicate 1 | 2.438 | 0.4024 | -0.1139 | 2.671 |
| LI replicate 2 | 2.420 | 0.3986 | -0.1754 | 2.235 |
| LI replicate 3 | 2.407 | 0.4361 | -0.3904 | 3.969 |

1.3.3 Experiment 3 Laboratory study of attachment and pull off strength of *mytilus edulis* to plastic mesh

The results for the laboratory experiment are shown in Table 12 and statistically analysed in Table 13. Table 13a shows the results of a Chi-squared analysis on the numbers of threads produced on each of the six substrata used in the experiment. There were significant differences in the numbers of threads produced comparing the six substrata ($\chi^2 = 14.119$, d.f. = 5, $0.05 > P > 0.01^*$). The numbers of threads produced on the plastic mesh also gave very interesting results. The comparison between thick mesh on sediment and thick mesh stretched over the opening of a dish gave very significant differences for numbers of threads produced ($\chi^2 = 7.556$, d.f. = 1, $0.01 > P > 0.001^{**}$). The same comparison for fine mesh gave no significant differences for numbers of threads produced ($\chi^2 = 0.138$, d.f. = 1, $0.80 > P > 0.70$ n.s.). The numbers of threads produced on mesh was also compared to the numbers of threads produced under control conditions on sediment only. The number of byssus threads produced on thick mesh stretched over the opening of a dish was the only significant comparison with the single mussel control on sand ($\chi^2 = 6.862$, d.f. = 1, $0.01 > P > 0.001^{**}$). Both the fine mesh on sediment and the thick mesh on sediment gave significant differences in the numbers of threads produced compared to the two mussel control condition ($\chi^2 = 4.2$, d.f. = 1, $0.05 > P > 0.01^*$ and $\chi^2 = 4.680$, d.f. = 1, $0.05 > P > 0.01^*$ respectively). The results show that there is no significant difference in the numbers of threads produced by mussels on fine mesh or thick mesh. There is therefore no significant advantage in choosing fine mesh in preference over thick mesh in a large-scale field-seeding programme.

Table 13b shows the statistical analysis of the data for pull-off force of mussel byssus threads, from five of the six substrata. The pull-off force was recorded per mussel not per byssus thread. The arrangement of the byssal attachment of *M. edulis* is complex from a structural engineering perspective. There are many threads acting in various directions thus making a detailed tensile investigation beyond the limits of this biological study. The pull-off measurement may be taken as a rough indication of the force required to dislodge the mussel from a firm substratum. The One-way Analysis of variance clearly shows that there is no significant difference in the pull-off force

comparing the five substrata. This suggests that there is no significant mechanical advantage for attachment between the five substrata

Table 12: Table showing the dimensions and force required to break the byssus attachment of mussels under six experimental conditions. F/B/SED = black 14mm square plastic mesh with fine 1mm diameter mesh laid onto 5cm of sand inside a 19mm ID circular dish. T/G/SED = green 14mm square plastic mesh with thick 2mm diameter mesh laid onto 5cm of sand inside a 19mm ID circular dish. C/1/ = control condition with 1 mussel laid onto 5 cm of sand in a 19mm ID circular dish. C/2/ = control condition with 2 mussels laid onto 5 cm of sand in a 19mm ID circular dish. F/B/SUS = black 14mm square plastic mesh with fine 1mm diameter mesh tied over the opening of a 19mm ID circular dish. T/G/SUS = green 14mm square plastic mesh with thick 2mm diameter mesh tied over the opening of a 19mm ID circular dish.

| Substrate | MUSSEL WET WEIGHT (g) | LENGTH (cm) | BREADTH (cm) | HEIGHT (cm) | Number of byssus threads | PULL OFF (kg) | FORCE (N) |
|-----------|----------------------------------|----------------|-----------------|----------------|--------------------------------|-----------------------|--------------|
| F/B/SED/1 | 17.19 | 4.9 | 2.2 | 2.2 | 9 | 0.16 | 0.004 |
| F/B/SED/2 | 24.45 | 5.6 | 2.5 | 2.7 | 3 | 0.04 | 0.001 |
| T/G/SED/1 | 27.05 | 5.3 | 2.6 | 2.4 | 13 | 0.22 | 0.055 |
| T/G/SED/2 | 17.22 | 5.1 | 2.2 | 2.1 | 5 | 0.12 | 0.003 |
| C/1/1 | 18.04 | 5.0 | 2.1 | 2.6 | 33 | -- | |
| C/1/2 | 19.97 | 5.3 | 2.1 | 2.5 | 20 | -- | |
| C/2/1 | 11.44 15.66 | 4.4 4.7 | 1.9 2.0 | 2.1 2.2 | Total 12 | 0.22 | 0.055 |
| C/2/2 | 14.23 12.01 | 4.5 4.2 | 1.9 2.0 | 2.4 1.9 | Total 18 | 0.28 | 0.007 |
| F/B/SUS/1 | 13.10 | 4.6 | 1.8 | 2.2 | 4 (5) | 0.10 | 0.025 |
| F/B/SUS/2 | 16.75 | 4.9 | 2.1 | 2.4 | 2 | 0.02 | 0.0005 |
| T/G/SUS/1 | 12.15 | 4.8 | 1.9 | 2.2 | 4 (10) | 0.08 | 0.001 |
| T/G/SUS/2 | 14.85 | 4.6 | 2.2 | 2.1 | 12 | 0.16 | 0.004 |

Table 13a: Table showing the Chi-squared (χ^2) statistical analysis of numbers of threads produced on the six experimental substrata in experiment 3. Notations for comparisons are the same as the notations in Table 12. d.f. = degrees of freedom. P = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non asterisked probabilities are not significant.

| comparison | χ^2 | d.f. | P |
|--------------------|----------|------|-------------------------|
| All six substrata | 14.119 | 5 | $0.05 > P > 0.01^*$ |
| F/B/SED Vs F/B/SUS | 0.138 | 1 | $0.80 > P > 0.70$ |
| T/G/SED Vs T/G/SUS | 7.556 | 1 | $0.01 > P > 0.001^{**}$ |
| C/1 Vs C/2 | 3.826 | 1 | $0.05 > P > 0.01^*$ |
| F/B/SED Vs T/G/SED | 0.028 | 1 | $0.90 > P > 0.80$ |
| F/B/SUS Vs T/G/SUS | 3.274 | 1 | $0.08 > P > 0.07$ |
| F/B/SED Vs C/1 | 0.694 | 1 | $0.50 > P > 0.40$ |
| F/B/SUS Vs C/1 | 0.045 | 1 | $0.90 > P > 0.80$ |
| T/G/SED Vs C/1 | 0.548 | 1 | $0.50 > P > 0.40$ |
| T/G/SUS Vs C/1 | 6.862 | 1 | $0.01 > P > 0.001^{**}$ |
| F/B/SED Vs C/2 | 4.2 | 1 | $0.05 > P > 0.01^*$ |
| F/B/SUS Vs C/2 | 1.44 | 1 | $0.30 > P > 0.20$ |
| T/G/SED Vs C/2 | 4.680 | 1 | $0.05 > P > 0.01^*$ |
| T/G/SUS Vs C/2 | 1.035 | 1 | $0.40 > P > 0.30$ |

Table 13b: Table showing the One-way analysis of variance statistical analysis of the force required to break the byssus attachments of mussels for six experimental substrata. Notations for comparisons are the same as the notations in Table 12. d.f. = degrees of freedom. *P* = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non-asterisked probabilities are not significant.

| comparison | Source of variation | d.f. | Sum of squares | Mean squares | <i>F</i> | <i>P</i> |
|--------------------|---------------------|------|----------------|--------------|----------|-------------------|
| All five substrata | Factor | 4 | 42800 | 10700 | 2.62 | $0.20 > P > 0.10$ |
| | Error | 5 | 20400 | 4080 | | |
| | Total | 9 | 63200 | | | |
| F/B/SED Vs F/B/SUS | Factor | 1 | 1600 | 1600 | 0.31 | $0.70 > P > 0.60$ |
| | Error | 2 | 10400 | 5200 | | |
| | Total | 3 | 12000 | | | |
| T/G/SED Vs T/G/SUS | Factor | 1 | 2500 | 2500 | 0.61 | $0.60 > P > 0.50$ |
| | Error | 2 | 8200 | 4100 | | |
| | Total | 3 | 10700 | | | |
| F/B/SED Vs T/G/SED | Factor | 1 | 4900 | 4900 | 0.80 | $0.50 > P > 0.40$ |
| | Error | 2 | 12200 | 6100 | | |
| | Total | 3 | 17100 | | | |
| F/B/SUS Vs T/G/SUS | Factor | 1 | 3600 | 3600 | 1.12 | $0.45 > P > 0.40$ |
| | Error | 2 | 6400 | 3200 | | |
| | Total | 3 | 10000 | | | |

1.3.4 Experiment 4 Intertidal field study of the attachment, loss and transportation of *Mytilus edulis* to plastic mesh introduced to a sandy shore

The results of this experiment are shown in Figure 32 and Tables 14 to 20. In Figure 32 the differences in both distance and direction of mussel transportation on day 18 is clearly shown. In the upper intertidal the mussels are transported smaller distances than in the lower intertidal environment (maximum distance: 211cm and 1182cm respectively). The pattern of transport is also a clear contrast in both environments. In the upper intertidal environment, there is a distinctly random pattern. In comparison, the lower intertidal environment shows a directional transportation pattern in the direction of the prevailing wind and currents. A series of One-way analysis of variance was conducted on the distances the mussels were transported at the UI and LI sites for day 11 and day 18 of the experiment (Table 14). The comparisons of UI and LI on day 11 and day 18 both showed highly significant differences in the distances that mussels were transported on the shore (Table 14: $F = 33.28$, d.f. = 1, $P < 0.001^{***}$ and $F = 37.37$, d.f. = 1, $P < 0.001^{***}$). The distances mussels were transported at the UI site on day 11 were compared with the distances mussels were transported on day 18. This comparison was also made with the LI data on day 11 compared to day 18. Only the LI site was found to have significant differences in the distances mussels were transported comparing day 11 and day 18 (Table 14: $F = 24.20$, d.f. = 1, $P < 0.001^{***}$). A further set of One-way analyses of variance were conducted for the UI and LI data comparing all of the four substrata used in the experiment. The LI site showed significant differences for the distances mussels were transported on the shore between the four substrata. The results were significant for both day 11 and day 18 (Table 14: $F = 3.53$, d.f. = 3, $0.05 > P > 0.01^*$ and $F = 3.28$, d.f. = 3 $0.05 > P > 0.01^*$ respectively).

Figure 32: Figure showing the movement and direction that mussels were transported on the shore at both the upper and lower intertidal in experiment 4. The upper intertidal low energy site displays a random pattern for the distance and direction that mussels were transported on the shore. The maximum distance that mussels were transported at the upper intertidal site was 211cm. The lower intertidal high-energy site displays a directional pattern for the distance and direction that mussels were transported on the shore. The maximum distance that mussels were transported at the lower intertidal site was 1182cm.

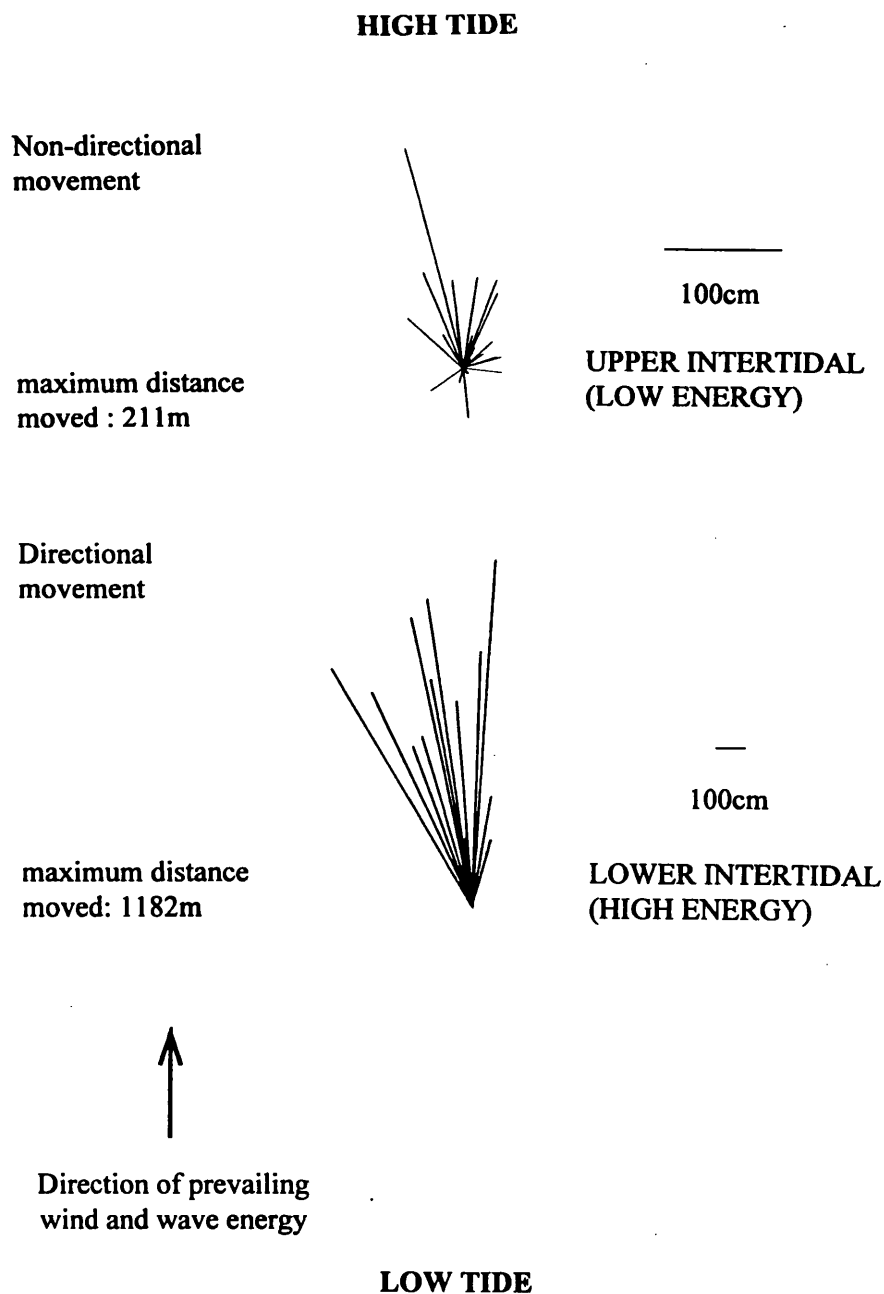


Table 14: Table showing the One-way analysis of variance statistical analysis of the distances that mussels were transported over the 18 day duration of experiment 4. UI = upper intertidal. LI = lower intertidal. d.f. = degrees of freedom. *P* = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non-asterisked probabilities are not significant.

| Comparison | Source of variation | d.f. | Sum of squares | Mean squares | F | P |
|----------------------------------|-------------------------------|-----------------|-------------------------------|------------------|-------|---------------------|
| UI day 11 Compared to LI day 11 | htd11/ltd11 Error Total | 1 138 139 | 569222 2360579 2929801 | 569222 17106 | 33.28 | $P < 0.001^{***}$ |
| UI day 18 Compared to LI day 18 | htd18/ltd18 Error Total | 1 59 60 | 2448314 3865203 6313517 | 2448314 65512 | 37.37 | $P < 0.001^{***}$ |
| UI day 11 Compared to UI day 18 | htd11/htd18 Error Total | 1 94 95 | 549 159299 159848 | 549 1695 | 0.32 | $0.60 > P > 0.50$ |
| LI day 11 Compared to LI day 18 | ltd11/ltd18 Error Total | 1 103 104 | 1425534 6066483 7492017 | 1425334 58898 | 24.20 | $P < 0.001^{***}$ |
| UI day 11 all substrata compared | htd11all Error Total | 3 56 59 | 9990 95038 105029 | 3330 1697 | 1.96 | $0.15 > P > 0.10$ |
| LI day 11 all substrata compared | ltd11all Error Total | 3 76 79 | 275582 1979968 2255550 | 91861 26052 | 3.53 | $0.05 > P > 0.01^*$ |
| UI day 18 all substrata compared | htd18all Error Total | 3 32 35 | 7558 46712 54270 | 2519 1460 | 1.73 | $0.20 > P > 0.10$ |
| LI day 18 all substrata compared | ltd18all Error Total | 3 21 24 | 1216631 2594302 3810933 | 405544 123538 | 3.28 | $0.05 > P > 0.01^*$ |

The numbers of mussels lost at both the upper and lower intertidal sites were analysed using Chi-squared analysis. Table 15a shows the comparison between the three mussel densities individual mussels, 5 mussels and 15 mussels at the upper intertidal and lower intertidal sites on day 11 of the experiment. Mussels were deemed lost only after a thorough search of a 10-metre diameter area with its centre at the centre of the quadrat. It is clear from the Table that more mussels are lost from the lower intertidal site than the upper intertidal site. Also at the termination of the experiment on day 18 (Table 15b) there were greater losses of mussels from both the upper and lower intertidal compared to day 11. Table 15c shows the Chi-squared analysis of individual mussels, 5 mussels and 15 mussels at the upper intertidal and lower intertidal sites comparing day 11 and day 18 of the experiment. The data for numbers of mussels lost from individual mussel quadrats, groups of 5 mussels and groups of 15 mussels comparing the upper intertidal and the lower intertidal sites on day 11 were significantly different (Table 15c: $\chi^2 = 24.670$, d.f. = 5, $P < 0.001^{***}$). The same comparison on day 18 was significantly different (Table 15c: $\chi^2 = 113.939$, d.f. = 5, $P < 0.001^{***}$). A comparison of numbers lost from individual mussels, groups of 5 and groups of 15 at the upper intertidal site comparing day 11 and day 18 data was found to be significant (Table 15c: $\chi^2 = 40.936$, d.f. = 5, $P < 0.001^{***}$). This comparison at the lower intertidal site was also found to be significant (Table 15c: $\chi^2 = 102.048$, d.f. = 5, $P < 0.001^{***}$). A further set of Chi-squared analyses was conducted comparing the different experimental substrata used. The data for the numbers lost on day 11 for the upper intertidal and the lower intertidal sites comparing the four substrata are shown in Table 16a. The data for the numbers lost on day 18 for the upper intertidal and the lower intertidal sites comparing the four substrata are shown in Table 16b. In general on both day 11 and day 18 there were greater losses of mussels at the lower intertidal site. The upper intertidal comparison of the data on day 11 for the four substrata was not significantly different (Table 16c: $\chi^2 = 3.018$, d.f. = 3, $P > 0.05$ n.s.). The lower intertidal comparison, however, showed significant differences for the numbers of mussels lost between the four substrata (Table 16c: $\chi^2 = 9.741$, d.f. = 3, $0.05 > P > 0.01^*$). The lower intertidal site lost significantly more mussels from the plastic mesh substrate than the upper intertidal (Table 16c: $\chi^2 = 25.247$, d.f. = 3, $P < 0.001^{***}$). The comparison of control substrata on sand between the upper and lower intertidal gave no significant difference in numbers of mussels lost (Table 16c: $\chi^2 = 5.250$, d.f. = 3, $0.2 > P > 0.15$ n.s.). The upper intertidal

comparison between the four substrata on day 18 at the end of the experiment was not significantly different for numbers of mussels lost (Table 16b: $\chi^2 = 5.875$, d.f. = 3, $0.15 > P > 0.10$ n.s.). The lower intertidal showed significant differences between numbers lost from all four substrata (Table 16b: $\chi^2 = 37.740$, d.f. = 3, $P < 0.001^{***}$). The comparison between numbers of mussels lost from mesh substrata comparing the upper and lower intertidal gave significant differences between the tidal levels (Table 16b: $\chi^2 = 58.862$, d.f. = 3, $P < 0.001^{***}$). The comparison between the two control substrata on sand was also significantly different for numbers of mussels lost comparing the upper and lower intertidal (Table 16b: $\chi^2 = 66.949$, d.f. = 3, $P < 0.001^{***}$). A comparison between day 11 and day 18 for the upper intertidal gave significant differences between both the two mesh substrata and the two control substrata for numbers of mussels lost (Table 16b: $\chi^2 = 17.850$, d.f. = 3, $P < 0.001^{***}$ and $\chi^2 = 10.769$, d.f. = 3, $0.05 > P > 0.01^*$). Both of these comparisons at the lower intertidal were significantly different for numbers of mussels lost (Table 16b: $\chi^2 = 47.008$, d.f. = 3, $P < 0.001^{***}$ and $\chi^2 = 78.073$, d.f. = 3, $P < 0.001^{***}$).

Table 16a: Table showing the numbers of mussels lost and remaining on day 11 from individual mussels, groups of 5 mussels and groups of 15 mussels (1, 5 and 15 respectively) from experiment 4. UI = upper intertidal. LI = lower intertidal.

| Day 11 | Numbers of mussels | | |
|-----------|--------------------|----|-----|
| | 1 | 5 | 15 |
| UI | | | |
| Remaining | 8 | 39 | 120 |
| Lost | 0 | 1 | 0 |
| Total | 8 | 40 | 120 |
| LI | | | |
| Remaining | 6 | 33 | 108 |
| Lost | 2 | 7 | 12 |
| Total | 8 | 40 | 120 |

Table 16b: Table showing the numbers of mussels lost and remaining on day 18 from individual mussels, groups of 5 mussels and groups of 15 mussels (1, 5 and 15 respectively) from experiment 4. UI = upper intertidal. LI = lower intertidal.

| Day 18 | Numbers of mussels | | |
|-----------|--------------------|----|-----|
| | 1 | 5 | 15 |
| UI | | | |
| Remaining | 4 | 37 | 109 |
| Lost | 4 | 3 | 11 |
| Total | 8 | 40 | 120 |
| LI | | | |
| Remaining | 3 | 5 | 58 |
| Lost | 5 | 35 | 62 |
| Total | 8 | 40 | 120 |

Table 16c: Table showing the Chi-squared (χ^2) statistical analysis of differences of numbers of mussels lost and remaining at the upper and lower intertidal for experiment 4, comparing Individual mussels, groups of 5 mussels and groups of 15 mussels (1, 5 and 15 respectively). UI = upper intertidal. LI = lower intertidal. d.f. = degrees of freedom. P = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non asterisked probabilities are not significant.

| Comparison | d.f. | χ^2 | P |
|--|------|----------|-------------------|
| 1, 5, 15 UI mussels Vs 1, 5, 15 LI day 11 | 5 | 24.670 | $P < 0.001^{***}$ |
| 1, 5, 15 UI mussels Vs 1, 5, 15 LI day 18 | 5 | 113.939 | $P < 0.001^{***}$ |
| 1, 5, 15 UI day 11 Vs 1, 5, 15 UI day 18 | 5 | 40.936 | $P < 0.001^{***}$ |
| 1, 5, 15 LI day 11 Vs 1, 5, 15 LI day 18 | 5 | 102.048 | $P < 0.001^{***}$ |

Table 16a: Table showing the numbers of mussels lost and remaining on day 11 from four different substrata treatments used in experiment 4. UI = upper intertidal. LI = lower intertidal. Mesh→mesh = mussels laid onto 0.25m² pieces of green plastic mesh with a square aperture of 14mm x 14mm and a 2mm diameter mesh in the laboratory, allowed to form clumps then transported with the mesh and laid into the field. Mesh = mussels laid as individuals with no space between the mussels laid onto 0.25m² pieces of green plastic mesh with a square aperture of 14mm x 14mm and a 2mm diameter mesh in the field. Sand→Sand = mussels laid onto sand in the laboratory, allowed to form clumps then transported and laid onto sand in the field. Sand = mussels laid as individuals with no space between mussels laid onto the field sand.

| Day 11 | Substrate treatment | | | |
|-----------|---------------------|------|-----------|------|
| | Mesh→Mesh | Mesh | Sand→Sand | Sand |
| UI | | | | |
| Remaining | 42 | 42 | 42 | 41 |
| Lost | 0 | 0 | 0 | 1 |
| Total | 42 | 42 | 42 | 42 |
| LI | | | | |
| Remaining | 39 | 31 | 38 | 39 |
| Lost | 3 | 11 | 4 | 3 |
| Total | 42 | 42 | 42 | 42 |

Table 16b: Table showing the numbers of mussels lost and remaining on day 18 from four different substrate treatments used in experiment 4. UI = upper intertidal. LI = lower intertidal. Mesh→mesh = mussels laid onto 0.25m² pieces of green plastic mesh with a square aperture of 14mm x 14mm and a 2mm diameter mesh in the laboratory, allowed to form clumps then transported with the mesh and laid into the field. Mesh = mussels laid as individuals with no space between the mussels laid onto 0.25m² pieces of green plastic mesh with a square aperture of 14mm x 14mm and a 2mm diameter mesh in the field. Sand→Sand = mussels laid onto sand in the laboratory, allowed to form clumps then transported and laid onto sand in the field. Sand = mussels laid as individuals with no space between mussels laid onto the field sand.

| Day 18 | Substrate treatment | | | |
|-----------|---------------------|------|-----------|------|
| | mesh→mesh | mesh | sand→sand | sand |
| UI | | | | |
| Remaining | 41 | 35 | 35 | 38 |
| Lost | 1 | 7 | 7 | 4 |
| Total | 42 | 42 | 42 | 42 |
| LI | | | | |
| Remaining | 32 | 11 | 8 | 13 |
| Lost | 10 | 31 | 34 | 29 |
| Total | 42 | 42 | 42 | 42 |

Table 16c: Table showing the Chi-squared (χ^2) statistical analysis of differences of numbers of mussels lost and remaining at the upper and lower intertidal for experiment 4, comparing the four different substrate treatments in experiment 4. LM = mussels laid onto 0.25m² pieces of green plastic mesh with a square aperture of 14mm x 14mm and a 2mm diameter mesh in the laboratory, allowed to form clumps then transported with the mesh and laid into the field. FM = mussels laid as individuals with no space between the mussels laid onto 0.25m² pieces of green plastic mesh with a square aperture of 14mm x 14mm and a 2mm diameter mesh in the field. LC = mussels laid onto sand in the laboratory, allowed to form clumps then transported and laid onto sand in the field. FC = mussels laid as individuals with no space between mussels laid onto the field sand. UI = upper intertidal. LI = lower intertidal. d.f. = degrees of freedom. *P* = probability. Probabilities given are asterisked significant according to the following system. 0.05>*P*>0.01*, 0.01>*P*>0.001** and *P*<0.001***. All non asterisked probabilities are not significant.

| Comparison | d.f. | χ^2 | <i>P</i> |
|------------------------------------|------|----------|-----------------------|
| UI day 11 LM/FM/LC/FC | 3 | 3.018 | <i>P</i> >0.05 |
| LI day 11 LM/FM/LC/FC | 3 | 9.741 | 0.05> <i>P</i> >0.01* |
| UI day 18 LM/FM/LC/FC | 3 | 5.875 | 0.15> <i>P</i> >0.10 |
| LI day 18 LM/FM/LC/FC | 3 | 35.740 | <i>P</i> <0.001*** |
| Day 11 UI LM/FM Vs LI LM/FM | 3 | 25.247 | <i>P</i> <0.001*** |
| Day 18 UI LM/FM Vs LI LM/FM | 3 | 58.682 | <i>P</i> <0.001*** |
| Day 11 UI LC/FC Vs LI LC/FC | 3 | 5.250 | 0.20> <i>P</i> >0.15 |
| Day 18 UI LC/FC Vs LI LC/FC | 3 | 66.949 | <i>P</i> <0.001*** |
| Day 11 UI LM/FM Vs Day 18 UI LM/FM | 3 | 17.850 | <i>P</i> <0.001*** |
| Day 11 LI LM/FM Vs Day 18 LI LM/FM | 3 | 47.008 | <i>P</i> <0.001*** |
| Day 11 UI LC/FC Vs Day 18 UI LC/FC | 3 | 10.796 | 0.05> <i>P</i> >0.01* |
| Day 11 LI LC/FC Vs Day 18 LI LC/FC | 3 | 78.073 | <i>P</i> <0.001*** |

1.4.5 JOINT FIELD EXPERIMENTS

The joint fieldwork was conducted on Ardmore bay. In total two experimental sets will be reported. Experiments in experimental set I were conducted with Suzanne McCulloch. Experiments in experimental set II were conducted with Patricia Whitaker.

1.4.5.1) Experimental set I: large scale analysis of mussel bed formation at successively greater distances from the rocky shore

The results of this experiment are shown in Figures 33 and 34 and Tables 17 to 20.

The mussel bed areas at both the upper intertidal site and the lower intertidal sites are shown in Figure 33. The upper intertidal site displays mussel beds with areas greater than 100cm² compared with the lower intertidal which has no mussel beds with areas over 100cm². A One-way Analysis of variance was conducted comparing mussel bed areas between the upper and lower intertidal (Table 17). The comparison of mussel bed area between the upper intertidal and lower intertidal sites was not significant (Table 17: $F=3.93$, d.f. = 1, $0.55 > P > 0.50$ n.s.). This result shows that mussel bed areas are not significantly different when comparing tidal levels.

Figure 33: Two plots showing the mussel bed areas found in the upper intertidal (A) and lower intertidal (B) 30m x 10m transects in experiment A. Mussel beds areas are given in cm². There were 35 boulders selected within each quadrat at the upper and lower intertidal sites.

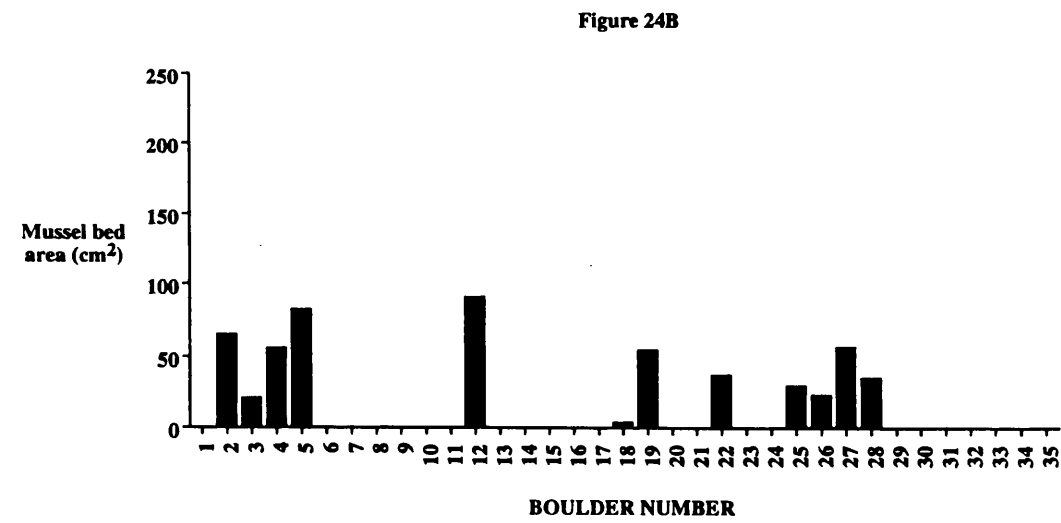
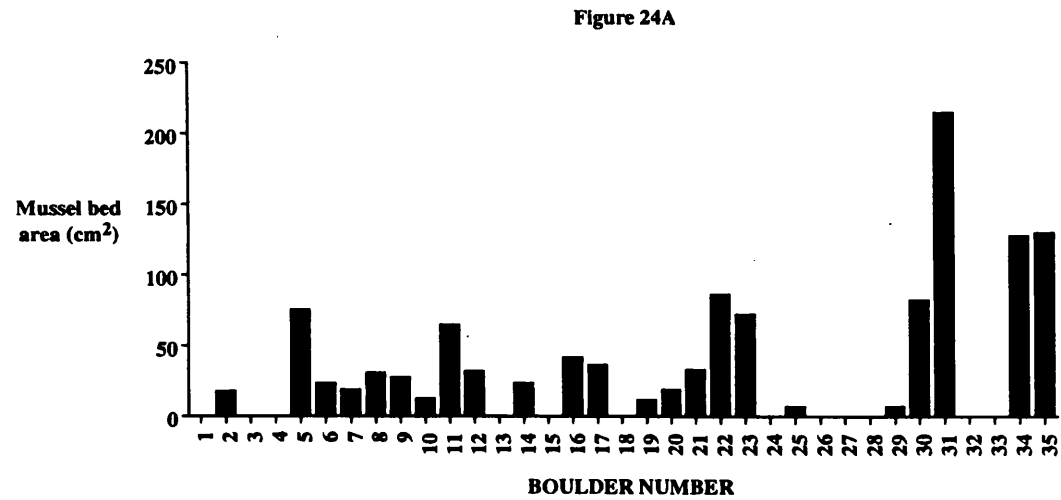


Figure 34 clearly shows the contrast between the upper and the lower intertidal sites for percentage mussel cover on boulders, percentage barnacle cover on boulders, percentage algal cover on boulders and percentage of bare patches on boulders. In general, both the barnacle cover and mussel cover appear to be greater at the lower intertidal site. A Chi-squared analysis was conducted on the frequency of the four different percentage cover criteria. The numbers of occurrences were recorded for percentage coverage of the four cover criteria at 0-25, 26-50, 51-75 and 75-100%. The results of the Chi-squared analysis are shown in Table 18. The comparison between all of the four cover criteria at the UI was found to be highly significant (Table 18: $\chi^2 = 27.212$, d.f. = 9, $P < 0.001^{***}$). The same comparison at the LI site was found to be highly significant (Table 18: $\chi^2 = 31.618$, d.f. = 9, $P < 0.001^{***}$). There were also significant differences in the comparison of percentage mussel cover of boulders between the UI and LI sites (Table 18: $\chi^2 = 16.356$, d.f. = 3, $0.01 > P > 0.001^{**}$). This illustrates the contrasting environments of the UI site and the LI site with respect to attachment of mussels.

The results of Experiment A investigating laying mussels onto boulders and monitoring their attachment success is shown Table 19. Mussels laid onto boulders at the LI site lost all mussels from boulders with surface covers of algae, barnacle, algae with mussel, algae with barnacle and algae with bare rock. This may be due to the LI site being more exposed to the tidal currents present in the bay. The upper intertidal lost most mussels from the boulder covered with algae and bare rock.

Figure 34: Eight plots showing the eight different percentage cover criteria. Eight boulders with the highest percentages of each of the eight percentage cover criteria were chosen from the upper intertidal transect and the lower intertidal transect. Figure 34A shows the percentage cover of algae on boulders at the upper intertidal site. Figure 34B shows the percentage cover of mussels on boulders at the upper intertidal site. Figure 34C shows the percentage cover of barnacles on boulders at the upper intertidal site. Figure 34D shows the percentage of bare rock on boulders at the upper intertidal site. Figure 34E shows the percentage cover of algae on boulders at the lower intertidal site. Figure 34F shows the percentage cover of mussels on boulders at the lower intertidal site. Figure 34G shows the percentage cover of barnacles on boulders at the lower intertidal site. Figure 34H shows the percentage of bare rock on boulders at the lower intertidal site.

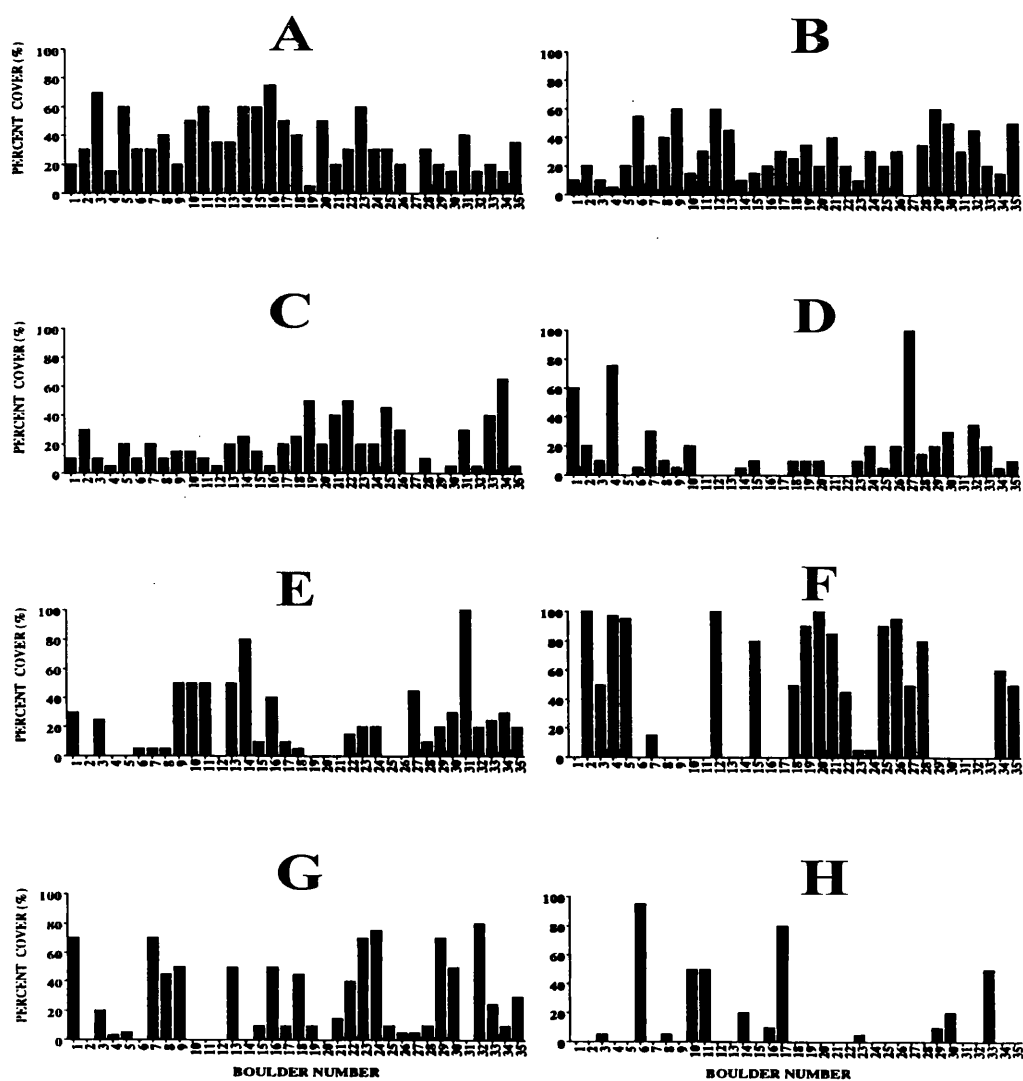


Table 17: Table showing the One-way analysis of variance comparing the mussel bed areas found around boulders within the upper and lower intertidal transects in experiment A. UImbA = upper intertidal mussel bed area. LImbA = lower intertidal mussel bed area. d.f. = degrees of freedom. F = variance ratio. P = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non asterisked probabilities are not significant.

| Source of variation | d.f. | Sum of squares | Mean squares | F | P |
|---------------------|------|----------------|--------------|------|-------------------|
| UImbA/LImbA | 1 | 5797 | 5797 | 3.93 | $0.55 > P > 0.50$ |
| Error | 68 | 100413 | 1477 | | |
| Total | 69 | 106210 | | | |

Table 20 and 21 clearly show the differences between the UI site and the LI site for Experiment B and Experiment C investigating attachment around boulders at different orientations. For experiment B the UI site in general lost fewer mussels than the LI site. Mussels were deemed lost only after a thorough search of a 10-metre diameter area with the boulder at the centre of the search area. Table 20 shows the percentages of mussels lost comparing the UI site and the LI site. The groups of mussels laid 15cm away from boulders with and without associated mussel bed at the upper intertidal lost fewer than those placed touching the boulder. At the lower intertidal the fewest mussels were lost from mussels laid touching boulders without mussel beds. Experiment C concentrated on mussels being laid in line with the prevailing current both in front and behind the boulder and to the left and right of the boulder (Table 21). In this experiment the upper intertidal clearly lost fewer mussels than the lower intertidal. The only losses at the upper intertidal were from mussels laid 15cm from boulders with mussel beds. All the mussels laid 15cm from boulders with and without mussel beds were lost.

Table 18: Table showing the Chi-squared (χ^2) statistical analyses comparing the eight different boulder percent cover criteria at both the upper and lower intertidal transects in experiment A. UI = upper intertidal. LI = lower intertidal. d.f. = degrees of freedom. P = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non-asterisked probabilities are not significant.

| data | comparison | χ^2 | d.f. | P |
|--|------------|----------|------|-------------------------|
| UI all cover criteria | UI all | 27.212 | 9 | $0.01 > P > 0.001^{**}$ |
| LI all cover criteria | LI all | 31.618 | 9 | $P > 0.001^{***}$ |
| UI % mussel Cover Vs LI % mussel cover | UI m/LI m | 16.356 | 3 | $0.01 > P > 0.001^{**}$ |

Table 19: Table showing the percentage of mussels lost from mussels laid onto the eight different boulder percent cover criteria at the upper and lower intertidal transects from experiment A.

| | Initial boulder attachment | | | | | | | |
|-------------------------|----------------------------|--------|----------|-----------|----------------|------------------|-------------------|----------------|
| | Algae | Mussel | Barnacle | Bare rock | Algae + mussel | Algae + barnacle | Algae + bare rock | Algae + mussel |
| Upper Intertidal % lost | 0 | 15 | 50 | 0 | 15 | 15 | 75 | 0 |
| Lower Intertidal % lost | 100 | 15 | 100 | 0 | 100 | 100 | 100 | 15 |

Table 20: Table showing the percentage of mussels lost from mussels laid at the eight orientations around boulders at the upper and lower intertidal transects from experiment B. N/S/E/W = orientations around the boulder of North, South, East and West respectively. NE/SE/SW/NW = orientations around the boulder of North East, South East, South West and North West respectively. UI = upper intertidal. LI = lower intertidal. +mb = boulder with mussel bed around it. No mb = boulder with no mussel bed around it. 0cm = mussels laid at 0cm touching the boulder. 15cm = mussels laid at 15cm away from the boulder.

| Initial boulder attachment | | | | | | | |
|----------------------------|-------------|-------------|-----------------|-----------------|-------------|-------------|-----------------|
| | N/S/E/ W | N/S/E/ W | NE/SE/S W/NW | NE/SE/ SW/NW | N/S/E/ W | N/S/E/ W | NE/SE/ SW/NW |
| | UI | UI | UI | UI | LI | LI | LI |
| | +mb | No mb | +mb | No mb | +mb | No mb | +mb |
| | 0cm | 0cm | 15cm | 15cm | 0cm | 0cm | 15cm |
| % lost | 50 | 50 | 25 | 25 | 50 | 25 | 100 |
| | | | | | | | 50 |

Table 21: Table showing the percentage of mussels lost from mussels laid at the eight orientations around boulders at the upper and lower intertidal transects from experiment C. 1 = orientations around the boulder in line with the prevailing current both in front and behind the boulder and to the left and right of the boulder respectively. UI = upper intertidal. LI = lower intertidal. +mb = boulder with mussel bed around it. No mb = boulder with no mussel bed around it. 0cm = mussels laid at 0cm touching the boulder. 15cm = mussels laid at 15cm away from the boulder.

| Initial orientation around boulder | | | | | | | | |
|------------------------------------|-----------------------|-------------------------|------------------------|--------------------------|-----------------------|-------------------------|------------------------|--------------------------|
| | 1 UI +mb 0cm | 1 UI No mb 0cm | 1 UI +mb 15cm | 1 UI No mb 15cm | 1 LI +mb 0cm | 1 LI No mb 0cm | 1 LI +mb 15cm | 1 LI No mb 15cm |
| % lost | 0 | 0 | 50 | 0 | 75 | 75 | 100 | 100 |

1.3.5.2 Experimental set II: artificial formation of mussel beds on fine sand sediment by clumping

This experimental section contains three experiments. Experiment I was a preliminary study of mussel attachment on fine sand sediment. Experiment II was a development of experiment I. Experiment III introduced pre-formed clumps into the experimental design.

The results of the preliminary experiment I reveal that there are differences in the numbers of mussels remaining on the shore after six days comparing the two initial mussel arrangements (Data not shown). There was no replication included in the experimental design as this was a preliminary experiment therefore no statistical analyses were conducted on the data.

The results of Experiment II are shown in Figure 35 and Table 22 to 23. In general, the UI site lost more mussels over the eight days of the experiment than the LI site. This is an interesting contrast to the results of experiment 2 page 24. Mussels were deemed lost only after a thorough search of a 10-metre diameter area with its centre at the centre of the quadrat. A series of Chi-squared analyses was conducted on the data. The comparison of mussels laid onto the shore touching at the UI site and at the LI site were found not to be significantly different for numbers of mussels lost (Table 23: $\chi^2 = 0.603$, d.f. = 1, $0.45 > P > 0.40$ n.s.). The same comparison using the data for mussels laid onto the fine sand spaced out was also not significant (Table 23: $\chi^2 = 0.693$, d.f. = 1, $P > 0.05$ n.s.). Both the comparison at the UI site and the LI site between mussels laid out touching and laid spaced out were not significantly different for numbers lost (Table 23: UI: $\chi^2 = 0.135$, d.f. = 1, $0.75 > P > 0.70$ n.s. and LI: $\chi^2 = 0.079$, d.f. = 1, $0.80 > P > 0.75$ n.s.).

Figure 35: Figure showing the movement and direction that mussels were transported on the shore at both the upper and lower intertidal in experiment II. The upper intertidal low energy site displays a random pattern for the distance and direction that mussels were transported on the shore. The maximum distance that mussels were transported at the upper intertidal site was 560cm. The lower intertidal high-energy site displays a partially directional pattern for the distance and direction that mussels were transported on the shore. The maximum distance that mussels were transported at the lower intertidal site was 710cm.

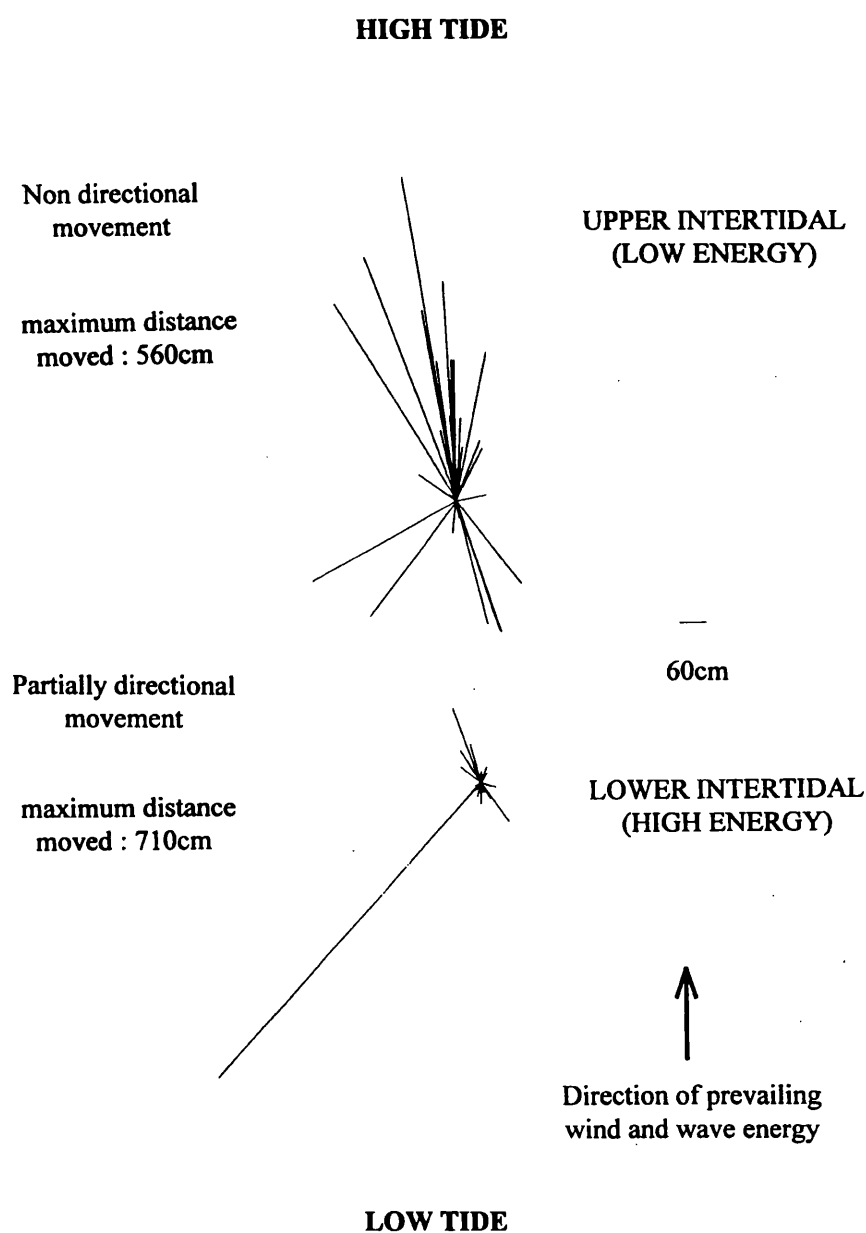


Table 22: Table showing the numbers of mussels lost and remaining after eight days for the two experimental conditions at both the upper and lower intertidal used in experiment II. Three different densities of mussels were used-individual mussels, groups of 5 mussels and groups of 15 mussels (1, 5 and 15 respectively). Touching = mussels laid onto field sand with no space between the mussels. Spaced = mussels laid onto field sand with 1cm space between mussels.

| | | Numbers of mussels | | |
|----------------|-----------|--------------------|----|----|
| | | 1 | 5 | 15 |
| UI Touching | Remaining | -- | 3 | 25 |
| | Lost | -- | 7 | 5 |
| | Total | -- | 10 | 30 |
| | | | | |
| UI Spaced | Remaining | 1 | 6 | 23 |
| | Lost | 1 | 4 | 7 |
| | Total | 2 | 10 | 30 |
| | | | | |
| LI Touching | Remaining | -- | 9 | 28 |
| | Lost | -- | 1 | 2 |
| | Total | -- | 10 | 30 |
| | | | | |
| LI Spaced | Remaining | 2 | 7 | 26 |
| | Lost | 0 | 3 | 4 |
| | Total | 2 | 10 | 30 |
| | | | | |

Table 23: Table showing the Chi-squared statistical analysis of the numbers of mussels lost and remaining for the two experimental conditions after 8 days for experiment II.

UI = upper intertidal. LI = lower intertidal. Three different densities of mussels used individual mussels, groups of 5 mussels and groups of 15 mussels (1, 5 and 15 respectively). Touching = mussels laid onto field sand with no space between the mussels. Spaced = mussels laid onto field sand with 1cm space between mussels. d.f. = degrees of freedom. *P* = probability. Probabilities given are asterisked significant according to the following system. 0.05>*P*>0.01*, 0.01>*P*>0.001** and *P*<0.001***. All non asterisked probabilities are not significant.

| Comparison | d.f. | χ^2 | <i>P</i> |
|--|------|----------|----------------------|
| UI touching Vs LI touching Vs UI spaced 5/15 Vs LI spaced 5/15 | 3 | 0.810 | 0.85> <i>P</i> >0.80 |
| UI touching Vs LI touching | 1 | 0.603 | 0.45> <i>P</i> >0.40 |
| UI spaced out Vs LI spaced out | 1 | 0.693 | <i>P</i> >0.05 |
| UI touching Vs UI spaced 5/15 | 1 | 0.135 | 0.75> <i>P</i> >0.70 |
| LI touching Vs LI spaced 5/15 | 1 | 0.079 | 0.80> <i>P</i> >0.75 |

The results of experiment III are shown in Figures 36 to 38 and Tables 24 to 29. Figures 36 to 38 show the distance and direction mussels were transported at the UI and the LI on day 6, day 10 and day 24 respectively. Figure 36 clearly shows that after only six days both the upper intertidal and the lower intertidal show directional patterns of mussel transport in the direction of the prevailing currents. This is a contrast to experiment 2 Figure 30 page 87. The maximum distances that mussels were transported at the upper and lower intertidal sites were 790cm and 1030cm respectively. Figure 37 shows the distances and directions that mussels were transported at both upper intertidal and the lower intertidal in experiment III on day 10 of the experiment. The directional pattern of mussel transport remains in the direction of the prevailing wind and wave energy. The maximum and minimum distances that mussels were transported at the upper and lower intertidal were, 1820cm and 1060cm, respectively. Figure 38 shows the distances and direction that mussels were transported at the upper and lower intertidal sites during experiment III by day 24. There is a stark contrast between the upper and lower intertidal. The upper intertidal has few mussels remaining and the maximum distance that the mussels were transported was 1060cm. There were no mussels remaining at the lower intertidal site.

A series of Chi-squared statistical analyses was conducted on the data of numbers of mussels lost and remaining at both the upper and lower intertidal sites on day 6, day 10 and day 24 of experiment III. Table 25 shows the Chi-squared statistical analysis comparing the numbers of mussels lost and the numbers of mussels remaining on day six of experiment III. Three experimental conditions were used. Condition 1 mussels laid onto field sand with no space between the mussels. Condition 2 mussels laid onto field sand with 1cm space between mussels. Condition 3 mussels laid onto sand in the laboratory, allowed to form clumps then the clumps were transported and laid onto sand in the field. The comparisons between mussels the three experimental conditions at the upper intertidal and at the lower intertidal were significant (Table 25. UI touching 5/15 Vs UI spaced 5/15 Vs UI laboratory 5/15 $\chi^2 = 15.054$, d.f. = 5, $0.05 > P > 0.01^*$ and LI touching 5/15 Vs UI spaced 5/15 Vs UI laboratory 5/15 $\chi^2 = 20.816$, d.f. = 5, $P < 0.001^{***}$). The only other significant comparison between numbers of mussels lost and numbers of mussels remaining was between laboratory clumped groups of five mussels and laboratory clumped groups

of 15 mussels comparing the upper and lower intertidal (Table 25 UI lab 5/15 Vs LI lab 5/15 $\chi^2 = 29.30$, d.f. = 3, $P < 0.001^{***}$). Table 27 shows the Chi-squared statistical analysis comparing the numbers of mussels lost and the numbers of mussels remaining on day 10 of experiment III. Only two of the comparisons were significant. The lower intertidal comparison between the three experimental conditions (Table 27: LI touching 5/15 Vs LI spaced 5/15 Vs LI laboratory 5/15 $\chi^2 = 13.908$, d.f. = 5, $0.05 > P > 0.01^*$). The upper intertidal/lower intertidal comparison of groups of 5 and groups of 15 laboratory clumped mussels (Table 27: UI laboratory 5/15 Vs LI laboratory 5/15 $\chi^2 = 14.815$, d.f. = 3, $0.01 > P > 0.001^{**}$). Table 29 shows the Chi-squared statistical analysis comparing the numbers of mussels lost and the numbers of mussels remaining on day 24 of experiment III. The comparison of the three experimental conditions at the upper intertidal was not significant for numbers of mussels lost and numbers of mussels remaining. The lower intertidal had no mussels left after 24 days exposure on the shore.

Figure 36: Figure showing the movement and direction that mussels were transported on the shore after six days at both the upper and lower intertidal in experiment III. The upper intertidal low energy site displays a directional pattern for the distance and direction that mussels were transported on the shore in the direction of the prevailing wind and wave energy. The maximum distance that mussels were transported at the upper intertidal site was 790cm. The lower intertidal high-energy site displays a directional pattern for the distance and direction that mussels were transported on the shore in the direction of the prevailing wind and wave energy. The maximum distance that mussels were transported at the lower intertidal site was 1030cm.

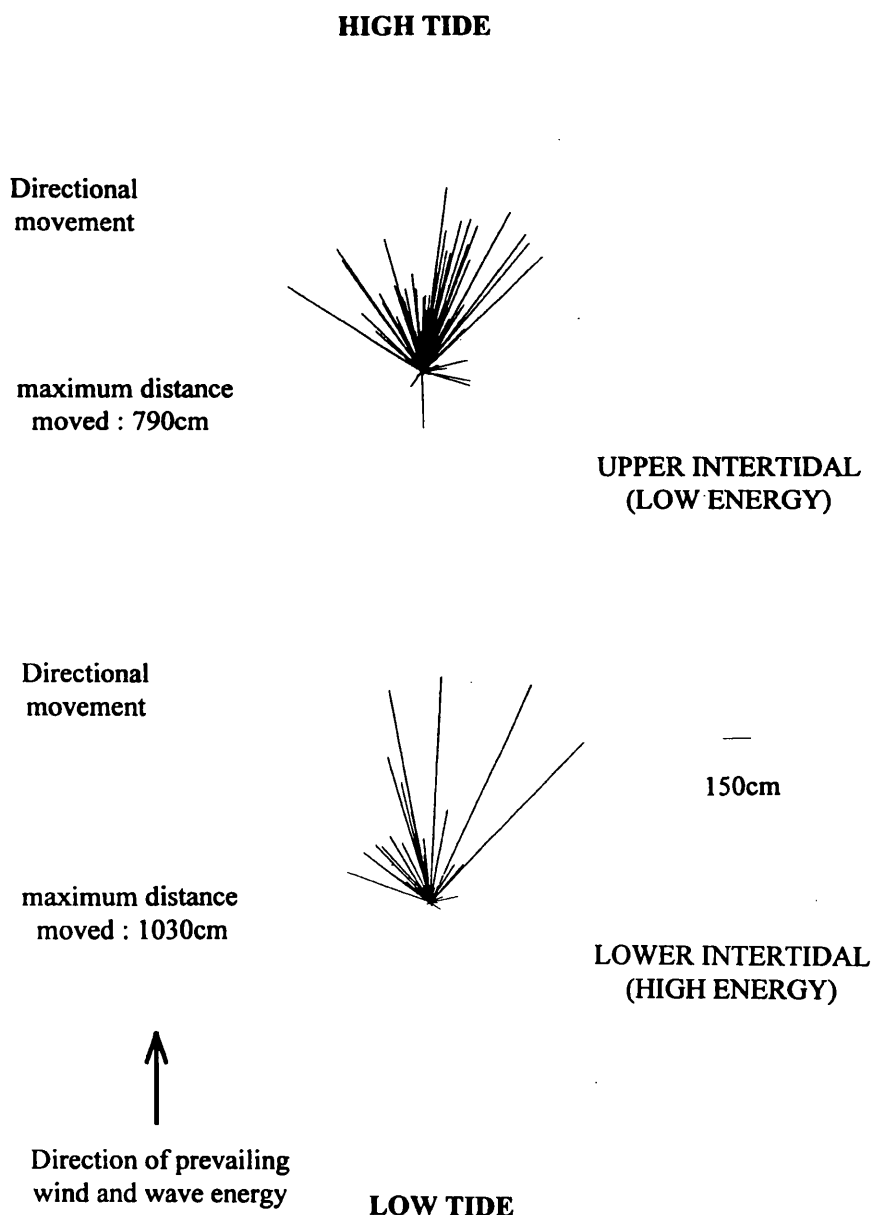


Figure 37: Figure showing the movement and direction that mussels were transported on the shore after 10 days at both the upper and lower intertidal in experiment III. The upper intertidal low energy site displays a directional pattern for the distance and direction that mussels were transported on the shore in the direction of the prevailing wind and wave energy. The maximum distance that mussels were transported at the upper intertidal site was 1820cm. The lower intertidal high-energy site displays a directional pattern for the distance and direction that mussels were transported on the shore in the direction of the prevailing wind and wave energy. The maximum distance that mussels were transported at the lower intertidal site was 1060cm.

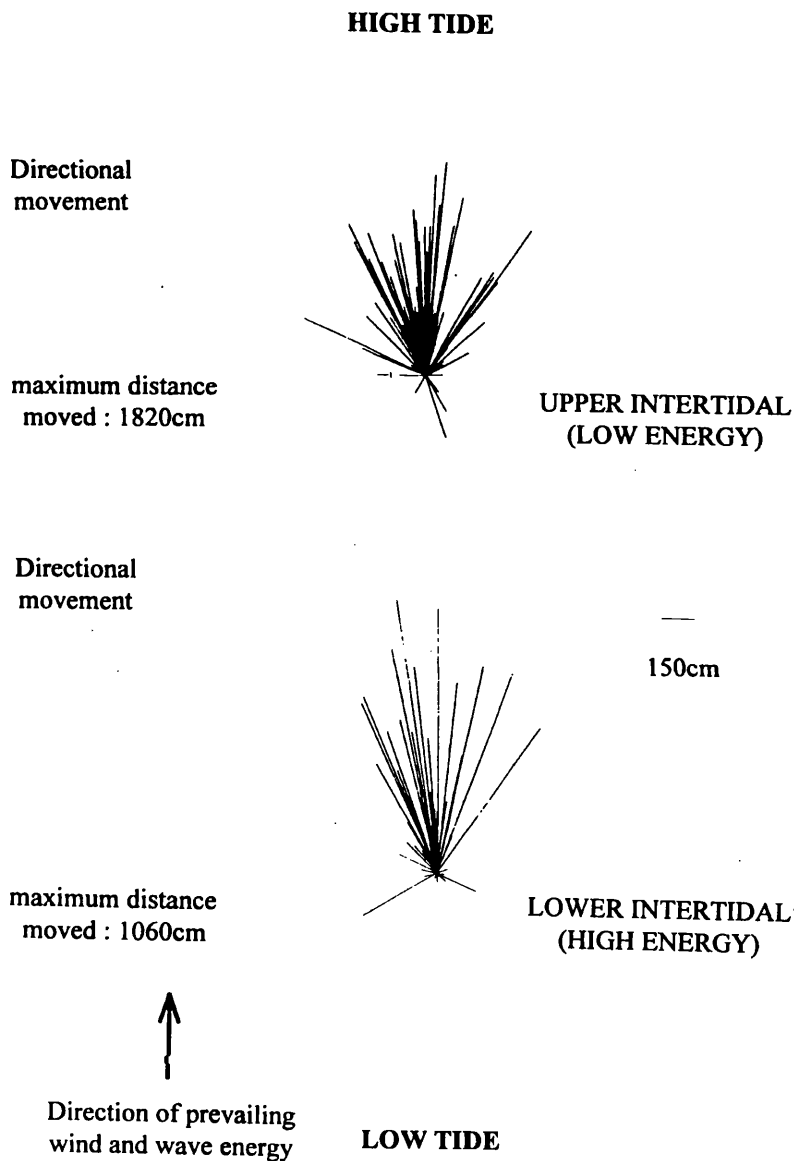


Figure 38: Figure showing the movement and direction that mussels were transported on the shore after 24 days at both the upper and lower intertidal in experiment III. The upper intertidal low energy site displays a directional pattern for the distance and direction that mussels were transported on the shore in the direction of the prevailing wind and wave energy. The maximum distance that mussels were transported at the upper intertidal site was 1060cm. There were no mussels remaining at the lower intertidal site.

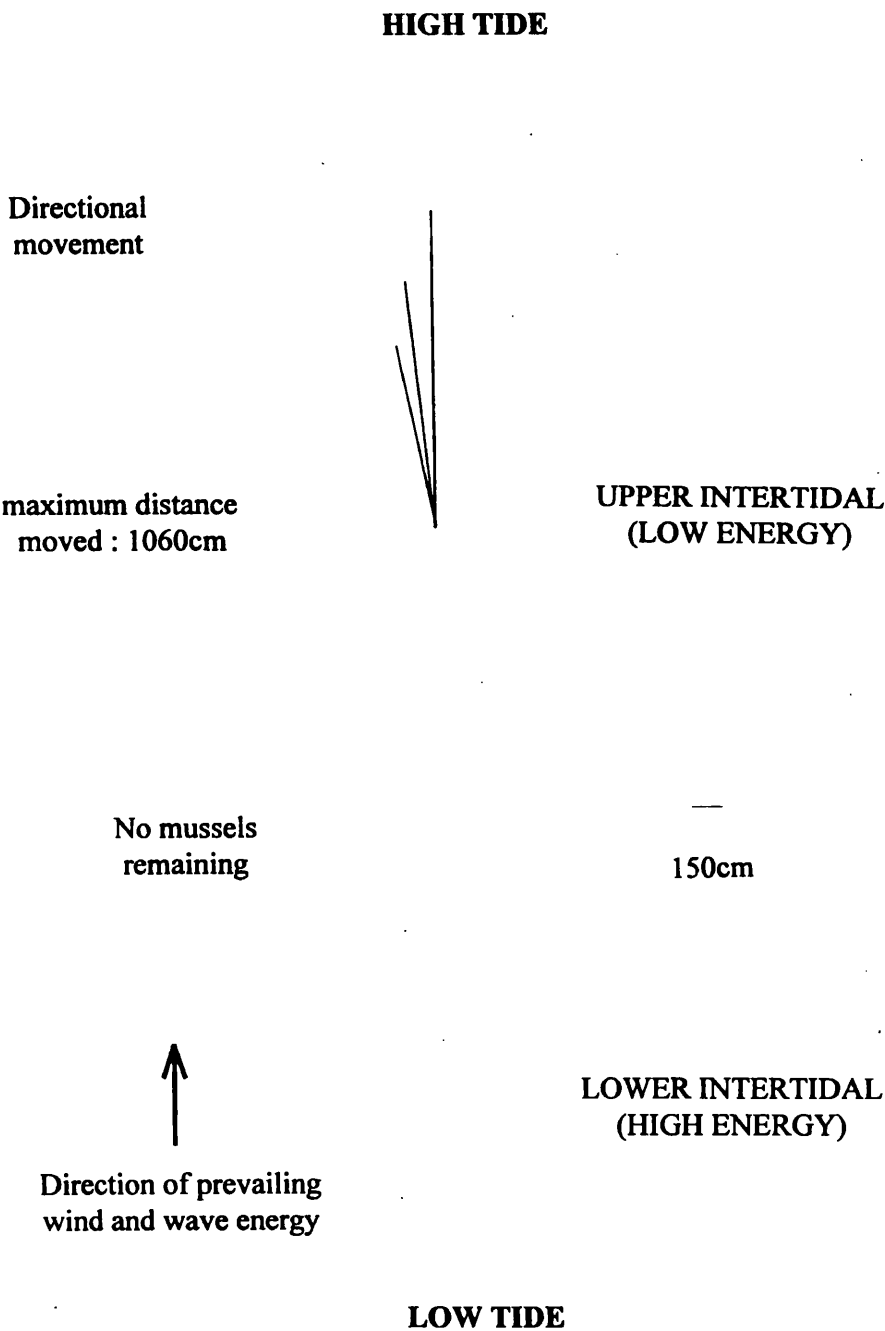


Table 24: Table showing the numbers of mussels lost and remaining after 6 days for the three experimental conditions at both the upper and lower intertidal used in experiment III. Three different densities of mussels used individual mussels, groups of 5 mussels and groups of 15 mussels (1, 5 and 15, respectively). Touching = mussels laid onto field sand with no space between the mussels. Spaced = mussels laid onto field sand with 1cm space between mussels. Laboratory = mussels laid onto sand in the laboratory, allowed to form clumps then the clumps were transported and laid onto sand in the field.

| | | Numbers of mussels | | |
|------------------|-----------|--------------------|----|----|
| | | 1 | 5 | 15 |
| UI Touching | Remaining | -- | 9 | 24 |
| | Lost | -- | 1 | 6 |
| | Total | -- | 10 | 30 |
| | | | | |
| UI Spaced | Remaining | 1 | 9 | 24 |
| | Lost | 1 | 1 | 6 |
| | Total | 2 | 10 | 30 |
| | | | | |
| UI Laboratory | Remaining | 1 | 3 | 24 |
| | Lost | 1 | 7 | 6 |
| | Total | 2 | 10 | 30 |
| | | | | |
| LI Touching | Remaining | -- | 10 | 21 |
| | Lost | -- | 0 | 9 |
| | Total | -- | 10 | 30 |
| | | | | |
| LI Spaced | Remaining | 1 | 7 | 19 |
| | Lost | 1 | 3 | 11 |
| | Total | 2 | 10 | 30 |
| | | | | |
| LI Laboratory | Remaining | 1 | 10 | 30 |
| | Lost | 1 | 0 | 0 |
| | Total | 2 | 10 | 30 |
| | | | | |

Table 25: Table showing the Chi-squared (χ^2) statistical analysis comparing numbers of mussels lost and remaining after 6 days for the three experimental conditions at both the upper and lower intertidal used in experiment III. Three different densities of mussels used individual mussels, groups of 5 mussels and groups of 15 mussels (1, 5 and 15, respectively). Touching = mussels laid onto field sand with no space between the mussels. Spaced = mussels laid onto field sand with 1cm space between mussels. Laboratory = mussels laid onto sand in the laboratory, allowed to form clumps then the clumps were transported and laid onto sand in the field. d.f. = degrees of freedom. P = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non asterisked probabilities are not significant.

| Comparison | d.f. | χ^2 | P |
|---|------|----------|---------------------|
| UI touching 5/15 Vs UI spaced 5/15 Vs UI lab 5/15 | 5 | 15.054 | $0.05 > P > 0.01^*$ |
| LI touching 5/15 Vs LI spaced 5/15 Vs LI lab 5/15 | 5 | 20.816 | $P < 0.001^{***}$ |
| UI touching 5/15 Vs LI touching 5/15 | 3 | 5.00 | $0.20 > P > 0.15$ |
| UI spaced 5/15 Vs LI spaced 5/15 | 3 | 3.723 | $0.30 > P > 0.25$ |
| UI lab 5/15 Vs LI lab 5/15 | 3 | 29.30 | $P < 0.001^{***}$ |

Table 26: Table showing the numbers of mussels lost and remaining after 10 days for the three experimental conditions at both the upper and lower intertidal used in experiment III. Three different densities of mussels used individual mussels, groups of 5 mussels and groups of 15 mussels (1, 5 and 15, respectively). Touching = mussels laid onto field sand with no space between the mussels. Spaced = mussels laid onto field sand with 1cm space between mussels. Laboratory = mussels laid onto sand in the laboratory, allowed to form clumps then the clumps were transported and laid onto sand in the field.

| | | Numbers of mussels | | |
|------------------|-----------|--------------------|----|----|
| | | 1 | 5 | 15 |
| UI Touching | Remaining | -- | 8 | 25 |
| | Lost | -- | 2 | 5 |
| | Total | -- | 10 | 30 |
| | | | | |
| UI Spaced | Remaining | 2 | 10 | 25 |
| | Lost | 0 | 0 | 5 |
| | Total | 2 | 10 | 30 |
| | | | | |
| UI Laboratory | Remaining | 1 | 6 | 26 |
| | Lost | 0 | 4 | 4 |
| | Total | 2 | 10 | 30 |
| | | | | |
| LI Touching | Remaining | -- | 10 | 23 |
| | Lost | -- | 0 | 7 |
| | Total | -- | 10 | 30 |
| | | | | |
| LI Spaced | Remaining | 2 | 7 | 26 |
| | Lost | 0 | 3 | 4 |
| | Total | 2 | 10 | 30 |
| | | | | |
| LI Laboratory | Remaining | 1 | 10 | 30 |
| | Lost | 1 | 0 | 0 |
| | Total | 2 | 10 | 30 |
| | | | | |

Table 27: Table showing the Chi-squared (χ^2) statistical analysis comparing numbers of mussels lost and remaining after 10 days for the three experimental conditions at both the upper and lower intertidal used in experiment III. Three different densities of mussels used individual mussels, groups of 5 mussels and groups of 15 mussels (1, 5 and 15, respectively). Touching = mussels laid onto field sand with no space between the mussels. Spaced = mussels laid onto field sand with 1cm space between mussels. Laboratory = mussels laid onto sand in the laboratory, allowed to form clumps then the clumps were transported and laid onto sand in the field. d.f. = degrees of freedom. P = probability. Probabilities given are asterisked significant according to the following system. $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non asterisked probabilities are not significant

| Comparison | d.f. | χ^2 | P |
|---|------|----------|-------------------------|
| UI touching 5/15 Vs UI spaced 5/15 Vs UI lab 5/15 | 5 | 6.240 | $0.30 > P > 0.25$ |
| LI touching 5/15 Vs LI spaced 5/15 Vs LI lab 5/15 | 5 | 13.908 | $0.05 > P > 0.01^*$ |
| UI touching 5/15 Vs UI touching 5/15 | 3 | 2.886 | $0.45 > P > 0.40$ |
| UI spaced 5/15 Vs UI spaced 5/15 | 3 | 3.660 | $0.35 > P > 0.30$ |
| UI lab 5/15 Vs LI lab 5/15 | 3 | 14.815 | $0.01 > P > 0.001^{**}$ |

Table 28: Table showing the numbers of mussels lost and remaining after 24 days for the three experimental conditions at both the upper and lower intertidal used in experiment III. Three different densities of mussels used individual mussels, groups of 5 mussels and groups of 15 mussels (1, 5 and 15, respectively). Touching = mussels laid onto field sand with no space between the mussels. Spaced = mussels laid onto field sand with 1cm space between mussels. Laboratory = mussels laid onto sand in the laboratory, allowed to form clumps then the clumps were transported and laid onto sand in the field.

| | | Numbers of mussels | | |
|------------------|-----------|--------------------|----|----|
| | | 1 | 5 | 15 |
| UI Touching | Remaining | -- | 0 | 1 |
| | Lost | -- | 10 | 29 |
| | Total | -- | 10 | 30 |
| UI Spaced | Remaining | 0 | 1 | 0 |
| | Lost | 2 | 9 | 30 |
| | Total | 2 | 10 | 30 |
| UI Laboratory | Remaining | 1 | 0 | 0 |
| | Lost | 1 | 10 | 30 |
| | Total | 2 | 10 | 30 |
| LI Touching | Remaining | -- | 0 | 0 |
| | Lost | -- | 10 | 30 |
| | Total | -- | 10 | 30 |
| LI Spaced | Remaining | 0 | 0 | 0 |
| | Lost | 2 | 10 | 30 |
| | Total | 2 | 10 | 30 |
| LI Laboratory | Remaining | 0 | 0 | 0 |
| | Lost | 2 | 10 | 30 |
| | Total | 2 | 10 | 30 |

1.4) DISCUSSION

The study of sediment stability concentrates on the stability of sediment slopes. There are many factors, which affect the stability of such slopes. The coastal zone is an area of great interest as beaches of soft substratum and gravel are formed from slopes of varying angles. The stability of sediments is of great concern in the coastal zones of the world especially as they are constantly under threat from the natural winter erosional processes of wind and tidal regimes. The predicted rise in global sea level will cause an increase in these erosional factors. Protection of the coastal zone by human intervention has been conducted predominantly in residential or industrial coastlines. Areas with low socio-economic importance may therefore be overlooked. This is paralleled in the terrestrial environment where engineering is used to stabilise sedimentary environments with socio-economic importance (Bromhead 1992, Brunsden & David 1984, Chandler 1991, Kraebel 1936, bowers 1950). There has been considerable interest and activity in the application of hard engineering methods to the problem of coastal erosion. Most of the work has been concerned with constructing structures that impede the waves before they interact with the shoreline. These structures include groins, artificial reefs, jetties, breakwaters and floating barriers (U.S. army, corps of engineers, 1984a & 1984b, Harlow 1990, Silvester 1990, Kelletat 1992, Simm 1996, Youdeowei & Abam 1997).

The structures, however, are constructed in shipping channels to prevent eroded sediment from being deposited in the channel which would then require dredging. Many coastlines are not connected with shipping and these still require some kind of protection. In these areas, the engineering methods are usually far too expensive as they do not directly affect industry. The development of cheaper and more environmentally acceptable methods of protecting these coastlines is becoming very important. Implementation of such methods has been conducted in developing countries as coastal environments directly affect the countries economy delta (Bennett & Reynolds 1993, Weinstock 1994, Youdeowei & Abam 1997). In developed countries, these coastlines are seen as areas of outstanding beauty, areas of special scientific interest or areas near housing developments.

The use of indigenous marine intertidal organisms is one method of environmentally acceptable coastal protection. Mussel beds formed by the species *Mytilus edulis* are known to form in a wide range of environments in the coastal zone (Kuenen 1942, Maas Geesteranus 1942, Suchanek 1978). These range from sheltered estuarine conditions to relatively high energy sandy shores to open coastlines. The mussel beds cover the underlying sediment and physically shield it from the action of waves and currents. This effect is paralleled by the use of geomembranes combined with organisms in engineering methods of reinforcing sediments (Gray & Leiser 1982, Agassi & Ben-hur 1992). The understanding of the mechanism by which these mussels form, maintain and repair these mussel beds is therefore of considerable importance. This is essential in the future development of mussel beds as environmentally acceptable methods of coastal zone protection. In this section, various aspects of the behaviour of mussels both in the laboratory and in the field have been investigated. More specifically in the field there was a focused investigation of the modification of these behaviours by contrasting high and low energy environments.

There have been a number of studies on the formation and strength of byssus threads by *Mytilus edulis*; these are relevant to the results found in this section. Mussels form threads quickly both in the laboratory and the field. They can produce between 5 and 40 threads per day (Allen *et al.*, 1976, Meadows & Shand 1989, Dolmer & Svane 1994, Personal observations). The rate of production can be affected by a number of factors animal size, salinity, oxygen and temperature (Reish & Ayers 1968, Allen *et al.*, 1976, Van Winkle 1979). Personal observations show that *Mytilus edulis* can produce and attach its first thread within one hour of being placed in a new environment. Byssus threads are then continuously produced which will increase the strength of the mussel's attachment over time. It is estimated that a mussel with 50 attached byssus threads would be able to withstand all but the most severe winter storms (Smeathers & Vincent 1979). The age and length of byssus threads is also of importance to overall strength. Older byssus threads are weaker than those more recently formed and longer threads are more resilient under strain (Price 1981). A more detailed study revealed that within the thread the end farthest from the mussel is stronger than the

end nearest the mussel (Allen *et al.*, 1976, Bell & Gosline 1996). The number and strength of byssus threads that mussel produce also varies in relation to changing environmental conditions. Greater numbers of byssus threads are formed in higher water velocities (Glaus 1968, Dolmer & Svane 1994). The strength of byssus attachment is found to be greater in exposed habitats with greater wave energy than in sheltered habitats (Price 1982, Witman and Suchanek 1984).

The upper and lower intertidal zones on Ardmore bay are very different in terms of their exposure to wave energy (Tufail *et al.*, 1989). This is because the prevailing winds blow directly into the bay from the west. The lower intertidal zone is a relatively high-energy area and the upper intertidal zone is a relatively low-energy area. This is most noticeable with a strong on-shore wind, where white capped waves are present only in the lower intertidal zone and not in the upper intertidal zone. Figure 30 page 87 and Figure 37 page 137, show the distinct contrast that this effect has on the distance and direction that mussels are transported at the upper intertidal and the lower intertidal. However, Figures 36 to 38 pages 136 to 138 show that there is also a distinct contrast between the north and south sides of the bay in terms of wave energy. This experiment shows that both the upper intertidal site and the lower intertidal site experience a higher energy environment. Very few mussels remained at the upper intertidal at the end of the experiment and no mussels remained at the lower intertidal. In all of the field experiments, more mussels were lost from single mussel quadrats. This was due predominantly to mussels being swept away by the tidal currents. However, some researchers discuss the role of predators in the loss of mussels from mussel beds and shorelines. Benthic predators (Cote & Jelnikar 1999, Rovero *et al* 1999). Seabirds (Hamilton 2000, Ost & Kilpi 2000). Personal observations also indicate some loss of mussels down feeding depressions of *Cardium edule*. The mussels are simply covered by sediment once drawn into the depression.

There is also a seasonal effect present expressed in the wave energy in the bay. Experiment 2 was conducted in October on the south side of the bay; this provided a specific pattern for the transport and displacement of mussels on the shore. After 18 days of the experiment mussels in the upper

intertidal were transported a maximum of 0.8m whereas mussels in the lower intertidal were transported a maximum of 3.8m. Experiment 4 was conducted in December when coastal areas typically experience storms. This is demonstrated by the greater distances that mussels were transported on the shore at both the upper and the lower intertidal sites. After 18 days the mussels had been transported a maximum of 2.11m and 11.82m for the upper intertidal and lower intertidal respectively. The contrasting regimes of energy and water currents are almost certainly responsible for the differences in the distances mussels were transported at the two tidal levels.

This correlates with work conducted by Price (1982) that showed that byssus threads are stronger in summer and autumn and weakest in winter and spring.

The joint experiment conducted with Patricia Whitaker uncovered a spatial dimension to the energy regimes on the shore. This experiment was conducted on the north side of the bay in contrast to the author's experiments conducted on the south side of the bay. The experiment was conducted in December. This work also showed the lower intertidal of this area of the bay to be a relatively high-energy zone. However after 10 days the upper and lower intertidal showed no obvious differentiation in pattern of distance and transport of mussels. At the end of the experiment after 24 days no mussels were left at the lower intertidal site and the few remaining at the upper intertidal showed a pattern for the mussels transported in the direction of the prevailing current. This is important because it clearly shows that mussels that were laid on the shore in areas of high energy without an introduced substrate will be washed away. There were significantly more mussels remaining after 18 days in experiment 4 that had been laid onto plastic mesh than onto the field sand itself (Table 16c: $\chi^2 = 35.740^{***}$ page 118). This is shown to a greater degree by the analysis of the numbers of mussels lost from the upper and lower intertidal sites from these contrasting experiments. Experiment 2 using an introduced gravel substrate showed that the gravel had a significant effect on the numbers of mussels lost (Table 9b page 94). Mussels that had been allowed to attach to gravel in the laboratory before being laid into the field were less likely to be lost than mussels laid directly into the field onto either sand or gravel. At the upper intertidal site,

2% were lost from the laboratory treated mussels and 14% were lost from mussels laid directly into the field. The equivalent percentages at the higher energy lower intertidal site were 6% and 31%. The number of gravel particles lost or gained by mussels during the experiment 2 were also analysed (Tables 10a to 10d pages 97 to 100). In general, individual mussels and clumps of five mussels lost significant numbers of gravel particles while clumps of 15 mussels gained significant numbers of gravel particles. Clumps of 15 mussels gained gravel particles at both the upper and lower intertidal sites. This is interesting because after 18 days more mussels were lost from clumps of 15 mussels at the lower intertidal site (Table 8a: 84%, 77% and 67%, for 15 mussels, 5 mussels and individual mussels respectively. Page 92). In contrast, at the upper intertidal site mussels gained a significant advantage from being in a clump. Only 6% were lost from clumps of 15 mussels compared with 15% from 5 mussels and 42% from single mussels. This difference was highly significant (Table 8b: $\chi^2 = 18.19^{***}$ page 92). From these results, therefore, mussels in clumps are protected from being lost in low-energy intertidal environments but not in high-energy intertidal environments. This result is somewhat surprising. The result may be due to large groups of mussels having a larger surface area for the energy of waves to act against. The mussels that survive at the lower intertidal site nevertheless increase the number of gravel particles that they pick up, to the same degree as the larger number of mussels at the upper intertidal site. Perhaps the higher energy regimes stimulate the mussels to seek more frequently for gravel particles that will then provide them with greater anchorage. This is partly verified by experiment 4 using introduced plastic mesh substrate. In this experiment, the mussels that were allowed to form attachments onto plastic mesh in the laboratory before being laid out into the field were at a significant advantage than these that did not have this treatment. They were in effect better adapted to the high energy lower intertidal environment as only 7% of mussels were lost on day 11 compared to 26% lost from mussels laid as individuals onto mesh in the field. This increased to 23% lost from laboratory mesh treated mussels and 73% lost from the mussels laid onto mesh in the field. The control conditions, mussels that were allowed to form clumps in the laboratory onto sand collected from the field and

mussels laid as individuals directly onto field sand, also lost more mussels than the laboratory mesh treated mussels.

Two laboratory experiments were conducted under static water conditions to investigate the responses of mussels to gravel, plastic mesh and sand. In experiment 1 the five mussels laid onto sand formed clumps by attaching byssus threads to the other mussel's shells. In the presence of sand + gravel mix or pure gravel, they produce more threads in total while attaching fewer threads to the other mussel's shells (Figure 25 page 79 and Table 4 page 78). This is corroborated by Shand (1991) who demonstrated that mussels attached to underlying gravel particles in a laboratory environment. Clumps of mussels that form onto sand containing gravel will be anchored to the sedimentary bed by threads being attached to gravel particles as well as to the shells of the other mussels. Under field conditions, these will have a great adaptive importance. Anchoring is needed mostly in high-energy environments, which is where gravel is found. This work shows the potential for mussels to be introduced into high-energy environments as a novel environmentally acceptable method of protecting such coastlines. The second laboratory experiment investigated attachment strength on plastic mesh. Plastic mesh may be considered as another artificial attachment surface for mussels. The attachment strength of mussel byssus to plastic mesh was compared to the attachment strength of mussels onto mussel shell. No significant difference was found between these two types of attachment. This itself is a most important result as it shows that the attachment from one mussel to another mussel's shell and the attachment to plastic netting are about the same. This could give rise to a viable substrate for sowing mussels onto in large quantities. Which could be laid onto soft sedimentary ecosystems as a method of shielding the sediment from wave energy.

For future experiments the mussels could be allowed to attach to the plastic netting in large holding tanks over a one-month period. This time would allow the mussels to become well established with firm attachments to the plastic mesh. The mesh could then be transported by container truck either in large sections or in a roll, which would be rolled out onto the shore. A second method would be simply to spread counted mussels onto the surface of the mesh on the shore. These mussels

however would require about one week to form attachments to the mesh under favourable tidal conditions. The netting would have to be fixed down onto the sediment in the same way as in experiments 2 and 4 in this section. The use of bamboo canes to fix down the mesh is firstly unobtrusive to the eye and secondly there is a small surface area, which will minimise erosion currents being formed. In simple terms the smaller the surface area exposed the easier the water passes around it. This means that large fast water currents are not formed as the water physically bends around an object. The project essentially needs a large-scale experiment to assess viability. On Ardmore Bay this would translate to three 50m by 1m areas of plastic mesh with mussels attached at both the upper and lower intertidal zones. There would be no specific groups of mussels, the surface of the mesh would simply be covered by mussels. The number of mussels on the mesh would be known and they would be of an average size between 4 cm and 6 cm in length. This experiment would benefit greatly from being set up in the summer months where both tides and winds are in general weaker. These weak tidal conditions would allow the mussels to become established by the appearance of the winter storms. The experiment would be left for one year. After this time the numbers of mussels on the netting would be checked. The sizes of the mussels attached to the netting would also be checked. This action would indicate whether recruitment of larvae was occurring. The particle size of the top 5 cm of the sedimentary column would be measured both in front of and behind each area of mesh at both tidal sites. This would give an indication of the degree of force dissipated from the waves interacting with the shore. The experiment would also be carried out with similar methodology on higher energy beaches as a comparison. This method of coastal zone protection is an improvement on engineering methods for a number of reasons. Firstly, this method would cost considerably less than an engineering construction. Secondly this method is less labour intensive than an engineering construction and requires less active maintenance. Finally, this is an environmentally friendly project using mostly natural materials. This is important where unsightly constructions may spoil an area of natural beauty or disturb natural wildlife. This method after a short time will simply resemble large natural mussel beds with the plastic mesh covered by sediment thus rendering the entire installation aesthetically and environmentally acceptable.

SECTION : II

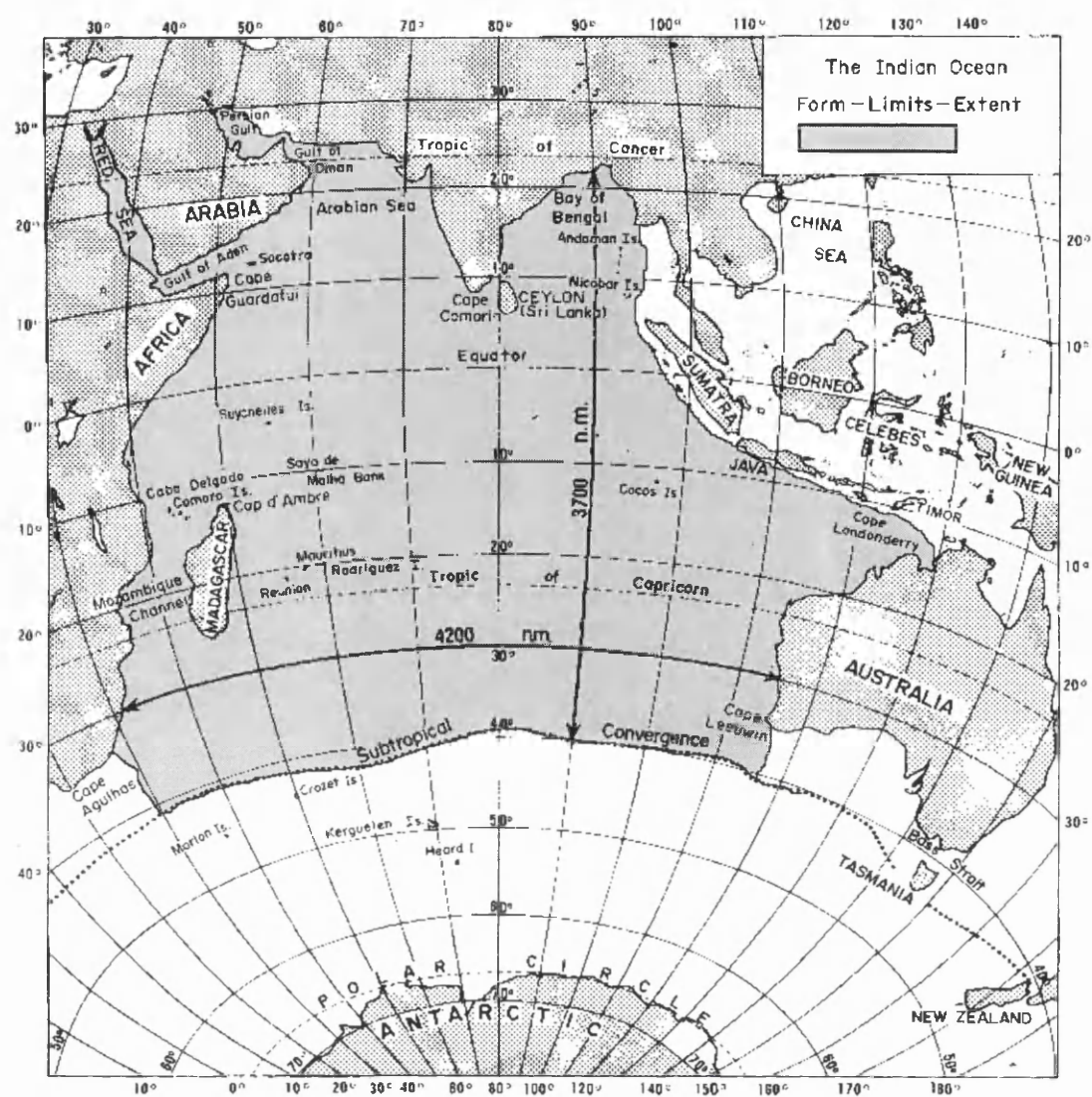
Effect of the oxygen minimum zone off the coast of Oman in the Arabian Sea on biological mixing, geochemistry and Geotechnics of sediments on the continental slope and abyssal plain

2.1 INTRODUCTION

2.1.1 Continental slope and abyssal plain environments of the Indian Ocean/Arabian Sea

The physical form and extent of the Indian Ocean along with the Arabian Sea are shown in Figure 39. The depth of the Indian ocean ranges from less than 200m to over 400m (Kolla *et al.*, 1981, Coumes & Kolla 1984). The continental slope is relatively small with significant depths of water close to the continents coast. The topography of the bottom of the Indian Ocean includes many ridges formed from the oceanic ridge system that encircles the globe. There are three principal ridges, the Southeast, the Southwest Ridge and the Carlsberg Ridge. These ridges are spreading ridges, formed when convection in the asthenosphere layer of the earth's core pushes molten magma to the surface of the earth. This emerges and cools to forming new oceanic crust. This action is complemented by subduction zones where tectonic plates are forced underneath each other. The spreading zones of the worlds oceans all have different spreading rates. The spreading rate of the Indian Ocean is 2 cm per year (Dietrich 1973). The western part of the Indian Ocean is more fragmented than the eastern part. The eastern part has three principal basins whereas the western part has eight principal basins. In both of these, the basins are separated by minor ridge systems formed from buckled oceanic plate. The Arabian Sea has a more simple topography. The sea has two main basins with the Murray ridge running between them (Kolla *et al.*, 1981). The SE basin is smoother due to large inputs of sediment from the Indus River (Kolla *et al.*, 1981, Coumes & Kolla 1984, Meadows & Meadows 1999). The Indian Ocean has restricted circulation to the north. This restricted circulation affects the circulation of the weather patterns. According to Wyrтки (1973), the Indian Ocean has three main circulation systems. Firstly there is a seasonal monsoon gyre, secondly a south hemisphere subtropical anticyclone gyre and finally the Antarctic waters with the circumpolar current. The latter two circulation patterns are found in the other oceans of the world. The Indian Ocean is however

Figure 39: Physical limits of the Indian Ocean (Wyrтки 1973)



unique in having a reversing monsoon gyre shown in Figure 40. The monsoon system is comprised of two seasonally separate components. The first of these the northeast monsoon forms in November and remains until April. The northeast monsoon is gentle with slight disturbance of the thermocline along with weak upwelling.

The SW monsoon begins in May and lasts until October. There is a general reversal in circulation of the northern Indian Ocean and Arabian Sea due to the formation of the SW monsoon. The current velocities are much stronger penetrating below the thermocline. Strong upwelling is also observed along the northern coastlines.

Upwelling forms when winds cause currents to flow from the shore displacing surface water which is replaced by deep water rising to the surface (Smith 1968). This deep water is nutrient rich compared to the nutrient depleted surface waters. This introduction of nutrients causes a burst of activity in the phytoplankton (Aruga 1973, El-Sayed & Jitts 1973). This in turn causes an increase in fish populations (Currie *et al.*, 1973). The Arabian Sea in particular shows strong areas of upwelling. Figure 41 shows a schematic representation of upwelling on the Oman margin of the Arabian Sea. The Arabian Sea is unique as it is essentially enclosed by land on three sides. This not only restricts the circulation patterns but also affects the types of sediment found on the seabed. The Arabian Sea also exhibits a layer of high salinity water deep in the water column. This affects circulation at intermediate depths, preventing water coming in from southern regions (Qasim 1998). An extensive study of the sediments of the Arabian Sea conducted by Richard *et al.*, (1965) shows that terrigenous sediment is predominant. Wind blown sediment carried by the monsoon contributes to a significant part of the sediment of the sea's basin.

Figure 40: Reversing seasonal monsoon system of the Indian Ocean/Arabian Sea (Wyrtki 1973).

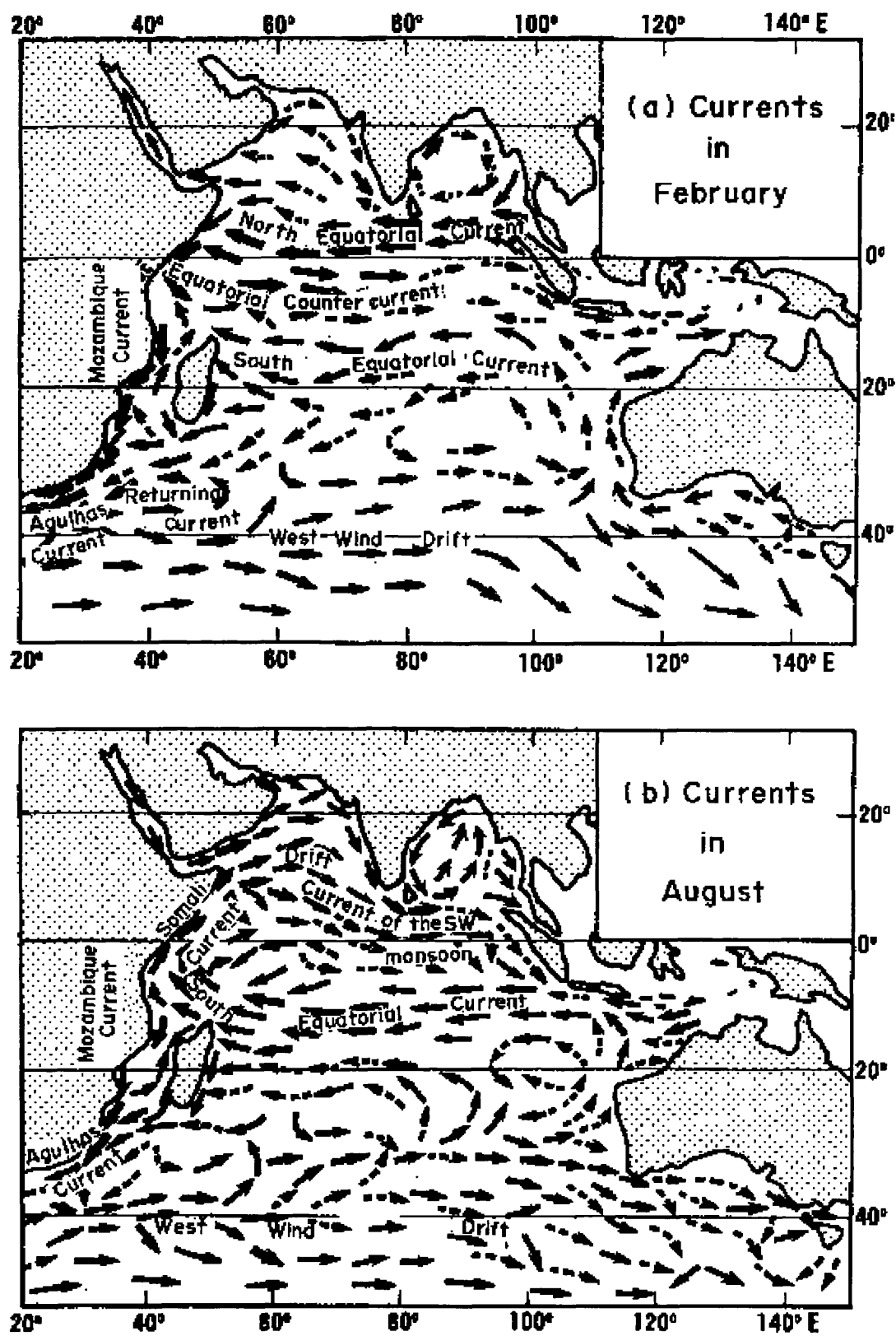
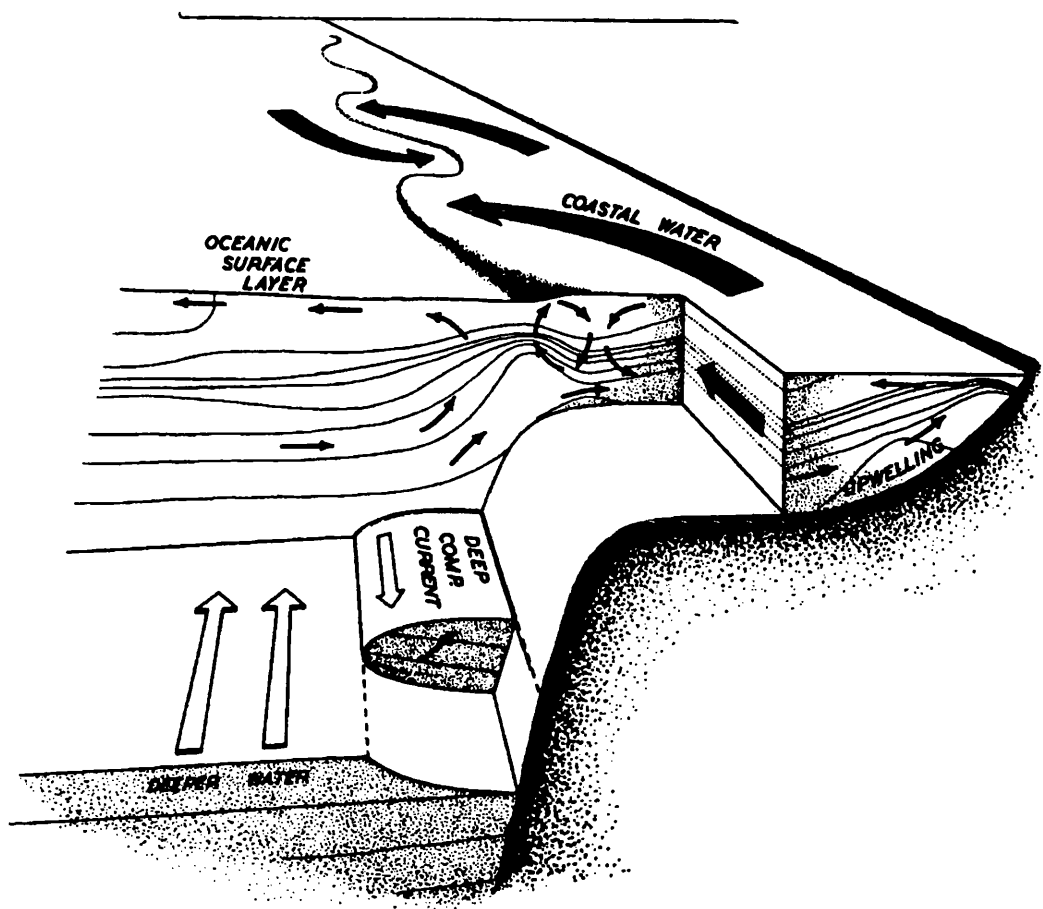
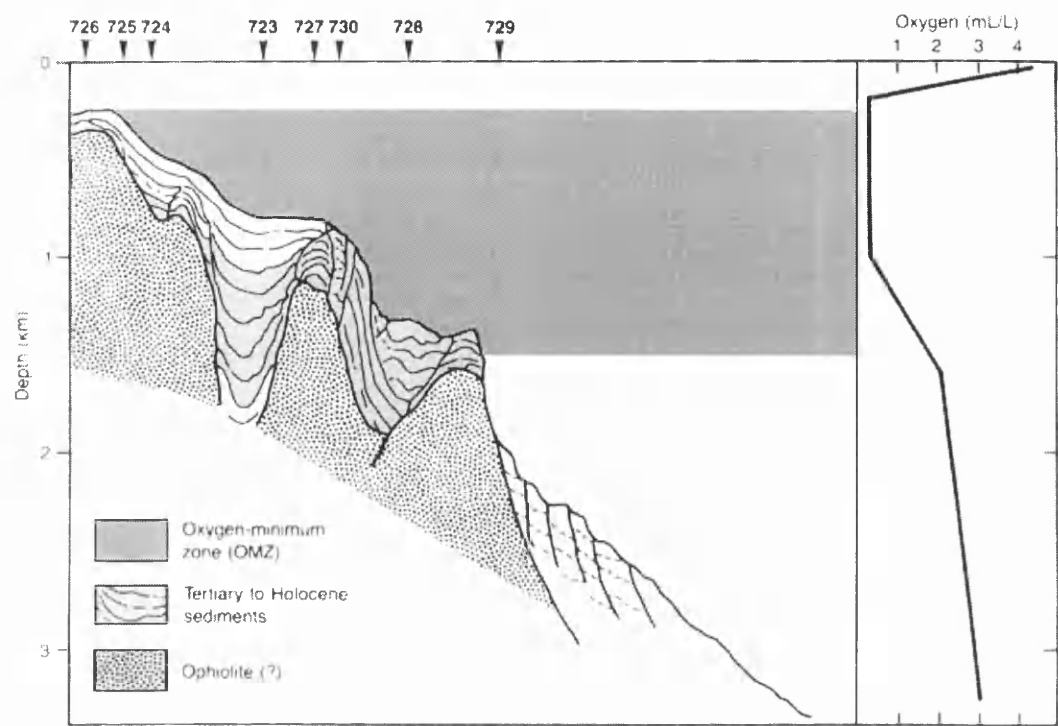


Figure 41: Schematic representation of the upwelling system of the Arabian Sea generated by the Benguela current (Currie *et al.*, 1973).



The sediments of the Oman Margin are of particular interest as they are exposed to the mid water oxygen minimum zone (OMZ). This extends from inshore waters horizontally out and with a depth range between 200 m and about 1000 m (Meadows & Meadows 1999). Figure 42 shows a schematic extent of the OMZ on the Oman margin. OMZ's are well-known phenomena in the worlds oceans. These phenomena have been found not only in the Arabian Sea but also in the Bay of Bengal, NW Pacific margin, eastern Pacific, the Philippines and off the SW coast of Africa (Kamykowski & Zentara 1990). OMZ's are thought to form because of high productivity and poor circulation (Rogers 2000). The Arabian Sea is an environment with high productivity and poor circulation created from the monsoon upwelling and land restricted circulation. It is suggested by Wyrski (1962), that organic matter sinking through the water column is degraded by microorganisms, which results in low oxygen localised at the depth of poorest circulation in the water column. Below this layer of low oxygen water the oxygen levels increase due to a decrease in the biological oxygen demand which in turn may be due to increased circulation at that depth in the water column. This increased circulation will bring oxygen into the area so controlling the populations of reducing microorganisms (Wyrski 1962, Sarimento *et al.*, 1988, Kamykowski & Zentara 1990). In certain areas, the OMZ impinges onto the continental shelf. This may impose stress on organisms living on or in the sediments of the continental slope (Childress & Seibel 1998, Lamont & Gage 2000). The particular site of study in the Arabian Sea off the Oman coast is reported to be inhabited by dense communities of polychaete worms from the Spionid, Cirratulid, Ampharetid and Paraonid families (Levin *et al.*, 2000). These benthic communities will experience stress due to low oxygen concentrations in the water and sediment. Investigations of this sedimentary ecosystem will expand our knowledge of the effects of stress presented by the unusual environmental conditions related to the area (Slater & Kroopnick 1984, Sardesai 1994, 1995, Von Rad *et al.*, 1996). This research may also give insights into the study of trace fossil which relates the behaviour and ecology of organisms to the structures found in sedimentary rocks (Bromley, 1990). A significant part of this thesis is published in the Journal of Deep Sea Research (Meadows *et al.*, 2000).

Figure 42: Schematic representation of the OMZ of the Oman Margin.



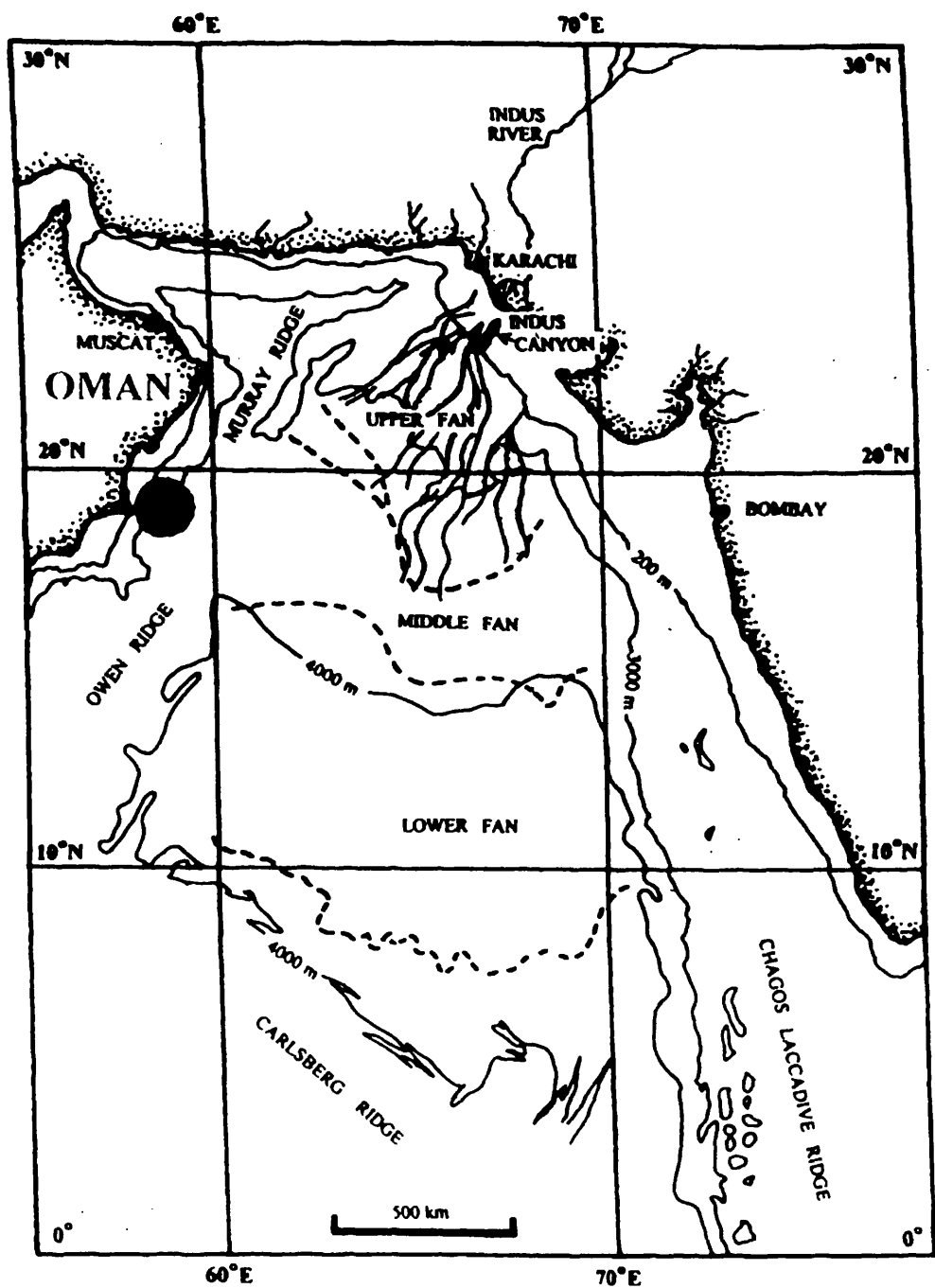
2.2 MATERIALS AND METHOD

This section deals with joint research conducted on an oxygen minimum zone in the Arabian Sea. The laboratory work estimating water content, carbonate and total organic matter was conducted by Fraser West at Glasgow University. The other parameters of Eh, pH, biological mixing and shear strength, were by conducted by Peter Meadows and Azra Meadows on board R.R.S. Discovery. The oxygen minimum zone is located off the coast of Oman the positions of the samples for this research are shown in Table 29. Oxygen minimum zones are bodies of water with a measurably lower oxygen concentration than that of the surrounding water. This low oxygen concentration affects pelagic organisms, also where the zone impinges onto the continental slope it will affect benthic organisms.

Aim of experiment

The specific objectives of the research were to quantify changes vertically into the sediment in biological mixing along with geotechnical and geochemical sedimentary parameters. The relationship between the different parameters was also of concern. The change in these relationships was studied from shallow water depths at the edge of the continental slope, across the OMZ and finally onto the abyssal plain. Sediment subcores were taken from box cores taken at 12 water depths between 391 and 3396 metres. Quantitative measurements were taken on the sedimentary parameters of water content, total organic matter, carbonate, biological mixing, Eh, pH and redox potential.

Figure 43: Map showing the location of the sampling site (solid circle) off the coast of Oman in the Arabian Sea (Meadows *et al.*, 2000).



2.2.1 Sediment preparation

Sediment samples were retrieved using spade box core equipment (50cm X 50cm) during the R.R.S. Discovery 211 cruise off the coast of Oman, south of Masirah Island, in the Arabian Sea, the position of the sampling area is shown in Figure 43. The box core was deployed at each station and was immediately processed onboard upon recovery. Two subcores were taken using PVC cylinders (Internal Diameter = 10.2cm, Length =50cm). One of the subcores was used for biological mixing, the second for the remaining parameters. The subcores were extruded by plunger and sectioned at 0-1cm, 1-2cm, 2-3cm, 3-4cm, 4-5cm, 5-7.5cm, 7.5-10cm, 10-15cm, 15-20cm, 20-25cm, 25-30cm, 30-35cm and 35-40cm. In some cores, the sediment depth was below 40cm.

2.2.2 Statistical analysis of data

The data were statistically analysed using MINITAB Release 11.21 computer software. The methods used were correlation analysis, linear regression analysis, One-way analysis of variance, and multivariate cluster analysis using single linkage and the Euclidean distance measure (Everitt, 1980; Shaw and Wheeler, 1985; Krebs, 1989).

2.2.3 Experimental design

2.2.3.1 Field measurements

Measurements of Eh and pH were taken on board R.R.S. Discovery at each of these depth sections, 0-1cm, 1-2cm, 2-3cm, 3-4cm, 4-5cm, 5-7.5cm, 7.5-10cm, 10-15cm, 15-20cm, 20-25cm, 25-30cm, 30-35cm and 35-40cm. Three replicate readings were taken. Eh was measured using a platinum combination redox electrode, series 1441-400 (Kent Taylor Ltd. England) and pH was taken using a spearhead combination pH electrode, series 1415-400 (Kent Taylor Ltd. England). Shear strength measurements were taken also taken at each of the core sections using a fall-cone penetrometer

(Geonor ROA, Oslo, Norway) (Hansbo 1957).

Biological mixing was also measured on board the research vessel. The measurements were taken visually on the horizontal plane, as each depth section of the subcore was extruded. The observations were recorded viewed from above as one to one scale drawings of vertical burrows. Burrow counts were made on open burrows and these were then converted into burrows per square metre.

2.2.3.2 Laboratory measurements

In the laboratory, water content, total organic matter and carbonate were measured for 14 stations. These parameters were measured using the loss of weight on ignition method (BS 1377 1975).

The carbonate loss on ignition technique was subject to quality control procedures using internal standards (spiked sediment) in the Biosedimentology Unit laboratory by a colleague (Murray, Pers. Res. Comm). Biogenic carbonate (bivalve mollusc shell material .4 mm fraction from Ardmore Bay) was crushed, and passed through a 1-mm sieve. This material was added to pre-ashed, pre-weighed sediment. A comparison was made between two temperatures (550°C and 950°C) and two combustion times (1hr and 4hrs) giving 4 treatments in total. The control was pre-ashed, pre-weighed sediment with no biogenic carbonate added. From this study it was concluded that combusting the sample at 950°C for 1hr gave 100% recovery.

Each station had a sectioned subcore from a box core sample. The subcore was sectioned in measured intervals as described above. From each section, a sample of sediment was taken in a 50mm Sterilin bottle for this analysis. The sediment in the bottle was then homogenised to ensure an even mixing of any water present. Ceramic crucibles were labelled with a unique mark using ceramic paint. The paint had to be fired at 900°C beforehand to fix it permanently. A careful

schedule of experimental procedures was worked out. This posed many logistical problems as timings of the various procedures had to be precisely monitored and three sets of crucibles were staggered through the procedures simultaneously. The experimental sets comprised of all the sediment depth sections from one station core. The maximum number of crucibles in a run was 26 as there were two replicates per sediment depth section. At the beginning of each run, a station was chosen randomly. The crucibles to be used in the next day's run were stored overnight in a desiccator. The top section of the core was dealt with first then moving down the core in sequence. The first empty crucible was weighed and placed onto a tray. Then the Sterilin bottle containing the top section was opened and the contents homogenised using a spatula. The crucible was half filled by volume with sediment. This was weighed again and placed on a metal tray. This process was then repeated for all depths including replicates. The lids of the bottles were replaced immediately after each sediment sample was removed, to prevent evaporation of any water. The full tray was then placed in an oven set at 80°C for 24 hours. After 24 hours, the tray was removed and the crucibles were placed into a desiccator for four hours to cool. The crucibles were then removed one by one from the desiccator and weighed. After weighing the crucibles were placed on the tray again and carried to the furnace. The crucibles were placed individually into the furnace which was then set to 480°C. Then after 4 hours at 480°C the furnace was allowed to cool to around 100°C. The crucibles were then removed to a tray. The crucibles were placed into a desiccator again to cool to ambient room temperature for four hours. The crucibles were then weighed individually and transported to the furnace. The crucibles were placed into the furnace, which was then set to 950°C and left for one hour. After the hour, the furnace was allowed to cool to 100°C and the crucibles were removed. The crucibles were individually weighed and the remaining sediment was retained. This procedure was repeated for all the sample stations, which involved three stations' crucible sets being in the desiccator, oven or furnace simultaneously.

2.3 RESULTS

The results are separated into six main sections. These sections compare differences in the effects

of the OMZ on the various sedimentary parameters measured. They compare differences of these both with increasing depth into the sedimentary column and increasing depth down the continental slope.

2.3.1 Down core profiles of sedimentary parameters for each station

Figure 44 shows the down core profiles for the sedimentary parameters of Eh, pH, percent TOM, and percent carbonate. Figure 45 shows the down core profiles for the sedimentary parameters percent water content, shear strength, and biological mixing (number of burrows). The parameters (Percentage water content, Percent total organic matter (TOM), Percent carbonate) were measured by Fraser J. C. West (FJCW). The remaining four were measured by Azra Meadows (AM), (Eh, pH, shear strength) and Peter S. Meadows (PSM) (burrow numbers/m²) respectively.

Eh: (AM)

There is a general decrease towards more reducing conditions with increasing sediment depth at all stations. This is most noticeable in the first 2 plots 391-406m and 840-854m water depth. Here, the Eh decreases towards 0-mV at sediment depths below 15cm. At water depths of 987 metres to 3396 metres, the Eh decreases in the top 5cm of the sediment. Below 5cm, it remains constant with a value around 300-mV.

Figure 44: Sediment depth profiles for Eh given in mV, pH, percentage total organic matter (% TOM) and percentage carbonate (% CARB.). The stations were split into five groups of water depths; stations A and B (391m-406m), stations C and D (840m-854m), stations E,F and G (987m-1008m), stations H and I (1265m-1285m) and stations J and K (3394m-3396m). Error bars are standard deviations.

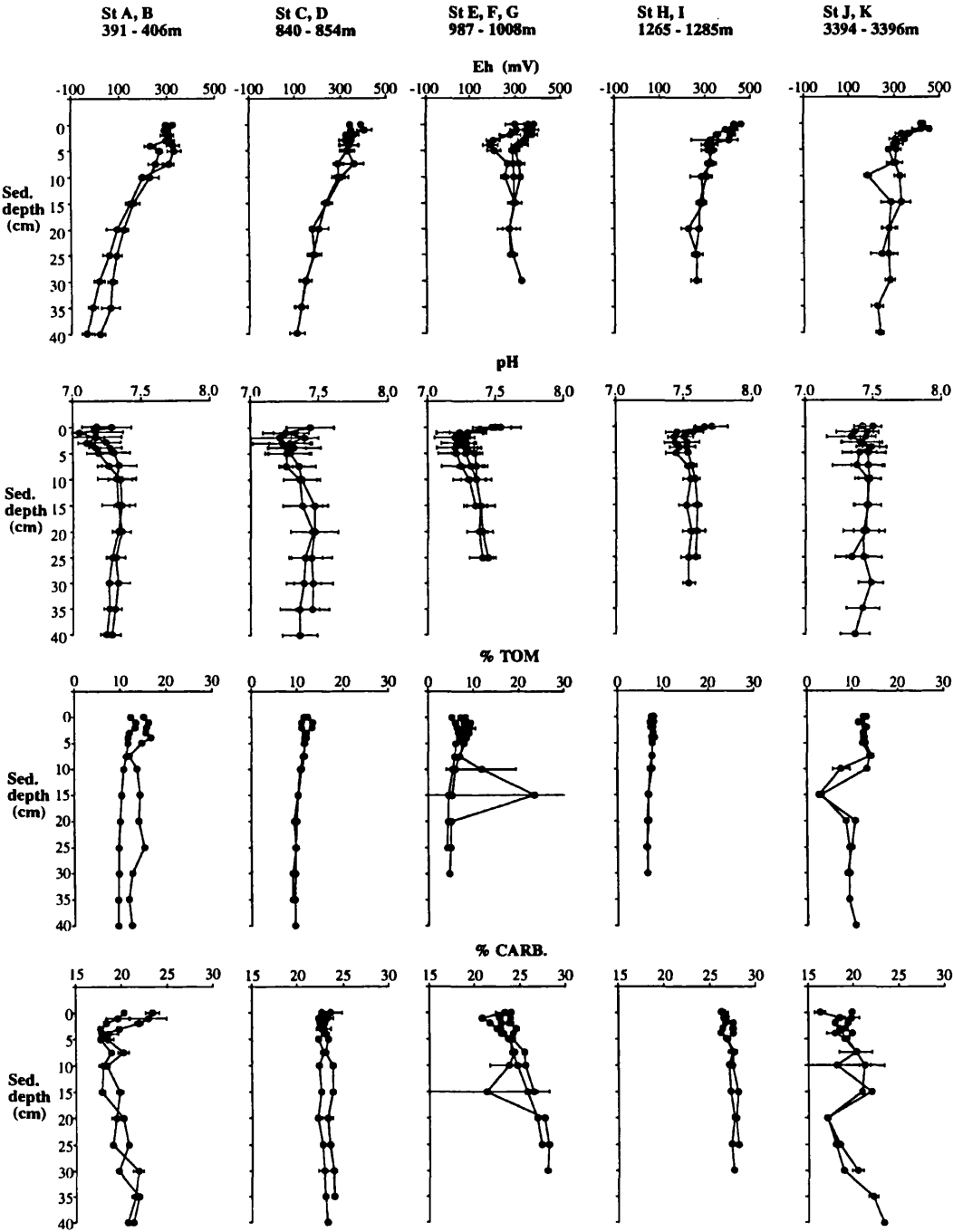
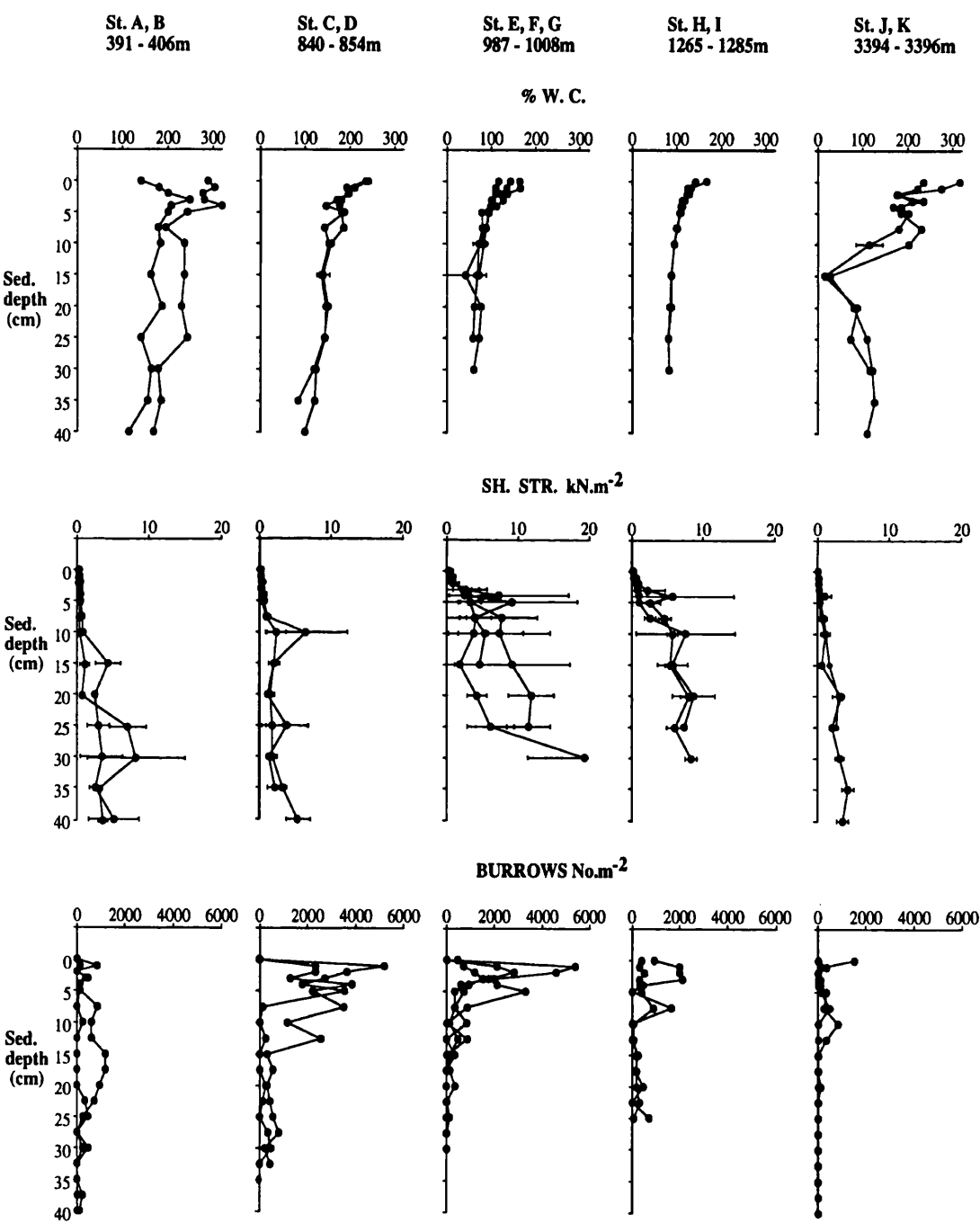


Figure 45: Sediment depth profiles for percentage water content (% W.C.), shear strength (SH. STR.) given in kN.m^{-2} and biological mixing (BURROWS) given as burrow numbers per m^2 . The stations were split into five groups of water depths; stations A and B (391m-406m), stations C and D (840m-854m), stations E,F and G (987m-1008m), stations H and I (1265m-1285m) and stations J and K (3394m-3396m). Error bars are standard deviations.



pH: (AM)

The pH shows an inverse of the relationship between Eh and sediment depth. There is a decrease in pH from the surface of the sediment to about 5 cm followed by slight increase deeper in the sediment at all stations. At water depths of 391-406 metres, the pH remains below 7.5 for the entire depth of the core. Water depths of 840-854 metres show pH values below 7.5 for the first 15cm of the sediment core then remain around 7.5 for the remainder of the core. The water depths in the range 987-3396m all exhibit pH values on or above 7.5 for the top 2cm of sediment depth. This effect highlights a difference in pH of the surface sediments between the shallower and deeper water depths.

Percentage Total Organic Matter (TOM): (FJCW)

There is a small decrease in percentage TOM for all stations as depth increases down the Sediment core. The decrease in most cases is smooth though some cores exhibit wide fluctuations around the 15cm depth. The investigation of the surface 0-1cm sediment depth of the core gave rise to two distinct groups. The first group has surface percentage TOM values higher than 10% at water depths of 391 metres to 854 metres, and again at water depths of 3394 metres and 3396 metres. The second group contains stations with intermediate water depths (987 metres to 1285 metres) where the percentage TOM at the surface of the sediment is lower than 10%.

Percentage Carbonate: (FJCW)

Changes in carbonate with sediment depth are variable at all of the water depths, however there are some recognisable trends. At water depths of 391 metres to 406 metres, there is a sharp decrease in carbonate in the top 4cm of the sediment. Conversely, at water depths of 3394 metres to 3396 metres, there is a tendency for the carbonate to increase slightly with sediment depth. Some water depths show wide fluctuations in carbonate between sediment depths of 10cm and 20cm, which

may possibly indicate biological mixing.

Percent water content: (FJCW)

There is a general decrease in percent water content as depth increases down the sediment core at all the water depths. The investigation of surface 0-1cm sediment depths shows two group trends. The first group containing the water depths of 391 metres to 854 metres, and water depths 3394 metres and 3396 metres, show very high water content values in the first centimetre depth of sediment. The second group containing water depths of 987 metres to 1285 metres show relatively low percent water content values in the first centimetre depth of sediment.

Shear Strength: (AM)

There is a general increase in shear strength as depth increases into the sediment. The shear strength of sediments is affected by a number of factors. The most obvious factor is water content. The more water the sediment contains the more the sediment particles are lubricated and so have a lower shear strength. It is therefore unsurprising that the data for shear strength of the surface 5cm of the sediment at most water depths has values of about 0 kN.m^{-2} . At many water depths, there is also a marked increase in the variability of the shear strength with increasing sediment depth. However, there is little variability at water depths of 3394 metres and 3396 metres. In some cores, the sediment was heterogeneous, consisting of two and occasionally three visually different types of sediment occurring at the same sediment depth (Fig. 46 III). Heterogeneity was observed at water depths of 992 metres, 1008 metres, and 1265 metres, also 3392 metres, 3394 metres and 3396 metres. Where possible, shear strength measurements were taken of the different sediments types at a given sediment depth. These were analysed by a series one way analyses of variance (Table 30). The results of the analyses showed that at two out of the four water depths (3392 and 3394 metres) there was a significant difference between the two types of sediment. The sandy mud had higher shear strength than the mud in each case.

Figure 46: Biological mixing and burrow patterns (I, II and III). Biological mixing and sediment heterogeneity (IV). Infilled burrows (V). Sediment heterogeneity (VI). Biological mixing shown as small circles; burrow patterns shown as patterns of small circles; sediment heterogeneity hatched Vs non hatched and infilled burrows stippled.

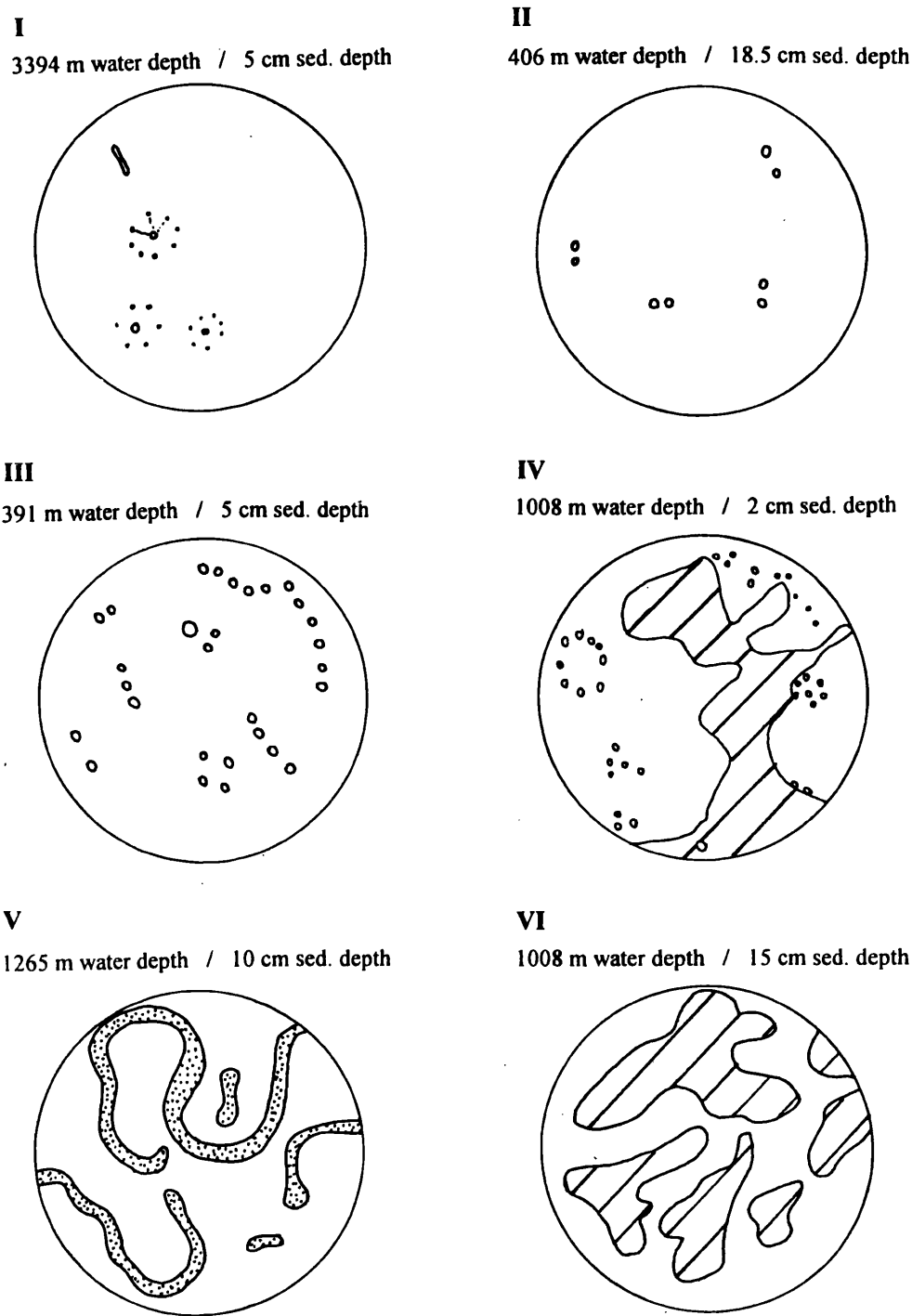


Table 29: Table showing the One-way analysis of variance comparing the shear strength (kN.m⁻²) of sandy mud and mud at different water depths and sediment depths. (Degrees of freedom = 3 except at 992m water depth where d.f. = 2). Probabilities given are asterisked significant according to the following system. 0.05>P>0.01*, 0.01>P>0.001** and P<0.001***. All non asterisked probabilities are not significant.

| Water depth (m) | Sed depth (cm) | Shear strength (kN.m ⁻²) Sandy mud mean ± s.d. | Shear strength (kN.m ⁻²) Mud mean ± s.d. | F ratio | Probability |
|-----------------|----------------|--|--|---------|---------------|
| 992 | 4 | 289.500 ± 383.20 | 2.200 ± 0.900 | 1.12 | 0.05>P>0.25 |
| 3392 | 25 | 13.060 ± 7.065 | 0.543 ± 0.086 | 9.42 | 0.05>P>0.025* |
| 3394 | 20 | 3.467 ± 0.336 | 2.197 ± 0.464 | 14.76 | 0.025>P>0.01* |
| 3396 | 10 | 7.396 ± 9.641 | 0.792 ± 0.181 | 1.41 | 0.50>P>0.25 |

Table 30: Table showing correlations of parameters within the sediment cores at each water depth. Degrees of freedom given in brackets (d.f.). Probabilities given are asterisked significant according to the following system, 0.05>P>0.01*, 0.01>P>0.001**, P<0.001***. All un-asterisked probabilities are not significant.

| water depth | 391m | 406m | 840m | 854m | 987m | 992m | 1008m | 1265m | 1285m | 3394m | 3396m |
|-------------------|-----------|----------|----------|-----------|----------|-----------|-----------|----------|-----------|-----------|----------|
| Total no. | 14 | 14 | 14 | 13 | 12 | 11 | 9 | 11 | 12 | 14 | 11 |
| Sed.depths (d.f.) | (12) | (12) | (12) | (11) | (10) | (9) | (7) | (9) | (10) | (12) | (9) |
| Eh / pH | -0.638* | -0.534 | -0.600* | -0.722** | -0.096 | 0.480 | 0.182 | 0.122 | -0.008 | -0.130 | 0.257 |
| Eh / %TOM | 0.599 | 0.624* | 0.921*** | 0.913*** | -0.040 | -0.573 | 0.609 | 0.851*** | 0.585* | 0.272 | 0.415 |
| Eh / %CARB | -0.652* | -0.044 | -0.308 | -0.730** | -0.502 | 0.284 | -0.675* | -0.799** | -0.726** | -0.551* | 0.321 |
| Eh / %WC | 0.611* | 0.761** | 0.803*** | 0.901*** | 0.814** | 0.014 | 0.887** | 0.942*** | 0.947*** | 0.521 | 0.756** |
| Eh / SH.STR | -0.892*** | -0.745** | -0.564* | -0.660* | -0.224 | -0.001 | -0.805** | -0.809** | -0.893*** | -0.818*** | -0.580 |
| Eh / BURR | 0.147 | -0.085 | 0.647* | 0.591* | 0.528 | -0.420 | 0.499 | 0.633* | 0.521 | 0.582* | 0.288 |
| pH / %TOM | -0.135 | -0.542* | -0.672** | -0.753** | 0.036 | -0.787** | -0.435 | -0.088 | -0.360 | -0.384 | 0.127 |
| pH / %CARB | 0.070 | -0.221 | 0.314 | 0.441 | 0.507 | 0.602* | 0.159 | -0.288 | 0.228 | -0.079 | 0.287 |
| pH / %WC | -0.138 | -0.544* | -0.239 | -0.490 | -0.359 | -0.354 | 0.310 | 0.302 | -0.047 | -0.336 | 0.432 |
| pH / SH.STR | 0.520 | 0.263 | 0.353 | 0.561* | 0.267 | 0.328 | -0.267 | 0.210 | 0.236 | 0.191 | -0.519 |
| pH / BURR | -0.323 | 0.546* | -0.747** | -0.712** | -0.751** | -0.739** | -0.638 | -0.260 | -0.145 | 0.104 | 0.144 |
| %TOM / %CARB | -0.694** | -0.077 | -0.232 | -0.705** | -0.622* | -0.920*** | -0.924*** | -0.803** | -0.790** | -0.220 | -0.268 |
| %TOM / %WC | 0.869*** | 0.951*** | 0.841*** | 0.795** | -0.237 | 0.722* | 0.655 | 0.805** | 0.610* | 0.899*** | 0.737** |
| %TOM / SH.STR | -0.547 | -0.364 | -0.608* | -0.582* | -0.382 | -0.687* | -0.330 | -0.799** | -0.540 | -0.352 | -0.511 |
| %TOM / BURR | 0.072 | -0.251 | 0.789*** | 0.685** | -0.023 | 0.828** | 0.839** | 0.491 | 0.552 | 0.387 | 0.352 |
| %CARB / %WC | -0.738** | -0.007 | -0.103 | -0.887*** | -0.532 | -0.892*** | -0.814** | -0.840** | -0.795** | -0.289 | 0.051 |
| %CARB / SH.STR | 0.477 | 0.067 | 0.078 | 0.695** | 0.703* | 0.881*** | 0.407 | 0.677* | 0.686* | 0.443 | -0.430 |
| %CARB / BURR | -0.049 | -0.003 | -0.516 | -0.383 | -0.575 | -0.772** | -0.696* | -0.227 | -0.177 | -0.312 | 0.264 |
| %WC / SH.STR | -0.661** | -0.551* | -0.599* | -0.668* | -0.531 | -0.904*** | -0.698* | -0.804** | -0.904*** | -0.568* | -0.801** |
| %WC / BURR | 0.180 | -0.237 | 0.571* | 0.382 | 0.621* | 0.656* | 0.453 | 0.442 | 0.493 | 0.511 | 0.328 |
| SH.STR / BURR | -0.185 | -0.076 | -0.538* | -0.412 | -0.400 | -0.621* | -0.220 | -0.555 | -0.484 | -0.383 | -0.355 |

Biological mixing: (PSM)

The parameter of biological mixing was determined by the measurement of numbers of open burrows in the horizontal plane of the sediment. The numbers of burrows in the sediments showed variability both in the sediment cores and at the different water depths. The numbers of burrows were low at the sediment surface and below about 15cm. Most burrows were discovered at depths of 2cm and 10cm. In general, numbers of burrows were highest at water depths of 840 metres to 1265 metres. Very few burrows were recorded at the shallowest water depths and at the deepest water depths. Figure 46 I and II show examples of biological mixing, and burrow pattern and size in the horizontal plane of sediment cores. In some cases burrows and heterogeneous sediment were found on the same horizon of the core and in other cases infilled burrows were observed (Schäfer 1972, Santos *et al.*, 1994).

2.3.2 Correlations between sedimentary parameters (FJCW, AM, PSM)

Correlations between pairs of sedimentary parameters were conducted in two ways. The first way examined correlations within the sediment cores at each water depth (Table 31). The second way examined correlations across all the water depths with seven different sediment core depths (Table 32). The following is a summary of the most important effects.

Down core correlations at each water depth (station) (FJCW, AM, PSM)

Shear strength was negatively correlated with water content that is as water content increases shear strength decreases (Table 31: 391, 992, 1265, 1285, 3396m). Shear strength was also negatively correlated with Eh (Table 31: 391, 406, 1008, 1265, 1285, 3394m). Water content was positively correlated with Eh which suggests that higher water content values will give rise to higher Eh values (Table 31: 406, 840, 854, 987, 1008, 1265, 1285, 3396m).

Table 31: Correlation coefficients between pairs of sedimentary parameters using all the water depths and at seven different sediment depths. Degrees of freedom = 8. Probabilities given are asterisked significant according to the following system, $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$, $P < 0.001^{***}$. All non asterisked probabilities are not significant.

| Sed depth | 0cm | 5cm | 10cm | 15cm | 20cm | 25cm | 30cm |
|--------------|---------|----------|---------|-----------|-----------|-----------|-----------|
| Eh/ TOM | -0.068 | 0.422 | -0.041 | -0.389 | -0.789** | -0.833** | -0.899*** |
| Eh/ WC | 0.147 | 0.395 | -0.248 | -0.903*** | -0.967*** | -0.887*** | -0.913*** |
| TOM/ CARB | -0.524 | -0.832** | -0.584 | -0.415 | -0.753* | -0.806** | -0.811** |
| TOM/ WC | 0.861** | 0.989*** | 0.815** | 0.355 | 0.875*** | 0.902*** | 0.996*** |

The positive correlation between water content and TOM suggests a similar effect (Table 31: 391, 406, 840, 854, 1265, 3394, 3396m). Carbonate was shown to be negatively correlated with water content (Table 31: 391, 854, 992, 1008, 1265, 1285m). Carbonate was also shown to be negatively correlated with TOM (Table 31: 391, 854, 992, 1008, 1265 and 1285m). These correlations generally reflected down core trends in the various parameters.

Correlations across all the water depths (stations) at seven different sediment depths (FJCW, AM, PSM)

Sediment depths of 0cm, 5cm, 10cm, 15cm, 20cm, 25cm, and 30cm were selected for these analyses (Table 32). There were significant negative correlations between Eh and TOM, Eh and water content and TOM with carbonate. These occurred mainly in the deeper sections of the sediment column. There were significant positive correlation's between TOM and water content at six out of seven sediment depths excluding 15cm. Few of the other correlations were statistically significant.

2.3.3 Relationship between sedimentary parameters, water depth and bottom water oxygen content (FJCW, AM, PSM)

The relationships between sedimentary parameters and water depth at sediment depths of 0 cm and 5 cm are shown in Figure 47 and at sediment, depths of 15 cm and 30 cm are shown in Figure 48.

In general, the data for the different sediment depths showed the same trends. Eh, pH and percentage carbonate showed an increase with water depth from 391 metres to about 1285 metres. Percent TOM and water content show a decrease with water depth to about 1285 metres. There was no clear relationship between shear strength and biological mixing with water depth. The data at the two deeper water depths of 3394 metres and 3396 metres did not relate to the trends observed at shallower stations.

Figure 47: Sediment parameters with increasing water depth at 0cm (solid circles) and 5cm (open circles) sediment depth. (Note regression lines not shown. Regression analysis carried out only on nine water depths: 391m, 406m, 840m, 854m, 987m, 992m, 1008m, 1265m, and 1285m).

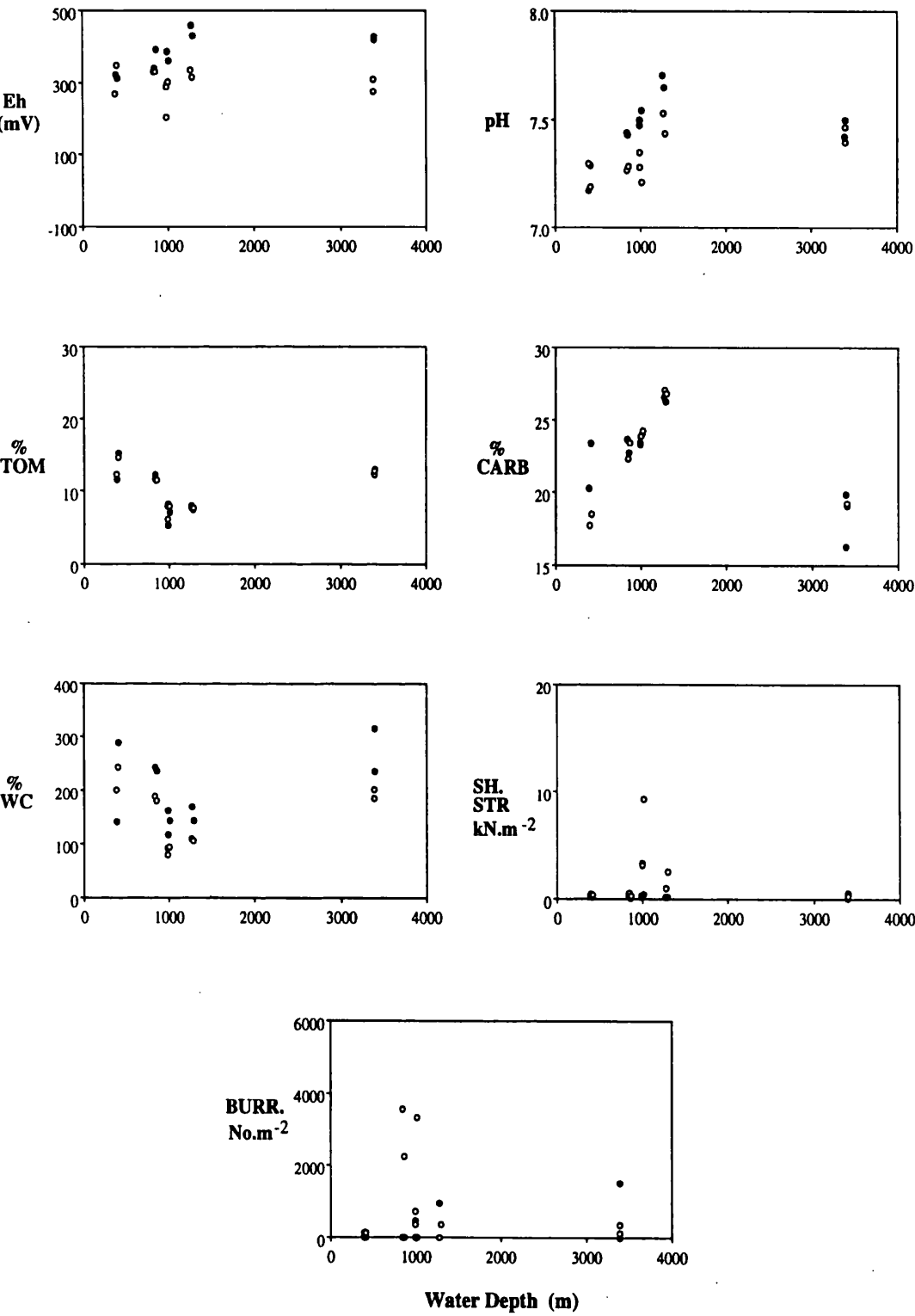


Figure 48: Sediment parameters with increasing water depth at 15cm (solid circles) and 30cm (open circles) sediment depth. (Note regression lines not shown. Regression analysis carried out only on nine water depths: 391m, 406m, 840m, 854m, 987m, 992m, 1008m, 1265m, and 1285m).

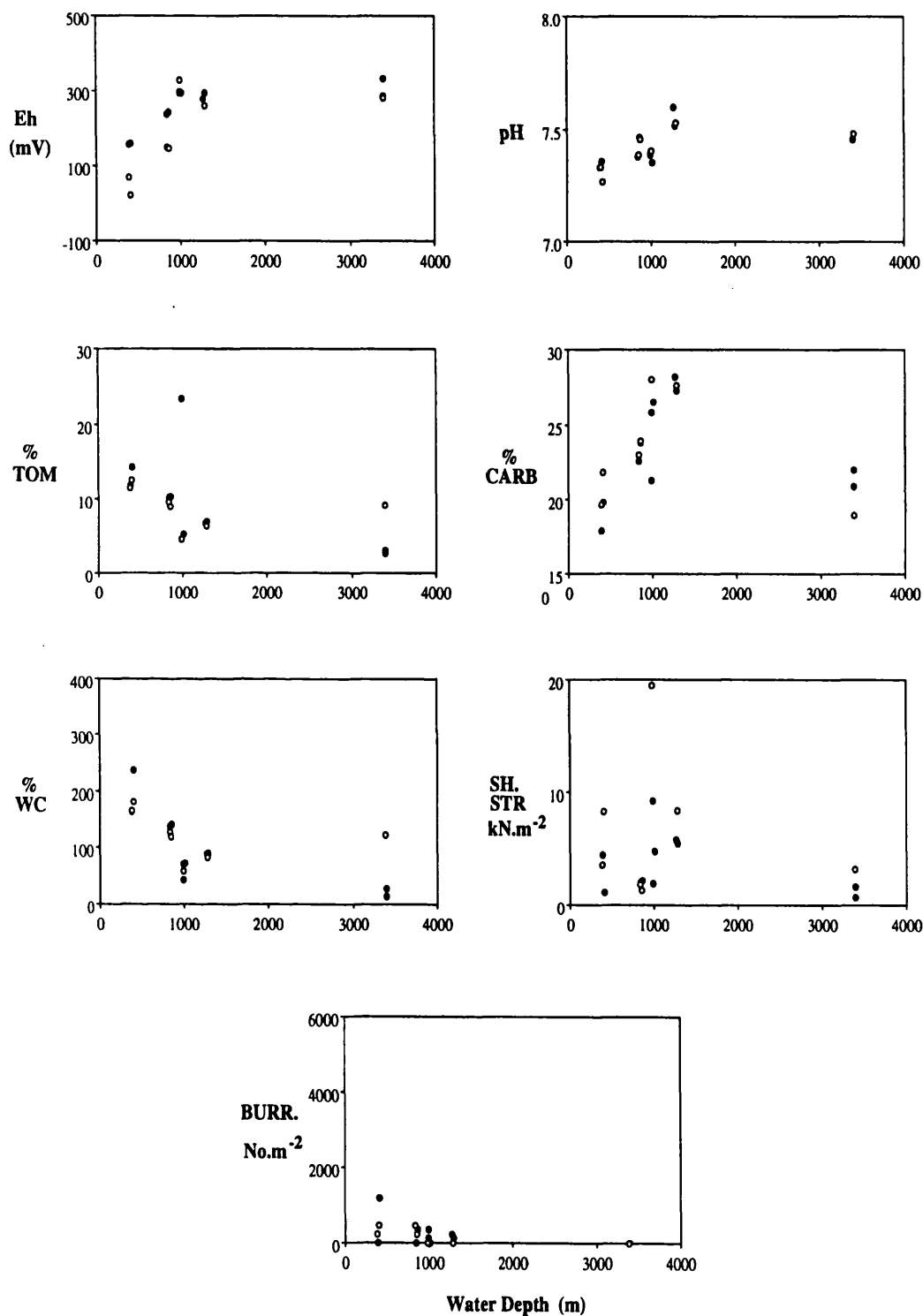


Table 32: Linear regression analysis of parameters (Eh = redox potential (mV), %TOM = percent total organic matter, %CARB = percent carbonate, %WC = percent water content, SH. STR = shear strength, BURR = no. of burrows per m²) against water depth and calculated bottom-water oxygen concentration (ml.L⁻¹) at sediment depths 0cm and 15cm (nine water depths: 391m, 406m, 840m, 854m, 987m, 992m, 1008m, 1265m, and 1285m). In oxygen concentration regressions (right hand side of table), y = ln (oxygen concentration (ml.l⁻¹)). Degrees of freedom = 17. Probabilities given are asterisked significant according to the following system, 0.05>P>0.01*, 0.01>P>0.001**, P<0.001***. All non asterisked probabilities are not significant.

| Water Depth (m) | | | Oxygen concentration (ml.L ⁻¹) | |
|----------------------|------------------------|-----------|--|----------|
| Regression equation | | F-ratio | Regression equation | F-ratio |
| Sediment depth: 0cm | | | | |
| Eh | y = 0.125x + 255 | 8.00* | y = 316x + 274 | 12.06** |
| pH | y = 0.0005x + 7.02 | 125.51*** | y = 1.14x + 7.13 | 87.94*** |
| %TOM | y = -0.00753x + 16.3 | 10.17* | y = -16.2x + 14.4 | 7.33* |
| %CARB | y = 0.00483x + 19.4 | 15.06** | y = 11.9x + 20.2 | 24.26*** |
| %WC | y = -0.0851x + 258 | 1.96 | y = -199x + 240 | 2.02 |
| SH. STR | y = -0.000067x + 0.303 | 0.26 | y = -0.186x + 0.298 | 0.38 |
| BURR | y = 0.657x - 390 | 4.63 | y = 1740x - 314 | 7.59* |
| Sediment depth: 15cm | | | | |
| Eh | y = 0.162x + 106 | 36.64*** | y = 332x + 153 | 13.47** |
| pH | y = 0.000202x + 7.24 | 8.06* | y = 0.519x + 7.27 | 13.62** |
| %TOM | y = -0.00647x + 16.1 | 1.00 | y = -16.0x + 15. | 1.15 |
| %CARB | y = 0.0101x + 14.7 | 32.80*** | y = 22.9x + 17.0 | 26.52*** |
| %WC | y = -0.150x + 249 | 12.03* | y = -303x + 204 | 6.58* |
| SH.STR | y = 0.0037x + 0.75 | 1.87 | y = 8.93x + 1.42 | 2.09 |
| BURR | y = -0.495x + 704 | 1.56 | y = -970x + 546 | 1.05 |

The clear trends in Eh, pH, percent TOM, percent carbonate, and water content in the water depth range of 391 metres to 1285 metres, led to a series of linear regression analyses (Table 33). Table 33 shows the linear regression equations and F ratios for each of the seven parameters in relation to water depth and bottom-water oxygen content at sediment depths of 0 cm and 15cm.

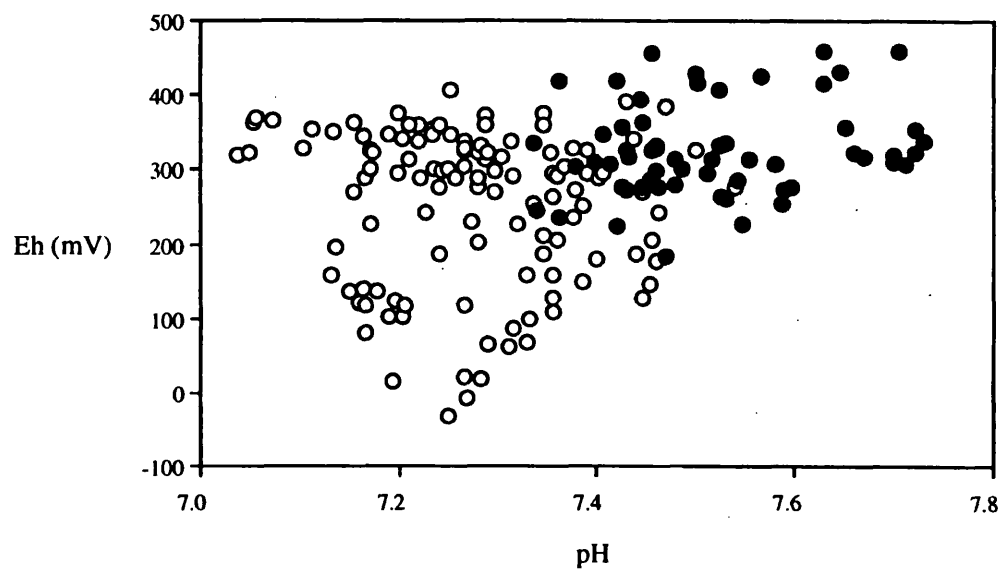
Relationship between sedimentary parameters and water depth

The relationships between sedimentary parameters and water depth are shown in Table 33. Eh, pH and percent carbonate all showed positive linear relationships with water depth at both sediment depths. At the 0cm sediment depth, percent TOM showed a negative linear relationship with water depth. Both shear strength and burrow numbers showed no significant relationship with water depth.

Relationship between sedimentary parameters and bottom-water oxygen

The relationships between sedimentary parameters and bottom-water oxygen are shown in Table 33 (Burkill 1998). These show similarities with the results of the relationship between the parameters and water depth. There was a significant positive linear relationship between Eh, pH, percent carbonate and bottom-water oxygen at 0 cm and 15 cm. There was also a significant negative linear relationship between percent TOM and bottom-water oxygen at sediment depth of 0 cm. There were no significant linear relationships between shear strength and bottom-water oxygen. Interestingly there was a significant positive linear relationship between burrow numbers and bottom-water oxygen at 0-cm sediment depth.

Figure 49: Eh – pH diagram for all water depths: 391m-1008m (open circles) and 1265m-3396m (solid circles).



2.3.4 Sediment heterogeneity (PSM)

Sediment heterogeneity, in the form of laminated structures and the presence of two or three visually different sediment types in surface view, was noted at a number of stations (Figure 46 III). When present, this usually occurred from 1-2 cm to the bottom of the core. The frequency of occurrence of sediment heterogeneity changed at different water depths. It was present at 391 metres, largely absent from 406 to 854 metres, and then present from 987 to 3394 metres. It almost certainly represents slump or turbidite activity (Santos *et al.*, 1994; Mulder & Cochonat, 1996), although there are occasional large infilled burrows.

2.3.5 Relationship between Eh and pH (FJCW, AM, PSM)

Figure 49 shows the Eh - pH diagram for all sediment depths at 15 water depths. There are four more stations in this data set than in the remainder of the data in the section, because Eh and pH was measured at four additional stations (610 metres, 630 metres, 688 metres and 3392 metres water depth). There is a marked increase in pH and a less obvious increase in Eh with increasing water depth. In addition, the data divide into two groups that are related to water depth. The first group lies between water depths of 391 metres and 1008 metres. This group has a pH range of 7.0 to 7.5 and an Eh range of -50 mV to +390 mV. The second group lies between water depths of 1265 metres and 3396 metres. This group has a pH range of 7.3 to 7.8 and an Eh range of +200 mV to +490 mV. It appears from these data that the first group has a relatively low pH with reducing conditions. The second group has a higher pH with distinctly lower reducing conditions.

2.3.6 Cluster analyses (FJCW, AM, PSM)

Two sets of cluster analyses were conducted. In the first set, all the sediment parameters were used to cluster sediment depths. This was done for all the water depths combined, and then for each water depth in turn (Fig. 50). In the second set, all the sediment parameters, and then each parameter in turn was used to cluster the water depths (Fig. 51).

Clustering of sediment depths

The results of the first set of cluster analyses (Fig. 50) show that in general, the upper part of the sedimentary column and the lower part of the sedimentary column form two distinct clusters. This occurs when all the stations are used in the analysis, and when the data from each of the water depths (stations) are used separately. This simply means that the upper part of the sedimentary column is distinctly different to the lower part of the sedimentary column.

There are three exceptions. These are at the shallowest water depth (391 metres), an intermediate water depth (987 metres) and the deepest water depth (3396 metres). It is interesting to note that the surface sediment (0cm) is distinctly different to all other sediment depths in eight of the 11 water depths and in the dendrogram for all stations combined.

Clustering of water depths

The results of the second set of cluster analyses (Fig. 51) show distinct clusters of different water depths. When all sediment parameters are used in the analysis, the water depths cluster in pairs with increasing water depth except for water depths 987 metres and 992 metres.

When each sedimentary parameter is analysed separately, slightly different patterns develop. However there is a general tendency for neighbouring water depths to cluster. This is most

Figure 50: Dendograms of sediment depth for all water depths (stations) and for each water depth (station) in turn.

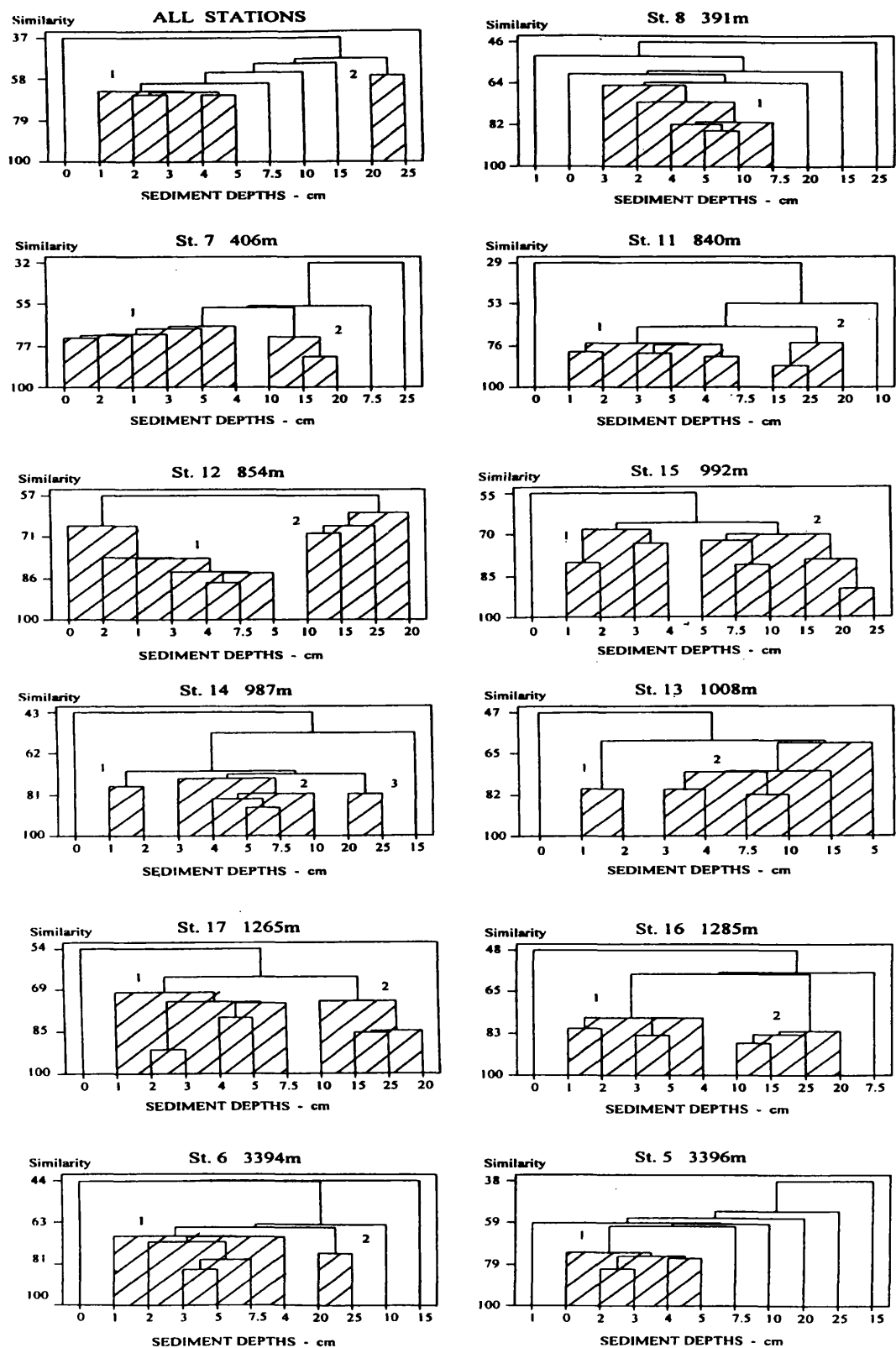
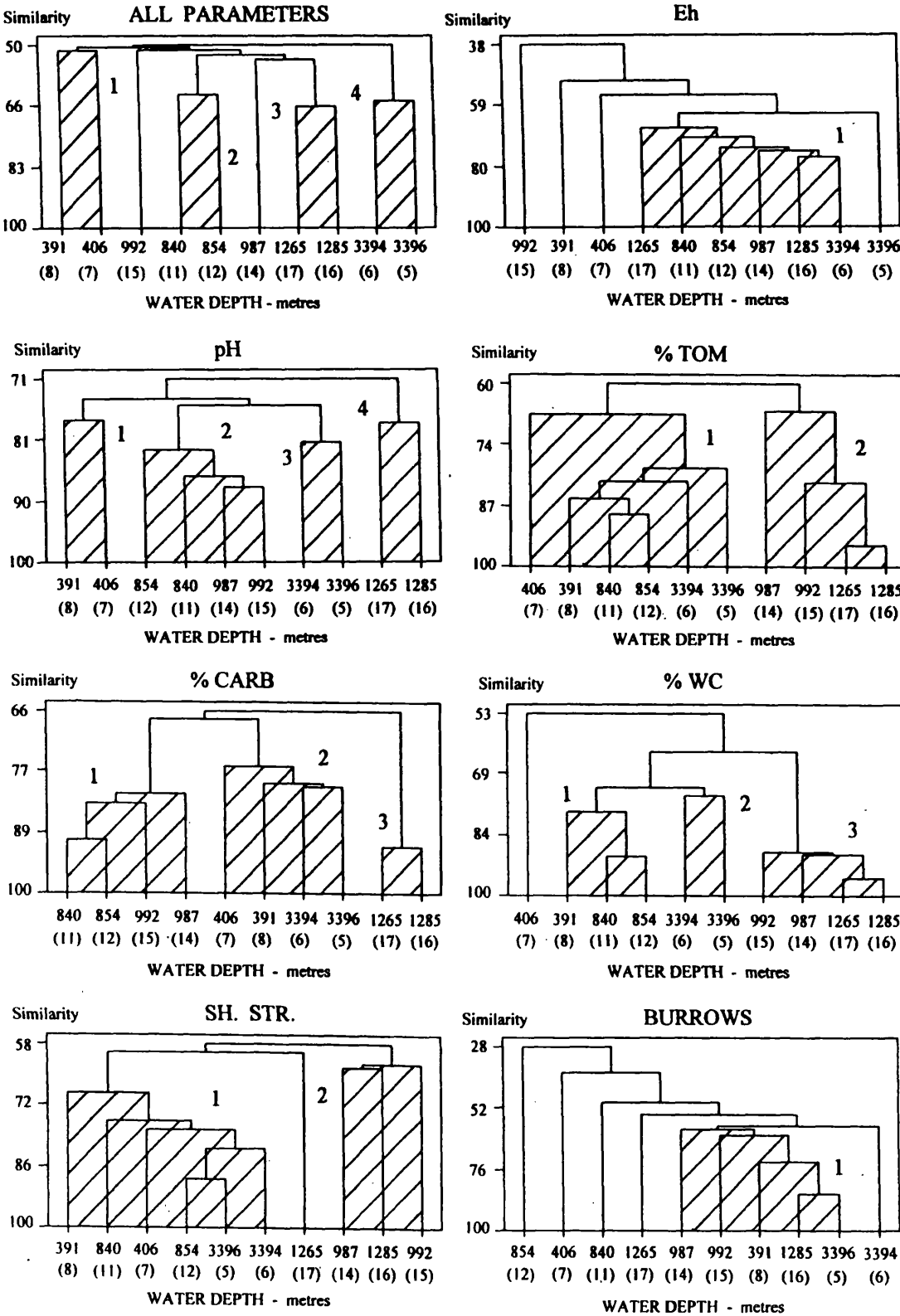


Figure 51: Dendograms of water depths (stations) using all sediment parameters and with each parameter in turn.



obvious with pH. It is also interesting that burrow numbers yield no clusters that are related to increasing water depth. The data for percent carbonate show a clear distinction between the water depths. The shallowest and deepest water depths cluster, the middle range of water depths (840-987 metres) cluster and the two transitional water depths (1265 and 1285 metres) cluster. The two transitional depths are thought to mark the limit of the OMZ and so they are found to exhibit interesting relationships for some parameters. The two water depths (1265m and 1285m) are linked in the clusters of pH, percent TOM, percent carbonate and percent water content clustering tightly in all four parameters. This gives rise to the possibility that these parameters could be secondary markers for the detection of Oxygen minimum zones.

2.4 DISCUSSION

In this section I will discuss my own work along with work conducted by Azra Meadows and Peter S. Meadows so that a complete picture of the data collected from the Discovery 211 cruise may be presented.

The mid-water OMZ is a significant feature of the Arabian Sea. It impinges on the continental slope of the surrounding landmasses at water depths ranging from a minimum water depth of about 100 metres to a maximum depth of about 1200 metres (Wyrski, 1973). Its potential influence on the sedimentary ecosystems and associated benthic epifauna and infauna is therefore likely to be important. There are, however, down slope and up slope processes that may intensify this influence. Turbidity currents of mud and water will periodically move down the continental slope carrying oxygen rich water with them. Seasonal upwelling of nutrient rich oxygenated water will move up the slope driven by the south-westerly monsoon winds in summer (Bruce, 1974; Prell & Streeter, 1981; Quraishie, 1988, 1989; Meadows & Meadows 1999). (These south-westerly monsoon winds extend from April/May until September/October. Cruise D211 took place during early October 1994 to early November 1994). The nutrient rich water leads to dramatic increases in primary productivity in the surface waters, and the resultant phytoplankton blooms off the coast of Somalia and Oman are large enough to be obvious in satellite imagery. These phytoplankton blooms then lead via secondary productivity to a large release of organic material that enters and enhances the OMZ as it is broken down. All of these processes are very active on the continental slope off Oman. Some of them are likely to increase the effect of the OMZ on the sedimentary ecosystems of the slope, while others are likely to reduce the effect. In this context, it is interesting that the western Arabian Sea has higher oxygen concentrations than the eastern region at the same latitude. The area probably experiences more down slope movement of sediment and winnowing because of its steeper bathymetric slope (Slater & Kroopnick, 1984; Paropkari *et al.*, 1992).

The net influence of the OMZ on the sedimentary ecosystems of the slope off Oman is therefore a

matter of debate, and represents the starting point for the investigations reported here.

2.4.1 Effects of the OMZ and of increasing water depth

The down core profiles of sedimentary parameters show a number of effects that may be attributable to the impact of the OMZ on the slope.

The down core profiles of Eh (Figure 44) clearly identify low redox conditions below the sediment surface. This was particularly noticeable at water depths of 391 metres to 854 metres. The Eh results at these water depths, which range from -50mV to $+400\text{mV}$, indicate that the sediments can be regarded as suboxic or dysaerobic as defined by Tyson (1995). However even at these water depths the Eh only approaches zero in the deepest parts of the core. Even within the OMZ, therefore, the surface layers of the sediment are not anoxic, and the sedimentary chemical processes will be operating at dysaerobic levels.

The down core profiles of pH (Figure 44) show pH values below 7.1 for sediment depths 0-5cm at water depths of 391 metres to 1008 metres. The values for the percentage TOM were above 10% at the sediment surface at water depths of 391 metres to 854 metres (Figure 44). There was a decrease in carbonate in the top 4 cm of the sediment at water depths of 391 to 840 metres (Fig. 44). Water content of the surface of the sediment was highest at water depths of 391 to 840 metres (Fig. 45). These changes in Eh, pH, % TOM, carbonate and water content are the most obvious potential effects of the OMZ within the water depth range of 391 to 1008 metres.

Similar effects can be identified in the down core correlations at water depths of 391 to 1265 metres, which implies that where the OMZ impinges on the slope the coupling between the parameters is more closely linked. Eh was frequently positively correlated with TOM, suggesting that freshly deposited TOM at the sediment water interface within the OMZ has not had time to be

mineralised by microbial action. This would reduce the Eh of the pore water in the sediment. TOM was negatively correlated with carbonate. Although this correlation is discussed in several texts (Stein, 1991; Tyson, 1995), it is not clear, why there is such a close inverse coupling within the OMZ.

In addition to the effects of the OMZ on the parameters identified above, there were interesting linear relationships between water depth and five of the seven parameters. The sedimentary parameters were Eh, pH, percentage TOM, percentage carbonate, and percentage water content, within the water depth range of 391 to 1285 metres. There were also linear relationships between bottom-water oxygen and the same sedimentary variables (Table 33). There were significant positive linear relationships between Eh, pH, and carbonate with increasing water depth and with increasing bottom-water oxygen. There were also significant negative linear relationships between percentage TOM and increasing water depth and with increasing bottom-water oxygen. One explanation for these relationships may be that the effect of the OMZ is most marked towards the shallower water depths - becoming more apparent as water depth increases to 1285 metres.

The Eh-pH diagram (Fig. 49) places the first group of water depths broadly in the transitional Eh-pH zone above the sulphide-sulphate boundary (Baas Becking *et al.*, 1960; Brookins, 1988). These findings support the hypothesis that the sediments on the Oman margin do not become entirely anaerobic, at least during late monsoonal and post-monsoonal conditions. This may be caused by upwelling of oxygen-rich deep water or by down slope slumping of sediment and oxygen-rich surface water.

The cluster analysis provided further information on the effects of the OMZ and of trends from shallower to deeper water depths (Fig. 50 & Fig. 51). When all parameters were used to cluster the water depths, four clusters were produced. These were 391 and 406 metres, 840 and 854 metres, 1265 and 1285 metres, and 3394 and 3396 metres.

The cluster analyses, the Eh-pH diagram, and the linear relationships between sedimentary parameters and water depth and bottom-water oxygen, are convincing evidence that the OMZ has its greatest effect on the shallower sedimentary environments of the Omani slope.

2.4.2 Changes in sedimentary parameters and bioturbation down the sedimentary column

The changes of sedimentary parameters and burrow numbers down the sedimentary column (Fig. 47, 48) in addition to being associated to the OMZ, could also be related to the activity of benthic invertebrates and micro-organisms (Berner, 1980; Burnett & Nealson 1981; Snider *et al.*, 1984; Pfannkuche 1985; Meadows *et al.*, 1994b). Levin *et al* (2000) investigates the community structure of benthic macrofaunal invertebrates. The Levin *et al* (2000) study was conducted at the same experimental sites as this study. It is highly likely therefor that the organisms sampled by Levin *et al* (2000) formed burrows discovered in this study. The organisms in question were surface feeding polychaete worms. “Spionids and cirratulids dominated at the 400- and 700-m stations, paraonids and ampharetids at the 850-m and 1000-m stations.”

At water depths of 391m to 854m and 3394m to 3396m high levels of percent TOM are present in the surface layers of sediment. These high levels of percent TOM will be mineralised by the feeding actions of micro-organisms and meiofauna. This microbial and meiofaunal activity probably accounts for the decrease in pH from 0cm to 5cm in the sedimentary column, as the organisms release external metabolites. Microbial and meiofaunal activity may also account for the marked reduction in Eh below 5 cm in the sediment column, as the organisms use up oxygen in the pore water.

The wide variation in shear strength with increasing sediment depth at some of the water depths may be related to the presence of burrows or to the marked sediment heterogeneity that was frequently noticed. Burrow numbers usually peaked at depths of 2 to 10 cm, and were often very variable even within one core. This is in agreement with data using the same technique, from deep-sea sediments obtained from the Atlantic and Pacific Oceans (Meadows & Tait, 1985; Meadows &

Meadows, 1994).

The cluster analyses shown in Figure 50 identify two important features of the sedimentary column. The first concerns the sediment water interface. The sediment water interface is represented by the 0cm data, and this usually does not form part of any cluster. This means that its characteristics are generally not similar to the remainder of the sedimentary column, and it highlights the sediment water interface as being the point at which the overlying water column interacts with the sedimentary column proper. The second feature concerns the sediment column itself. At most water depths, the data divide the sediment column into two clusters - an upper cluster and a lower cluster. This might be caused by the depth to which biological mixing extends; however, burrows are often recorded considerably deeper than this in the sediment column. A second explanation may relate to meiofaunal and microbial activity being more intense at shallower sediment depths (Burnett & Nealson, 1981; Snider *et al.*, 1984; Meadows & Tait, 1985; Pfannkuche, 1985). This may be comparable with a high abundance of both groups of organisms close to the sediment water interface found in Pacific abyssal plain sediments (Burnett, 1981; Meadows *et al.*, 1994b).

2.4.3 Relationship of the results to geochemical processes within and above the sediment

The results are of interest in relation to geochemical processes within the sedimentary column and immediately above the sediment-water interface. There is a considerable literature concerning the processes involved in organic matter oxidation, carbon dioxide evolution and carbonate dissolution in slope and deep sea sediments, also the relationship of these processes to sediment Eh, and the oxygen content of the overlying water (Emerson & Bender, 1981; Emerson *et al.*, 1982; Emerson & Archer, 1990; Archer & Meierreimer, 1994; Hales *et al.*, 1994; Jahnke *et al.*, 1994; Jahnke, 1996; Jahnke *et al.*, 1997). The degradation of organic carbon by micro-biological activity causes the release of carbon dioxide. This release at and near the sediment water interface is likely to reduce sediment Eh, and so reduce oxygen content along with inducing dissolution of carbonate in

the sedimentary column.

The effects of these processes can be identified in many of the results. Both TOM and Eh decrease down the sedimentary column, while carbonate increases (Fig. 44, 45). As bottom-water oxygen increases towards the lower margin of the OMZ at 1200m (Burkill, 1998), Eh, pH, and carbonate increase, and TOM decreases. These effects are linear and statistically significant both near the sediment-water interface, and deeper in the sedimentary column - except for TOM (Table 33). In addition, there are highly significant inverse correlations between Eh and TOM, between TOM and carbonate, deeper in the sedimentary column at 20 cm, at 25 cm, and at 30 cm (Table 33). Biological mixing, however, does not appear to play a significant role at least when measured by numbers of burrows, as there are few significant correlations with other parameters. This may suggest microbiological rather than macrobenthic activity is the main biological driving force in affecting the above processes.

This study would benefit significantly from a comparative study of the Indus Fan, located off the coast of Pakistan. The details of a proposed cruise plan can be found on the World Wide Web (Cowie *et al.*, 1999). The document outlines the study area of interest. The area is rich in organic matter that has been expelled from the mouth of the river Indus. The organically rich fan is fairly simple in its topography which will make studying and retrieving satisfactory samples much easier. The oxygen minimum zone in this area is extremely well developed. This is shown up by sediments underlying the waters up to approximately 1000 m being well laminated with high organic carbon contents. This area shows little biological activity, a distinct contrast to the Oman Slope. Moving down in water depth the effects of the OMZ lessen and as they do the sediments show more biological mixing and also are somewhat depleted in organic matter. The new study would include a repeat of the work conducted in this study. The new study would also be conducted at the same time of the year as the first study to allow more meaningful comparisons.

SECTION : III

Preliminary investigation of radioactive uptake on to

Marine invertebrate mucus in intertidal sediments

3.1 INTRODUCTION

3.1.1 Marine radioactive pollution

In recent years marine environmental pollution has received much attention, as the detrimental effects of increasingly higher levels in the environment become more evident. Pollutants are present as various compounds with equally variable residence times in the oceans and sediments. Conservative pollutants such as radioactive isotopes have the longest potential threat to marine ecosystems, as half-lives of some nuclides are thousands of years long. They are not degraded by micro-organisms and so are persistent in the environment. Radionuclides present in the oceans originate from various sources. Some are undoubtedly natural though a significant amount is from anthropogenic sources. The North Sea has been well studied as it exhibits high levels of radionuclides. Inputs of man-made radioactive pollution began in the middle of the 1960's after the atmospheric nuclear bomb tests in the Pacific Ocean. The next major input specifically in the North Sea came from the Sellafield nuclear reprocessing plant, Cumbria and finally the last major input was from the Chernobyl reactor incident in the 1980's (Kautsky 1988). Small inputs also occur from naval installations berthing nuclear vessels, for example nuclear submarines though these inputs are well below regulatory levels (Fuller & Casey 1989). When the radionuclides enter the oceans, they are incorporated by various mechanisms into sedimentary environments on the ocean floor (Duursma & Hoede 1967, Duursma & Bosch 1970, Aston & Stanners 1981). The sediment water interface in these environments will be of particular interest. This interface allows exchanges between solid and aqueous phases of various compounds (Bokuniewicz *et al.*, 1975). This is particularly relevant for radionuclides as some adsorption reactions into sediments are potentially reversible (Patel *et al.*, 1978, Hess *et al.*, 1978, Stanners & Aston 1982).

3.1.2 Intertidal pollution

In intertidal environments, the effects of pollution will be most evident where residence times in the water column or in sediment are extended. This situation will occur in intertidal bays or estuaries. An estuary or intertidal bay can be defined as a semi-enclosed coastal body of water with a free connection to the open sea and the seawater contained within is measurably diluted by fresh water runoff (Pritchard 1967). This definition highlights one important factor of an estuary or intertidal bay, that is a restricted exchange of seawater. This means that any pollution carried in by currents may not be carried out for some time, thus increasing the chances of the pollutant being adsorbed into the sediment. Some intertidal bays and estuaries will have salt marshes associated with them. These are built up partly from the deposits of the bay and so may be a sink for pollutants (Beefink & Rozemaj 1988).

3.1.3 Biosedimentological interactions and invertebrate mucus production

The oceanic sediments provide a habitat for a vast diversity of organisms, both vertebrates and invertebrates. The marine invertebrates are by far the most prolific. Most marine invertebrate organisms produce mucus, which is used by the organisms for various functions (Hunt 1967, Kideys & Hartnoll 1991, Bretz & Dimock 1983). Studies have also been conducted investigating the molecular composition of mucus (Rahemtulla & Løvtrup 1974, Denny 1980, Grenon & Walker 1980, Denny 1983).

Many organisms bioturbate or mix sediments through the action of feeding or the excavation of burrows where the sediment particles are bound together using mucus (Cadée 1976, Trevot 1977, Grant *et al.*, 1982 Bromley 1990, Meadows *et al.*, 1990, Meadows & Meadows 1991). The action of bioturbation and burrowing of marine invertebrates may provide a route by which radionuclides

are incorporated into sediments (Kershaw *et al.*, 1983, Kershaw 1985, Swift & Kershaw *et al.*, 1986, Jones *et al.*, 1988, Swift 1993). The mucus secreted by marine invertebrates during burrowing and tube building has been shown to adsorb radionuclides (Beasley & Fowler 1976, Miramand *et al.*, 1982, Vangenechten *et al.*, 1983). Mucus produced by marine nematodes was shown by Howell (1982) to exhibit uptake of heavy metals. This work demonstrates a possibility that the mucus of these nematodes may experience uptake of radionuclides. This is rather a disturbing hypothesis as marine nematodes occur in very high abundance in benthic environments globally.

Despite a vast literature on radionuclide uptake onto sediments, very little attention has been focused onto the uptake by marine invertebrate mucus specifically. This experiment was designed to investigate the uptake of two radionuclides that are known to be constituents of Sellafield nuclear processing plants outputs. Americium (Am^{241}) and Caesium (Cs^{137}) are present in the outputs from Sellafield and react with the environment in characteristically different ways. Americium is very particle reactive and so it is removed from solution rapidly. Caesium is a biologically active element and behaves in a similar way to potassium, which is essential in many life processes of organisms. There has been a great deal of work conducted on the Sellafield plant and its discharges (Baxter *et al.*, 1979; McKinley *et al.*, 1981; Mackenzie *et al.*, 1987; Baxter *et al.*, 1988, McKenzie *et al.*, 1999).

The organism chosen for this experiment was the polychaete worm *Hediste diversicolor* because it produces large quantities of mucus.

3.1.4 Natural history of *Hediste diversicolor*.

Hediste diversicolor is an errant polychaete worm of the phylum Annelida. *H. diversicolor* can be found in the Northeast and Northwest Atlantic Ocean, the Mediterranean Sea, the Black Sea and the Caspian Sea (Chambers & Garwood 1992).

H. diversicolor belongs to the family Nereididae of the class Polychaeta of the Phylum Annelida. The external morphology of *H. diversicolor* consists of a long tubular segmented body tapering to the posterior. The body is divided into three main sections, the head or prostomium, the segmented trunk or metastomium and the rear or pygidium.

The prostomium bears four eyes in a trapezoidal arrangement, the prostomial tentacles and a pair of antennae. There are also two crevices present behind the eyes called the nuchal organs. Immediately behind the prostomium lies the peristomium. Both the peristomium and the prostomium are un-segmented. The peristomium bears two pairs of cirri on each side. The mouth or pharynx is located on the ventral side of the peristomium. The pharynx consists of three main parts, the oral ring, the maxillary ring and the mouth bearing a pair of jaws. The pharynx can be extended by the action of the oral and maxillary rings.

The metastomium consists of numerous segments that can number up to 100. Each segment bears a pair of fleshy extensions called parapodia, which bear chaetae. The parapodia are active in both the respiration and the locomotion of the organism. There are two main types of parapodia. The first two segments of posterior of the peristomium bear uniramous parapodia. These parapodia bear only one group of chaetae. The second type of parapodia is found on all the remaining segments of the metastomium. These uniramous parapodia bear two groups of chaetae. The chaetae can be either simple, composed of one piece or compound composed of two or more pieces.

On the shore, *H.diversicolor* is found in estuaries and bays with large expanses of soft sediment. These areas can be prone to fluctuating salinities and *H.diversicolor* is known to tolerate fully marine to brackish salinities on the shore (Smith 1955a, Smith 1955b, Smith 1956). The distribution in higher salinities is reduced by competition from *Nereis virens*, which can tolerate much greater salinities (McLusky 1989). In sediments, *H.diversicolor* forms a tube in the substratum, which it irrigates by body undulations. *H.diversicolor* forms a simple burrow when there is a low density of organisms. When there is a high density of organisms, the burrows formed are more complex with many side tunnels and openings (Davey 1994).

3.1.4.1 Circulatory system

Hediste diversicolor displays a closed circulatory system with blood flowing within blood vessels as shown in Figure 52. There is no separate heart to pump blood, the dorsal blood vessel has a muscular lining allowing contractions which transport the blood forward toward the head region. The ventral blood vessel does not possess a muscular lining and conveys blood to the rear of the organism. The blood flowing in the dorsal vessel travels dorsally passing through circular vessels, this supplies blood to the parapodia. Figure 54 shows the highly vascularised parapodia and circulatory system. Gas exchange is conducted in the parapodia and waste is excreted through the nephridia. The blood continues into the ventral vessel where the blood is transported to the intestine via the subintestinal vessel where nutrients are adsorbed into the blood. To complete the circulation, the blood flows back to the dorsal vessel conveyed by the suprainintestinal vessel. The blood contains haemoglobin dissolved in the blood plasma and the cells of the blood are nucleated. The coelomic fluid has a minor role in circulation. The fluid transports nutrients to tissues and collects waste that is excreted via the nephridopore which deals with functions comparative to the kidneys.

Figure 52: Lateral view of *Hedistes diversicolor* showing the digestive system, the circulatory and nervous systems. 1. Ventral vessel; 2. Nerve chain; 3. Subpharyngeal ganglia; 4. Circumpharyngeal connective; 5. Brain; 6. Mouth; 7. Buccal cavity; 8. Pharynx; 9. Oesophagus gland; 10. Oesophagus; 11. Intestine; 12. Retractor; 13. Dorsal vessel.

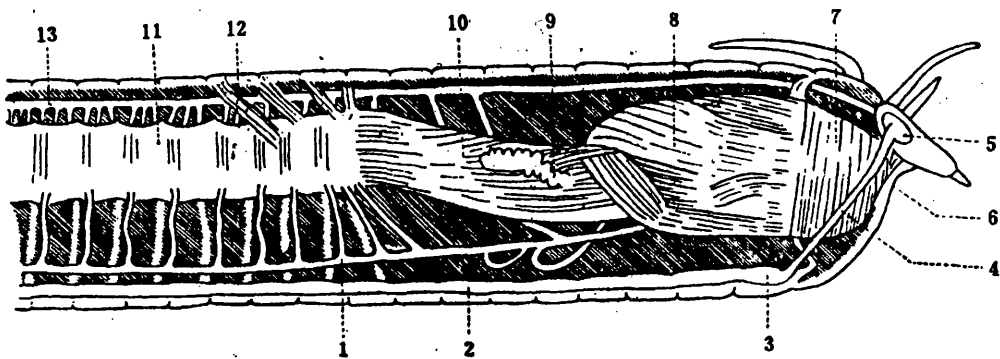
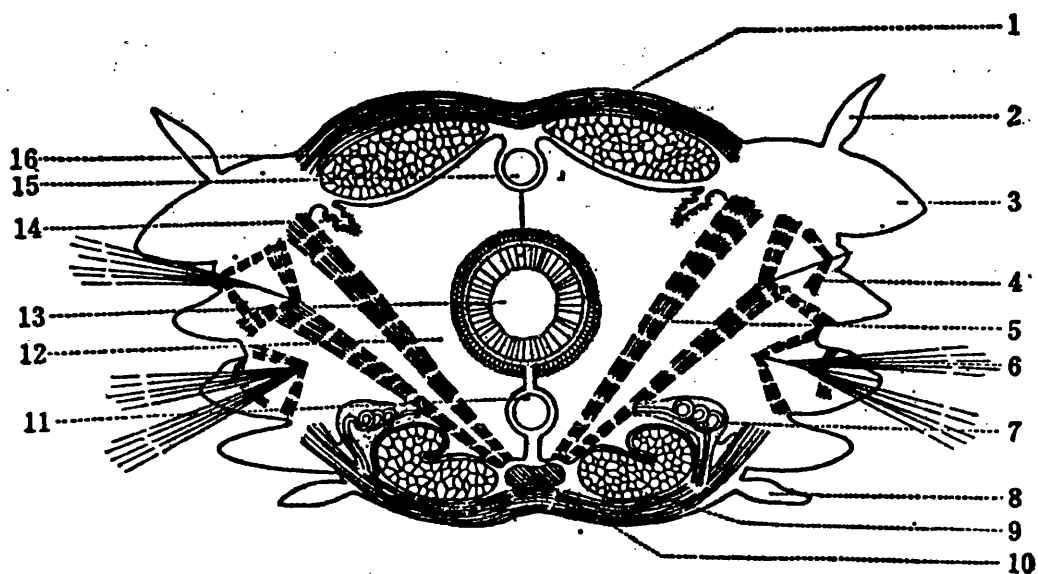


Figure 53: Cross section of *Hediste diversicolor* showing muscular system. 1. Circlum muscle; 2. Dorsal cirrus; 3. Notopodial ligule; 4. Chaetae retractor; 5. Oblique muscle; 6. Chaetae (Setae); 7. Nepheridia; 8. Ventral cirrus; 9. Ventral longitudinal muscle; 10. Nerve cord; 11. Ventral vessel; 12. Coelom; 13. Intestine cavity; 14. Dorsal ciliated organ; 15. Dorsal vessel; 16. Dorsal longitudinal muscle.



1. Circlum muscle;
3. Notopodial ligule;
5. Oblique muscle;
7. Nepheridia;
9. Ventral longitudinal muscle;
11. Ventral vessel;
13. Intestine cavity;
15. Dorsal vessel;

2. Dorsal cirrus;
4. Chaetae retractor;
6. Chaetae (Setae);
8. Ventral cirrus;
10. Nervous;
12. Coelom;
14. Dorsal ciliated organ;
16. Dorsal longitudinal muscle.

3.1.4.2 Feeding mechanism

H. diversicolor feeds by two main methods. The first feeding method is active predation of smaller invertebrates. The second is a suspension feeding method using a mucus-netting bag through which water is passed (Harley 1950, Riisgård 1991). Using body undulations, *H. diversicolor* irrigates the burrow and the flow through the mucus net allows plankton to be trapped and conveyed to the mouth (Fretter & Graham 1976). The digestive system of *H. diversicolor* consists of eight main parts. Food passes from the mouth through the buccal cavity to the pharynx, then esophagus, stomach and intestine with waste being passed through the rectum and expelled from the anus. The remainder of the waste produced by the body is expelled through pairs of nephridia found in each segment. The nephridium comprises of a compact mass of fine ciliated nephridial tubules. The opening that waste enters the nephridia by is called the nephridostome and opens into the coelom. The waste products are evacuated to the outside via the nephridiopore located at the base of the ventral cirrus.

3.1.4.3 Nervous system and sense organs

H. diversicolor has a well-developed chain nervous system. This chain nervous system consists of a brain, paired circumpharyngeal connectives, a subpharyngeal ganglion and a ventral nerve chain. The brain is bilobed and is located in the dorsal area of the prostomium. The pair of circumpharyngeal connective nerves emerges from either side of the brain, pass dorsally to fuse together forming the subpharyngeal ganglion. The subpharyngeal ganglion is located in the ventral region of the first pair of parapodia. This then connects to the ventral nerve chain. The ventral nerve chain is derived from a fusion of the right and left lateral nerves. In each segment the nerve chain possesses a swollen ventral ganglion giving the appearance of a chain.

The peripheral nervous system consists of three main parts. The first part concerns the optic nerves which serve the two pairs of eyes. The eyes are constructed in a ring formation and comprise of a

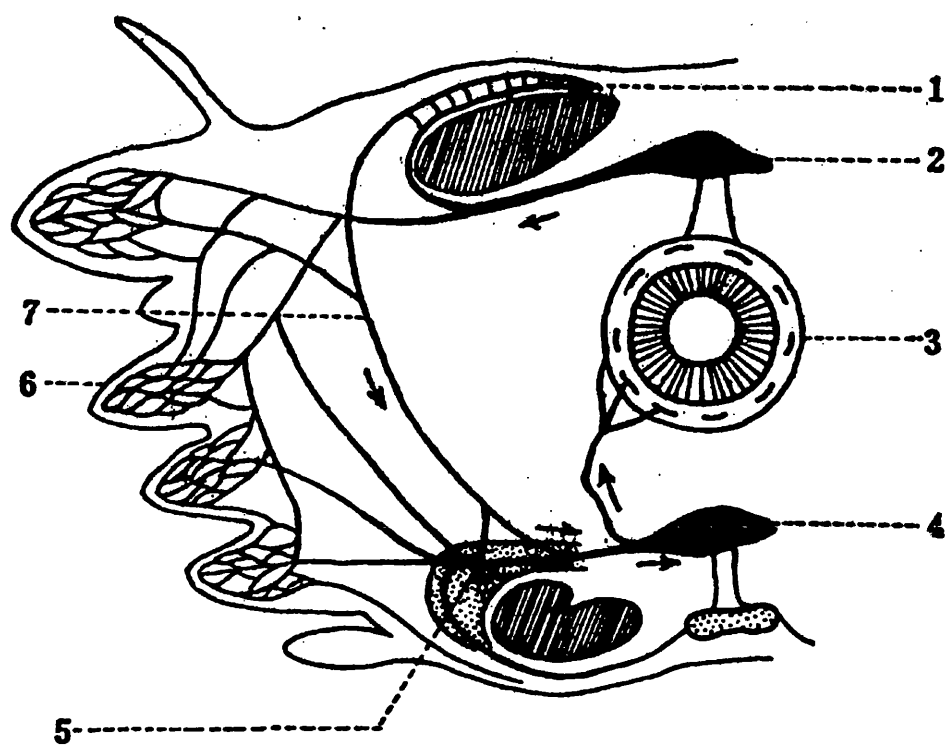
cornea, a pigment cell layer, a photoreceptor layer and a lens of crystalline jelly. The second part of the peripheral nervous system concerns the palpal nerves, which serve the four pairs of prostomial palps. The third part of the peripheral nervous system concerns the nerves that serve the remainder of the body. Each of the main body segments has three pairs of segmental nerves. The first two segments have only two pairs.

3.1.4.4 Musculature and locomotion

The internal structure including the musculature of *Hediste diversicolor* is shown in the cross section of one body segment (Figure 53). There are four main layers, which form the internal structure of the metamere. The cuticle is the outermost layer, which is in contact with the substratum. This layer is comprised of scleroprotein secreted by glandular cells found in the epidermis. The second layer is the epidermis. This is constructed from columnar epithelial cells which have the glandular cells interspersed between them. The glandular cells are not only active in cuticle production but also in the production of mucus. The next layer of the body is the muscular layer. This consists firstly of a layer of circular muscles, which run along the inside circumference of the metamere. A secondary layer of muscle lies inside the layer of circular muscle. This secondary layer of transverse muscle runs the length of the segment on both the dorsal and ventral sides. There is one pair of dorsal longitudinal muscles and one pair of ventral longitudinal muscles arranged in diagonal opposition. The oblique muscles control the movements of the parapodia. The oblique muscles run from the ventral median to the parapodia. The innermost layer of the body is the coelom. The coelom in simple terms is a fluid filled cavity. The coelomic fluid gives the worm its hydrostatic skeleton, against which the bodies muscles act. Transverse septa divide the coelom into its respective metameres.

The locomotion of *Hediste diversicolor* is effected by two mechanisms. These mechanisms are the activity of longitudinal muscles and secondly the action of the parapodia against the substratum (Gray 1939, Trueman 1970). Gay (1939) describes in detail the following locomotory process of *H. diversicolor*. When moving on the substratum slowly only the parapodia are used. When moving

Figure 54: Circulatory system found in the parapodia active in gas exchange. 1. Subepiderm plexus; 2. Dorsal vessel; 3. Gut plexus; 4. Ventral vessel; 5. Nephridial plexus; 6. Parapodia plexus; 7. Circle vessel.



faster or swimming, the contractions of the longitudinal muscles are employed. A cascading waveform was recognised flowing anterior to posterior. This wave is effected by the alternate longitudinal muscles of each segment contracting and relaxing. The parapodia also work in opposition. When one parapodium faces forward the opposite parapodia faces backwards. *H.diversicolor* is a poor swimmer as the effort exerted into the high frequency and velocity of muscular contractions is not translated into rapid progression through the water. There is little information concerning the role of the nervous system in the control of locomotion. The head can be removed leaving the body free to maintain a swimming action (Dales 1963). This suggests local control possibly by the ventral nerve chain.

3.1.4.5 Reproductive system

Hediste diversicolor reproduces sexually.

The female coelom fills with great numbers of coelomic corpuscles. This forms a parenchyma (false tissue) which then disappears leaving the mature oocytes to fill most of the coelom. Specialised coelomic corpuscles phagocytose the muscle layers of the body wall. The oocytes are formed in the ventral part of the coelom by germinal epithelial cells. After spawning, the oocytes not released are broken down into a milky compound in the coelom.

In the male, sperm mother cells are formed in the ventral part of the coelom from germinal epithelial cells. These sperm mother cells break loose into the coelom in small groups. These cells then develop into the fully motile sperm.

In both the male and the female, the breeding season brings about a change in colour that differs from the normal adult colouration. The female develops a dark green colouration and the male develops a bright green colouration.

Spawning has not been observed (Dales 1950). There are four possible mechanisms of gamete

evacuation from the body, through nephridia, from coelomic ducts, through the anus or from a rupture of the body wall. *H.diversicolor* is thought to spawn in burrows on the surface of the substratum without swarming behaviour displayed by many other polychaete worms.

3.1.4.6 Development and growth

The development of *Hediste diversicolor* can be separated into four main stages.

Firstly the embryonic development. Cleavage of the embryo into 2-cell, four cell and eight cell is followed by the development of a blastula developing into a gastrula one day after fertilisation.

Secondly, the trochophore stages. The larvae is now pear shaped with an apical cilia tuft, there is also a pair of red eye-spots present. The chaetae begin to develop and the pharynx also appears.

Thirdly the notochaete stage. The chaetae begin to extend outside the larval body on the parapodia after five days. Significant cephalisation occurs in the anterior of the larvae. The digestive system also fully develops at this stage.

Finally, the setiger juvenile. One month after fertilisation the larvae resembles an adult worm both morphologically and ecologically although not sexually mature.

3.2 MATERIALS AND METHOD

The objective of this experiment was to determine whether the mucus secretions of marine invertebrates could exhibit uptake of radionuclides. The experiment was a preliminary experiment designed to give viable results in a limited time scale therefore no replication was implemented.

Aim of experiment

The aim of this experiment was to test the following hypothesis phrased as questions:

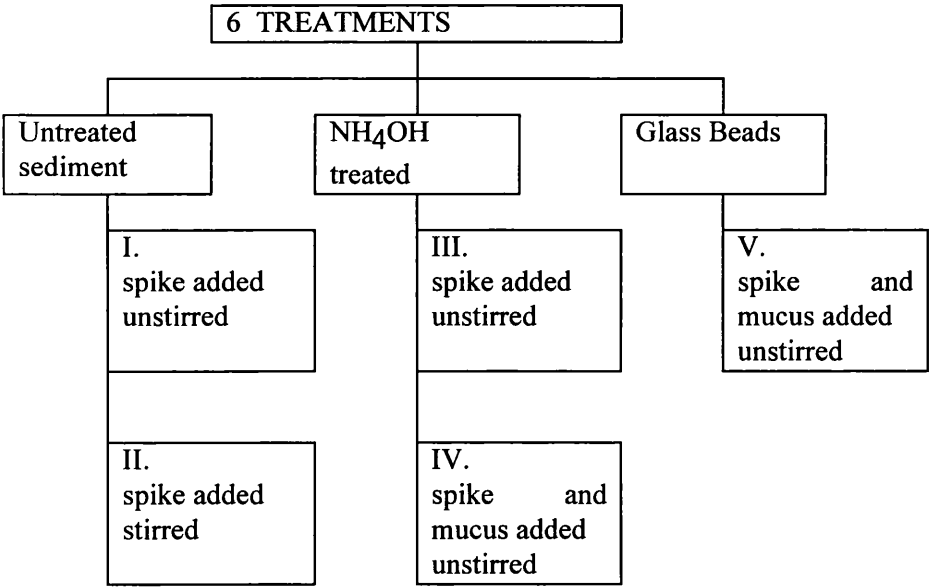
1. Does the uptake of the radionuclides into the sediment or water vary over the duration of the experiment?
2. Is there any difference in the uptake of radionuclides comparing the different substrata?
3. Does the introduction of mucus affect the uptake of the radionuclides?

Design and conduct of the experiment

Sediment, sea water and animals for this experiment were collected from an intertidal bay Ardmore Bay (Figure 9, page 35) in the Clyde estuary (Latitude 55° 58' 32" N Longitude 4° 41' 29" W).

The sediment was fine sandy mud collected from the mid tide region of the bay. A spade was used to gather the top 3 cm brown coloured aerobic layer of sediment. The experimental design is shown in Figure 55.

Figure 55: Experimental design showing the six experimental treatments. Treatment I using natural field sediment with an Americium (Am^{241}) and Cesium (Cs^{137}) radioactive spike solution added and left standing. Treatment II using natural field sediment with an Americium (Am^{241}) and Cesium (Cs^{137}) radioactive spike solution added and stirred. Treatment III using field sediment treated with NH_4OH to remove organic matter with radioactive spike solution added and left to stand. Treatment IV using field sediment treated with NH_4OH to remove organic matter with radioactive spike solution added and marine invertebrate mucus then left to stand. Treatment V using 1cm-glass beads as a substrate with radioactive spike solution added and marine invertebrate mucus then left to stand.



3.2.1 Sediment preparation

In the laboratory, the sediment was washed through three times using seawater from the seawater storage tank in the Graham Kerr building Glasgow University as in section I. This was done to remove very fine particles and so give a more uniform particle size. The sediment was then sieved through a 500µm British Standards sieve to remove very large particles and macrofauna. This sediment was used in treatments I and II. For treatments III - VI the Sediment was treated with NH_4OH to remove organic matter.

Removal of organic matter from sediment

The total weight of wet sediment required for treatments III - VI was 400g, 200g per treatment. In total 600g of treated sediment was prepared. The 600g of wet sediment was weighed on pre-weighed tin foil and then washed into to a 1-litre glass beaker with about 20ml of de-ionised water. To this 50 ml of 35% concentration NH_4OH was added in a fume cupboard stirred thoroughly and left to stand for 15 minutes. This was then washed through six times with de-ionised water, once with dilute hydrochloric acid and finally washed twice more with de-ionised water. The pH of the wet sediment was taken to check for a value between seven and eight.

The control sediment used here was comprised of 1mm spherical glass beads. This was chosen for simplicity as the process of obtaining a perfectly clean sediment would involve combustion of the sediment at very high temperatures to remove organic matter and carbonate.

3.2.2 Sea water preparation

Seawater was collected from Ardmore Bay was transported to the laboratory in large polythene containers. The seawater was then pressure filtered through GFA filter membrane. It was then passed through a resin column containing Potassium hexacyanocobalt II Ferate II complex. This procedure was conducted to remove ^{137}Cs . Radioactive counts conducted on the resin found the ^{137}Cs activity to be within natural levels.

3.2.3 Animal choice and collection

Preliminary experiments were conducted on invertebrates of the phylum Annelida. The species selected was *Hediste diversicolor* because it produces large quantities of mucus. These were collected from Ardmore Bay at low tide zone using a garden fork because a spade could have damaged the animals. The animals were transported to the laboratory in seawater and were stored in fresh seawater in the laboratory.

3.2.4 Mucus production and collection

Mucus from *H.diversicolor* was introduced into sediments to be used in the experiment in the following way. The 200 g of sediment and 480 ml seawater were added to the I-litre beaker. Then 20 *H.diversicolor* were added to the beaker and left for 48 hours to produce mucus. The *H.diversicolor* were then removed using a small plastic mesh net. Mucus could be observed intermixed with the sediment on the removal of *H.diversicolor*.

3.2.5 Preparation of the radioactive spike solution

The radioactive compound used in treatments III to VI was a solution of Americium and Caesium nuclides. The Caesium used was the ^{137}Cs nuclide from a stock solution with 45.3 kBq/g on 01/08/1986. The half life or time taken for the activity to reach half the original level for ^{137}Cs is 30.17 years. This means that a decay calculation must be applied, as the activity will have decreased in the 9.6 years from 01/08/1986 to 14/02/1996.

Radioactive decay calculation for ^{137}Cs

$$N = N_0 \times 1/e^{((\lambda / t_{1/2}) \times t)}$$

Where

N = Activity now

N_0 = Activity at time 0

e = exponent

λ = *Lambda* 0.693

$t_{1/2}$ = Half life of nuclide

t = Time for decay

$$N = 45.3 \text{ kBq/g} \times 1/e^{((0.693 / 30.17 \text{ y}) \times 9.6 \text{ y})}$$

$$= 45.3 \text{ kBq/g} \times 1/e^{(0.02297 \times 9.6 \text{ y})}$$

$$= 45.3 \text{ kBq/g} \times 1/e^{0.22051}$$

$$= 45.3 \text{ kBq/g} \times 1/1.2467$$

$$= 45.3 \text{ kBq/g} \times 0.80212$$

$$= 36.34 \text{ kBq/g}$$

Activity = 36.34 kBq/g on 14/02/1996.

The Americium used was the ^{241}Am nuclide from a stock solution with 5999.4 Bq/ml on 17/02/1995. The half life or time taken for the activity to reach half the original level for ^{241}Am is 432 years. The activity of the nuclide will not have dropped significantly in 1 year. Therefore no correction calculation was strictly necessary though it is given here to give a complete calculation.

$$N = 5999.4 \text{ Bq/ml} \times 1/e^{((0.693/432 \text{ y}) \times 1 \text{ y})}$$

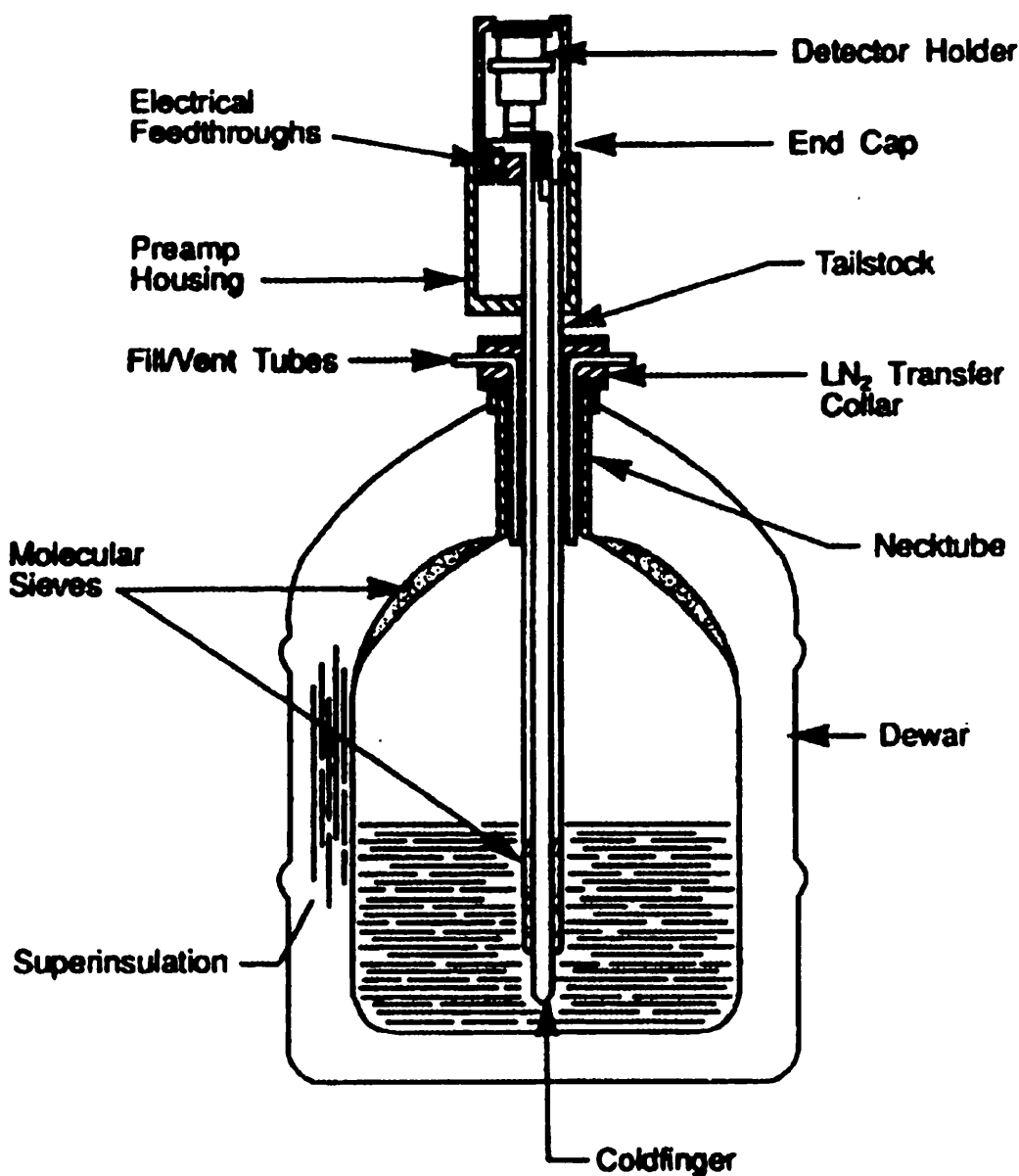
$$N = 5989.8 \text{ Bq/ml on 14/02/96.}$$

Fine calibrated pipettes were used to extract accurate measures of both nuclide solutions for the spike solution. For ^{137}Cs , 6 ml was taken and the actual concentration in the spike solution was calculated as follows activity given in Becquerels per millilitre:

$$\text{Activity of } ^{137}\text{Cs stock solution} = 36.34 \text{ kBq/g}$$

$$6 \text{ ml} = 0.0570 \text{ g}$$

Figure 56: Cross section of the Tennelec Germanium Lithium crystal Gamma radiation detector. The cryogenic Dewar flask lies bellow the detector head containing the electronic monitoring hardware. The Dewar flask is filled with liquid Nitrogen to cool the crystal and keep it within proper operating temperature. Modified from the Tennelec manufacturers operational manual.



$$0.0570\text{g} \times 36.34 \text{ kBq/g}$$

$$= 2.07138 \text{ kBq/g}$$

$$\text{In } 250 \text{ ml} = 2.07138 \text{ kBq/g} / 250 \text{ ml}$$

$$= 0.00829 \text{ kBq/g}$$

$$= 8.29 \text{ Bq/ml on } 14/02/1996$$

For ^{241}Am , 0.6 ml was taken, stock solution = 5.9898 kBq/ml

$$0.6 \text{ ml}$$

$$1 \text{ ml} = 5.9898$$

$$0.6 \text{ ml} = 0.6 \times 5.9898 \text{ kBq/ml}$$

$$= 3.59388 \text{ kBq/ml}$$

$$\text{In } 250 \text{ ml} = 3.59388 \text{ kBq/ml} / 250 \text{ ml}$$

$$= 0.0144 \text{ kBq/ml}$$

$$= 14.4 \text{ Bq/ml}$$

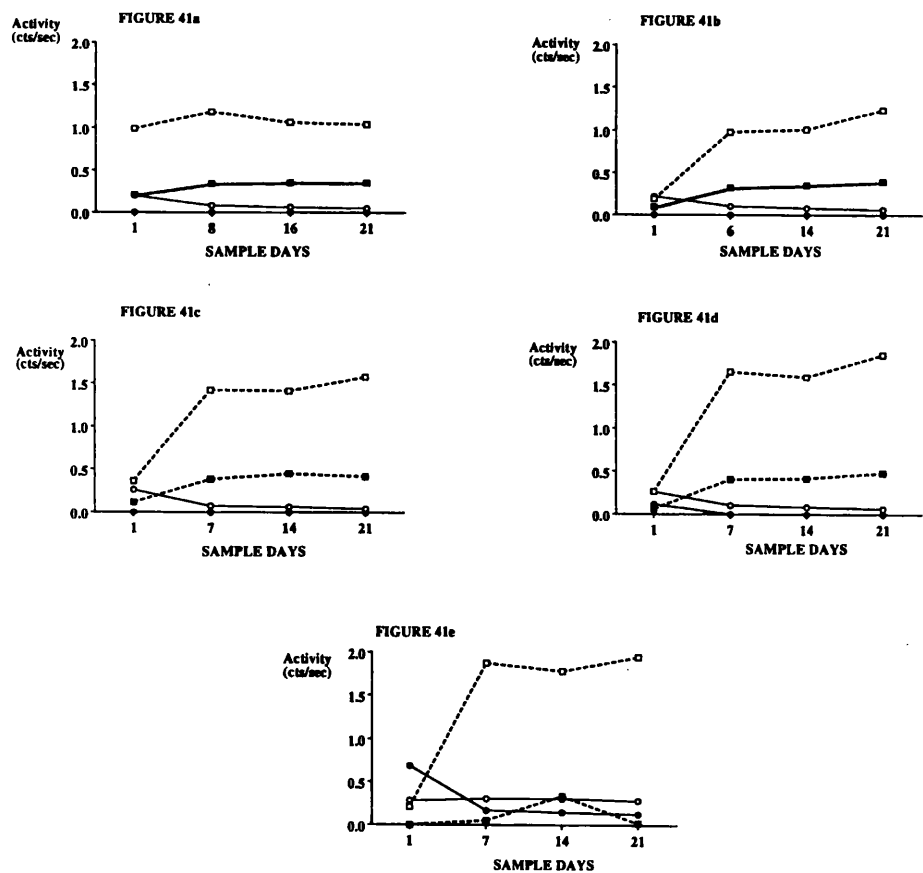
The remainder of the 250ml of the spike solution was made up with de-ionised water and 2 ml of 9M hydrochloric acid.

One of the particular species of radiation that these nuclides emit is gamma (γ) radiation. The detector used in these experiments was a Tennelec Germanium Lithium Crystal detector. A cross section of the detector assembly can be seen in Figure 56 modified from the Tennelec manufacturer's operational manual.

Before any experiments were conducted, calibration measurements were taken of radioactive samples of known activity. The sediment from Ardmore Bay was also measured for background

Figure 57: Plots of Americium and Caesium in water and sediment sub samples taken from the five experimental treatments. Figure 57a shows the plot for experimental treatment I, natural sediment plus radioactive spike solution and left to stand. Figure 57b shows the plot for experimental treatment II, natural sediment plus radioactive spike solution and stirred. Figure 57c shows the plot for experimental treatment III, NH_4OH treated sediment with sea water and spike added not stirred. Figure 57d shows the plot for experimental treatment IV, NH_4OH treated sediment with sea water and spike added plus mucus not stirred. Figure 57e shows the plot for experimental treatment V, glass beads with seawater, spike and mucus added not stirred. Activity given in counts per second (Cts/s). The plot lines in the graph are identified as follows;

- = Americium in water
- = Caesium in water
- - □ - - = Americium in sediment
- = Caesium in sediment



radiation. A comparison was made between high, mid and low tide sediment three replicates were analysed for each tidal level. The activity of ^{137}Cs and ^{241}Am was undetectable in all the samples therefore no correction factor was necessary in the experiment.

The experiments were carried out in a radiation clean room at the Universities Research and Reactor Centre East Kilbride. The room had artificial ventilation natural and artificial lighting and a temperature of 19°C. The experiments were conducted using a 1-litre glass beaker carefully observing all radiation safety procedures. Samples were taken from the 1-litre beaker over a 21-day period. On each sampling day, firstly a water sample was taken followed immediately by a sediment sample. The water samples were taken by pipette; 50 ml was measured into a graduated measuring cylinder. The sediment sample was extracted using a wide aperture pipette. This was then deposited onto a Buchner filter paper in the ceramic holder, which was mounted onto the Buchner vacuum flask. The vacuum then removed the water from the sediment sample. There was a level marked on the inside of the ceramic holder to ensure that uniform samples were taken. The samples were placed into plastic sample bottles. These were placed onto a framework just above the detector crystal.

For treatments V and VI the 20 animals were added to and removed from each experiment before the spike solution was added. The animals were left to produce mucus in the substratum for 24 hours then removed.

3.3) RESULTS

The results for this section are shown Figure 57. The data are statistically analysed in Tables 34 and 35.

3.3.1 Plots of counts per second over time for five treatment conditions

Treatment I, natural sediment with sea water and spike added not stirred:

There are clear trends for both Americium and Caesium over the 21 days. There is no detectable Americium in the water phase throughout the experiments' duration. The trend for Americium in sediment appears to show a peak around day 8 then falls back to the starting value. This could be due to random sampling error, as the treatment was not mixed. The pattern for Caesium in water shows a steeper decrease from initiation to day five then a more gradual decrease to day 21. The Caesium in sediment is the inverse of this trend.

Table 33: Table showing the One-way analysis of variance comparing the five experimental treatments for Americium in water samples, Americium in sediment samples, Caesium in water samples and Caesium in sediment samples. Degrees of freedom (d.f.). F = variance ratio. P = probability. Probabilities given are asterisked significant according to the following system, $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non asterisked probabilities are not significant.

| Comparison | Source of variation | d.f. | Sum of Squares | Mean Squares | F | P |
|---|---------------------|------|----------------|--------------|------|-------------------------|
| Americium in water for experiments 1-5 | Factor | 4 | 0.2461 | 0.0615 | 4.46 | $0.05 > P > 0.01^*$ |
| | Error | 17 | 0.2343 | 0.2343 | | |
| | Total | 21 | 0.4804 | | | |
| Americium in sediment for experiments 1-5 | Factor | 4 | 0.959 | 0.240 | 0.77 | $0.60 > P > 0.50$ |
| | Error | 17 | 5.272 | 0.310 | | |
| | Total | 21 | 6.231 | | | |
| Caesium in water for experiments 1-5 | Factor | 4 | 0.10811 | 0.02703 | 5.32 | $0.01 > P > 0.001^{**}$ |
| | Error | 17 | 0.08633 | 0.00508 | | |
| | Total | 21 | 0.19444 | | | |
| Caesium in sediment for experiments 1-5 | Factor | 4 | 0.1602 | 0.0400 | 2.18 | $0.20 > P > 0.10$ |
| | Error | 17 | 0.3122 | 0.0184 | | |
| | Total | 21 | 0.4723 | | | |

Table 34: Table showing the One-way analysis of variance comparing day one and day 21 for the five experimental treatments for Americium in water samples, Americium in sediment samples, Caesium in water samples and Caesium in sediment samples. Degrees of freedom (d.f.). F = variance ratio. P = probability. Probabilities given are asterisked significant according to the following system, $0.05 > P > 0.01^*$, $0.01 > P > 0.001^{**}$ and $P < 0.001^{***}$. All non-asterisked probabilities are not significant.

| Comparison | Source of variation | d.f. | Sum of Squares | Mean Squares | F | P |
|--|---------------------|------|----------------|--------------|-------|---------------------|
| Americium in water for day one and day 21 for experiments 1-5 | Factor | 1 | 0.0465 | 0.0465 | 1.01 | $0.40 > P > 0.30$ |
| | Error | 8 | 0.3699 | 0.0462 | | |
| | Total | 9 | 0.4164 | | | |
| Americium in sediment for day one and day 21 for experiments 1-5 | Factor | 1 | 2.882 | 2.882 | 24.54 | $P < 0.001^{***}$ |
| | Error | 8 | 0.939 | 0.117 | | |
| | Total | 9 | 3.821 | | | |
| Caesium in water for day one and day 21 for experiments 1-5 | Factor | 1 | 0.05109 | 0.05109 | 9.06 | $0.05 > P > 0.01^*$ |
| | Error | 8 | 0.04514 | 0.00564 | | |
| | Total | 9 | 0.09623 | | | |
| Caesium in sediment for day one and day 21 for experiments 1-5 | Factor | 1 | 0.1375 | 0.1375 | 7.53 | $0.05 > P > 0.01^*$ |
| | Error | 8 | 0.1461 | 0.0183 | | |
| | Total | 9 | 0.2836 | | | |

Treatment II, untreated sediment with sea water and spike added stirred:

Treatment 2 shows very similar trends to those of treatment 1. The Americium in water is not detectable. The pattern of Americium in sediment is slightly different; this may be due to the effect of mixing. The patterns of Caesium in sediment and water are again an inverse of each other.

Treatment III, NH_4OH treated sediment with sea water and spike added not stirred:

The trends of Americium and Caesium data in treatment III appear to mirror the trends in treatment II. This is very interesting, as the substrates here are different. It is also important to note that the values for Americium in sediment are significantly higher for treatment III than for those in treatment I.

Treatment IV, NH_4OH treated sediment with seawater, spike and mucus added not stirred:

The trends in this treatment are very interesting. There is a measurable amount of Americium in the water phase decreasing from initiation to day seven. The trend for Americium in the sediment shows a steep increase from initiation to day seven, then another increase from day 14 to day 21. The Caesium in water and in sediment shows an inverse trend.

Treatment V, glass beads with seawater, spike and mucus added not stirred:

This experiment shows interesting trends for both Americium and Caesium. Americium in water shows a decreasing trend from initiation to day seven, then levels off. This trend is the inverse of the Americium in substrate data, which has a steep increase from initiation to day 7 and again from

day 14 to day 21. Caesium in the water phase appears to show a flat trend throughout the experiment. The Caesium in substrate peaks at day 14 then decreases to day 21. This appears to be Caesium re-entering the liquid phase though the Caesium in water data do not register this increase.

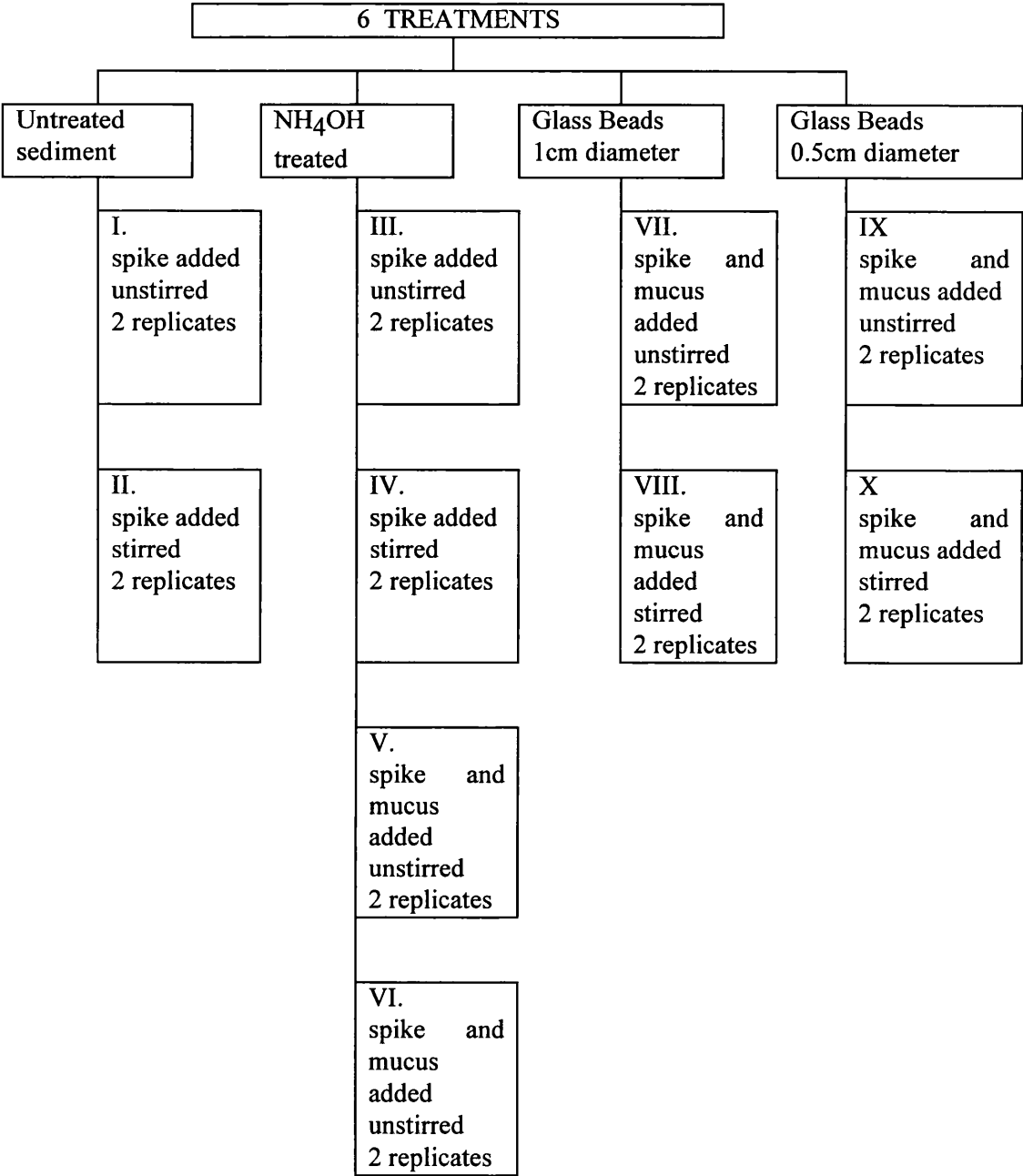
3.3.2 Amounts of americium and caesium in water and sediment comparing all five experiments

The results of the one way analysis of variance are shown in Table 34. The results show significant differences between the five experiments for both the Americium and Caesium activity in the water fraction ($F = 4.46$, d.f. = 4, $0.05 > P > 0.01^*$ and $F = 5.32$, d.f. = 4, $0.01 > P > 0.001^{**}$ respectively). The sediment fraction for Americium and Caesium shows no significant differences between the five experiments ($F = 0.77$, d.f. = 4, $0.60 > P > 0.50$ n.s. and $F = 2.18$, d.f. = 4, $0.20 > P > 0.10$ n.s. respectively).

Amounts of Americium and Caesium in water and sediment comparing day 1 data and day 21 data:

The results of the one way analysis of variance for day 1 and day 21 data are shown in Table 34. Three out of the four analyses were significant. The analysis comparing the five experiments over the 21 days for Americium in water was not significant. This means that for all the experiments there was no difference for Americium detected in the water. Conversely, the analysis of Americium in sediment was highly significant comparing the five experiments over the 21-day period. This means that the Americium uptake into the sediment was very different between the five experiments over the 21-day period. This highlights the different uptake processes of

Figure 58: Proposed experimental design.



radionuclides by the different substrates. This also suggests that marine invertebrate mucus may be

important in the uptake process. The Caesium counts in both the sediment and the water are significant between the five experiments over the 21-day period. This means that the amounts of Caesium in the water differs between the five experiments over the 21-day period. Sampling error may contribute to this as only two treatments were stirred.

3.4) DISCUSSION

Radioactive pollution is becoming more evident in the sedimentary deposits of the world's oceans. A large proportion of the radiation is an artefact of nuclear power stations, waste dumping and weapons testing around the globe (Aarkrog 1978, Figueira & Cunha 1998, Egrov *et al.*, 1999). The Sellafield nuclear fuel reprocessing plant UK regularly discharges controlled amounts of radionuclides into the Irish Sea (BNFL 1974-85). Due to the inputs of Sellafield, the Irish Sea has become the focus of a vast amount of research. This research monitors various aspects of the mechanisms of entry into and movement through the marine environment. The water column is an obvious point to start, as this is the gateway to the marine environment. A considerable amount of research has been conducted in monitoring the various radioactive elements that constitute the Sellafield discharges (Baxter *et al.*, 1979, McKinley *et al.*, 1981, Mackenzie *et al.*, 1987, Baxter *et al.*, 1988, Dunster 1998, Wolstenholme *et al.*, 1998, Leonard *et al.*, 1999). Work conducted by Hamilton (1999) suggests that the distributions of the actinide compounds of the Sellafield discharges are influenced by nearby industrial waste discharges. Discharges of iron compounds such as Magnetite and Hematite derived from mining and iron ore smelting seem to be active in adsorbing the radionuclides. When adsorbed to these compounds the radionuclides will be transported by currents and deposited with the iron compounds elsewhere in the environment. This is interesting when considering recent research that has uncovered a rather worrying trend in the transport of radioactive elements with water currents northwards (Kershaw & Baxter 1995). This will potentially have a detrimental effect on the essentially pristine environment of the Arctic. Contamination has not only been found in the water column (Buraglio *et al.*, 1999), it has also been detected in organisms of the many trophic levels of the environment (Klungsoyr *et al.*, 1995). Further research has carried on from the studies of the radionuclides present in the water column and focuses on the transfer of these radionuclides into the sedimentary environment. Wolstenholme *et al.*, (1998) found that radiocarbon from Sellafield discharges was present in sub-tidal sediment

cores associated with the organic matter fraction of the sediment. This is of potential importance to my work as I have focused on radionuclide uptake onto organic compounds specifically marine invertebrate mucus. Other studies have identified inorganic compounds active in the uptake of radionuclides. Kershaw *et al.* (1999) describes the concentration and distribution of Plutonium and Americium in sub tidal sediments of the Irish Sea. The activities of both of the compounds show increasing variability as depth increases into the sediment. The study shows an apparent 40% loss of discharged isotopes, which was corrected for half-life decay. The loss is thought to be due to inability to sample effectively the surrounding coarse-grained sediment that lies around the Irish Sea mud patch. These coarse grained sediments according to Hamilton (1989) contain large complex iron-rich matrices combined with sediment grains. The large particles usually measuring >0.5mm diameter, attract radionuclides which bind to the black iron-rich outer coating. This will locally intensify concentrations of radionuclides in that particular area. The outer coatings, however, can be detached by the abrasive nature of the sediment and thus leaving the radionuclides mobile in the environment again. The remobilization of the radioactive compounds deposited from Sellafield discharges have also been reported by Cook *et al.* (1997). The activity ratios of Plutonium isotopes were found to be inconsistent with discharges from Sellafield and so could only be derived from the isotopes already deposited re-entering the solution. These radioisotopes on re-entry to the water column have the potential to be transported on-shore during storm events (Wilkins *et al.*, 1999). Radioisotopes will also appear in intertidal sediments due to the natural on shore currents present in the tidal regimens. With respect to this, studies have been conducted on intertidal salt marsh sediments, which give a good record of radioactive deposition over time (Oldfield *et al.*, 1993, Pulford *et al.*, 1998, Tyler 1999). However, these areas may be subject to disturbance from infaunal invertebrates present in salt marsh environments (Hughes 1999). This would release the radioactive substances back into the intertidal environment.

The intertidal environments of the Irish Sea and West Coast of Scotland support a vast community

of diverse benthic invertebrates. Many of these marine invertebrates are infaunal, living beneath the sediment surface where they mix the sediment during burrowing or feeding (Taylor & Moore 1995, Astall *et al.*, 1997). Work conducted by Swift (1993) showed that the area off Sellafield experienced high biological mixing by marine invertebrates. This is interesting when considering research that showed radioisotopes being incorporated into the sediment by biological mixing (Kershaw *et al.*, 1983, Suchanek 1983, Kershaw *et al.*, 1984, Kershaw 1985, Hamilton *et al.*, 1991).

Complementary terrestrial work by Brown & Bell (1995) on earthworms, showed an uptake of radioactive compounds through the ingestion of sediment. Some of these radioactive compounds will pass out with the faeces and be left inside the worm's burrow thus increasing the activity of the sediment.

The second experimental condition in this section comprised of stirred natural sediment shows the effect of mixing the sediment. This may imitate the biological mixing by marine invertebrates in the field.

Further research in the marine intertidal has investigated the uptake of radioactive compounds by marine invertebrates (Beasley & Fowler 1976, Vangenechten *et al.*, 1983). The studies investigated whole body uptake and identify the areas of the invertebrates with greatest uptake. Both the studies showed that the mucus secretions of the organisms are important in the uptake of radionuclides. However, they did not specifically analyse isolated mucus secretions with respect to uptake. Howell (1982) suggests that mucus secreted from marine nematodes may increase the uptake of heavy metals into sediments. None of the studies appears to isolate mucus secretions to determine their role in radioisotope uptake. This is the starting point of the study reported in this section. This preliminary study attempts to develop the hypothesis that marine invertebrate mucus does take up radioisotopes. Experimental conditions IV and V show the effect of the addition of mucus from the

marine polychaete *Hediste diversicolor*. This was isolated mucus, that is the worms were left in the substrate to produce mucus and then removed from the sediment. The substrates used were devoid of organic matter so that the mucus was the only source of organic material in the sediment. This work shows clearly that radionuclides, predominantly Americium are adsorbed by marine invertebrate mucus.

The study was focused on two of the radioisotopes present in the Sellafield discharges namely, Americium (Am^{241}) and Caesium (Cs^{137}). The nature of these two radionuclides must be taken into account when investigating their respective levels of uptake into sediments. Americium is present in a tertiary oxidation state (Am (III)) which is very particle reactive on encountering good electron donors (Kershaw *et al.*, 1999). In other words Americium is removed rather quickly from the water column by being adsorbed onto the surface of particles settling through the water column, though de-sorption has been recorded (Stanners & Aston 1982). Caesium is less particle reactive. In nature it experiences fluxes and may be adsorbed onto particles and then de-sorbed back into the water column (Patel *et al.*, 1978, Stanners & Aston 1982). Caesium is transferred to the sediment by being adsorbed onto settling clay particles by potassium ion exchange. The reversible reaction can however be blocked by organic matter and sediment oxides (Evans *et al.*, 1983). Under highly reducing conditions of anoxic sediments, NH_4^+ provides the exchange thus releasing the Caesium back into solution (Evans *et al.*, 1983, Sholkowitz & Mann 1984).

The experiment in this section may be considered as a preliminary study. The experiment was designed under tight time restrictions and was designed to give a simple indication of the viability of the project. Replication was not included for the aforementioned reason. This means simply that no statistical analyses can be conducted on the data. However, I do believe that the work is not only valid but also forms the bases from which to develop further research that is more definitive. I

include here an outline of an experiment that may be considered definitive.

The proposed experimental design is shown in Figure 58. The experiment consists of 8 experimental conditions with two replicates per condition. The methods for preparing the sediments are identical to those in the preliminary experiment. There are two size categories of glass beads (1cm diameter and 0.5cm diameter) to investigate the role of particle size in the uptake of the radionuclides when mucus is present. The spike solution will be identical to the solution used in the preliminary experiment. The detector will be the Germanium/Lithium detector used in the preliminary experiment. The experiment would run over 8 to 10 months to complete.

GENERAL DISCUSSION

The three sections of this thesis address three facets of the interaction between marine benthic invertebrates and sedimentary environments that they inhabit. Sections one and three are concerned with interactions of benthic invertebrates and sediments in the intertidal zone. Section two is concerned with the interactions of benthic invertebrates and sediments in continental slope environments.

Section one investigates the protection of coastal zone environments in light of the predicted sea level rise in the 21st century (Hoffman 1984, Jelgersma & Tooley 1992, Shennan 1992, Bird 1996, Raper *et al.*, 1996, Saizar 1997, Olivo 1997). Engineering methods utilising man made constructions (US Army corps of engineers 1984 I & II, Harlow 1990, Silvester 1990, Kelletat 1992, DeRond 1996, Simm 1996, Youdeowei & Abam 1997) has traditionally protected these areas. These structures are predominantly employed in areas of human settlement or navigable bodies of water. Where sites of special scientific interest (SSSI) or regular coastlines are subject to erosion heavy engineering methods may not be applicable. In these cases a more subtle environmentally friendly method may be required to protect the coastline.

Naturally occurring coastal protection is afforded by mangrove swamps in tropical countries (Bennett & Reynolds 1993, Weinstock 1994). Sea or terrestrial grasses are also used to stabilise sand dunes and sediments in both tropical and temperate coastal zones (Seliskar 1995, kaul 1996, Lancaster & Baas 1998, Bruno 2000). The use of intertidal benthic invertebrates may be a more flexible method of coastal protection as they are resilient to the intertidal environment.

Mytilus edulis has an extensive range in both the northern and southern hemispheres on intertidal coastlines. Experiments conducted in section one showed that mussels will attach to some types of substrata introduced into the coastal environment. More mussels were lost from the higher energy lower intertidal zone as compared to the upper intertidal. The experiments in essence show that mussels could be used as a natural form of coastal zone protection. This is shown by a significant

number of mussels left attached to the introduced substratum after several tidal cycles. This means that there will be mussels left *in situ* to physically armour the underlying sediment. Mussels were also found to remain attached after early winter storms. The long-term effectiveness of this method could only be fully tested by a series of large-scale field experiments. This would employ the use of the introduced netting substratum from the field experiments in both sheltered and exposed shores. Larger numbers of mussels would be used to maximise the armouring coverage of the mussels and to simulate large natural mussel beds. For a fully comprehensive test, the mussels would have to be exposed to tidal cycles for at least one year to experience the full range and strength of tides.

Section three studies the interaction between marine benthic invertebrates and their mucus secretions and the incorporation of radionuclides into sediments. Radionuclides are becoming more prevalent in the Irish Sea due to discharges from the Sellafield nuclear reprocessing plant. Two biologically active constituents of the discharges are Americium²⁴¹ and Cesium¹³⁷. Both these radionuclides react with the substratum in different ways. Americium is more particle reactive, which means it is lost from suspension in the water column very quickly. Cesium is very mobile, which means that the radionuclide may be attached to particles in sediment though this may be reversible thus releasing the Cesium back into suspension.

Marine invertebrates are present in high numbers in most sedimentary environments. In the intertidal zone, this is particularly evident. The majority of marine invertebrates produce mucus, which is used for various functions in the life cycles of the organisms. There have been numerous research works conducted on heavy metal uptake by marine invertebrates. There has also been much study done on the radioactive uptake by marine invertebrates. These research investigations show that high concentration of both heavy metals and radionuclides are associated with mucus related areas of the organisms. There has however been little research conducted on the uptake of radionuclides by mucus itself. The work conducted in section three revealed that the radionuclides in question did experience uptake into sediment substratum and mucus. Americium was taken up very quickly and Cesium was taken up in negligible amounts. Natural sediment was stripped of

organic matter, mucus was then added along with the radionuclides. The results showed an uptake of Americium, which suggests uptake by the mucus as there should be little left in the sediment to cause uptake. This result is strengthened by an experiment where there was uptake of the radionuclides onto glass beads covered in mucus.

Future studies would benefit from a lengthy extended research program building on the results of the existing experiments. There would be replication of all experimental conditions. In addition, several experimental sediments without mucus added would be included. These would be in effect control conditions for the experiment.

The continental slope displays an environment that marine benthic invertebrates and sediments interact. This environment is the mid water oxygen minimum zone which impinges on the sediments of the continental slope. The continental slope of the Oman coast in the Arabian Sea displays a particularly strong post-monsoonal OMZ. The OMZ is thought to extend from 100m to 1200m. Where the OMZ is in contact with the sediments of the continental slope it may impose stresses onto the organisms living on or within the sediment. Work conducted by Lamont & Gage (2000), Levin *et al.* (2000) and Smith *et al.* (2000) show that there are many benthic species living within the OMZ. These species will bioturbate the sediment mixing layers as they move the sediment. The amount of bioturbation displayed in the sediment can be an indication of biological activity. Sediment core samples retrieved from Reinek box cores from various stations off the Oman coast during Discovery Cruise 211 revealed bioturbation patterns (Meadows *et al.* 2000). The patterns occurred mostly in the first 15cm of the core. There was also high organic matter present in the surface few centimetres of the sedimentary column. This may be due to low rates of decomposition of organic matter in the water column by microorganisms. The Eh measurements indicated an oxygen-depleted environment from 300m to 1200m water depth, which is consistent to the OMZ range quoted in the literature.

A natural comparative study for future research would be a study of the Indus fan region of the Arabian Sea/Indian Ocean. Here there would be high organic inputs from the Indus River itself. This may interfere with the nutrient bringing cycle of the monsoon.

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