Studies on the Impact of a Water-based Drilling Mud Weighting Agent (Barite) on some Benthic Invertebrates

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Abstract

A comparative study was carried out to observe effects of standard grade and fine grade barite on the filtration rates of four suspension feeding bivalves, *Modiolus modiolus, Dosinia exoleta, Venerupis senegalensis* and *Chlamys varia.* Standard grade barite, the most commonly used weighting agent in water-based drilling mud, was responsible for altering the filtration rates of the four bivalve species and damaging the gill structure. The four bivalves were exposed to 0.5mm, 1.0mm and 2.0mm daily depth equivalents of standard grade barite, which permanently remained in suspension. All three barite levels altered the filtration rates leading to 100% mortality. The horse mussel, *Modiolus modiolus* was the most tolerant to standard barite with the scallop, *Chlamys varia* the least tolerant. Fine grade barite, at a 2mm daily depth equivalent, also altered the filtration rates of the four bivalve species, but only affected mortality of *Venerupis senegalensis*, with 60% survival at 28 days. *In-vivo* studies showed damage to the gills, ranging from displaced inter-lamellar junctions to the deletion of large parts of demibranch. *Post-mortem* microscopy studies showed damage to individual filaments with a marked reduction in the active surface area of the gill. Field studies have shown that the presence of standard grade barite is not acutely toxic to seabed fauna but does alter benthic community structure when it is persistent.

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Chapter One:

Introduction to the North Sea Drilling Operations

1.0. Background to Research

Preliminary research into the physical effects barite may have towards bivalves was conducted from undergraduate studies by the author and from previous studies by Barlow and Kingston, 2001. These preliminary studies coincided with a requirement of the offshore oil and gas industry to study water-based drilling mud. Most research to date has focused on the toxic effects of offshore oil and drilling wastes towards the marine environment. There has been very little research into the physical effects of offshore wastes. Oil and Gas UK had just completed a research and development programme in 2002, called the Drill Cutting Initiative. The main aims of this project were to determine the environmental impact of drill cuttings piles, how the characteristics of individual piles change over time, and to find the best solution in dealing with them. Water-based drilling mud will always be introduced into the marine environment in the initial stages of drilling when no riser is in use. Unlike cuttings piles, where they mainly accumulate under the platforms, drilling mud can be distributed over vast distances.

1.1. The North Sea

The North Sea is located on the continental shelf of North West Europe. The North Sea opens to the North into the Atlantic Ocean, to the Southwest via the English Channel, and to the East through the Baltic Sea. The total area of the North Sea is approximately 750,000km^2 with a water volume of approximately 94,000km^3 (North Sea Task Force, 1993).

1.1.1. North Sea Oil and Gas Production

The North Sea is the most extensive oil and gas province in the world and currently supplies 70% of the United Kingdom's energy supply (Oil and Gas UK, 2008). The total recovery of indigenous oil and gas in the North Sea to date exceeds 37 billion barrels (Blackwood, 2007).

In 1964 the first drilling licences were granted to offshore UK. The first gas field discovered within the North Sea was in the South at West Sole in 1965 with production starting in 1967. Oil was initially discovered within the North Sea in the Arbroath field in 1969 but it was not until 1975, within the Argyll oil field, that oil production first occurred. By the end of 1975 one thousand wells had been drilled within the UK waters of the North Sea. In 1978 the UK North Sea oil production exceeded one million barrels per day with production exceeding consumption within the UK in 1981. The production of oil achieved a new record of 18 million barrels (127.5 million tonnes) in 1985 (Oil and Gas UK, 2007).

In 1991, one hundred fields were in production in the UK sector of the North Sea. By the end of 1997 a record number of production fields were in use; 98 oil, 75 gas and 13 condensate. With relation to the field numbers, the combined offshore oil and gas production reached a new record of 29 million barrels of oil equivalent (203.8 million tonnes of oil equivalent). By 1999 oil and gas production had peaked to 18 million barrels (125 million tonnes) and 105 billion cubic metres respectively. Gas production continued to rise to 115 billion cubic metres in 2000 unlike oil production, which declined to 16 million barrels (115 million tonnes). By the year 2006 oil and gas production in the North Sea had declined to 2.9 million barrels of oil equivalent. UK oil and gas production had declined by 5% from 2006 to 2007 (Oil and Gas UK, 2007). Although production has been in decline in the recent years there is still an estimated remaining reserve of up to 25 billion barrels in the North Sea (UKOOA, 2007) and the UK will rely on this oil and gas to provide 40% of its energy needs by 2020 (Blackwood, 2007). Table 1.1 shows the amount of oil and gas produced within the North Sea from 2006 to 2008.

Table 1.1: Oil and gas production figures from the North Sea from 2006 to 2008. N.B. boepd – barrels of oil and gas equivalent per day

	2006	2007	2008 forecast
Total Production (million boepd)	2.9	2.8	2.6-2.7
Oil Production (million boepd)	1.6	1.6	1.6
Gas Production (million boepd)	1.3	1.2	1.1
Exploration Wells Drilled	29	34	-
Appraisal Wells Drilled	41	77	-
Development Wells Drilled	192	163	-
Volumes Discovered (Millions boe)	500	300-400	-
New Field Approvals	13	15	-
New Field Start ups	14	20	15

Figure 1.1 shows the total yearly production of oil produced in the North Sea from 1994 to 2008. In 1997 there was a peak of oil production with a total of 5,271,485 million m^3 produced. There was a general decline in oil production within the North Sea since from 1997 to 2008 with a slight peak in 2003 when 4,607,320 million m^3 was produced.

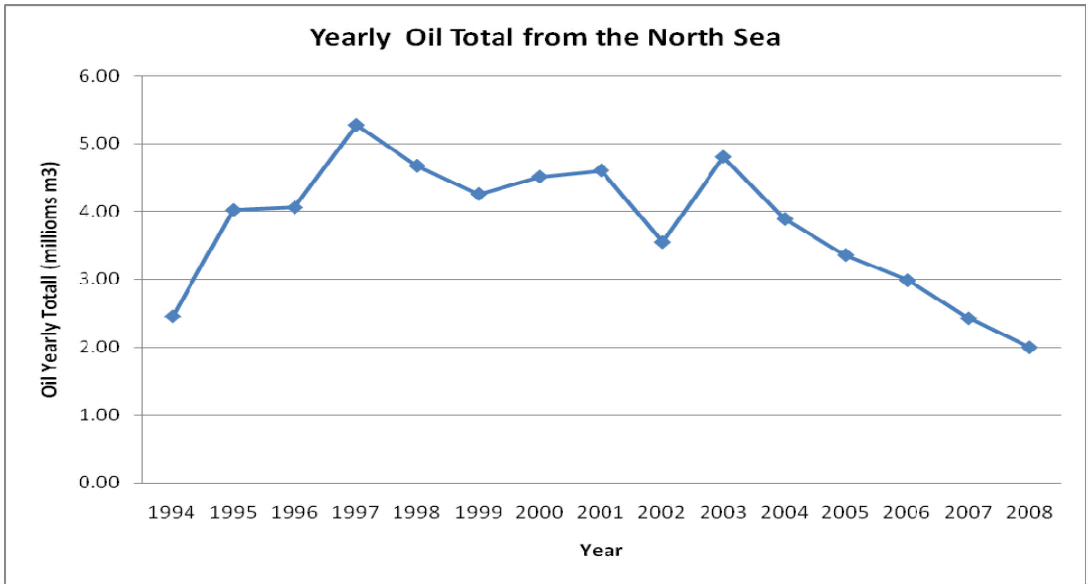


Figure 1.1: Yearly oil total produced in the North Sea from 1994 to 2008 (BERR, 2008).

1.1.2. *Production Platforms*

Structures built to extract oil and gas from the North Sea include subsea equipment fixed to the ocean floor as well as platforms. These range from smaller structures in the Southern and Central North Sea to very large structures in the Northern North Sea built to withstand the harsh weather conditions in deep waters. Platforms were developed using either concrete, because their great weight held them firmly on the seabed and they provided convenient space for integral storage of oil, or steel jackets pinned to the seafloor with steel piles. The Brent D is an example of a concrete platform. It weighs over 200, 000 tonnes and was built to store over a million barrels of oil. In 1999 there were 420 platforms in the North Sea with 210 of the platforms in the UK sector (UKOOA, 1999a). Global subsea well installations in the UK waters grew to 300 units in 2006 with a further 500 units forecast by 2011 (ITF, 2006).

Within the North Sea in recent years the oil and gas industry has reached maturity. Most new developments do not require massive production platforms and instead existing structures are used. To develop offshore fields as economically as possible, numerous directional wells radiate out from a single platform to drain a large area of reservoir. Deviated wells, which exceed 80° from the vertical, are known as horizontal wells. Horizontal wells maximise contact with reservoirs and reduce the logistic constraints of drilling many vertical wells in one area. More than one horizontal section can be drilled in one well as a multilateral well. This technique maximises the number of wells that can be drilled from small platforms.

Smaller fields are drilled from subsea clusters rather than being drilled from a large central platform. Production here occurs from several wells which all join up at a subsea manifold. The manifolds are often linked to a nearby platform.

1.2. Drilling Wastes

During offshore exploration a wide variety of wastes are produced on the platform, some of which are discharged onto the seabed. In the initial phases, extensive drilling operations are undertaken resulting in the major discharge of drill cuttings and drilling mud (Gerrard *et.al.,* 1999).

1.2.1. Produced Formation Water

Produced formation water is the oily water usually discharged from a platform after separation from oil. It consists of formation water and potentially includes water, which was injected into the reservoir to maintain pressure (Holdway, 2002). Produced waters are mainly salt solutions with a highly complex mixture of organics, radionuclides and metals which may be present in higher levels than natural seawater including barium, cadmium, chromium, iron and lead. After treatment produced water is usually discharged into the sea. It has been estimated that there were 234 million tonnes of produced water released into the UK sector of the North Sea alone in 1997 (Holdway, 2002). In recent years the total volume of produced water discharged into the North Sea has declined from 2005 to 2007 from 235 million m^3 to 203 million m^3 respectively. The decline is due to an increase in the number of installations re-injecting the produced water. The volume of produced water re-injected by installations from 2005 to 2007 was 24.8 million m^3 to 40.5 million m^3 (BERR, 2008). Components of produced water can either diffuse into the atmosphere, adsorb onto and settle out onto the bottom sediments, disperse due to water currents, or be taken up and metabolised by both pelagic and benthic marine organisms (Holdway, 2002).

1.2.2. Drill Cuttings

Drill cuttings contain mainly particles of the rock substratum being drilled through. In the northern North Sea, most discharged material from offshore drilling accumulates in distinct ‘cuttings piles’ (Breuer *et.al.*, 2004). The strong currents of the southern North Sea prevent the formation of these cuttings piles. When oil based mud drill cuttings are discharged, the large particles, about 90% of the cuttings, are inclined to flocculate together and settle onto the seabed, forming piles. The remaining 10% of the cuttings are usually fine grained clay particles that are diluted out by prevailing currents (Neff, 2005). Water based mud cuttings can still flocculate together but are less inclined to do so.

Drill cutting piles are very heterogeneous and their content and volumes are difficult to forecast (UKOOA, 2002). Cuttings are either discarded into the surface waters, where they will be dispersed over a wide area and eventually settle out to form small piles, or are dumped onto the seabed where larger cuttings piles will be formed (Neff, 2005). The exact form of drill cuttings discharged will alter depending on the rock formation, mud used, depth of the well etc (Gerrard *et.al.*, 1999). They can contain a range of sizes from fine clay particles to coarse gravel (ERT, 1992). Barium, zinc and lead are the most abundant metals present within cutting piles (UKOOA, 2002).

Drill cuttings are brought up to the platform, separated from the drilling mud and then discharged into the sea, although the dumped cuttings will still contain the insoluble portion of the drilling mud that coats the cuttings (Breuer *et.al.*, 2004). Between 1964, when drilling first occurred, and 1993 it has been estimated that 7 million m^3 of drilling cuttings had accumulated on the seabed around platforms in the whole of the North Sea. In 2000, it was estimated that there were $500{,}000m^3$ and $700{,}000m^3$ cuttings present in the northern and central North Sea respectively. In 2004, it was estimated that the volume of disposed cuttings has risen to 12 million m^3 (Breuer *et.al.*, 2004). UK drill cuttings volumes from multi-well installations alone are about $700{,}000m^3$ in the central North Sea and $500{,}000m^3$ in the northern North Sea (UKOOA, 2002). Oil-based mud cuttings are no longer discharged into the North Sea and are instead returned to shore for treatment and disposal.

Cuttings piles generally have 20-60% water content, a bulk density of 1.6-2.3t/m³, and a particle size ranging from 10µm to 2cm (Breuer *et.al.,* 2004). Table 1.2 shows the water content and bulk density of drill cutting piles from different platforms in the North Sea.

Table 1.2: Water content and bulk density of drill cutting piles (From UKOOA drill cutting programme, 1999a).

Platform	Water Content, %	Bulk Density t/m³
NW Hutton	14-70	1.3-2.0
Heather Alpha	22-33	1.89-2.4
Clyde	17-34	1.68-1.79
Beatrice	20-29	2.3-2.7
Fulmar A	20-54	1.49-1.94

The long-term fate of piles is determined by the dumping method and the physics of ocean currents and wave movement (UKOOA, 1999a). In areas of shallow water (<50m) and strong currents (e.g. Southern North Sea) the cuttings will rapidly disperse once dumped. In areas with relatively weak currents (e.g. basins of central and northern North Sea) the cuttings, depending on the drilling mud used, may flocculate and accumulate under and around platforms and form extensive piles. Once the piles are formed they can accumulate under platforms for very long periods of time, mainly in the northern North Sea, and remain relatively undisturbed. The threshold fluid stress necessary to generate transport of cuttings is around 0.1-0.2Nm^{-2}. The North Sea tidal bed shear stress is an order of magnitude lower and therefore the transport of cuttings in-situ is unlikely (Breuer *et.al.,* 2004).

1.2.3. Drilling Mud

Drilling mud, also known as drilling fluid, is a vital component of any drilling operation. In the early days of rotary drilling, the primary function of drilling mud was to bring the drill cutting from the bottom of the hole to the surface. Today it is recognised that drilling mud has many more important functions (Engelhardt *et.al.*, 1983; Caenn and Chillingar, 1996; Wills, 2000; Barlow and Kingston, 2001).

Drilling mud must:

1. Cool and lubricate the drill bit and drill string
2. Assist in removal of drill cuttings from the well bore
3. Control subsurface pressure to prevent any blowouts from the well
4. Maintain bore hole stability by protecting produced formations by minimising formation/fluid interactions and sealing the wall of the bore hole with an impermeable cake.
5. Control corrosion of the metal components of the drilling tools, casing and rig facilities that are exposed to the corrosive marine environment
6. Maximise drilling penetration rates

Drilling mud is pumped from the platform through the drill string. The mud exits the drill string through nozzles in the drill bit, and returns to the surface through the annular space between the drill string and the walls of the hole. As the drill bit grinds rock into drill cuttings, the cuttings become trapped within the mudflow and are carried to the surface. In the initial stage of drilling, the mud and cuttings are directed onto the seabed. Once a riser has been installed the mud and cuttings are directed to the platform where they are separated. To return the mud to the re-circulating mud system, separation of the solids from the drilling mud is vital. It has been roughly estimated that drill cuttings can become coated with drilling mud at a quantity equal in volume to the cuttings (UKOOA, 1999a).

1.3. Types of Drilling Mud

Drilling mud contains a base fluid and a mixture of chemical additives manufactured to perform a variety of functions during drilling (Davies and Kingston, 1992). The mud can be classified into different categories according to their base fluid (Caenn and Chillingar, 1996). In the 1960s, water-based mud (WBM) was initially used, however, certain formations with WBM can prove difficult primarily due to hole instability caused by the swelling of water-absorbing rock such as hydrophilic shales. These problems were overcome by replacement of the base fluid with non-aqueous alternatives (Davies *et.al.*, 1984). Throughout the 1970s and very early 1980s the cheapest and most common lubricant used by the drilling industry was diesel oil. During the early 1980s, diesel oil was replaced by mineral oils of lower toxicity towards the marine environment (Davies *et.al.*, 1984; UKOOA, 1999). The early oil-based mud reduced friction and allowed development of advanced drilling techniques to extend the range and precision of the wells, enhancing the recovery of hydrocarbon reserves.

Since 1990, a large number of extended-reach and horizontal wells have been drilled. The mud technology required to drill high-angle holes is more demanding than that for drilling straight holes. Water-based mud is not as successful through the drilling process of high angle holes as oil-based mud. In 1990 there was an introduction of synthetic based mud. This was designed to be less toxic and degrade faster in marine sediments than oil-based mud while providing similar advantages in drilling difficult wells. In 1991 the use of oil-based mud was restricted and was banned from being discharged onto the seabed and in 2000 the use of oil-based mud was effectively banned.

1.3.1. Water-Based Drilling Mud (WBM)

Water-based drilling mud is an aqueous suspension of clay or polymeric substances with a viscosity higher than water. It uses either freshwater or saltwater as its carrier fluid and is involved in most of the worlds drilling operations (Caenn and Chillingar, 1996). Water-based mud can be classified by its specific shale drilling fluid formulations and the effect of these mud systems on water content, swelling pressure and pore-pressure.

Water-based mud continually disperses drill-cutting particles into the drilling fluid because of their water content. To offset this, the mud is often diluted and therefore, excess amounts of drilling mud are released during drilling operations (UKOOA, 1999a). Therefore the use of WBM produces larger amounts of drilling waste than oil-based mud. Although water-based mud is found to be friendlier to the environment than OBM and SBM, it has been reported that WBM does contain significant levels of hydrocarbons from the drilling process, particularly when it passes through the reservoir (UKOOA, 1999a).

1.3.2. Diesel Oil-Based Drilling Mud (DOBM)

Diesel oil was used to replace water as the base fluid for mud systems designed for certain applications. Diesel oil-based mud is composed of various molecular-weight-range cuts refined from crude oil for fuel. Its composition varies with the original crude composition and the distillation process.

Oil-based drilling mud and diesel-based drilling mud are selected for their superior temperature stability, lubricity and the hole stabilizing attributes (UKOOA, 1999b). Oil-based mud is used in high temperature formations, formations containing water sensitive minerals, clays or reactive gases, and in wells where a high level of lubrication is required. Oil-based drilling mud can be classified as either low fluid loss or relaxed fluid loss system:

- **Low fluid loss** – to limit the fluid lost, asphalt or lignitic agent is added depending on the temperature range that is to be confronted.
- **Relax fluid loss** – emulsifiers and organophilic clay viscosifiers are added to exhibit low fluid loss.

In 1981 a total of 212 wells were drilled on the UK continental shelf and the Department of Energy estimated that 76 of the wells were drilled using DOBM resulting in a total of approximately 7000t of diesel oil being discharged attached to the drill cuttings. In 1983, a total of 223 wells were drilled and it is believed that about 65% were drilled using OBM, and the total quantities of oil discharged were about 7700t of diesel oil (Davies *et.al.*, 1984).

The toxicity of diesel oil, due to its high aromatic content (Caenn and Chillingar, 1996), led to the decision by the UK Government that the use of diesel as a base fluid was effectively prohibited from the 1st January 1987, and could only be used under certain circumstances. DOBM can still be used as a spotting fluid for stuck pipe. The Norwegian Government banned the use of diesel oil-based mud in October 1986 (Gerrard *et.al.*, 1999), leading to the development of alternative sources of oil based drilling mud.

1.3.3. Low Toxicity Oil-Based Drilling Mud (LTM)

There was a drive to replace diesel oil-based drilling mud due to the environmental concerns associated with the toxic high aromatic content of the diesel oil. Because of the perceived high toxicity of DOBM (Wills, 2000; McCosh and Getliff, 2002), a mud was developed in which the high aromatic content was eliminated (Caenn and Chillingar, 1996; McCosh and Getliff, 2002).

Drilling activities continued to increase with 25,800t of oil associated with drill cuttings being discharged into the North Sea in 1985 (UKOOA, 1999b). Although low toxicity mud was designed to reduce the environmental impact of the mud (Wills, 2000; Neff, 2005), cuttings from the LTM still seemed to have a substantial impact towards the marine environment. In order to combat this, on 1st January 1989, a discharge limit for oil on cuttings was set at 15%. The oil on cuttings ratio was then reduced to 10% and after 1992 it was reduced further down to 1%. It was not practicable to reach these levels, so the discharge of oil based-cuttings was effectively banned.

1.3.4. Synthetic-Based Drilling Mud (SBM)

In the mid 1990s the offshore drilling industry began phasing out the use of oil-based mud replacing it with light synthetic-based mud. Synthetic-based drilling mud is a water in oil emulsion intended to replace OBM as a low toxicity readily biodegradable alternative to mineral oil-based muds (Burke and Veil, 1995). These muds are a synthetic material as the carrier fluid is more readily biodegraded, unlike conventional oil-based mud (Engelhardt *et.al.*, 1983). SBM contain lubricants synthesised from products such as ethylene (Breuer *et.al*., 2004). They contain double bonds or functional groups promoting rapid environmental breakdown in water (Caenn and Chillingar, 1996). SBM only differs from OBM with their base fluids. The base fluid is replaced with esters, ethers, polyalphaolefins (PAOs) or linearalphaolefins (LAOs) and also vegetable oils.

These additives were intended to provide SBM with the same drilling advantages as OBM but with the handling and disposal characteristics of WBM. Although synthetic-based mud is much less toxic than hydrocarbon-based oils, it is still not completely non-toxic (Caenn and Chillingar, 1996) and the commonly used synthetic fluids bio-degradation rates were found to be similar to the OBM they replaced. Government regulations called for a reduction in the discharge of SBM to zero by 31st December 2000 (Breuer *et.al.*, 2004). The cuttings associated with synthetic-based drilling mud are either re-injected back into the well or taken ashore for treatment.

1.3.5. Alternative Water-Based Mud (AWBM)

Alternative based mud has been developed to cause less harm towards the marine environment. Replacement mud systems currently in use as possible replacements for OBM and SBM are:

- **Polymer mud**

Fresh-water polymer mud systems have been used throughout the horizontal drilling process. They are cost effective systems but do not cope with unstable shales (Caenn and Chillingar, 1996).

- **Salt/polymer mud**

In areas with wellbore stability problems, polymers are usually supplemented with a salt that supplies a cation to help stabilize the formation (Caenn and Chillingar, 1996).

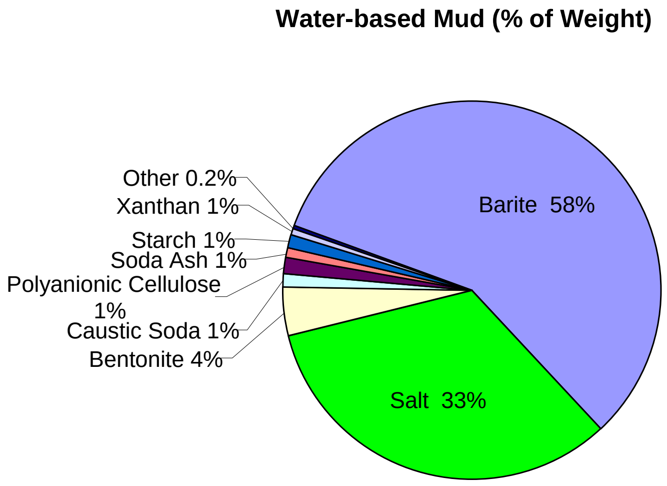
- **Cationic Mud**

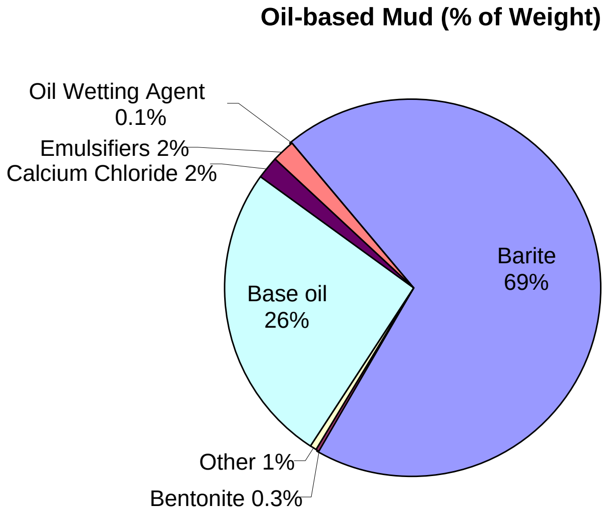
Cationic mud is a drilling fluid with a predominately cationic chemical nature (Caenn and Chillingar, 1996) and is designed to disperse less in comparison to other mud types (Orszulik, 2008). Cationics can be extremely inhibitive to shale or clay hydration. This mud uses non-reactive sepiolite or attapulgite clay, a cationic polymeric extender and cationic inhibitors. These ensure that the solids in suspension are positively charged which reduces the dispersal properties (Orszulik, 2008). The most common difficulty with these systems however, is the fluid loss control (Caenn and Chillingar, 1996).

1.4. Components of Drilling Mud

Although the type of drilling mud used has changed over the years due to the perceived toxicities and the damage they may cause towards the marine environment, the toxicities for each type of mud cannot be stated. The mud itself is formulated depending on the drilling job and its location (Wills, 2000). Drilling companies are not required to release details of the mud formulations, this confidentiality rule is upheld by OSPAR, and therefore there is no actual way of determining the toxicities of the individual drilling mud used.

Drilling mud often contains a variety of chemicals which are formulated as required from a generally limited list of additives (Holdway, 2002). The type and amount of chemical additives included in the mud formulation varies according to the required characteristics of the mud depending on the well to be drilled. Around 2,014 products are sold to offshore drillers (Wills, 2000). In general, the quantities of additives for OBM/SBM are less than that added to WBM (UKOOA, 1999a). Figures 1.2a and 1.2b show an example of a typical water and oil-based drilling mud. The composition of drilling mud is continually being altered throughout the drilling process to solve particular down-hole problems that may be encountered (Neff, 2005).





Figures 1.2a and b: example of Typical Water and Oil Based Drilling Mud Composition (Adapted from UKOOA drill cutting programme, 1999a)

Products added to the mud for creating the physical-chemical properties required are divided into several categories according to their function and composition (Terzaghi *et.al.*, 1998). Each category may contain several alternative materials with different properties (Neff, 2005).

1.4.1. Weighting Agents

Weighting agents are the most abundant component of drilling mud (Neff, 2005). Weighting agents are used to prevent blowouts by maintaining the borehole pressure (Sadiq *et.al.*, 2003). The weighting agent is mixed with water and other materials then pumped into the drill hole. The weight of the mixtures counteracts the force of the oil and gas when it is released, which allows the oil and gas rig operators to prevent the explosive release of the oil and gas from the ground. Barite (barium sulphate) is the main and preferred weighting agent, due to its relatively high specific gravity of 4.5. Ilmenite, haematite (iron oxide), siderite, dolomite and calcium carbonate are also used as weighting agents (Caenn and Chillingar, 1996). Haematite sometimes replaces barite for use in deep drilling within a high density mud (Neff, 2005). Ilmenite has a specific gravity of 4.5 to 5.0 and is mainly used within the Norwegian sector of the North Sea (Neff, 2005).

1.4.2. Viscosifiers

Viscosifiers are used with all types of drilling mud and are generally added in the form of clay. Viscosifiers build viscosity through complex interactions with the emulsions (Caenn and Chillingar, 1996). The clay forms a thick gel in the well bore preventing the settlement of drill cuttings and barite (Neff, 2005). There are commonly two types of clay components in drilling mud, the most used being sodium montmorillonite, commonly called bentonite. Bentonite also helps to prevent fluid loss by coating the wall of the borehole (Neff, 2005). The other clay in general use is attapulgite, commonly known as salt gel (Caenn and Chillingar, 1996). With WBM, organic polymers derived from cellulose and natural biopolymers are also in general use (UKOOA, 1999a). These act as a replacement for clay when drilling in soft formations (Neff, 2005).

1.4.3. Surfactants

These can be used for different purposes such as defoamers, detergents, lubricants and emulsifiers. Surfactants help to maintain wet ability throughout the drilling process (Wenger *et.al.*, 2004). Various additives have been used to lower the friction factor in water-based mud and include modified vegetable oils and refined polyols. A WBM with a sufficient amount of polyol added behaves more like an oil mud in its wetting characteristics (Caenn and Chillingar, 1996). This greatly improves the lubricity, shale stability and formation return permeability of the mud (Caenn and Chillingar, 1996). To reduce friction and resistance of the drill string, lubricants such as vegetable or mineral oils, may be added to WBM, predominantly when drilling a deviated well (Neff, 2005).

1.4.4. Shale Stabilisation Agents

The stability of clay-rich shales is fundamentally affected by their complex physical and chemical interaction with drilling fluids. It has been shown that shale-fluid interactions can be manipulated to enhance cuttings and wellbore stabilisation as well as improving hole-making ability in shale formations (vanOort, 2003). Asphalt-based shale stabilisers are added to aid well-bore proficiency and prevent sticking drill pipe. Polyalkylated glycols are added to prevent the formation of gas hydrates in the drilling mud (Wenger *et.al.*, 2004). Soltex is a asphalt produced as a residue in petroleum refining and made water-soluble by a special sulphonation process that is used primarily for shale control (Terzaghi *et.al.*, 1998).

1.4.5. Fluid Loss Control Agents

These agents are added to reduce the loss of fluid from the mud into the drilled formation. The added compounds include bentonite, because of its fine particle size, lignite and polymers. Lignite is an important additive for WBM because it also acts as an emulsion stabiliser (UKOOA, 1999a). The polymers can include modified starch and polyanionic cellulosic polymer (Caenn and Chillingar, 1996).

1.5. Offshore Regulations

Pollution problems, related to the oil and gas industry, have been recognised since the development of North Sea oil and gas fields, and have become a major political problem in Western Europe (Wills, 2000). To prevent damage to the marine environment a number of regulatory frameworks exist. The North Sea legal systems originate mainly from the Oslo Convention on the Prevention of Marine Pollution by Dumping from Ships and Aircraft (1972), the Paris Convention on the Prevention of Marine Pollution from Land-based Sources (1974), and the Bonn Agreement for Co-operation in Dealing with Pollution of the North Sea by Oil and other Harmful Substances (1983). A new Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR), signed in Paris in 1992, has now replaced the earlier Oslo (1972) and Paris (1974) conventions (Oil and Gas UK, 2003).

OSPAR, is the foundation for national laws governing the discharge of offshore drilling wastes in the Waters of the OSPAR signatory states which include the United Kingdom, Norway, the Netherlands and many more (Wills, 2000). The main criteria that OSPAR uses to regulate offshore discharges take into account the persistence, toxicity and other poisonous properties of the chemicals in drilling wastes. These principles can be seen in *OSPAR. 1996. PARCOM Decision 96/3 on a Harmonized Mandatory Control System for the Use and Reduction of the Discharge of Offshore Chemicals. Oslo*.

UK authorities require extensive details of every proposed drilling well to ensure complete regulation of the operator’s doings, see: *United Kingdom Department of Trade and Industry, Oil and Gas Directorate. 1999. Guidance Notes on the Offshore Petroleum Production and Pipelines (Assessment of Environmental Effects) Regulations 1999. Edition 2, March 1999, London).* For most developments to occur an environmental statement must be published by the operator.

1.6. Aims and Objective

The main purpose of this thesis is to determine the physical effects suspended barite may have towards suspension feeding bivalves at levels that might be encountered during the discharge of water based drilling muds during offshore oil exploration and production operations. The main study focuses on the impact of suspended barite on the filtration rates of four bivalve species, *Modiolus modiolus, Dosinia exoleta, Venerupis senegalensis* and *Chlamys varia.* Based on previous studies (Barlow and Kingston 2001) it is hypothesised that suspended barite is likely to inhibit the filtration rates of the suspension feeding bivalves, may result in damage to the filtration mechanism and ultimate mortality of the individuals. In this study it is intended to subject the bivalves to carefully controlled levels of suspended barite under laboratory experimental conditions, to measure the effect a range of concentrations has on gill filtration efficiency and to determine the nature and extent of damage to the structure of the ctenidia by detailed microscopy. Field trials will also be conducted to attempt to relate laboratory findings to conditions in the natural environment.

Chapter Two:

Impact of Suspended Barite on Bivalve Feeding Rates

2.1. Introduction

Research within the oil sector has focused mainly on cutting piles and associated oil-based drilling mud and hydrocarbon levels (Neff *et.al.*, 1980; Neff, 1981; Neff *et.al.*, 1989; Olsgard and Gray, 1995; Grant and Briggs, 2002; Rezende *et.al.*, 2002; Sadiq *et.al.*, 2003). However, since the 1st January 1997, the discharge of oil-based cuttings onto the seabed has effectively been banned. They are described as 'effectively' banned as a limit was of 1% oil on cuttings was set and this level is not practical to gain. Focus and concerns have now moved onto the impact of water-based drilling mud on the marine environment.

The main focus of this study is on the physical effects that barite, barium sulphate, may have on marine bivalve molluscs. Suspension feeding bivalves are generally immobile and are likely to be the most vulnerable to a change of suspended solid levels within the water column. Barite is chemically inert and therefore has an extremely low solubility in seawater and will have a low bioavailability and toxicity on marine animals. Once discharged onto the seabed, barite will be re-suspended and re-distributed in the water column where suspension feeders obtain their food and oxygen supply. Fine particulates from drilling wastes (cuttings and mud) have been detected mixed within the benthic boundary layer up to a distance of 8km from an active drill platform (Cranford *et.al.*, 1999; Muschenheim and Milligan, 1996).

2.1.1. Barite

As mentioned earlier, barite is a naturally occurring mineral and is a major component of all drilling mud (Holdway, 2002). Barite is very dense and found when mined from layers of sedimentary rock. It is primarily used in drilling mud as a weighting agent because of its relatively high specific gravity of 4.5; it is unusually heavy for a non-metallic mineral. The levels of barite within a drilling mud increase from use at the seabed surface to the bottom of a deep well, increasing from roughly 6.3kg/m^3 to 2000kg/m^3 respectively (Neff, 2005).

During a drilling operation, barite can be found in significant quantities in discharged cuttings, and is also present in produced water. Although there are calls to prevent all offshore dumping of cuttings and mud, this is operationally impractical within the initial drilling stage, before a riser has been installed. Once a riser has been placed in position, drilling fluid and cuttings are carried directly to the platform where drilling mud is separated from the cuttings, which may be discharged directly onto the seabed. A certain quantity of drilling mud (and barite) adheres to the cuttings and therefore forms part of the discharged cuttings pile. Figure 2.1 shows the total quantity of barite discharged into the North Sea from 1987 to 1996. Barite levels following 1996 could not be obtained. The volumes of discharged barite were very high, especially in the early 1990s. Following this, the number of offshore drilling operations decreased, which coincides with the decrease in barite levels discharged into the North Sea.

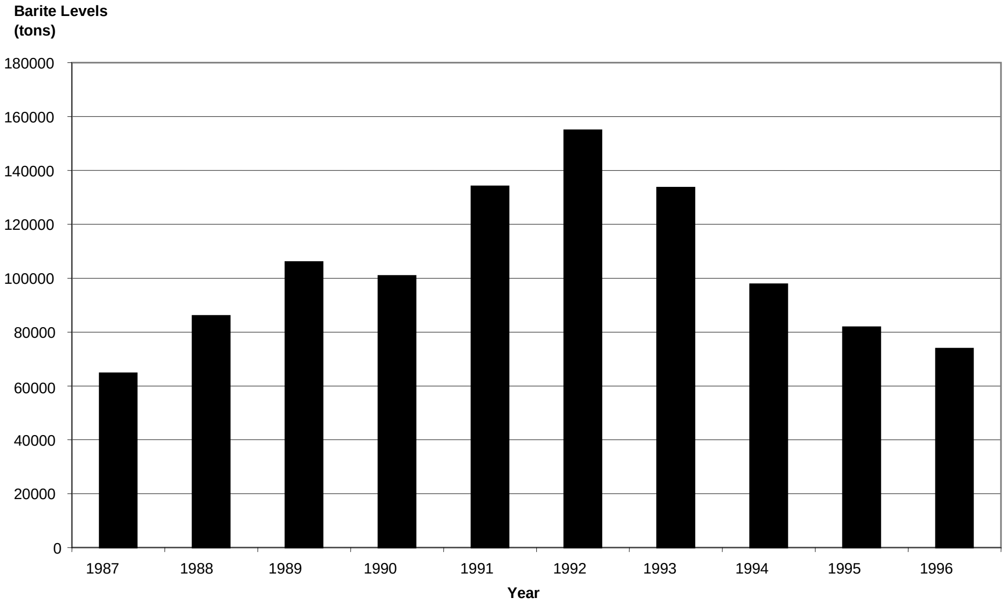


Figure 2.1: Levels of Barite Discharged into the North Sea from 1987 to 1996 (UKOOA 1999).

Barite represents a substantial constituent of most drilling mud, irrespective of which type of base fluid is used, and because of its inertia it is often used as a tracer for the spatial dispersal of discharged material (Daan and Mulder, 1996; Holdway, 2002). Elevated concentrations of barite relative to the natural sediment have been found in the North Sea around drilling platforms and the cuttings piles, as shown in table 2.1 (Breuer *et.al.*, 2004).

Table 2.1: Total Barite Concentrations found within 100m of selected North Sea Cuttings Piles (Modified from Breuer *et.al.*, 2004).

Location	Barite (µg/g)	Year Recorded
Cuttings Piles Levels		
Heather A	213, 000	1994
NW Hutton	2778	1993
NW Hutton	175, 038	2000
Beryl Alpha	2080	1999
Beryl Alpha	228, 557	2000
Mean data from several piles	21, 300	2000
North Sea Background Levels		
Sand	14	1995
Muddy sand/sandy mud	125	1995
Mean Mud (0-1cm)	149	1995

Differences in the concentration of barite over time can largely be explained by the patchy distribution of material near to platforms. Barite particles, once in suspension, can be transported considerable distances from the discharge source (Cranford and Gordon, 1992). Barite concentrations have been found substantially beyond background levels up to 300m from well locations. Concentrations between 500m and 1000m are still usually significantly elevated and traces of discharged material have been detected up to 2000m from drill platforms (Daan and Mulder, 1996). Barite concentrations in the water column have been found to increase throughout the drilling operation and be distributed up to 6500m from the drilling platform (Gordon *et.al.*, 2000).

2.1.2. Environmental Concerns

Drilling wastes, such as barite, the main component of drilling muds, have been shown to produce a detrimental effect on marine organisms. Reductions in somatic and/or tissue growth have been recorded with chronic exposure leading to mortalities of the adult sea scallop, *Placopecten magellanicus* (Cranford *et.al.,* 1999). These effects depend on the waste itself, environmental conditions present and the benthic organisms inhabiting the surrounding area (Grant and Briggs, 2002). Benthic organisms rely on the water-column and sediment for access to food. The macrobenthic diversity of the North Sea is approximately 1400 species (ICES, 2000). Benthic communities mainly consist of suspension and deposit feeders and predators. A suspension feeder is an organism that feeds by capturing particles suspended in the water column and a deposit feeder is an organism that obtains nutritional material from sediment (Levinton, 2001).

Beneath production platforms, where cutting piles accumulate, environmental conditions may be characterised by anaerobic sediments, devoid of macrofauna, for which smothering by the cutting pile appears to the main reason (Neff, 1981). Around the Forties and Beatrice platforms, in the northern North Sea, there is a zone of smothering extending out to around 200m, within which there are major changes to the benthic fauna (Davies *et.al.,* 1984). A peak of opportunistic species may occur between the severely depleted zone beside the cuttings pile and the progressively more diverse zone further away from the discharge. Further away from the platform, when cuttings piles have been derived from the discharge of oil-based drilling mud, faunal diversity may be similar to that in the surrounding area, but with a detectably different species composition (Davies *et.al.,* 1984). Field studies in the North Sea have demonstrated that any effect of barite on benthic communities is likely to be confined to within a 1000 - 2000m radius of platform sites (Breuer *et.al*., 1999). However, the nature of the effect is not fully understood and this impact may be caused by factors other than the presence of barite. Bamber (1980) noted that the addition of fly-ash onto the seabed was responsible for an increase in sediment instability leading to reduction in the porosity and permeability and therefore the suitability of the sediment for infaunal species.

The disposal of solid drilling wastes from oil and gas-drilling activities has been shown to be responsible for affecting suspended solid levels in the vicinity of offshore developments (Gordon, 1988). The existence of fine particulate material from drilling operations in the benthic boundary layer could interfere with the normal feeding behaviour of macrobenthos (Cranford and Gordon, 1992). Water-based drilling mud is more prone to re-suspension and re-distribution than any other type of drilling mud (Englehardt *et.al.*, 1983).

The focus of these exposure experiments is to determine the effect(s) barite may have on suspension feeding bivalves. Barite is the main component of water-based drilling mud and use of this type of mud is on the increase due to a drive towards a friendlier approach to drilling. Exposure to barium caused developmental problems for *Mytilus californicus* larvae. Abnormal shell calcification and embryo morphology were recorded. Gastrulae larvae were more sensitive to barium than blastula and trochophore larvae (Spangenberg and Cher, 1996). Any impact on larvae will affect future adult stocks. The distribution of barite can be affected by the current regime, waves and storms, water depth and the settling velocity (Gordon *et.al.*, 2000); which may result in the re-suspension of the particles. This may cause the prolonged residence of barite in the water-column from which suspension feeders obtain their food. Fine barite, barite ground down for longer in the mills, may remain in suspension for longer and be distributed over a greater distance than standard barite, due to the smaller particle size.

2.1.3. Bivalve molluscs

Bivalves belong to the phylum Mollusca, the second largest animal phylum, and contain around 8000 described existing species, with approximately 6700 being marine (Ruppert *et.al.*, 2004). Bivalves are ecologically important for both humans, as a food source, jewellery and decoration, and the marine environment, as food, habitat and nutrient re-cycling. Bivalves remove particles from the water-column and discharge them as either faeces or pseudofaeces, also known as biodeposits (Beninger *et.al.*, 1997; Vaughn & Hakenkamp, 2001). The biodeposits, once settled onto the seabed, may have an effect on the productivity and biodiversity of the benthic ecosystem (Navarro & Thompson, 1997).

Bivalves are adapted to being either epibenthic, living on the surface or to occupy the infaunal habitat in soft sediments (Ruppert *et.al.*, 2004). Many bivalve species have become adapted to life attached to the surfaces of hard substrata such as rock, shell and wood. A major group adapted to this are the marine mussels. Horse mussels, *Modiolus modiolus* live partially buried in mud gravel sediments and attach their byssal threads to small stones. A large number of bivalve species live on the surface without attaching to it, for example, the scallops.

2.1.3.1. Suspension Feeding Bivalves

Suspension feeding bivalves rely on the water-column for oxygen and food supplies. Suspension feeding bivalves contain an extensive mantle, containing a gill system, where particulate matter, usually phytoplankton, is filtered out (Ruppert *et.al.*, 2004). Bivalves are able to adapt their filtration rate to accommodate for a change in suspended silt and clay levels in the surrounding environment (Cranford and Gordon, 1992). The ability of bivalves to modify their feeding and digestive behaviour in response to changes in the quantity and quality of the particulate matter in suspension is well known (Urrutia *et.al.,* 2001). It is normal for a bivalve mollusc to encounter fluctuations in the quantity and quality of suspended particulate matter to which they are exposed (Navarro *et.al.*, 2004). In the North Sea, these fluctuations will probably be due to the re-suspension of bottom sediments by current and wave action. The suspended solid levels in the North Sea are roughly $51.9\text{-}62.4\text{x}10^6$ton/year. It is estimated that about 70% of the sediments and associated substances remain in sedimentation areas such as the Wadden Sea, Kattegat-Skagerrak area and the Norwegian Trench (Salomons, 2005).

Suspension feeders are particularly susceptible to a rise in suspended solids levels due to their filtration of the water column. They could be affected by suspended solids through abrasion of their gills, reduction in feeding rates and change in behaviour through energy and health loss (Cheung and Shin, 2005). The gill membranes are very delicate structures and are vulnerable to suspended particulate fractions, particularly particles of sharp profile. Suspension feeders therefore must be able to avoid clogging of the gills from heavy particle loads. Some infaunal bivalves have become adapted to living in areas of high sediment loads; for example, a number of eulamellibranchs contain a ring of papillae on the opening of the inhalant siphon. The inhalant siphon also allows expulsion of water and sediment overload in most eulamellibranchs. Foster-Smith (1975) found that the eulamellibranch *Venerupis senegalensis* can co-ordinate movement between the gills and siphons to clear the gills. Most marine bivalves are able to regulate their rate of food consumption and digestion allowing them to endure highly changeable trophic areas (Ibarrola *et.al.,* 2000). In areas of high sediment loads, bivalves have the ability to coat particles filtered out from within the water column in mucus, either accepting or rejecting them. Mucus production is a constant within the filtration process (Urrutia *et.al.*, 2001). The mucus-coated particles rejected from an organism are known as pseudofaeces.

2.1.3.2. Pseudofaeces

Bivalves have the ability to enhance the quality of their diet by means of particle selection and pseudofaeces production (Urrutia *et.al.*, 2001; Brillant and MacDonald, 2000; Velasco and Navarro, 2002). Pseudofaeces are rejected particles, usually inorganics, encased in mucus, produced by the gills and labial palps, and do not pass through the gut. Figure 2.2 below shows the internal anatomy of the bivalve *Mercenaria mercenaria*. Large unwanted particles are trapped by a primary mucous net and transported along the mantle to be accepted by the mouth or rejected. Smaller particles pass through the first mucous net and remain on the gill. These particles are then passed onto the tips of the gill filaments and gather there as a mucous string and are either passed onto the mouth or discarded out of the bivalve as pseudofaeces. This latter route is common for suspension feeding bivalves. A further route by which pseudofaeces can be produced bypasses the food pouch. Here, the particulate matter is trapped in a mucous net at the inhalant mantle cavity and rejected. This route is only temporary and is used when exposed to high particulate concentrations. The rejection of pseudofaeces results in the loss of energy, in the form of mucus, to the external environment.

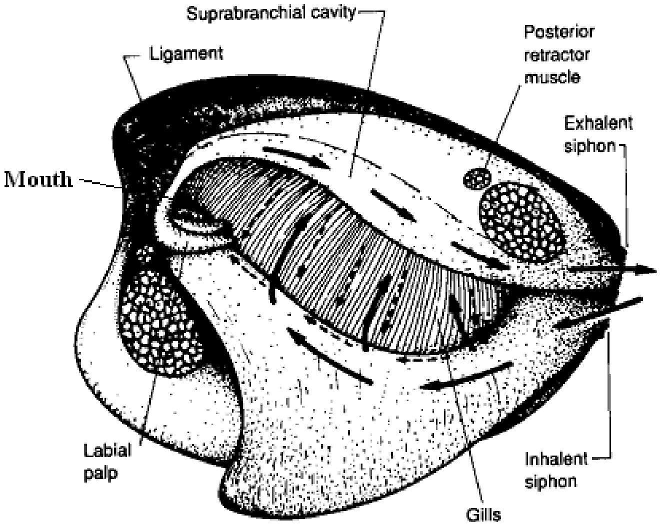


Figure 2.2: Internal Anatomy of Clam *Mercenaria mercenaria*. Interior of right valve. Arrows show direction of food particles over the gills. Adapted from Barnes 1980.

Kooijman (2006) suggests that pseudofaeces production equals silt consumption. When bivalves are exposed to an increase in particle density, the mucous strings on the demibranchs (half gills) become thicker (Chaparro *et.al.,* 2004). It has been discovered that the relationship between pseudofaecal mucus production and total rejection rate was partly dependent on the organic content of the feeding suspension (Urrutia *et.al*., 2001). Velasco and Navarro (2002) showed that pseudofaeces production increased as the quantity of seston increased and with a decrease in organics present. Re-suspension and redistribution of sediments will cause a mixture of organic and inorganic particles to be present within the water-column. Bivalve species that are better adapted to turbid environments regulate ingestion mainly by the production of pseudofaeces (Velasco and Navarro, 2002). *Argopecten purpuratus* has the ability to select organic over inorganic particles for ingestion (Navarro *et.al.,* 2004). *Mytilus edulis* also showed the ability to increase the proportion of rejected material when seston concentrations increased (Bayne *et.al.,* 1993), as has *Mytilus chilensis* and *Mulinia edulis* (Velasco and Navarro, 2002).

2.2. Material and Methods

Numerous experiments were conducted to determine the effects barite has on suspension feeding bivalves. An experimental set-up was developed, over 12 months, to expose controlled known amounts of barite to four species of suspension feeding bivalves. Three doses of barite (0.5mm, 1.0mm and 2.0mm) were chosen to reflect the approximate levels of barite accumulation that could be expected 100m to 500m from the point of an active discharge (Barlow and Kingston, 2001). Although these are daily depth equivalents it was ensured that the barite remained in suspension at all times. Barite concentrations have been found to be substantially beyond background levels up to 300m from well sites. Concentrations between 500m and 1000m are usually still significantly elevated and traces of discharged material have been detected up to 2000m from platforms (Daan and Mulder, 1996).

2.2.1. Experimental Animals

The bivalve species being used in this project are representative of species that can be found within the North Sea. They are found at different depths and within different sediment types. The four species chosen were *Modiolus modiolus*, *Chlamys varia*, *Dosinia exoleta* and *Venerupis senegalensis.* *M. modiolus* can be found part buried within soft sediments or standard grounds or attached to hard substrata, forming clumps or extensive beds. Fast growing populations of ten year old *M. modiolus* have been recorded attached to platform jackets in the North Sea (Holt *et.al.* 1998) and have all been recorded in the immediate vicinity of production platforms. *C. varia* lives sublittorally to depths of about 100m, either free living or attached by a byssus usually on rocky substrata. *D. exoleta* and *V. senegalensis* both live buried within the top ten centimetres of sand gravel and mud bottoms.

All four bivalve species, for the laboratory experiments, were collected from the west coast of Scotland. *V. senegalensis* and *D. exoleta* were collected from the Cregan Narrows in Loch Creran, 56°32.839'N and 5°17.260'W. *V. senegalensis* were also collected from the low shore in South Shian, Loch Creran, 56°31'273'N and 5°24'006'W. *M. modiolus* was collected from either Loch Linne, 56°33.840'N and 5°24.825'W, or near North Ballachulish, Loch Leven, 56°41.269N and 5°10.216'W.

C. varia was collected from Loch Creran or near North Ballachulish, Loch Leven, 56°41.269'N and 5°10.216'W. After collection they were taken back to the aquarium and left to acclimatize. The water was constantly aerated and the bivalves were fed the cultured algae, *Tetraselmis chui*, daily. The seawater was changed at regular intervals.

2.2.2. Development of Test Rig

In order to determine the effects of chronic low-level inputs of particulates such as barite, it was necessary to devise some means of administering controlled amounts of suspended material over extended periods of time.

Barite is extremely dense and is difficult to keep in suspension, so a re-circulation system, figure 2.3, needed to be developed in which carefully metered amounts of barite could be introduced into the water flow and then distributed evenly over a test tank. This was done by conducting a series of trials in which the properties of barite were determined in various metering configurations.

Figure 2.3: Recirculation System and Barite Dosing Rig

2.2.2.1. Barite Dosing Rig

A rig was constructed in which controlled amounts of barite could be introduced into a treatment tank. A schematic diagram (Figure 2.4) shows the design of the dosing rig, which provided a constant supply of filtered seawater and allowed an easy and reliable system of dosing of bivalves with different components of drilling muds. The flow through system design consisted of a test tank, settlement tank, sump, filter and pump.

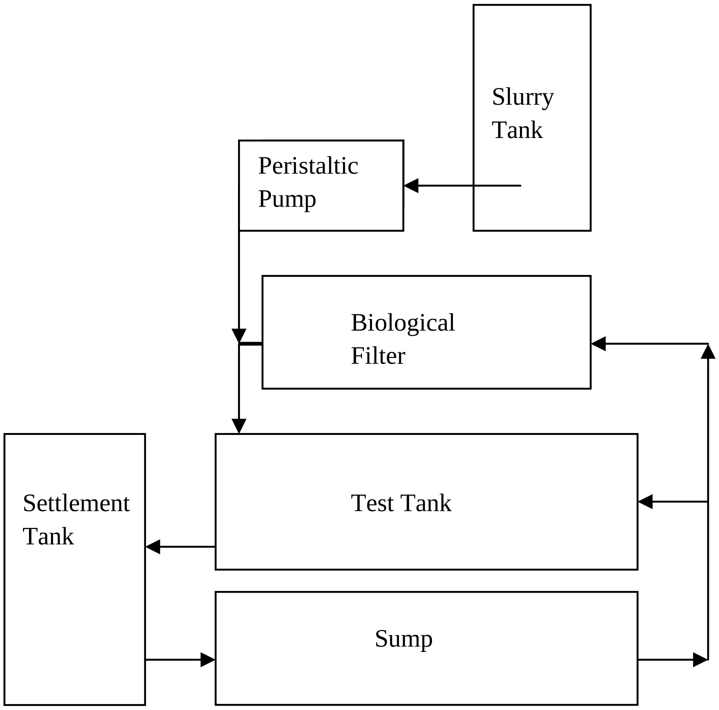


Figure 2.4: Schematic diagram of experimental exposure set-up

- **Flow-through System**

The main test tank (figure 2.5) was made from pvc plastic, was 100 x 100 x 50cm^2, and provided suitable living conditions for the bivalve species. Fresh seawater was introduced into the tank by a spray system placed below water level. The seawater from the test tank overflowed into a settlement tank, which allowed the dosing material to settle out and prevented it from continuing on into the rest of the recirculation system. The seawater from the settlement tank flowed into a sump. The sump acted as a water storage tank. Attached to the sump was a pump that pushed seawater around the whole system. The pump drove seawater up through two biological filters and back into the test tank. The whole system was plumbed using upvc plastic pipe work.

Figure 2.5: Test tank containing barite

- **Dosing System**

A dosing system was attached above the flow-through system. A barite slurry consisting of 50L of water to 1250g of dry barite was mixed together in a header tank. To minimise settlement, the barite was kept in suspension using a EUROSTAR power B stirrer set at 700 rpm. A Watson Marlow 520U peristaltic pump transferred a known amount of barite slurry into the exposure system by means of Watson Marlow 6.4 mm-internal diameter Marprene tubing. This was used for all experiments. The Marprene tubing was attached to Teflon tubing, which delivered the barite to the test tank water inlet. Teflon tubing has a non-stick surface to allow substances to pass through the tubing freely. The small bore size, internal diameter of 2mm and an external diameter of 4mm, allowed a high-pressure transfer of the barite slurry to prevent settlement and any sticking to the tubing. The barite slurry was then introduced into the system at a rate of 30 ml/min, to provide an even spread of a 2mm covering on the tank floor over a 24-hour period. Over three separate 24-hour periods sediment traps were placed in the tank. This determined that the distribution of barite was as predicted.

2.2.2.2. *Trial Experiments*

To ensure the whole system worked correctly and that the barite could be introduced in the levels required a number of trial experiments were conducted. To determine if the flow through system could cope with running 24 hours/day, seven days a week it was turned on and left to run for a week. After that week the system was found to be still running sufficiently. Sediment traps were again placed in the tank and left over three separate 24-hour periods to ensure a correct level of barite distribution.

Preliminary trials compared the survival of the cockle *Cerastoderma edule* and mussel *Mytilus edulis* kept in the test tank and static holding tanks. The preliminary trails were conducted using *C. edule* and *M. edulis* because they are robust intertidal animals and were easily accessible in large number. The trials indicated that whilst the bivalves could survive indefinitely in static tanks, they would not survive more than a few days in the experimental system, even without the administration of barite. This was a major setback as the intention of this experimental programme was to use sub-littoral species which are generally more sensitive to rapid fluctuations in environmental conditions than the intertidal test bivalves.

To determine the cause of bivalves' deaths, all possible stress factors were considered and steps were taken to identify and minimise them as far as practicable. Stress factors that were common between the test tank and the holding tanks (light, temperature and salinity) could be eliminated. Other possible factors were noise (from the spray system over the test tank) and vibration (from the re-circulation and filter system). After a considerable amount of trial and error the problem was solved by: lowering the spray system below the test tank water level to reduce surface noise; insulating the mountings of the main circulation pump to reduce vibration; insulating the pipe-work from the scaffolding structure of the test rig and finally; physically separating the test tank mounting from the rest of the test rig. Survival of the test bivalves, *C. edule* and *M. edulis,* was not affected following the corrections to the barite dosing rig.

2.2.3. Algal Readings

The main purpose of the exposure experiments was to determine the effect of barite on the filtration rates of a range of bivalve species that are commonly found in the vicinity of offshore drilling operations in the North Sea. A method had to be devised to calculate the filtration rates of the four experimental bivalve species used. The bivalves were placed in a known concentration of algae on a daily basis and the difference between the initial and final cell density was calculated.

The algae used were *Tetraselmis chui*, cultured using f/2 media (Stein, 1973). The algal cell density was routinely determined using a Malvern Mastersizer 2000 laser particle counter. An optical density approach was used to determine the cell density, in which alteration of the laser beam intensity (obscuration) was used as the detector. The Mastersizer 2000 is designed to measure the distribution of different sizes of particles within a sample. The optical unit captures the actual scattering pattern from the field of particles (Malvern, 1999). The obscuration measures the amount of laser light lost due to the introduction of the sample within the analyser beam, so the more algal cells present in suspension the greater the obscuration.

The Mastersizer 2000 settings were kept constant for each reading taken and for each experiment. The dispersant was set on water with a refractive index of 1.33. The Mastersizer pump speed was kept at 2500 rpm, the ultrasonic displacement was set at 10.00 and the light energy was kept below 300 units. Each algal sample was exposed to ultrasonic displacement for exactly one minute to ensure all particles were dispersed.

The initial concentration of algal cells was determined by direct counts using a modified-Fuchs Rosenthal haemocytometer. The haemocytometer consists of an etched square of 1.0mm^2 divided into 16 sub-units giving 0.2mm clearance that provides a counting chamber of 0.2mm^3 total volume. A series of algal concentrations were calibrated against the obscuration on the Mastersizer 2000.

Each bivalve was placed in seawater containing a pre-determined number of algal cells daily, and readings of the algal cells were taken an hour and half after the bivalves had begun to filter. After the feeding period, each algal sample was taken to the Mastersizer 2000 for the remaining algal concentration to be determined. It was ensured that there was no contamination of the samples before readings on the Mastersizer 2000. Between each sample, the system was cleaned and seawater was used for a background reading. The measurement data from a particle field could be contaminated by background electrical noise and also by scattering data from dust on the optics and contaminants floating in the 'clean' seawater. Measuring the background between each reading made a measurement of the system with only clean dispersant as well as measurement of the electrical background (Malvern, 1999). The background information was automatically subtracted from the sample measurement i.e. the algae, in order to 'clean' the data. The number of cells removed from suspension was calculated by subtracting the number left from the original inoculum.

2.2.3.1. Validation of Algal Readings

A control experiment was run to determine that the algae were not capable of increasing cell density over 1.5 hours in aquarium conditions. An algal suspension of known concentration was added to two one-litre beakers. One beaker was taken to the Mastersizer 2000 immediately and the algal cell concentration was recorded. The remaining beaker was left in the aquarium with conditions remaining constant. After 1.5 hours the beaker was taken to the Mastersizer 2000 and the algal cell concentration was recorded. The Mastersizer 2000 settings were kept constant, as described above in section 2.2.3. This was repeated over a six day period.

2.2.4. Control Experiments

The main flow-through system did not have the capacity to hold both the control (un-dosed) and test bivalves, so separate smaller control tanks were used. The control experiments were run simultaneously to the barite exposure experiments. The control tanks were 56cm x 36cm x 22cm^2 and were constantly aerated. The water was changed on a daily basis. The control experiments not only allowed an insight into the test bivalves 'natural' filtration rates but also provided observation of seasonal changes within their filtration rates. Salinity, temperature and surrounding sediment movements change seasonally (Ducrotoy *et.al.,* 2000) which influence the filtration activity of bivalves (Rajesh *et.al.*, 2001).

2.2.4.1. Control of Controls

It was important to determine if the bivalves in the main test tank and the smaller control tanks filtered at the same daily levels. A comparison experiment was performed to ensure that the main test rig did not have an effect on the filtration rates of the bivalves and that any effect was caused by the drilling mud component itself. Each individual control and test bivalve was placed in a 1000ml beaker in sea water containing 100,000 cells/ml/1.5hr of *Tetraselmis chui* for an hour and half. Their algal uptake was calculated, as described in section 2.2.3. This was repeated over a period of five days.

2.2.4.2. Sediment Control Experiment

Natural sediment was used to act as a control against the man-made barite particles. Sediment was collected from Torry Bay, Torryburn, Fife. Initially a sample of sediment was oven dried to remove excess water and then placed in a furnace, allowing the calculation of total organic matter within the sediment. Sediment with high organic content could interfere with the filtration results, but the percentage found within the sediment was deemed a suitable level (Table 2.2). The sediment was passed through a 64μm sieve to provide a similar particle size as barite. The sediment particles were then sized using the Mastersizer 2000.

Table 2.2: Organic content of Torryburn Sediment

Sediment Samples	Wet Weight (g)	Oven Dried (60 °c)	Weight Loss (g)	Furnace heated (650 °c)	Weight Loss (g)	% Organic matter
Sample 1	153.487	110.049	43.438	106.059	3.99	2.6
Sample 2	173.26	116.675	56.585	111.369	5.286	3.05
Sample 3	175.21	124.514	50.696	119.836	4.678	2.67

The four different bivalve species were placed in separate cages within the test tank and exposed to a 2mm daily depth equivalent of sediment over a 28-day period. The sediment remained in suspension throughout the test period. Each individual was placed in a separate 1000ml beaker, containing an algal suspension of

100,000cells/ml/1.5hr of algae for an hour and a half each day. The daily uptake of the algal suspension was calculated as described above in section 2.2.3.

2.2.5. Standard Barite Exposure Experiments

The experiment was repeated over three separate 28-day periods to allow a comparison of three different daily barite depth equivalents. As previously mentioned, three separate doses of 0.5mm, 1.0mm and 2.0mm (695ppm, 1390ppm and 2780ppm respectively) of barite were chosen to reflect the approximate levels of barite accumulation that could be expected 100m to 500m from the point of an active discharge (Barlow and Kingston, 2001). The peristaltic pump was set to 7.5 ml/min, 15 ml/min and 30 ml/min for the 0.5mm, 1.0mm and 2.0mm doses respectively. Although these doses are depth equivalents, the barite was continually kept in suspension. The four species were kept in the test tank in four cages and their daily filtration rate was calculated as described in section 2.2.3.

2.2.6. Fine Barite Exposure Experiment

Although standard barite is most commonly used in offshore drilling operations, fine barite can be used as a replacement when deemed suitable. Fine barite is the same material as barite, but is ground down for longer to produce smaller particles. The standard barite particles ranged in size from 0.7µm to 90µm with the highest particle volume being of the size 45µm. The fine barite particles ranged in size from 0.6µm to 63µm, with the largest number of particles found to be 15µm. Fine barite replaced barite at the highest daily depth equivalent of 2.0mm. The daily filtration rates of *Modiolus modiolus*, *Dosinia exoleta*, *Venerupis senegalensis* and *Chlamys varia* were calculated in the same way as the sediment and standard barite experiments.

2.2.7. Pseudofaeces

Bivalve species survive in turbid environments by regulating their ingestion mainly by the production of pseudofaeces (Velasco and Navarro, 2002). It has been reported that bivalves produce pseudofaeces when the surrounding particle concentration reaches a certain threshold (Kiørboe and Møhlenberg, 1981; Jørgensen, 1996), although, because the behaviour of different species varies in regards to particle processing (Hawkins *et.al.,* 1998) and different particles vary with regards to their probability of being rejected as pseudofaeces, the threshold will differ between species (Kiørboe and Møhlenberg, 1981). It was necessary to device a method of calculating the filtration rates of the bivalve species with the possibility of the introduction of unknown quantities of pseudofaeces into suspension.

2.2.7.1. Determination of the Nature of Pseudofaeces

Extra individuals of each of the four bivalve species were placed in the main test tank throughout each of the three barite treatments. They were fed in the same manner as described previously. Instead of taking the algal suspension to the Mastersizer after the filtration period, the pseudofaeces were collected and studied using a Leica MZ7_5 microscope. Photographs were taken using a Leica DC300 camera.

2.2.7.2. Correction for Pseudofaeces Production

When using the Mastersizer 2000 the algal cells in suspension are calculated using a light obscuration. A reduction in the number of algal cells in suspension will result in a reduced obscuration reading. However, the introduction of the dense mineral barite into the algal suspension will increase the obscuration. If the number of algal cells in suspension has reduced due to the bivalves' filtration, the decrease in number of suspended particles may be masked by the introduction of the waste barite resulting in a perceived filtration rate that is reduced. To provide a correction factor to compensate for the production of pseudofaeces, a series of experiments were conducted to estimate the contribution of pseudofaeces to the suspended particulate load during filtration rate determination.

Initially, the four bivalve species were exposed to the highest daily dose (2mm) of barite. All conditions were kept constant as in the previous exposure experiment, with seawater replacing the algal suspension. The seawater was taken to the Mastersizer 2000 and readings were taken based on the obscuration again. On the Mastersizer 2000 all settings were kept constant as before, with a pump speed of 2500 and the ultrasonic displacement at 10.00 and left to run for 60 seconds. As before, normal seawater was used for the background reading. This technique was repeated with sediment, fine barite and standard barite, at the reduced 0.5mm and 1.0mm levels.

2.2.8. Statistical Analysis

A two-way ANOVA (Analysis of Variance) was run to compare the four control bivalve species filtration rates and also to determine if there was a statistical difference between days. To determine which species differed a one-way ANOVA was run followed by a multi-comparison Tukey test. These statistical tests were also repeated on the sediment exposure experiment.

To compare the effects different daily doses of barite had on each individual test bivalve, a one-way ANOVA followed by a multi-comparison Tukey test was performed. It was run on the first and last day that all individuals for each barite level remained alive. This was necessary because of the unpredictable death rates of the bivalves within each barite treatment. It would be invalid to compare treatments that contained individuals of different numbers. Certain days were omitted due to negative values within the raw data. When this occurred, then the next useable (no negative values) days data was used as a replacement. This technique was repeated on the comparison between the different particulate matter experiments at the 2mm levels.

2.3. Results

2.3.1. *Validation of Algal Readings*

Figure 2.6 shows that the algal cell density did not differ between 0 and 1.5 hours in aquarium conditions. This illustrates that any change in the number of cells in suspension was therefore down to the bivalves themselves rather than by some unidentified artefact.

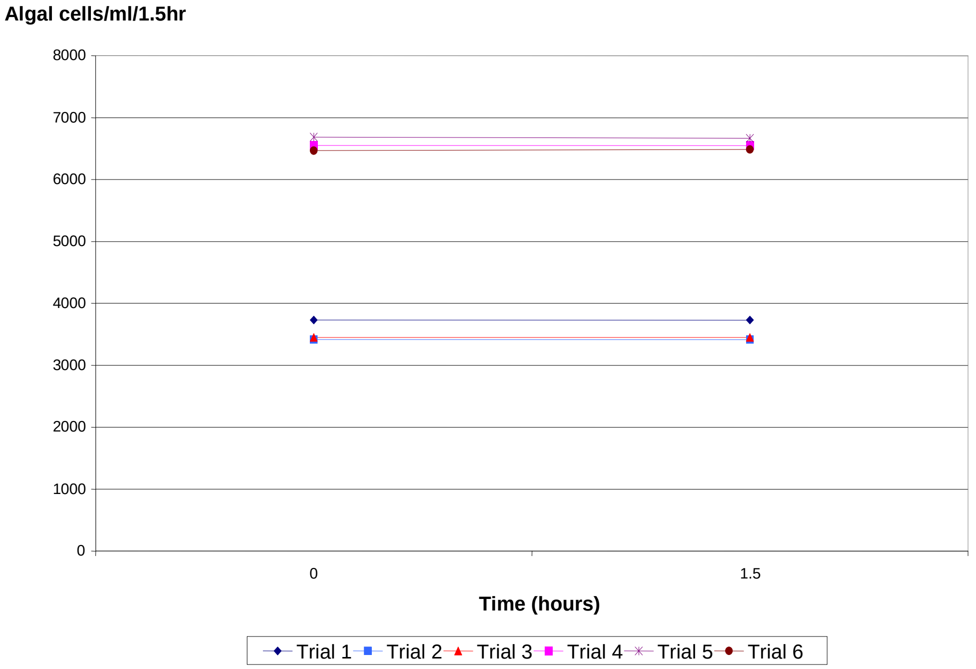


Figure 2.6: Cell density of algal suspension at 0 and 1.5 hours in aquarium conditions.

2.3.2. Preliminary experiments

2.3.2.1. Comparison of Controls

Figure 2.7 shows the mean filtration rates of *Modiolus modiolus*, *Dosinia exoleta*, *Venerupis senegalensis* and *Chlamys varia* over a 28-day period. All four bivalve species followed the same filtration pattern throughout the entire test period. At the beginning of the experiment there were fluctuations within the filtration of the algal suspension, on day four rising to roughly 5000 cells/ml/1.5hr and on day seven rising to about 11, 000 cells/ml/1.5hr. The peak on day seven could relate to a spring tide. There is a smaller peak around 14 days later indicating another spring tide. Bivalves retain their endogenous rhythm after being removed from natural conditions.

After day seven the filtration rates of all the bivalve species showed a steady pattern only peaking slightly around day 22. Running a two-way ANOVA, it was found that the feeding rates differed between days (p-value 0.000) and between species (p-value 0.000). A one-way ANOVA showed that there was a significant difference between species on 16 out of the 28 days. The main difference was found between *C. varia* and *D. exoleta*, significantly differing in their filtration rates from both *V. senegalensis* and *M. modiolus.*

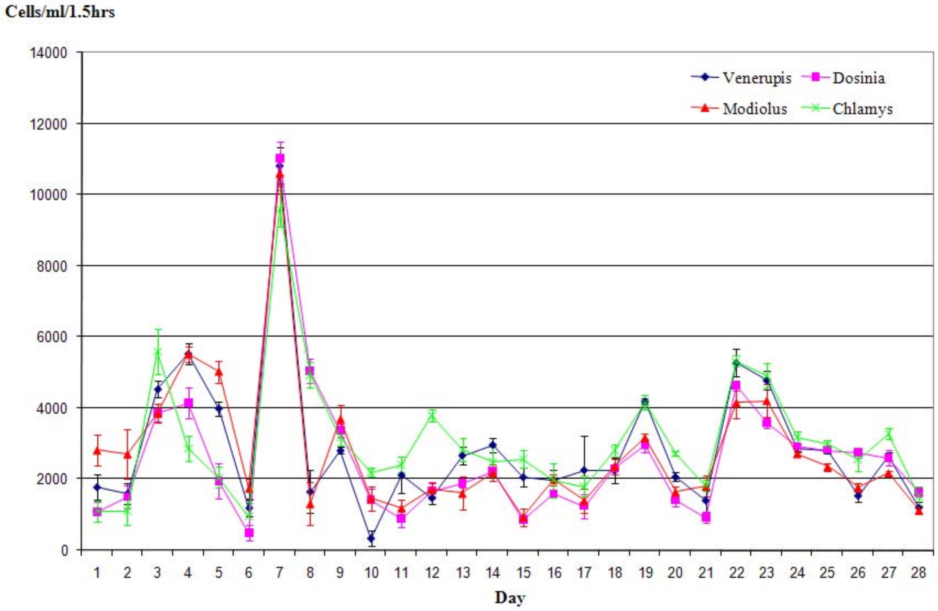


Figure 2.7: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by four species of suspension feeding bivalves (n=5) kept under control conditions.

Figure 2.8 shows the mean filtration rates of *Modiolus modiolus*, *Chlamys varia*, *Dosinia exoleta* and *Venerupis senegalensis*. The four bivalve species followed a similar filtration pattern throughout the experimental period. These control bivalves, Figure 2.8, were run at a separate time of the year from the control bivalves shown in Figure 2.7. A two-way ANOVA showed that a significant difference lay between both species and days. The one-way ANOVA showed that a significant difference was present between species on 16 out of the 28 days. Overall, *C. varia* and *M. modiolus* usually significantly differed in regards to their filtration rates, as did *D. exoleta* and *V. senegalensis*. The statistical difference between the four species will probably be related to size and gill structure. The four species differ in size, with *Modiolus modiolus* being the largest and *Chlamys varia* the smallest. The results displayed in Figures 2.7 and 2.8 allow a comparison into seasonal variations within a bivalve's filtration rate. The results in Figure 2.7 were produced in the months September to October and the results in Figure 2.8 were produced in the months of June to July.

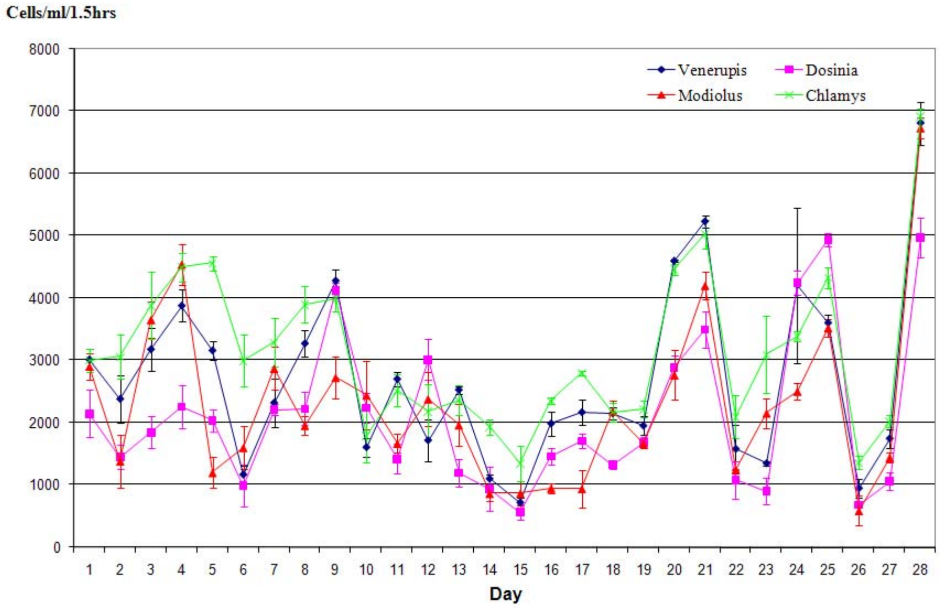


Figure 2.8: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by four species of suspension feeding bivalves (n=5) kept under control conditions.

2.3.2.2. Control of Controls

The filtration rate of *Venerupis senegalensis*, in both the control and test tanks can be seen below in Figure 2.9. The filtration rates of *V. senegalensis*, in both tanks, followed the same pattern through all five days. A one-way ANOVA followed by a multicomparison Tukey test was performed on each day and found that the only significant difference was on day two (p-value 0.013). The remaining days, 1, 3, 4 and 5 had no significant difference with p-values of 0.235, 0.110, 0.348 and 0.95 respectively. Different p-values were produced on each day due to the filtration rates changing on a daily basis. The filtration rates of *Mytilus edulis*, *Dosinia exoleta* and *Chlamys varia* can be seen below in figures 2.10, 2.11 and 2.12 respectively.

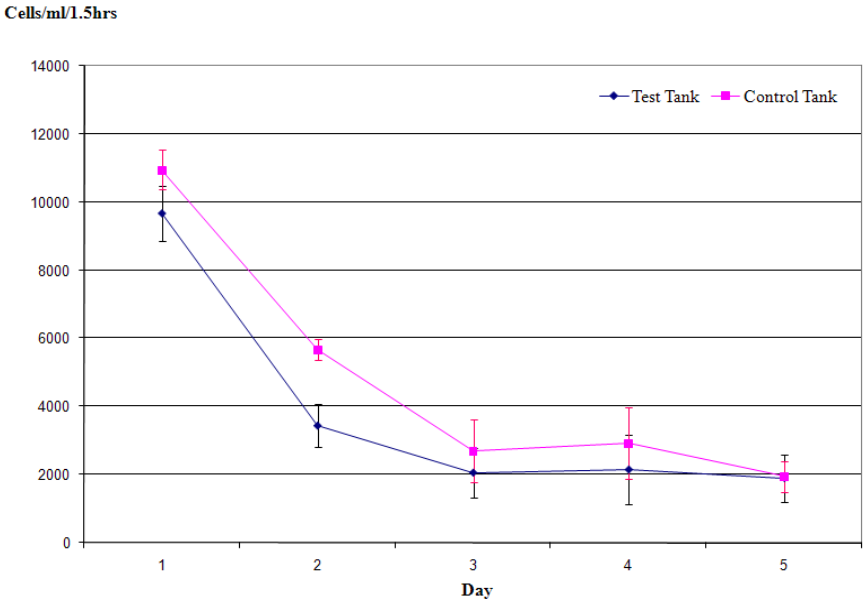


Figure 2.9: Comparison of filtration rates as indicated by mean number of algal cells removed after 1.5hrs by *Venerupis senegalensis* (n=5) kept in the test tank and the control tank.

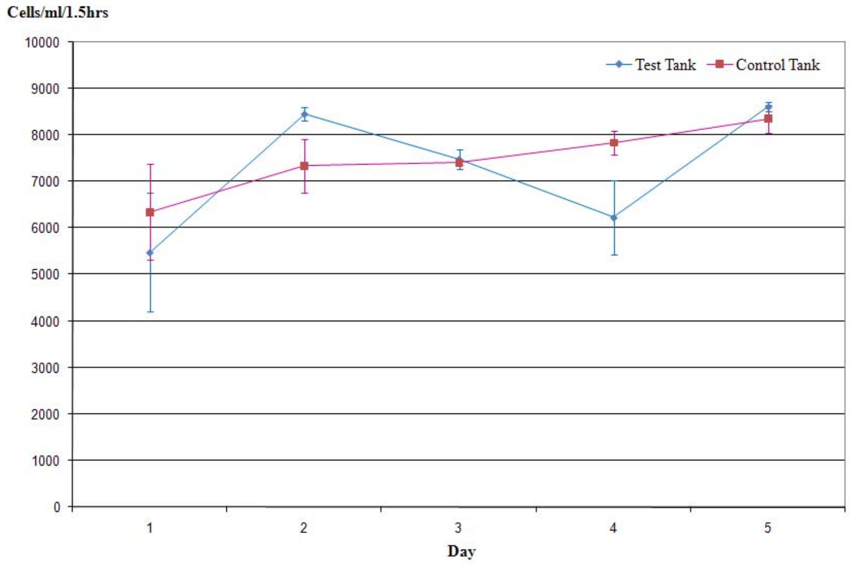


Figure 2.10: Comparison of filtration rates as indicated by mean number of algal cells removed after 1.5hrs by *Modiolus modiolus* (n=5) kept in the test tank and the control tank.

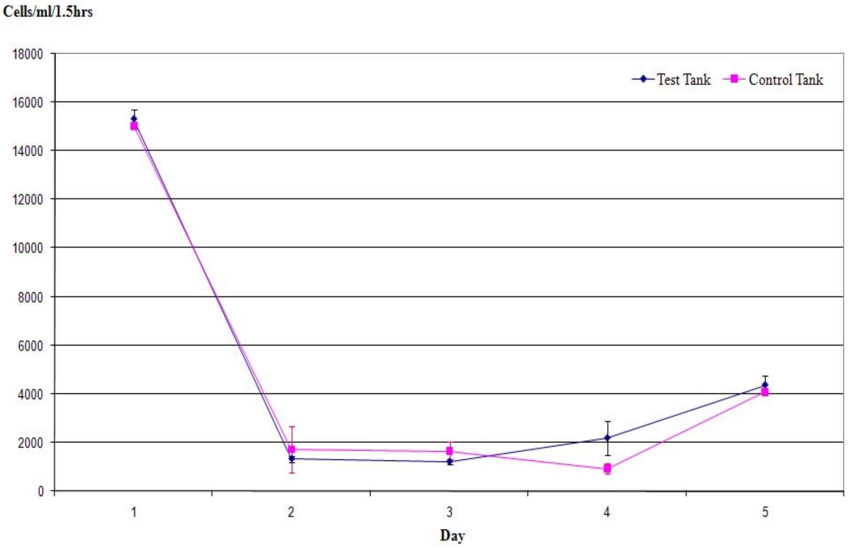


Figure 2.11: Comparison of filtration rates as indicated by mean number of algal cells removed after 1.5hrs by *Dosinia exoleta* (n=5) kept in the test tank and the control tank.

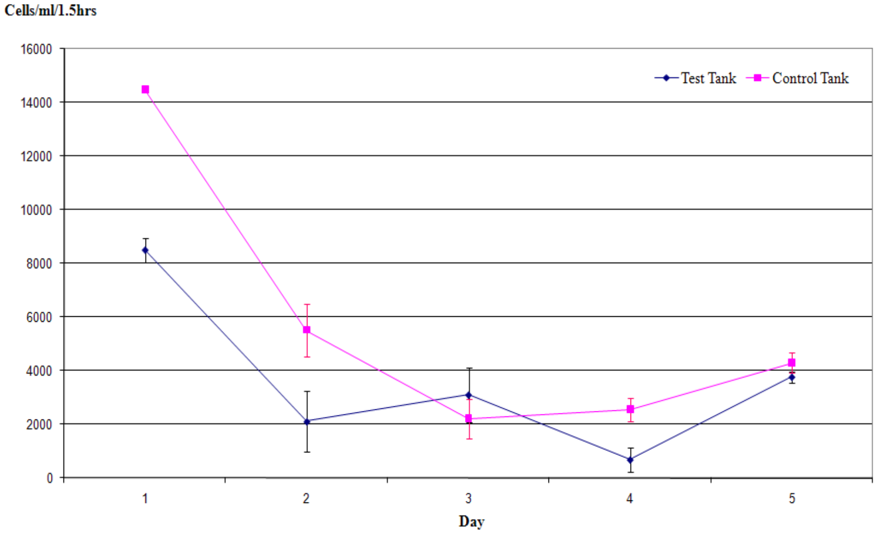


Figure 2.12: Comparison of filtration rates as indicated by mean number of algal cells removed after 1.5hrs by *Chlamys varia* (n=5) kept in the test tank and the control tank.

2.3.2.3. Sediment Control Experiment

Figure 2.13 shows the mean filtration rates, over 28 days, of the four experimental suspension feeding bivalves, when exposed to a daily 2mm depth equivalent of natural sediment. All four species followed a similar filtration pattern over the whole test period. There was a steady decrease in the uptake of the algae, from 10,000cells/ml/1.5hr, on day one, to between 2000 and 4000cells/ml/1.5hr on day seven. This was probably a response to the initial introduction of sediment into the test tank. After the initial decrease the filtration rates became constant indicating that the four bivalve species had become acclimatized to the sediment and had adapted to the influx of the particulate matter. All individuals survived the full 28-day test period.

Running a two-way ANOVA showed a significant difference between both days and species. Six days had to be omitted from the statistical test due to the raw data containing negative readings, caused by a high pseudofaecal production. A one-way ANOVA followed by a multicomparison Tukey test showed that 21 days out of the 22 (day one) showed a significant difference between the filtration rate of the suspension feeding bivalves. A p-value of 0.000 was produced on 19 of those days. Within the first 10 days of sediment exposure, there was continued change to which species the difference lay between. After day 10, the difference mainly lies between both *C. varia* and *D. exoleta* significantly filtering at different levels to both *V. senegalensis* and *M. modiolus.* The difference found between the filtration rates of the four species cannot be related to size. *Venerupis senegalensis* and *Dosinia exoleta* are very close in size but filtered at different levels with about 3000cells/ml/1.5hr difference on a daily basis.

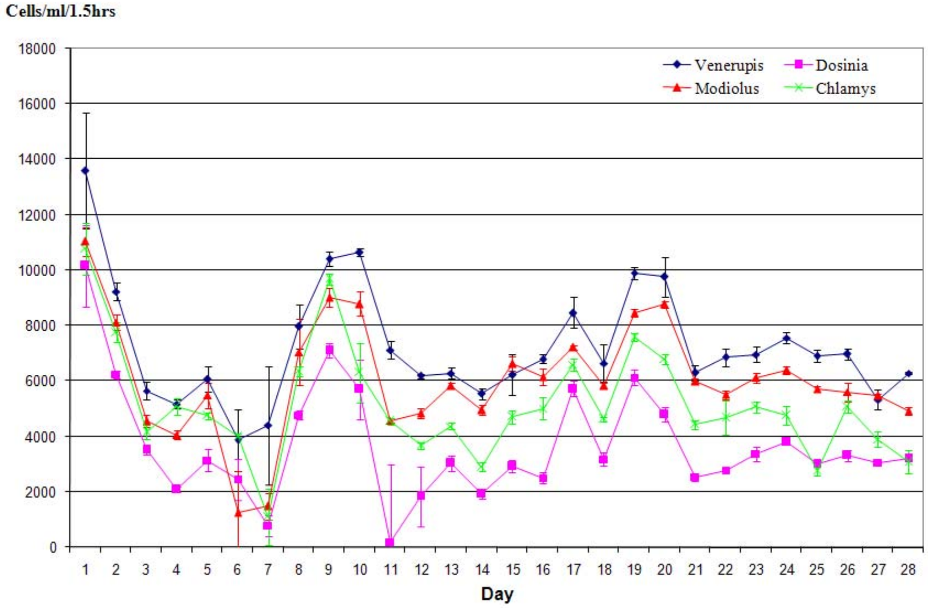


Figure 2.13: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by four species of suspension feeding bivalves (n=5) in the presence of suspended natural sediment.

2.3.2.4. Initial Barite Exposure Results

A comparison was made between the mean filtration rates of each species when exposed to the three different daily barite depth equivalents of 0.5mm, 1.0mm and 2.0mm. All four species reacted differently to each barite level. Figure 2.14 shows the results obtained for *Modiolus modiolus.* When exposed to the lower levels, 0.5mm and 1.0mm barite, the filtration rate of *M. modiolus* followed a similar pattern, with a large decrease on day five to approximately -8500 cells/ml/1.5hr. When exposed to the highest daily barite level of 2.0mm, the filtration rate varied largely within the first eight days before settling down. None of the barite exposed individuals survived the full test period. The controls survived the full duration of the experiment. Similar results were found for *Dosinia exoleta* and *Venerupis senegalensis*, seen below in Figures 2.15 and

2.16 respectively, with filtration rates going into the negative. However, it is not feasible to have a negative filtration rate.

The bivalves were continuously exposed to barite, with the exception of the filtration period when they were removed from the test tank and placed into beakers containing an algae suspension. The initial results showed negative values on certain days, suggesting that barite particles may have been introduced to the seawater, either on the surface of the test animals or via the introduction of pseudofaeces. It was ensured that the animal's surface was washed clean from barite so therefore the introduction must be through pseudofaeces (see Section 2.2.7).

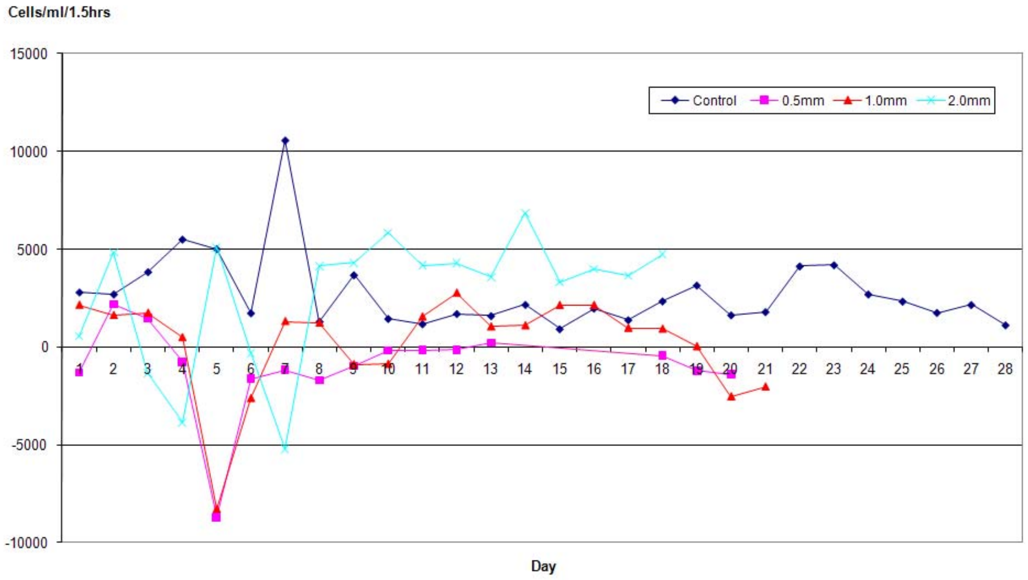


Figure 2.14: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Modiolus modiolus* (n=5) in the presence of different barite levels.

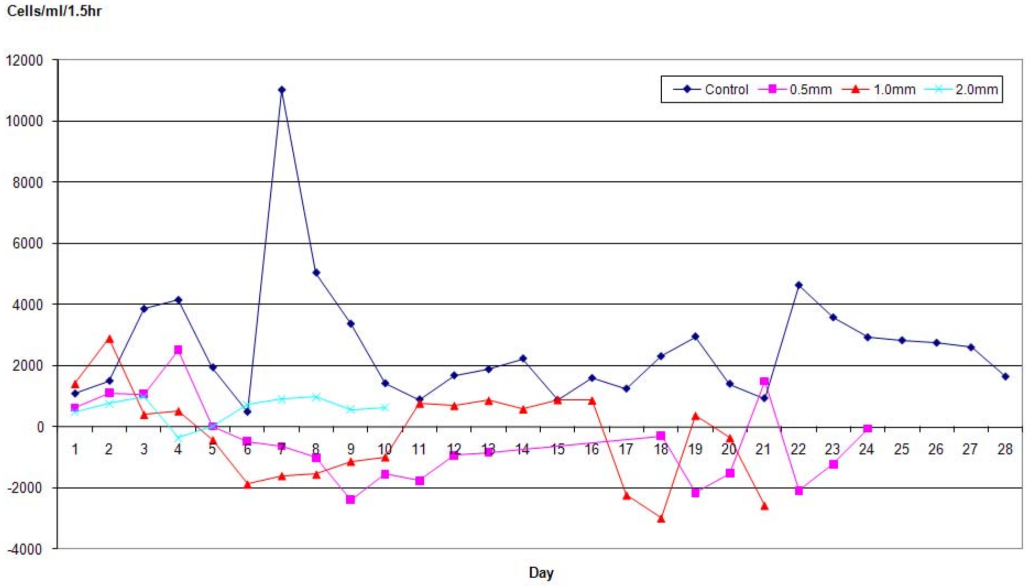


Figure 2.15: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Dosinia exoleta* (n=5) in the presence of different barite levels.

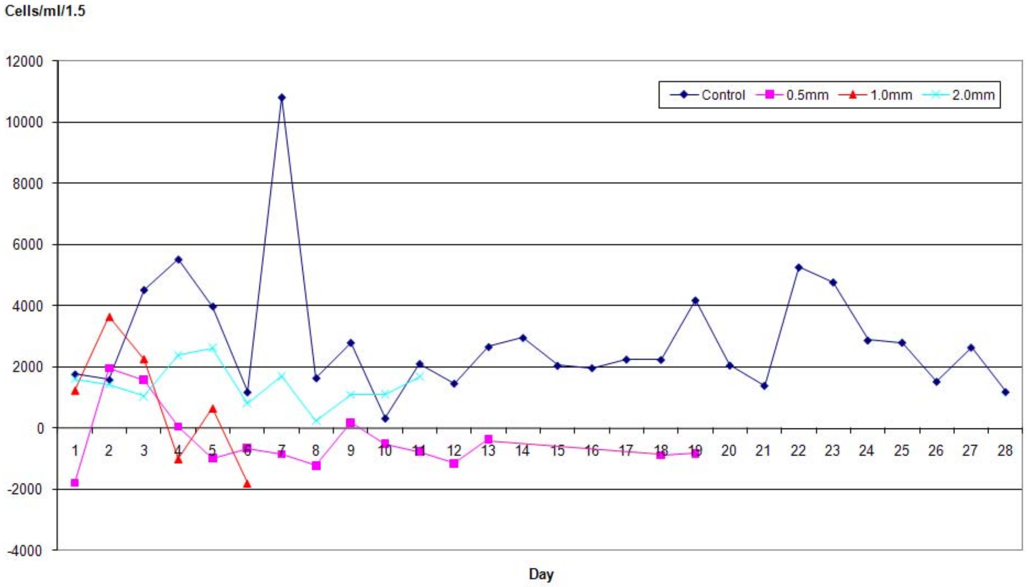
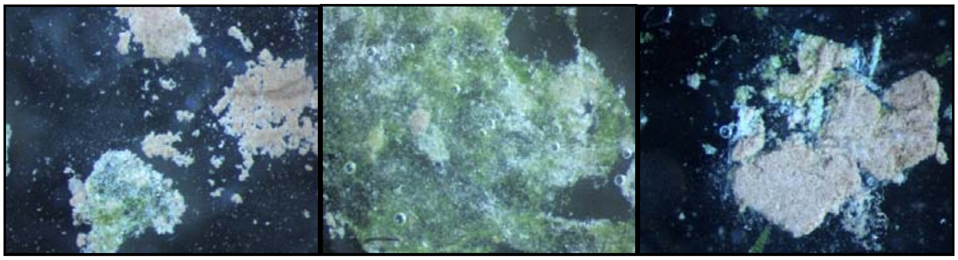


Figure 2.16: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Venerupis senegalensis* (n=5) in the presence of different barite levels.

2.3.2.5. Pseudofaeces Production

The results in section 2.3.2.4 showed the filtration rates of four bivalve species that were constantly exposed to barite. Negative readings were recorded although it is not feasible to gain a negative filtration rate. The filtration rate, as mentioned earlier, was calculated from the difference between the initial algal cell concentration and the remaining cell suspension after filtration. If no filtration was occurring then a result of zero should be recorded. The blank controls, shown in section 2.3.1, showed that the algal cell density did not change over the 1.5 hours in aquarium conditions. Therefore, the negative readings were an indication that something (probably pseudofaeces) had been added into the suspension and interfered with the initial results, thus not showing the actual filtration rates of the bivalves.

To prevent the introduction of additional particles, the bivalves were rinsed in clean seawater to ensure that no barite was present on their external surfaces prior to addition of the algal suspension. However, this could not prevent the introduction of pseudofaeces expelled from within the bivalve’s mantle cavity. Pseudofaeces collected and examined were found to contain a mixture of algal cells and barite particles. The algae are the green substance in figures 2.17a-c with the barite the brownish substance. This suggests that, under the test conditions, the bivalves were unable to separate the organic algal cells from the inorganic barite particles, and so expelled them together.



Figures 2.17a-c: *Modiolus modiolus* Pseudofaeces

2.3.2.6. Correction for Pseudofaeces Production

The average number of particles derived from pseudofaeces added to the algal suspension values for each species was calculated as previously described in section 2.2.7.2. The results are shown in Table 2.3. It was essential to correct for pseudofaeces production as it is a constant throughout the filtration process acting as a defence mechanism and energy saving process.

Table 2.3: Average number of foreign particles added to suspension by four suspension feeding bivalve species.

Treatment	Particles/ml added into suspension			
	Modiolus modiolus	*Venerupis senegalensis*	*Dosinia exoleta*	*Chlamys varia*
Sediment	3615	4620	1506	2063
Fine Barite	5772	2461	2228	3999
Barite 0.5 mm	3423	1609	3086	-
Barite 1.0 mm	4928	1836	4076	-
Barite 2.0 mm	3471	4131	1330	787

The results displayed in table 2.3 were subtracted from the original filtration rate counts. This method removed the interference by pseudofaeces on the filtration rates of the four suspension feeding bivalves. This allowed a corrected filtration rate for each species to be calculated. The amendment is a constant rate, as shown below in figure 2.18, because the mean number of particles added into suspension was used as the correction factor. It was not suitable to calculate the correction factor for each individual animal (n=5) for each species (n=4) on a daily basis due to time and resource constraints.

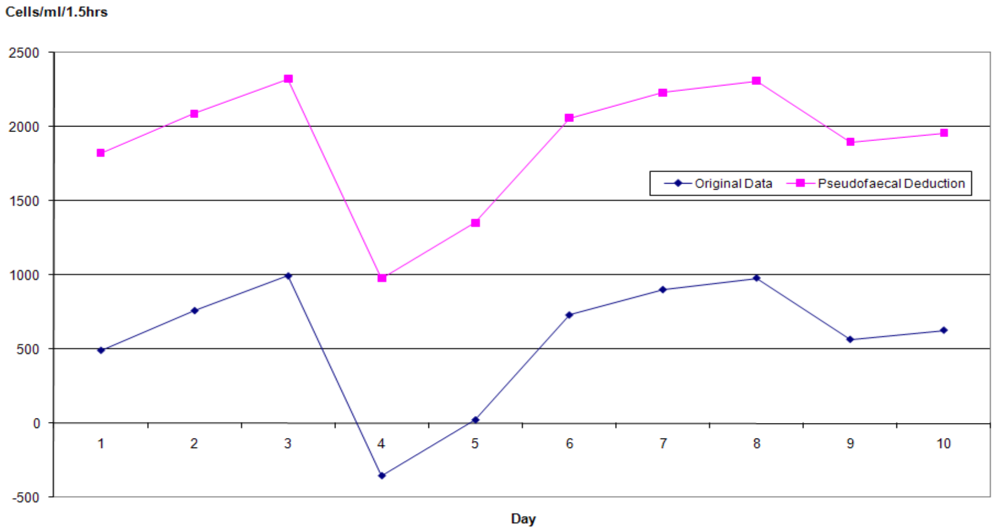


Figure 2.18: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Dosinia exoleta* (n=5) corrected for pseudofaeces production.

2.3.3. Final Barite Exposure Rates

The initial filtration readings were all amended for pseudofaeces production and the corrected results for each species exposed to barite can be seen below. On days that the pseudofaeces production is higher than the average, as shown in section 2.3.2.6, which is causing an interference with the readings and making them negative, the results have been displayed as zero, as it is not possible to gain a negative filtration rate. The filtration rates of each species and their tolerance in the presence of different barite concentrations is compared below. Their filtration rates when in the presence of the different particulate material at the 2.0mm daily depth equivalent is also analysed. Lethal time, LT_{50}, was calculated and compared for all four species. This is shown below in section 2.3.3.5. LC_{50} could not be calculated because of the lack of repetition for each barite exposure level.

2.3.3.1. Dosinia exoleta

Standard barite had an effect on the filtration and survival rates of *Dosinia exoleta*, see Figure 2.19. When exposed to the lowest daily levels of barite (0.5mm) there was a gentle increase in the filtration rate within the first few days followed by quite a sharp decline, from 5602 cells/ml/1.5hr remaining on day four to 701 cells/ml/1.5hr remaining on day nine. After day nine, there was a steady increase until after day 18, where there were fluctuations until day 23. When a daily rate of 1.0mm of barite was added to the experimental system, the uptake of algae by *Dosinia exoleta* showed a similar pattern to the 0.5 mm daily barite level. Figure 2.19 illustrates a large decrease in the filtration of the algal cells removed from suspension, from 6978 cells/ml/1.5hr/ on day two to 2204 cells/ml/1.5hr on day six. After day six, there was a steady incline within the filtration rate until it stabilised over days 11 to 16. After day 16, there were fluctuations within the filtration rate of *D. exoleta* until there was no more survival after day 21.

There was the least fluctuation within the filtration rates of the *Dosinia exoleta* that were present within the 2.0mm daily levels of barite. The volume of algal cells filtered on day one was 1822 cells/ml/1.5hr with 1958 cells/ml/1.5hr being filtered on day ten, the last day of survival. Throughout the experimental period, for this daily barite dose, there was only an obvious change on day four, with the removal of algal cells from suspension decreasing to 977 cells/ml/1.5hr. A one-way ANOVA run on the first and last days that all individuals remained alive, (see figure 2.20 below for survival rates) within each barite exposure level, found a significant difference (p-value of 0.000) between the filtration rates of the *D. exoleta* living in the presence of different levels of barite. Each day the treatments changed that significant difference was found between.

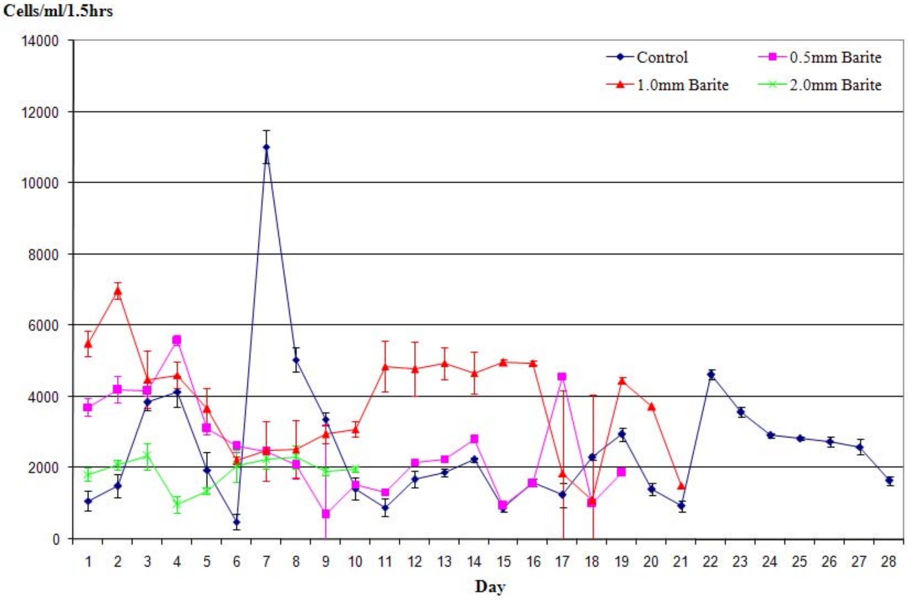


Figure 2.19: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Dosinia exoleta* in the presence of different barite levels.

Figure 2.20 shows that the survival of the control, sediment and fine barite treated *Dosinia exoleta* was not compromised throughout the test period. No *Dosinia exoleta* survived in the presence of standard barite for the full test period, surviving for 24 days, 22 days and 11 days when exposed to the daily barite levels of 0.5mm, 1.0mm and 2.0mm respectively. Although the 1.0mm treated *D. exoleta* died off sooner, (on day 22) than the 0.5mm standard barite dose, there is an indication that the lower dose had a more detrimental effect. The 0.5mm standard barite dose began to effect survival on day five unlike the higher 1.0mm dose which did not compromise survival until day 11. The lower 0.5mm dose continued to affect survival before the higher 1.0mm dose until day 20. The 2mm dose of standard barite had the worst effect on the survival rates where *D. exoleta* began to die off on day four. After day four there was an 80% survival rate until none were left alive on day 11.

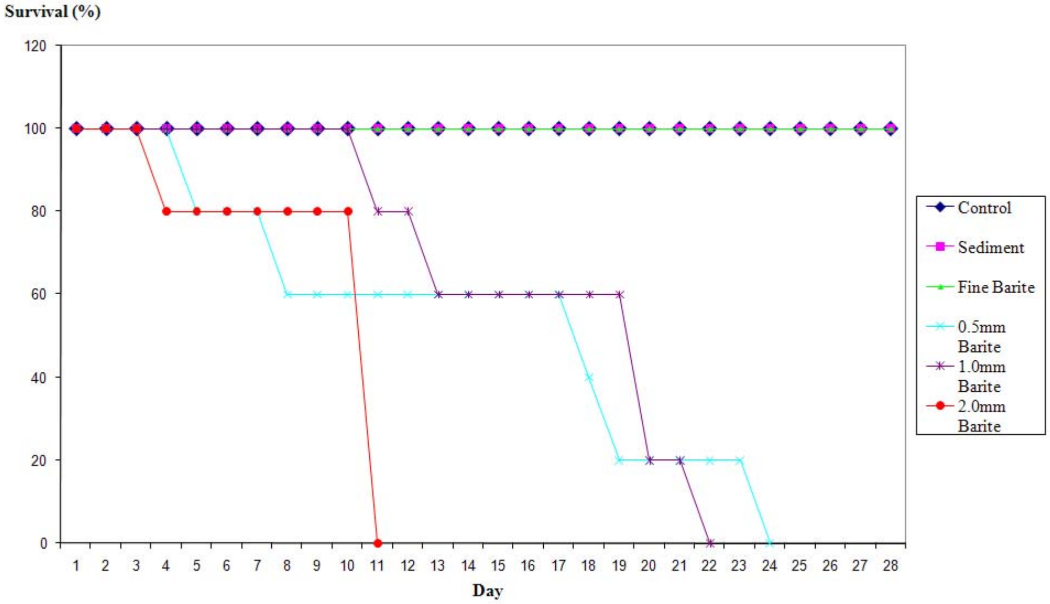


Figure 2.20: Survival rates of *Dosinia exoleta* in the presence of different particulate material.

Figure 2.21 shows the mean number of algal cells removed from suspension by *Dosinia exoleta* when living in the presence of a 2mm daily depth equivalent of different material. The control, sediment and fine barite treated *D. exoleta* all filtered the algae suspension through out the 28-day test period and had a 100% survival rate, as shown in Figure 2.20. The filtration of these three treatments fluctuated throughout the whole test period. The *D. exoleta* living in the presence of standard barite had quite a steady filtration pattern but survival was affected. Figure 2.20 shows that the *D. exoleta* exposed to the standard barite started dying off on day four and none survived past day eleven. The one-way ANOVA found a significant difference (p-value 0.000) between the different treatments with regard to *D. exoleta's* filtration rate.

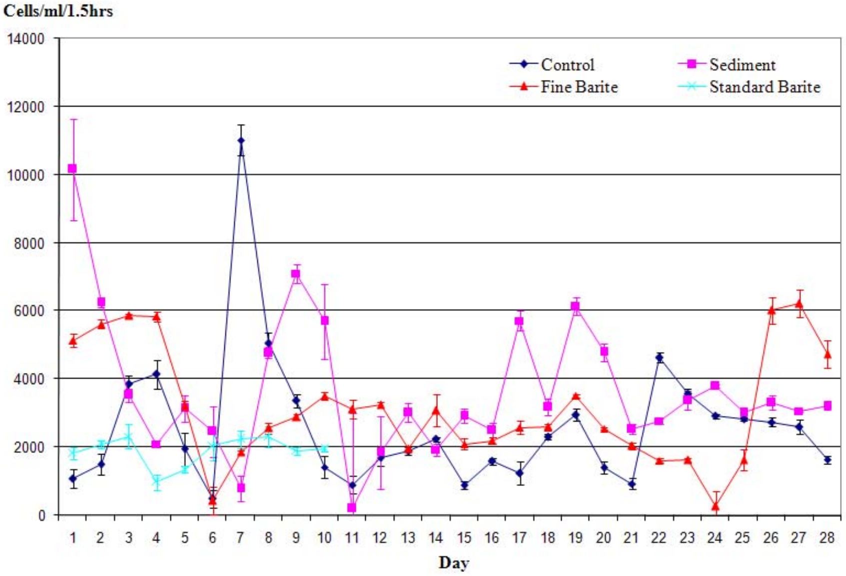


Figure 2.21: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Dosinia exoleta* in the presence of a 2mm daily depth equivalent of particulate material.

2.3.3.2. Venerupis senegalensis

Standard barite altered the filtration rates of *Venerupis senegalensis* and had a detrimental effect on their survival rates. A one-way ANOVA was performed on the first and last day that all individuals were still living, although day one had to be omitted due to a negative value interfering within the raw data, so day two was used as the first day. A p-value of 0.000 was produced on all days of comparison showing a definite statistical difference between treatments. The barite treatment that the significant difference was found to lie between varies daily.

Figure 2.22 shows there is a clear difference between the filtration rates of *V. senegalensis* when exposed to the highest daily dose (2.0mm) in comparison to the controls and other two barite levels. *V. senegalensis* lived for the longest period when exposed to the lowest dose of barite, 0.5mm. After an initial increase of the filtration of the algal suspension there was a general decrease from day two to day eight. After day eight there was slight fluctuation within the filtration readings with a steady decrease towards the end of survival, on day 19.

The *V. senegalensis* exposed to the 1.0mm barite level lived for the shortest period of time, only surviving for six days. This barite level caused large fluctuations within a short survival period. From days two to four there was a filtration decrease of 4641 cells/ml/1.5hr. There was another sharp decrease from day five to day six of roughly 2000 cells/ml/1.5hr. The first day that *V. senegalensis* had a daily level of 2.0mm added into the experimental tank, the average number of algal cells removed from suspension was 5742 cells/ml/1.5hr. On day 11, the last day, the filtration of the algae did not change much from the beginning with a reading of 5814 cells/ml/1.5hr being removed from suspension. Small fluctuations occurred within the filtration rate from day one to the last day on day 11.

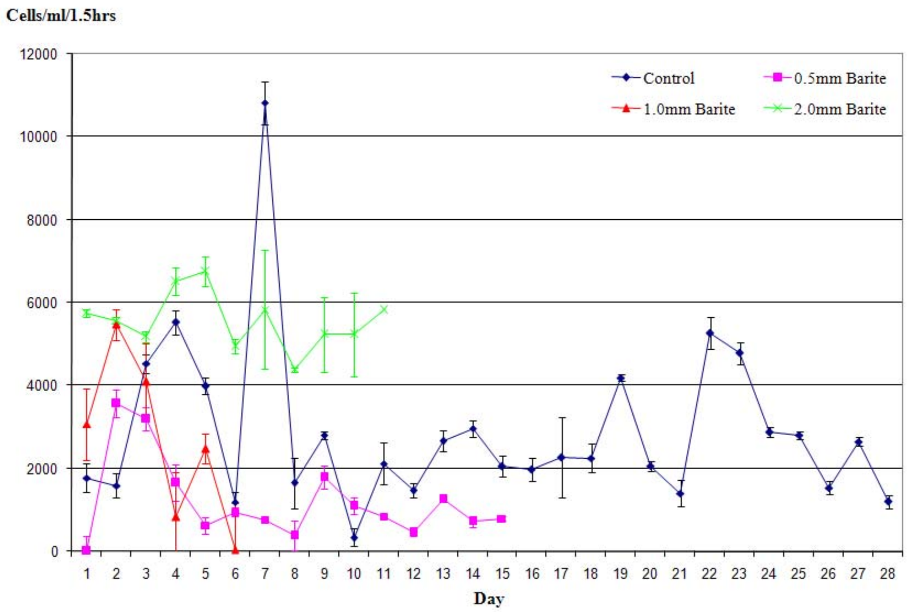


Figure 2.22: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Venerupis senegalensis* in the presence of different barite levels.

Figure 2.23 shows the survival rates of *V. senegalensis* in the presence of different particulate material. No *V. senegalensis* survived for the full test period when exposed to standard barite with survival periods of 20 days, 7 days and 12 days when in the presence of the 0.5mm, 1.0mm and 2.0mm daily depth equivalents respectively. *V. senegalensis* had the lowest tolerance to the 1.0mm daily level of standard barite with survival being compromised on day three. From day three onwards there was constant reduction within the survival of *V. senegalensis* up until none were left alive on day seven. The fine barite and 0.5mm standard barite treatment exposed *V. senegalensis* both started to die off on day five and survival for both remained at 80% until day 11. After day 11 the survival of the fine barite exposed animals dropped down to 60%, where it remained until the experiment period ended. Although survival of the 2.mm standard barite exposed *V. senegalensis* was compromised a day later than the 0.5mm exposed animals, they died off eight days earlier, on day 12 in comparison to day 20.

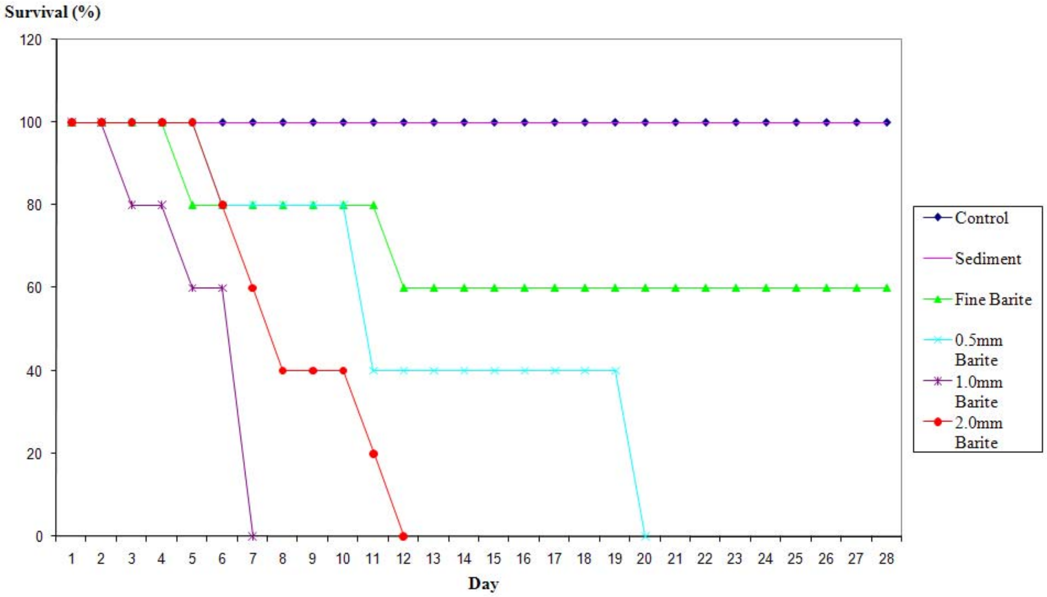


Figure 2.23: Survival rates of *Venerupis senegalensis* in the presence of different particulate material.

Figure 2.24 displays the mean algal cells removed from suspension by *Venerupis senegalensis* that had been living in the presence of a 2mm daily depth equivalent of different particulate material. Filtration of the algal suspension by *V. senegalensis* exposed to standard barite only had minor fluctuations between each day in comparison to the other three treatments, but survival was only for 11 days. The control and sediment exposed *V. senegalensis* both had a 100% survival rate for the full test period as displayed in Figure 2.23. The *V. senegalensis* living in the presence of natural sediment generally had the highest mean filtration rate over the test period, apart from in days 4-7. The filtration of the algal suspension by the fine barite exposed *V. senegalensis* fluctuated considerably, with certain days producing a zero, due to obscuration to the data by high levels of pseudofaeces being produced. Figure 2.23 shows that the *V. senegalensis* living in the presence of fine barite only had a 60% survival rate at the end of the 28-day test period. The one-way ANOVA showed that on all days of comparison a p-value of 0.000 was found indicating a high level of significance between the different treatments. The sediment treated *V. senegalensis* differed more than the other treatments with regards to the filtration of the algal suspension.

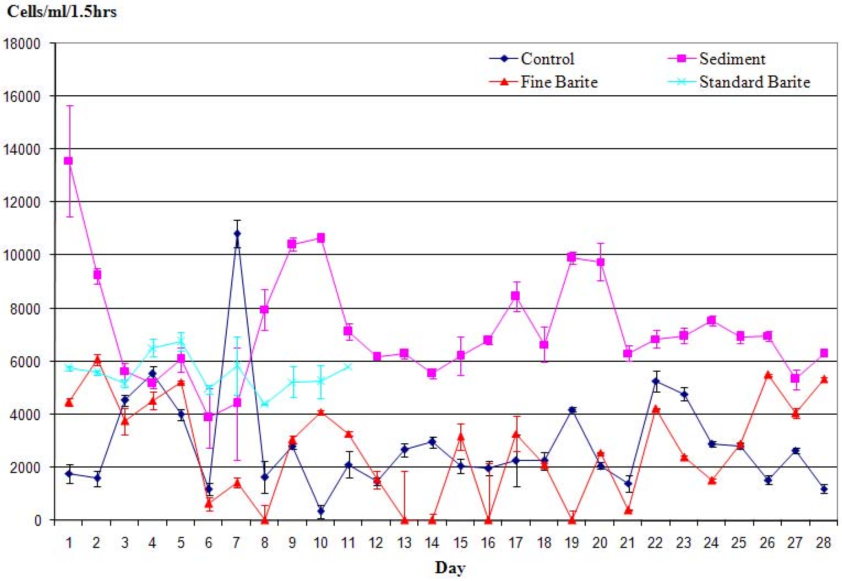


Figure 2.24: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Venerupis senegalensis* in the presence of a 2mm daily depth equivalent of different particulate material.

2.3.3.3. Modiolus modiolus

The filtration rates of *Modiolus modiolus* were affected by the presence of different daily depth equivalents of barite (Figure 2.25). *M. modiolus* showed the largest variations within the filtration rates in the first eight days of the test period. After day eight the barite-exposed bivalves filtered a larger number of algal cells than the controls. The barite 0.5mm daily dose had a survival period of 20 days for *M.*

modiolus. There was a decrease of the filtration from 5614 cells on day two to 1810 cells/ml/1.5hr on day six. After day six there was a gradual increase within the filtration of the algal suspension until day 13. After day 13 their filtration of the algae began to decrease until day 20 where the experiment ended for this dose, due to the death of all the *M. modiolus*.

The 1.0mm barite dose allowed the longest survival period for *M. modiolus* with 21 days. There was a decrease within the filtration within the first six days dropping from 7076 cells/ml/1.5hr on day one to 2307 cells/ml/1.5hr on day six. Between day six and 16 there was a steady fluctuation within the filtration of the algal suspension. After day 16 the filtration decreased until day 21, the last day of survival. The largest barite dose, 2.0mm, produced large fluctuations, within the first few experimental days. After day eight the fluctuations within the filtration rate were smaller but still existed until day 18, when the *M. modiolus* died off. Whilst running a one-way ANOVA followed by a Tukey test, a number of days within the initial stages of exposure had to be omitted due to negative values within the raw data. The results from the ANOVA (p-values of 0.000) showed a significant difference between the filtration rates of the barite exposed *M. modiolus*. No constant barite level was responsible for the differences found.

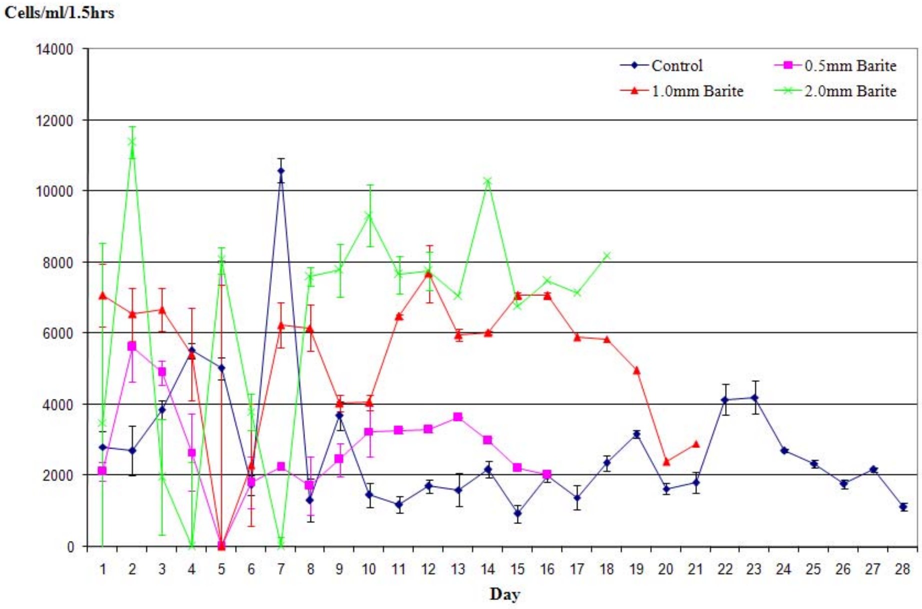


Figure 2.25: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Modiolus modiolus* in the presence of different barite levels.

Figure 2.26 displays the survival rates of *M. modiolus* that had been living in the presence of different particulate matter. The control, sediment and fine barite exposed *M. modiolus* all had a 100% survival rate. *M. modiolus* survived for 21 days, 22 days and 19 days when living in the presence of the daily depth levels of 0.5mm, 1.0mm and 2.0mm of standard barite.

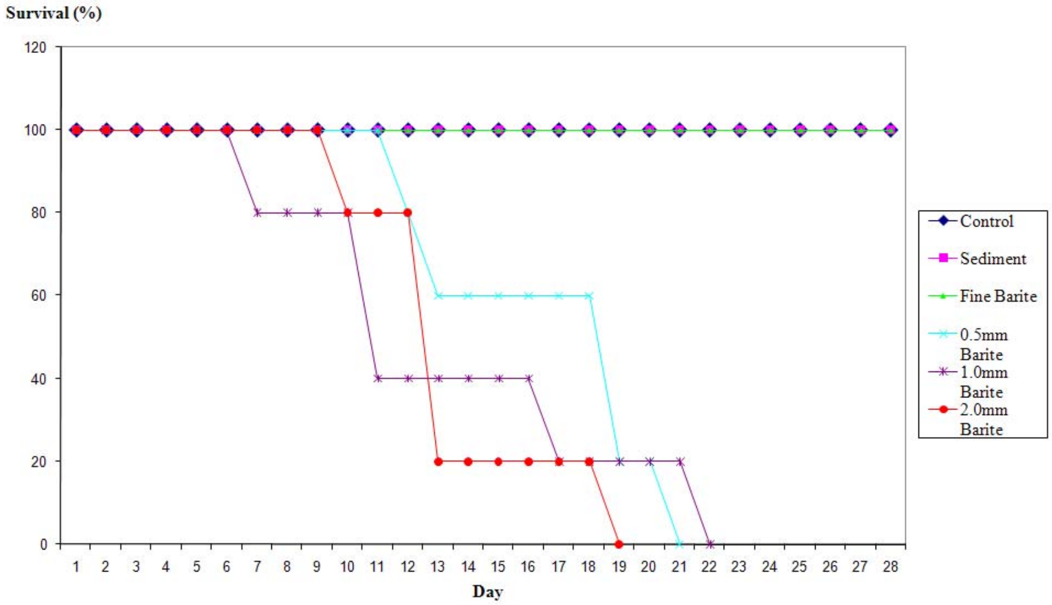


Figure 2.26: Survival rates of *Modiolus modiolus* in the presence of different particulate material.

Figure 2.27 displays the survival rates of *M. modiolus* living in the presence of a 2mm daily depth equivalent of different particulate substances. Within the first eight days of exposure, the relationship between the different treatments differed between days. After day eight, the controls filtered at a lower level than the different treatments. The one-way ANOVA showed that on all days tested a significant difference was found (p-value 0.000) with the control constantly differing from all other treatments.

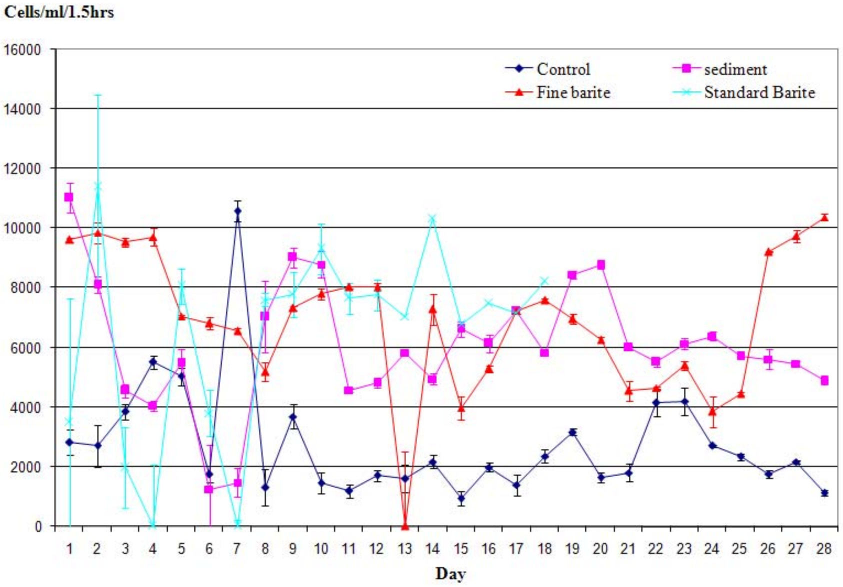


Figure 2.27: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Modiolus modiolus* in the presence of different particulate material.

2.3.3.4. *Chlamys varia*

Chlamys varia had the lowest tolerance to the presence of barite out of all four bivalve species. No statistical analysis could be run on the filtration rates of *C. varia* due to their poor survival. Figure 2.28 shows the severe results that barite had on *C. varia*. None of the *C. varia* exposed to the 0.1mm barite level survived past day one with the filtration on day one being quite low at 1212 cells/ml/1.5hr. The daily level of 0.2 mm barite produced a slightly longer survival period of only three days. The filtration of the algal suspension declined from 3749 on day one to 987 on day three.

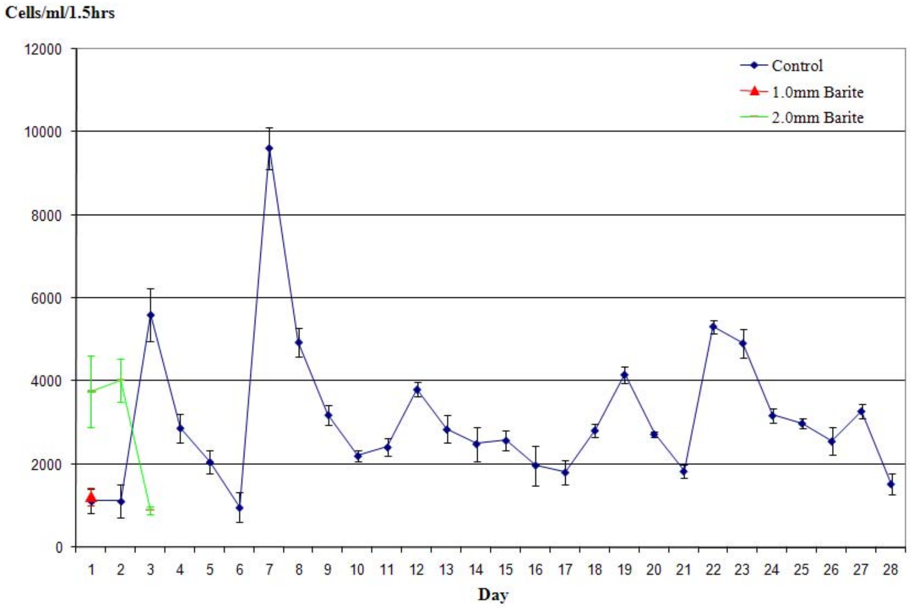


Figure 2.28: Filtration rate as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Chlamys varia* in the presence of different barite levels.

Figure 2.29 shows the survival rates of *C. varia* living in the presence of a 2mm daily depth equivalent of different particulate material. The control, sediment and fine barite exposed *C. varia* all had a 100% survival rate for the full 28-day test period. In less than 24 hours, there was only a 60% survival rate for the *C. varia* exposed to the 1.0mm standard barite treatment. By day two there was no survival. The *C. varia* living in the presence of the highest standard barite dose (2mm) had a slightly better survival rate, lasting for four days.

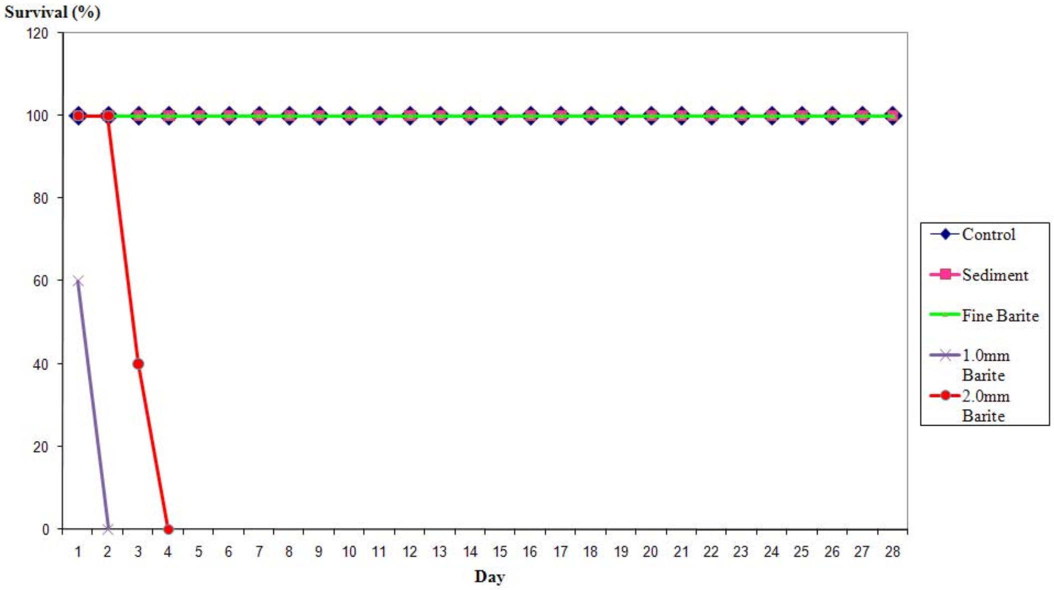


Figure 2.29: Survival rates of *Chlamys varia* in the presence of different particulate material.

Figure 2.30 shows the mean number of algal cells removed from suspension by *C. varia* living in the presence of a 2mm daily depth equivalent of different particulate material. The sediment exposed filtered more algal cells than the control *C. varia* apart from on days 3, 7 and 22. The filtration rate of the fine barite exposed *C. varia* drops dramatically from day one to day eight. After day eight there were quite large fluctuations throughout the rest of the test period. The fine barite exposed to *C. varia* produced high levels of pseudofaeces as indicated by the readings of zero. The standard barite caused the worst reaction by *C. varia.* The one-way ANOVA showed a significant difference (p-value 0.000) with no one specific treatment causing the difference.

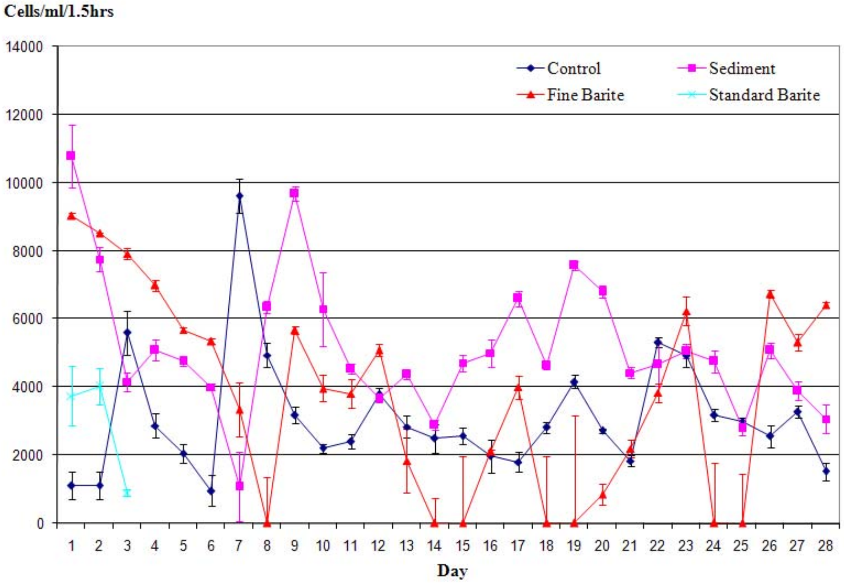


Figure 2.30: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by *Chlamys varia* in the presence of different particulate material.

2.3.3.5. Lethal Time

Lethal time, LT_{50}, determines the duration for 50% of the animals to die at a particular exposure concentration. It can also be called the median time to death. LT_{50} is not a measure of toxicity as the answer is time and not amount. The LT_{50} was calculated for each species and each barite exposure concentration and are shown below in Table 2.4. The results show that no pattern is repeated through out all species for each barite exposure level. All species, apart from *Dosinia exoleta*, the middle barite dose (1.0mm) was more toxic than the higher barite dose.

Table 2.4: Lethal time, LT_{50}, for bivalves in different barite concentrations

Barite Concentration	*Modiolus modiolus*	*Dosinia exoleta*	*Venerupis senegalensis*	*Chlamys varia*
0.5mm	19 days	18 days	11 days	-
1.0mm	11 days	20 days	7 days	2 days
2.0mm	13 days	11 days	8 day	3 days

2.4. Discussion

The findings suggest that standard barite and fine barite both appear to affect the filtration rate of four bivalve species. However, it cannot be determined whether their filtration ability caused their poor survival rate. Feeding within bivalves is physiologically regulated to maximised net energy gains in response to the quantity and quality of suspended particulate matter within the surrounding environment (Jørgensen, 1996). The gape of bivalve valves reflects the physical conditions of the ambient water including the presence of suspended particles (Riisgård, 2004). Prevalent conditions regulate the rhythm of the valves on a daily basis (Englund and Heino, 1994). Valve-opening behaviour is known to range from closed valves with retracted mantle edges to fully open valves with extended mantle edges (Jørgensen, 1996).

Standard barite is responsible for the alteration to the filtration rates of the suspension feeding bivalves, at all three daily depth equivalents (0.5mm, 1.0mm, 2.0mm). Standard barite had a lethal effect on the four species of suspension feeding bivalves with none surviving the duration of the experiment. *Chlamys varia* had the worst reaction towards barite with effects being almost instant. The survival periods of the remaining three species differed, with *Modiolus modiolus* generally being able to withstand the standard barite better than the other bivalve species. *M. modiolus* has a greater ability to remove particles from the water column over a wide size range (Navarro and Thompson, 1997) with pumping capacity proportional to gill area (Jørgensen *et.al.,* 1986).

Lethal time, LT_{50}, showed that the middle barite exposure dose, 1.0mm, gave the highest mortality for three out of the four bivalve species (Table 2.4). Bivalves filter the immediate surrounding water at a maximum rate with a fully open valve under optimal conditions. Sub-optimal conditions lead to a reduced valve gape and mantle edges (Riisgård, 2004). It cannot be stated for definite, due to the suspended barite obscuring site, but the valve gape may be responsible for *M. modiolus, V. senegalensis* and *C. varia* surviving the shortest time in the 1mm barite dose. The bivalves may have kept their valve gape more reduced in the 0.5mm and 2.0mm barite doses than when in the presence of the 1.0mm level. Sub-optimal conditions that control valve gape include the presence of suspended solids in very high or very low concentrations, lack of oxygen and foul water (Jørgensen, 1996).

A comparison of the filtration rates of the control bivalves gave an indication of what their 'natural' filtration rates were, although the rates were unlikely to be fully gained within an artificial environment. All control bivalves followed the same filtration pattern over the 28-day experimental period. There was a general instability within the filtration rates of the controls, showing that a bivalve's filtration rate naturally changes, as observed for many other species (Cranford and Gordon, 1992; Navarro and Velasco, 2003; Navarro *et.al.*, 2004; Vaughn & Hakenkamp, 2001). The one-way ANOVA showed that a significant difference was found on 16 of the 28 days. *C. varia* and *D. exoleta* both significantly differed from *M. modiolus* and *V. senegalensis* with regards to their filtration rate. This indicates that different species have different filtration rates regardless of size. *D. exoleta* and *V. senegalensis* are of similar size but their filtration rates significantly differed. The bivalves' filtration rates when in the presence of natural sediment were higher than the control results. Although this was the case, all four bivalve species followed the same filtration pattern over the 28-day test period.

Fine barite had a measurable effect on the filtration rates of all four species and only affected the survival of *Venerupis senegalensis* (with a 60% survival rate), suggesting that the bivalves were able to cope with the influx of the smaller particles. For examples the sea scallop, *Placopecten magellanicus*, can distinguish between particles of different sizes and densities, retaining larger particles for longer than smaller ones and lighter particles longer than denser ones (Brillant and MacDonald, 2000). Fine barite may have been expelled more quickly and easily than the standard barite, therefore producing larger quantities of pseudofaeces on a more constant basis. Silt, re-suspended frequently in the natural environment, is composed mainly of small inorganic particles, and therefore the process of expelling small particles sooner than larger ones may be a technique to save energy by avoiding digestion of poor quality particles (Brillant and MacDonald, 2000). Ingestion of barite by the bivalves was not analysed so it cannot be said if barite was consumed and present in their guts. Future studies would have to be performed to determine if bivalves do ingest barite and if so have the ability to survive with it in the gut.

The initial results gained from the exposure of the bivalve species to barite indicated that the control animals filtered a larger volume of the algal suspension than the standard barite dosed animals. Once the results were corrected for pseudofaeces production, the filtration rates of the test bivalves increased, with the standard barite exposed bivalves generally having a higher filtration rate than the control animals, as did the sediment exposed bivalves. Bivalves have the ability to distinguish between particles of different chemical composition and react by changing their clearance rate and their selectivity (Levinton *et.al.,* 2002; Laing, 2004).

The filtration rate of bivalves was found to increase with an increase in seston concentration and a decrease in organic content (Bayne *et.al.* 1993; Navarro and Velasco, 2003). Urrutia *et.al.,* (2001) showed that the cockle *Cerastoderma edule* increased its pumping rate when in the presence of a diet of low organic content. Combined with this increased filtration rate is an increase in pseudofaeces production (Bayne *et.al.,* 1993; Foster-Smith, 1975). Foster-Smith (1975) noted that three bivalves, *Mytilus edulis*, *Cerastoderma edule* and *Venerupis pullastra*, have the ability to restrict the volume of ingested material, whilst raising their filtration rate, as the volume of suspended material increases. The production of pseudofaeces results in the loss of energy into the external environment in the form of mucus and the increased filtration rate could be to compensate for this.

It can be assumed that the experimental bivalves were surviving on reduced energy levels. The presence of particles with a reduced organic content are linked to metabolic faecal losses, including products of secretion and/or abrasion during normal digestive processes (Hawkins *et.al.,* 1998). The production of pseudofaeces coinciding with an increased filtration rate will be responsible for energy loss within the bivalves. This cannot be said for definite as energy consumption was not determined. Energy loss can be calculated by oxygen consumption, decrease in dry flesh weight, and by the energy in faecal pellets (Gray and Elliott, 2009).

Bivalves are inclined to reject large or dense particles before smaller or lighter material (Foster-Smith, 1975). The reason behind this technique could be that organic material is not as dense as inorganic (Navarro and Velasco, 2003). As mentioned previously in chapter one, barite is a dense mineral with a specific gravity of 4.5. Since bivalves have the ability to reject particles based on load, they should be able to discard the standard barite and utilise the algae. The fact that none of the four species survived the full test period, even though they seemed to be filtering high quantities of the algae suspension, indicates that they were not able to utilise the algae for the purposes required. Since pure barite is virtually insoluble the observed effects are likely to result from a mechanical interference within the filtration rate.

Chapter Three:

Effects of Exposure to Suspended Barite on Bivalve Gill Structure

3.1. Introduction

The aim of this chapter is to identify what factor(s) may be responsible for the results gained within the main laboratory exposure experiments. Standard barite had a lethal effect at all three daily depth equivalents with none of the bivalve species surviving the 28-day test period. Fine barite had a measurable effect towards the algal uptake by suspension feeding bivalves, but survival was not affected, suggesting that they were able to cope with the influx of the smaller particles. Natural sediment did not harm the filtration rates of the bivalves, indicating that the suspension feeding test bivalves are particularly vulnerable to the man-made barite particles, especially the larger standard sized particles. Since pure barite is virtually insoluble, the observed effects are likely to result from a mechanical interference within the filtration rate.

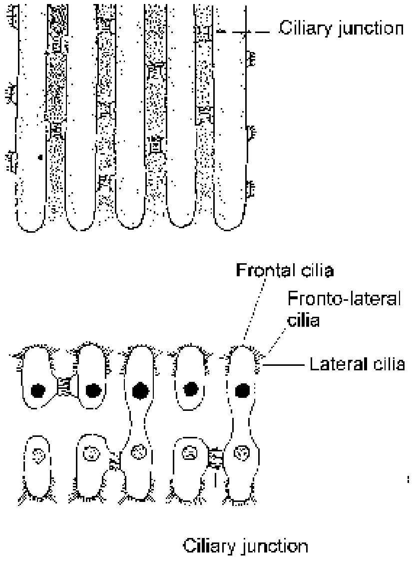
Suspension feeding bivalves rely on large gill systems to filter out particulate matter, mainly phytoplankton and detritus material (Barnes *et.al.,* 1996; Ruppert *et.al.,* 2004). The gill membranes are very delicate structures and are vulnerable to suspended particulate fractions (Drent *et.al.,* 2004; Navarro and Velasco, 2003; Silverman *et.al.,* 2000). Bivalves are mainly sedentary and will always be exposed to suspended material within the water column, the particles of which may vary in size, shape and composition. The bivalves are able to distinguish between food particles and those refractory to digestion and have the ability to dispose of unwanted particles, pseudofaeces, before they are taken into the gut. A bivalve's filtration rate relies on the whole gill structure, therefore the gills, along with the individual particle shapes were studied, using both different light and scanning electron microscopy techniques.

3.1.1. Bivalve Gill Structure

To determine if any damage may have occurred on or within the gills throughout the laboratory experiments, it is important to understand some aspects of the gill, for example, how it is structured and functions. The four bivalve species used within the main exposure experiments are classified as lamellibranches and are adapted to being suspension feeders (Barnes *et.al.,* 1996; Ruppert *et.al*., 2004). The bivalves in this study all possess a large mantle cavity that houses an extensive gill system. This is responsible for drawing water through the system, filtering out particulate matter (Ruppert *et.al*., 2004). The gill system allows a controlled response to the quantity and quality of material transferred from the water current to the mouth (Churchill and Lewis, 1924).

All bivalves contain two gills, usually positioned either side of their body organs (Ruppert *et.al.,* 2004). Each gill, or holobranch, is separated into one pair of outer and one pair of inner demibranchs (Cheung and Shin, 2005). The demibranchs separate the gill structure into inhalant and exhalant chambers (Bayne, 1976). Each demibranch bears many inter-connected junctions and are covered cilia (Cheung and Shin, 2005). Cilia are a very important component of the gill and are responsible for creating the respiratory current and removing unwanted particles from the gill surface (Ruppert *et.al*., 2004).

Lamellibranchs can be separated into different groups depending on their gill structure and the degree of development within them. Of the species used in the laboratory exposure experiments, *Modiolus modiolus* and *Chlamys varia* are fillibranchs, and, *Dosinia exoleta* and *Venerupis senegalensis* are eulamellibranchs. Figures 3.1 and 3.2 illustrate the difference within the two gill structures. In fillibranchs the individual filaments within the gills remain mainly independent of each other. Eulamellibranchs have the most specialised gill structure; here the filaments contain permanent tissue connections that extend over the entire length of the lamellae.



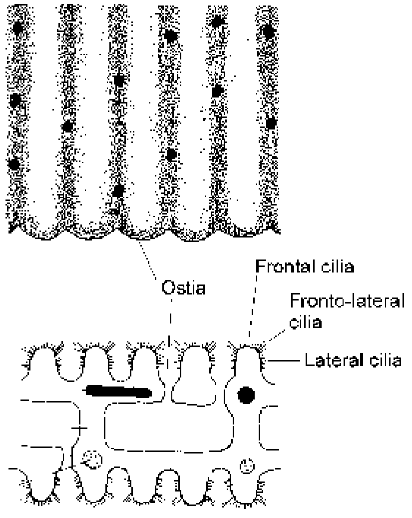


Figure 3.1: Fillibranch Gill

Figure 3.2: Eullamelibranch Gill

(Figures copied from Barnes, 1980).

Eulamellibranchs rely mainly on inhalant and exhalent siphons for water transport (Ruppert *et.al.,* 2004). Siphons allow bivalve species to gain access to a fresh supply of food and water whilst remaining buried within the sediment.

3.1.2. Sorting of Particulate Matter

Particle processing mechanisms in suspension feeding bivalves can be divided into the following: encounter, capture, transport, selection and ingestion (Silverman *et.al.,* 2000). Bivalves are known to entrap particles of various sizes, ranging from approximately >10 μm to 0.5 μm (Riisgård, 1988). The utilisation of particulate matter requires the ciliary sorting fields that are responsible for the separation of organic food from indigestible mineral particles (Ruppert *et.al.*, 2004). The cilia are separated into three categories, lateral, latero-frontal and frontal (see Figures 3.1 and 3.2). The lateral cilia uphold a water-flow through the demibranchs (Cheung and Shin, 2005) which creates a feeding current (Ruppert *et.al.,* 2004).

The fronto-lateral cilia are responsible for removing particles from the water current. These particles are then transported by the frontal cilia (Cheung and Shin, 2005) where they are coated in mucus and transported to ciliated food grooves. The cilia in the grooves transport the food either to the labial palps or reject them out of the bivalve (Ruppert *et.al.,* 2004). Lamellibranchs have five grooves for transporting particles. The use of each groove depends on the bivalve species. Scallops transfer food particles up into three food grooves that transport food to the labial palps and onto the mouth. Sediment particles are moved down two rejection tracts by other cilia. In mussels, such as *Mytilus edulis* and *Modiolus modiolus*, food and sediment are transported through any of the five food grooves, with little or no sorting occurring on the gills (Ruppert *et.al.,* 2004).

3.2. Materials and Methods

3.2.1. Particle Shape Analysis

The individual grains of sediment, standard barite and fine barite were studied using light and scanning electron microscope techniques. Barite, from used water-based drilling mud (WBM) was also analysed. Barite was extracted from used WBM by taking advantage of its high specific gravity. The used WBM was added to distilled water, and by the application of Stokes Law; the settling velocity was used to extract barite. To confirm that the extracted particles were barite, an elemental analysis was carried out on the Scanning Electron Microscope and displayed on the spectrum viewer.

3.2.1.1. Light Microscopy

The separate particles were initially observed using the Zeiss Axiophot microscope. The different particles were mixed with a drop of distilled water, placed on a microscope slide with a cover slip and analysed. Photographic images were taken by means of an Axiocam MRm black and white camera. Images were captured by Axiovision imaging software.

3.2.1.2. Scanning Electron Microscopy

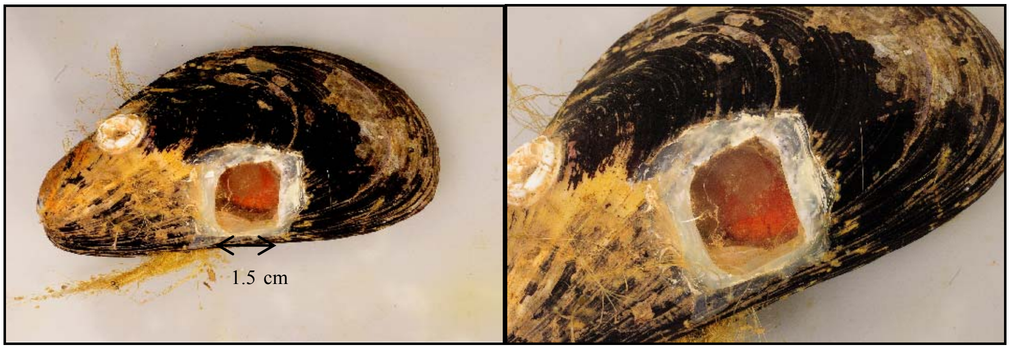
The scanning electron microscope (SEM) images the sample surface by scanning it with a high-energy beam of electrons. It allows the surfaces of specimens to be examined at high magnification whilst retaining a great image depth of field. The SEM was used to identify accurately the individual particle shapes of each substance in close detail. The particles were glued to stubs and analysed under low vacuum. The elemental analysis was run on the environmental scanning electron microscope, XL series 30, and displayed by the spectrum viewer software.

3.2.2. Gill Studies

There were two approaches to studying the effects of barite on the gill structure. Samples of gill tissue were taken from animals exposed to the maximum barite settlement rates used in the experiments and examined using light and scanning electron microscopy. *In-vivo* studies were also carried out in which direct observation of the gills was made during exposure to suspended barite particles.

3.2.2.1. In-vivo Studies

A square hole of about 1.5cm² was cut into the shell of the bivalve. The mantle tissue was removed and a glass cover slide was attached using araldite rapid resin (Figures 3.3a-b). This technique allowed the bivalves to be returned to seawater within 30 minutes, reducing stress. The animals were left for a week to allow the bivalves to acclimatise to their new conditions.



Figures 3.3a-b: Whole animal observation of *Modiolus modiolus* in vivo study

Photos were taken of the natural gill structure, before and during barite exposure, using a Leica DC300 camera attached to a Leica $MZ7_5$ microscope. The filtration rates of the fenestrated individuals (containing a window) were calculated, as described in chapter two, section 2.2.3, and compared to control un-fenestrated individuals before exposure. The filtration rate was also compared between fenestrated species kept in only seawater and barite exposed fenestrated individuals. The fenestrated animals were placed in the experimental system with the highest daily dose

(2mm) of barite. This daily level was chosen to determine the worst scenario that may occur from the laboratory exposure experiments. This procedure was tested originally on the blue mussel *Mytilus edulis.* It was then repeated on *Modiolus modiolus* (Figures 3.3a-b), *Venerupis senegalensis* and *Dosinia exoleta.* Ten individuals were used for each species. *Chlamys varia* was not used due to its intolerance towards the standard barite in the main laboratory experiments, surviving for only one day at the 2mm daily depth equivalent.

3.2.2.2. Microscopy Studies

When preparing the gill tissues for the SEM, four main steps were followed;

1. Fixation
2. Dehydration
3. Critical Point Drying
4. Conductive Coating

The surface of biological specimens is usually covered with extracellular materials, which are considered contaminants; they form an opaque layer on the surface of specimens in the SEM. During specimen preparation, fluids necessary for fixation, dehydration, and intermediate steps may stabilize and harden some of the extracellular material. These surface materials obscure the vision of the samples and will alter the results so removal before the fixation process is essential. The excised gill tissue samples were rinsed with distilled water before undergoing the fixation process.

When preparing samples for the SEM it must be noted that the internal and external surface morphology of tissue can be affected by fixation procedures (Hayat, 1981), so extreme care was taken throughout the procedure. Table 3.1 shows the procedures that were followed to prepare the gill tissues for critical point drying.

Table 3.1: Fixation and Dehydration Steps

	Chemical	Temperature	Time	Repetition
Wash	Buffer	Room	10-20 minutes	1
Primary Fixation	2% Gluteraldehyde in distilled water	Room	1-2 hours	1
Wash	Buffer	Room	10-20 minutes	3-5
Secondary Fixation	1-4% Osmium Tetroxide in distilled water	Room	1-2 hours	1
Wash	Buffer	Room	10-20 minutes	3-5
Dehydration	25% ethanol 50% ethanol 75% ethanol 90% ethanol 96% ethanol 100% ethanol	Room	10 minutes 10 minutes 10 minutes 10 minutes 10 minutes 10 minutes	1 1 1 1 1 2
Transition Solvent	Amyl Acetate: ethanol 25:75 50:50 75:25 100:0	Room	15 minutes 15 minutes 15 minutes Until Point Dried	1

The gill specimens were primarily fixed in 2% gluteraldehyde solution, which is an effective fixative in preserving cellular proteins, and brings about rapid fixation (Dykstra and Reuss, 2003). The specimens were then carefully washed in distilled water before being placed into 2% osmium tetroxide, which chemically hardens the fragile gill specimens. The hardening of the specimen ensures it is less vulnerable to damage during subsequent handling and better withstands the critical point dryer and damaging effects of the electron beam and vacuum in the column of the SEM (Hayat, 1981).

The specimens were then washed in distilled water to remove buffer salts and before being dehydrated. The dehydration process consisted of the gill specimens being placed in graded ethanol concentrations. It was important to take time over this process because rapid dehydration causes shrinkage of tissues. Following dehydration, substitution to amyl acetate was necessary for the critical point dryer. The substitution was not carried out directly but through graded baths of the two liquids. The samples were then preserved in 100% amyl acetate until critically point dried.

The critical point dryer, E3000, is designed for complete dehydration of biological tissue prior to examination in the scanning electron microscope (SEM). The chemically dehydrated specimens were placed into three specimen baskets held in a transfer boat. The transfer boat is an aluminium dish with an integral drain valve and holds the specimens, immersed in the substitution fluid (amyl acetate), during transfer to the pressure chamber. Cooling water was applied to the water jacket that surrounds the chamber, to reduce the chamber temperature to below 20°c.

The specimens were loaded and the chamber was filled with liquid CO_2. A series of flush cycles completely purged the specimens of dehydration fluid, replacing with liquid CO_2. The specimens were then left for over an hour to soak in the liquid CO_2. This was followed by another series of flush cycles. Once the specimens were completely saturated in liquid CO_2, hot water was applied to the water jacket to increase the temperature of the chamber. The chamber pressure increased as the temperature rose, taking the CO_2 through its critical point. The temperature and pressure were raised to about 35°c and 1200 psi respectively. The chamber was then slowly decompressed and the dried specimens removed and placed in a desiccator. It is essential to have a slow decompression to avoid damage to the specimens. The fixed and dried gill tissues were glued to stubs. The attached gills were sputter coated with gold. The prepared samples were studied under the SEM using high vacuum. The specimens remained in a desiccator at all times when not in use in the ESEM to prevent re-hydration.

3.2.3. Eulamellibranch Labial Palp Sizes

The labial palps were carefully removed from ten individuals of *Dosinia exoleta* and *Venerupis pullastra* under a dissection microscope and the lengths recorded. The labial palps were extracted from these two species only because of the palps involvement with particle sorting within eulamellibranchs. Unlike fillibranchs, which rely on their large gill system to separate and sort particles filtered out of suspension, eulamellibranchs also make use of their labial palps. Within eulamellibranchs, the labial palps help aid selection of organic particles over inorganic ones. Suspended particles are filtered out from the surrounding waters, passed over the surface of the gills towards the labial palps (Yonge and Thompson, 1976).

3.3. Results

3.3.1. Particle Shape Analysis

3.3.1.1. Light Microscopy

Figures 3.4 to 3.7 show an overview of sediment, fine barite, standard barite and barite from used water-based drilling mud. The sediment grains in Figure 3.4 show a variety of shapes and sizes. Most of the particles however, contain rounded edges. The fine (Figure 3.5) and standard (Figure 3.6) barite both contain a majority of quite angular sharp edges. The barite from the used WBM, shown in Figure 3.7, contains particles of similar shape to the standard and fine barite. Overall, the barite grains are quite irregular in shape.

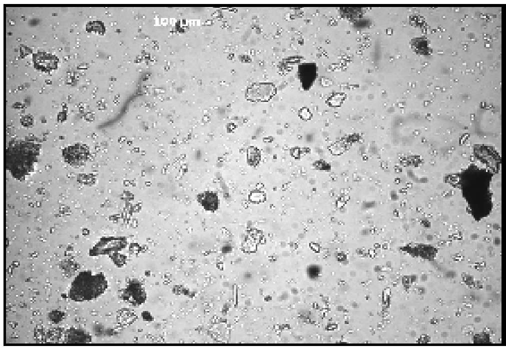


Figure 3.4: Sediment

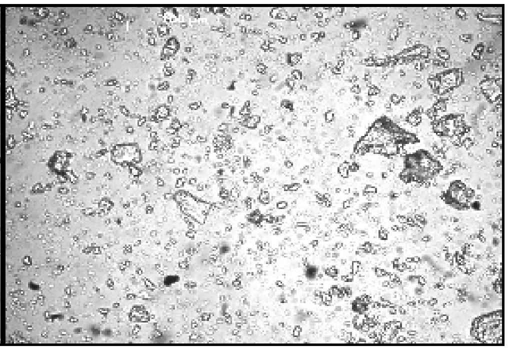


Figure 3.5: Fine Barite

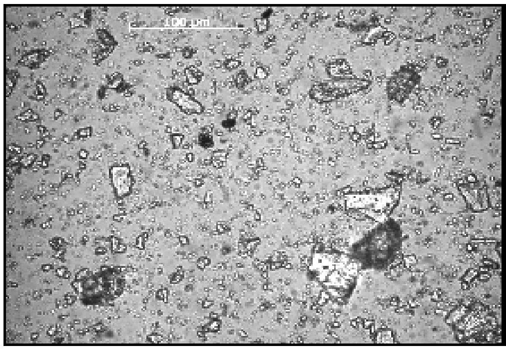


Figure 3.6: Barite

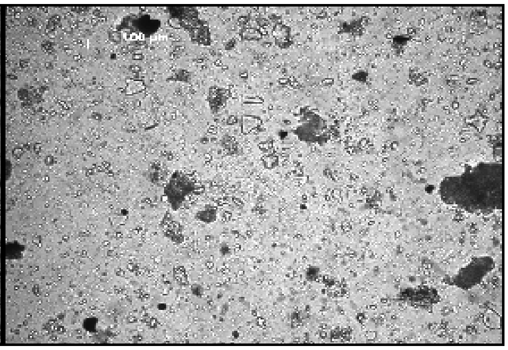


Figure 3.7: Barite from used WBM

3.3.1.2. Scanning Electron Microscopy

The SEM enabled more detailed analysis of each individual particle allowing the study of their shape and texture.

Sediment

Figures 3.8a and 3.8b show that the individual particles from Torryburn each contain different shapes. The sediment contains a mixture of particles ranging from grains with rounded edges to sharper fragments. The particles themselves look quite grainy and uneven in texture.

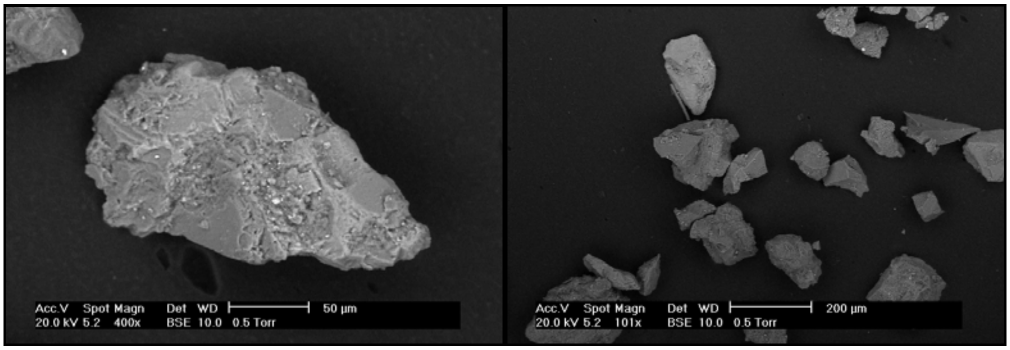


Figure 3.8a-b: Torryburn Sediment

Standard Barite

The particles have a distinct shape with sharp and angular edges, quite different to sediment particle shapes. Shown in Figures 3.9a-b are large barite particles with smooth sides unlike the rough sediment grains.

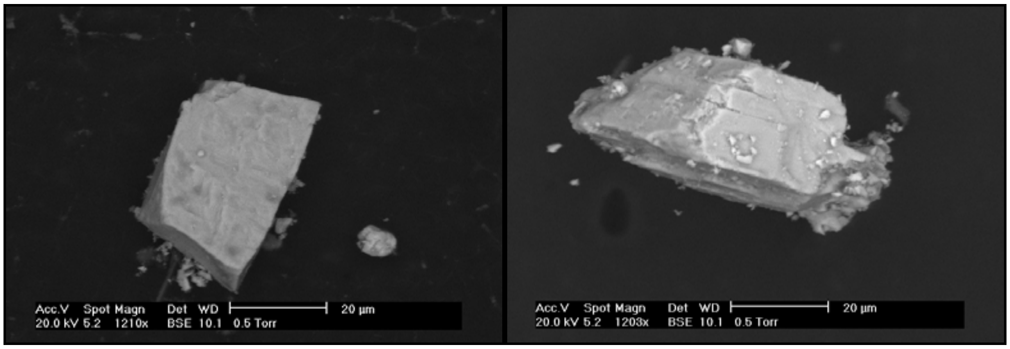


Figure 3.9a-b: Standard Barite

Fine Barite

Displayed in Figures 3.10a-b are fine barite particles which contain a similar shape and texture to standard barite. The particles are mainly sharp and angular, with smooth sides.

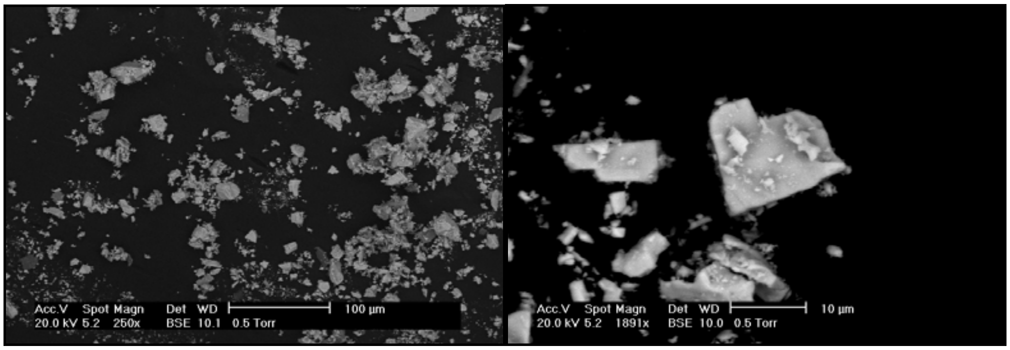


Figure 3.10a-b: Fine Barite

Barite from WBM

The barite particles extracted from used water-based drilling mud are very similar in shape to the freshly milled standard barite (Figure 3.11a-b). The sides of the barite are still quite smooth with sharp edges. Figure 3.12 displays the elemental analysis on the individual particles confirming that the grains extracted from used water-based drilling mud are barium sulphate, *i.e.* barite.

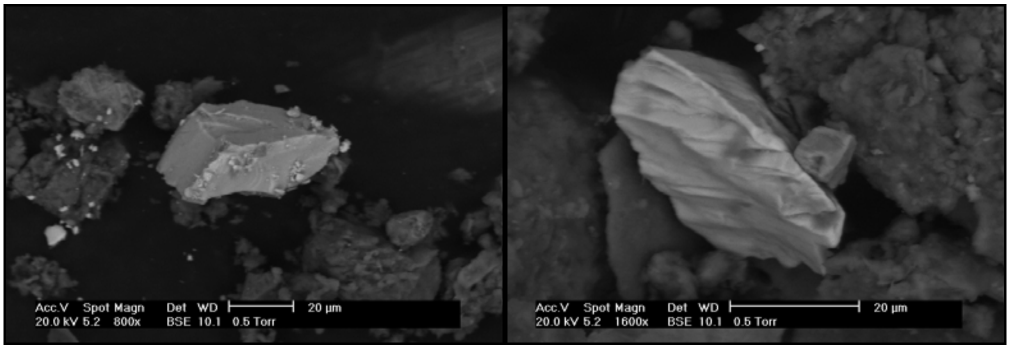


Figure 3.11a-b: Barite from used WBM

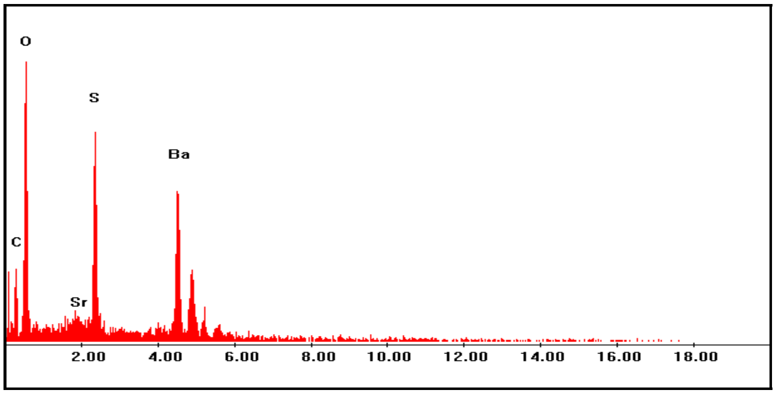


Figure 3.12: Elemental Analysis of Barite from used WBM

3.3.2. Gill Studies

3.3.2.1. In-vivo Studies

The original trial on *Mytilus edulis* was successful with all ten individuals surviving for around four months and six individuals remaining alive for over six months, with their mantle tissue and shell re-forming. *Modiolus modiolus* survived for four months allowing pre and post barite exposure photos to be taken. *Venerupis senegalensis* and *Dosinia exoleta* survived for just over a week. The invasive nature of the *in-vivo* studies precluded the use of large numbers of individuals in the experiments. The results are thus qualitative and primarily observational.

Figure 3.13 presents a comparison between the filtration rates of *Mytilus edulis* and *Modiolus modiolus*. It compares the filtration rates of un-fenestrated control individuals with the filtration rates of fenestrated individuals that have had a glass slide attached to allow *in-vitro* observations. No barite was introduced to the bivalves at this point. The results show that the attachment of a glass slide to the side of the shell does not interfere with the filtration rate of the two suspension feeding bivalves. Both the control and test species follow a similar filtration pattern over the seven days.

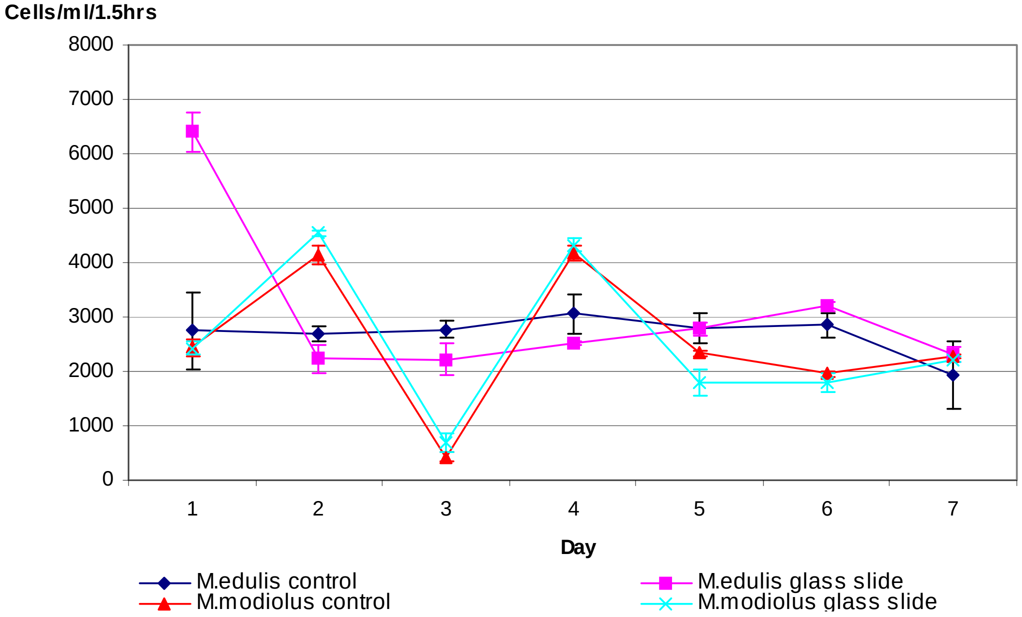


Figure 3.13: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by two species of suspension feeding bivalve.

Figure 3.14 displays the filtration rates of *Mytilus edulis* and *Modiolus modiolus* that have been exposed to a 2mm daily depth equivalent of barite. Both the control and test individuals had a glass slide attached for in-vivo observations. These results showed that the presence of the attached glass slide did not affect the filtration rates of these two suspension feeding bivalve species. The control *M. edulis* and *M. modiolus* filtered over 2000 cells/ml daily over the test period. The *M. edulis* exposed to barite had a filtration rate that was always lower than 1000 cells/ml. The filtration rate of *M. modiolus* exposed to barite fluctuated much more than both the controls and test *M. edulis.*

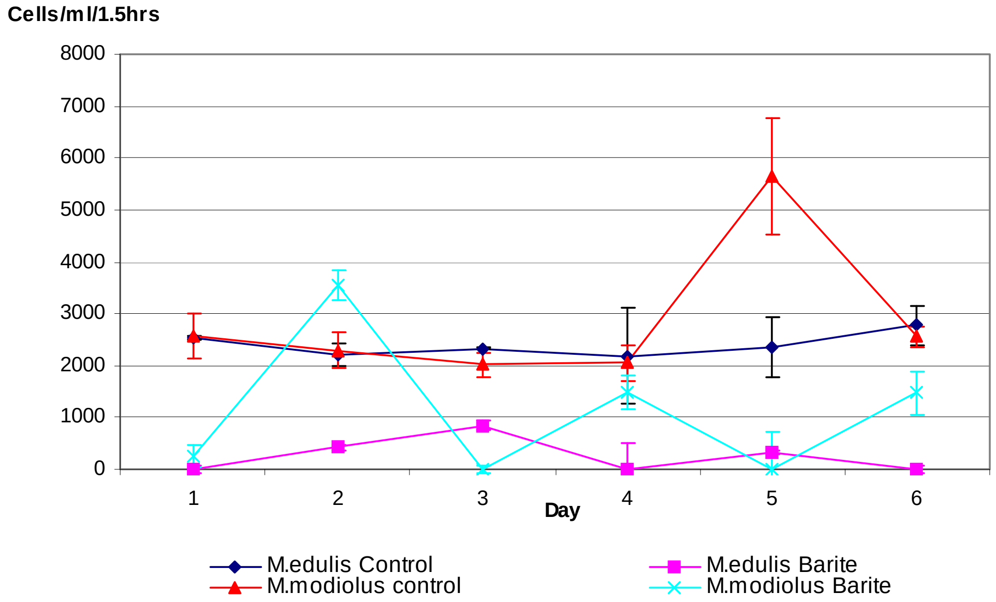
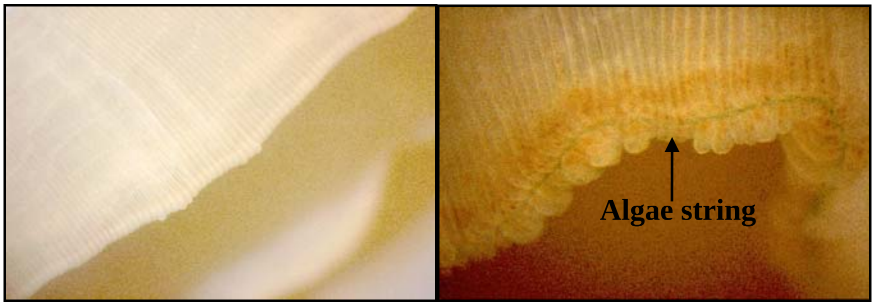


Figure 3.14: Filtration rates as indicated by mean number of algal cells removed after 1.5hrs filter feeding by two species of suspension feeding bivalves in the presence of barite.

Mytilus edulis

The re-formed shell of *Mytilus edulis* is shown in Figure 3.15. The control gill filaments of *M. edulis* are healthy looking and are shown in Figures 3.16a-b. They show regular comb like patterns with filaments of even length. In Figure 3.16b a string of algae is seen passing between the tips of the inner and outer demibranch. Figure 3.17a shows an overview of an outer demibranch that has been exposed to barite. There is damage at the tip of the gill filaments in several locations. The largest damaged area is displayed in closer detail in Figure 3.17b. Between the damaged gill filaments is a faint mucus string, which contained no algae.

Figure 3.15: Shell re-growth of *M.edulis*



Figures 3.16a-b: Control *M.edulis* Gill

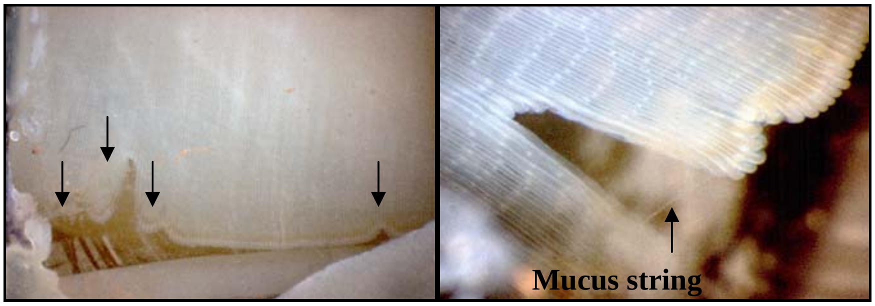
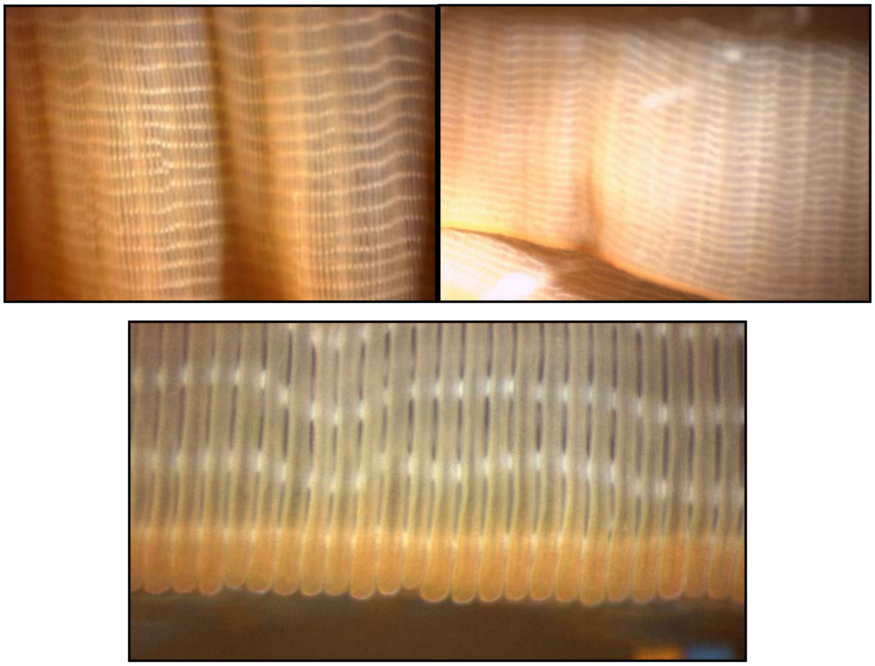


Figure 3.17a-b: Barite Exposed *M.edulis* Gill

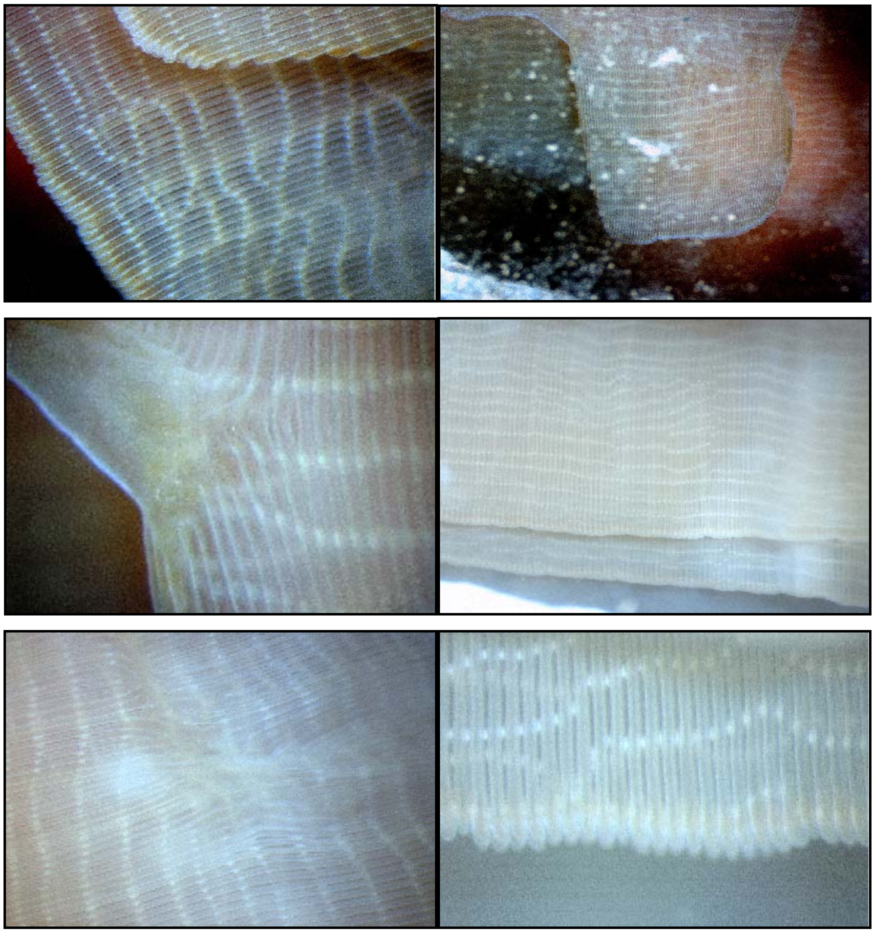
Modiolus modiolus

There is a noticeable difference between the gill tissues of the control and barite exposed *Modiolus modiolus.* The control *M. modiolus* gill tissues are in good condition. Figures 3.18a-b displays an overview of the outer demibranch with the inter-lamellar junctions, between each gill filament, positioned at a similar vertical height between each gill filament. Figure 3.18c shows a close-up of the gill filament tips of the outer demibranch. The filament tips are have a rounded appearance and are horizontally in line with each other.



Figures 3.18a-c: Control *M. modiolus* gills

The barite exposed *M. modiolus* gills are shown below in Figures 3.19a-f. The barite has caused visible damage to both the individual filaments and the whole outer demibranch. Figures 3.19a-c are from one *M. modiolus* individual and Figures 3.19d-f are from three separate *M. modiolus.* Figure 3.19a shows the inner demibranch and only part of the outer demibranch. Figure 3.19b shows the damaged outer gill which has been badly destroyed, with the surface area being reduced by roughly half. A close up view of the top of the filaments, in Figure 3.19c, indicates the gill was shredded or cut. Figures 3.19a, d, e and f, show that the inter-lamellae junctions appear to be vertically displaced.



Figures 3.19a – f: Barite exposed *M. modiolus* gills

3.3.2.2. *Scanning Electron Microscopy Studies*

Owing to difficulties in the fixation process, a comparison between the control, fine barite and barite treatments could only be made for *Modiolus modiolus* and *Dosinia exoleta*. The gills of *Chlamys varia* and *Venerupis senegalensis,* once fixed, were very brittle and did not allow satisfactory analysis of the filaments. As with the light microscopy work, the results were qualitative, based on observations of a few representative individuals. The gill tissue extracted from the control *M. modiolus* and *D. exoleta*, provided evidence, with their 'full' appearance (Figures 3.20a-b and 3.22) that the fixation process did not interfere with the main purpose of this study, determining the extent of damage barite has on the individual gill filaments.

Modiolus modiolus

The figures below compare control *Modiolus modiolus* gills to standard barite exposed gill tissues. Each gill filament of the control *M. modiolus* is straight and quite full bodied, as seen in Figures 3.20a-b. The 'fluffy' appearance of the filaments is likely to be cilia but unfortunately closer detail could not be observed. The gills extracted from *M. modiolus* exposed to the standard barite (Figure 3.21a) differ quite markedly, with a crinkled appearance, and lack the full body of the control gills. A tear in one of the gill filaments roughly 25µm x 40µm, a similar size to standard barite grain (average being 45µm in diameter) is shown in Figure 3.21b.

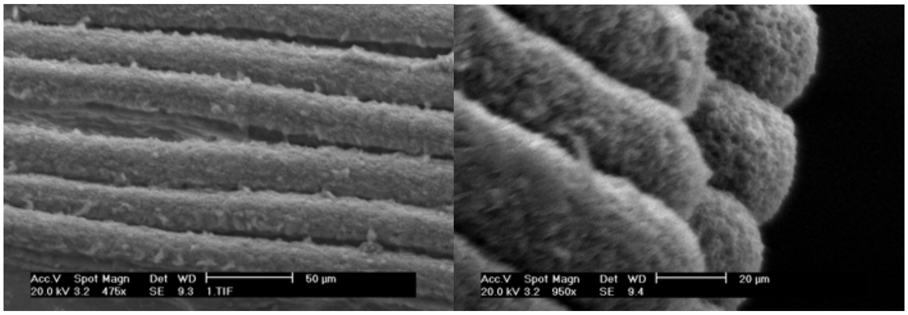


Figure 3.20a-b: Control *M.modiolus* Gill

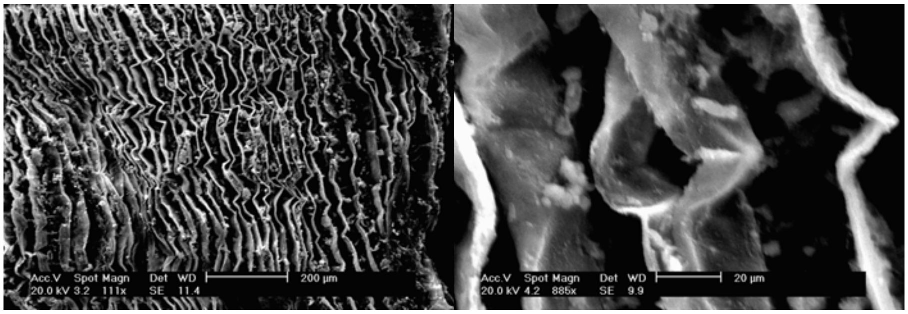


Figure 3.21a-b: Standard Barite Exposed Gill

Dosinia exoleta

Figures 3.22 to 3.24b show the difference between the gill tissues of control *Dosinia exoleta* and gills that had been in the presence of standard and fine barite. The control gill filaments are quite full bodied. The gills from *D.exoleta* exposed to standard barite (Figure 3.23) contain the same 'crinkled' appearance as the *M.modiolus* gills (Figure 3.21a).

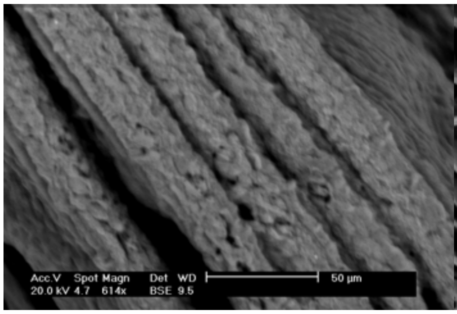


Figure 3.22: Control *Dosinia* Gill

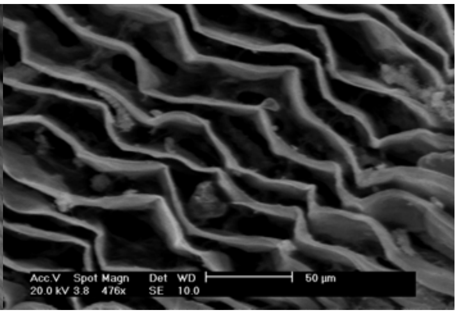


Figure 3.23: Standard Barite Exposed Gill

Figures 3.24a-b below show gill tissue from *D. exoleta*. They have been exposed to fine barite. Although these gill tissues look bare the filaments remain straight like the control gills and many inter-lamellar junctions are present. These connections are absent from the gill tissues taken from *D. exoleta* living in the presence of standard barite.

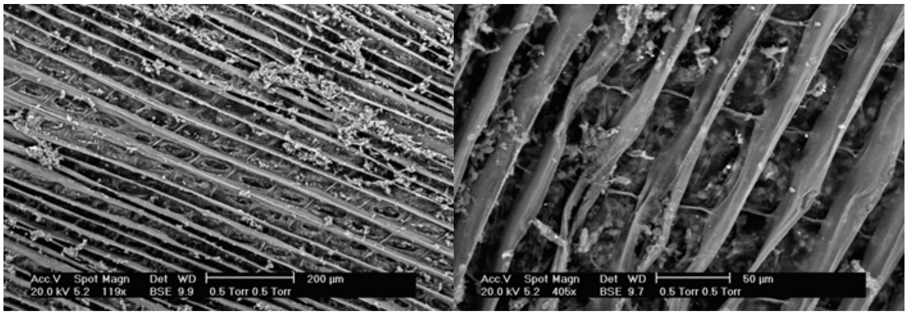


Figure 3.24a-b: Fine Barite Exposed Gill

Particle Shape in Relation to Gill Damage

Figure 3.25 shows standard barite grains of various sizes within the gill filaments of *Dosinia exoleta.* The largest grain, roughly 60µm in length, has damaged and cut through two individual filaments. Two-gill filaments in figure 3.26 encase a round sediment grain. There seems to be no damage caused to the filaments.

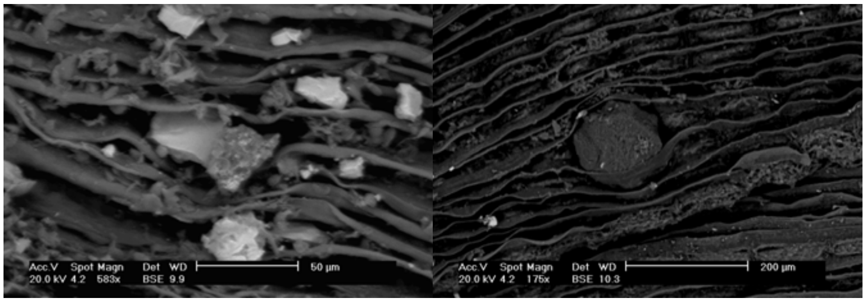


Figure 3.25 & 3.26: Gills Containing Barite and Sediment Particles Respectively

3.3.3. Eulamellibranch Labial Palp Sizes

Labial palp sizes vary between different species and the same species living in areas of different turbidity. It has been reported that in areas of highly turbid waters, bivalves contain palps of a larger size than the same species in areas of a reduced turbidity (Thiesen, 1977). The *Dosinia exoleta* and *Venerupis senegalensis* used here were both collected from the same site and were of the same size. The labial palp lengths, taken from ten individuals of *Dosinia exoleta* and *Venerupis senegalensis*, are shown below in Table 3.2. The mean palp lengths of *Dosinia exoleta* and *Venerupis senegalensis* are 10.4mm and 5.9mm respectively. The sizes of the palps indicate that *Dosinia exoleta* should be able to withstand an influx of increased particle concentration greater than *Venerupis senegalensis.*

Table 3.2: Labial Palp Sizes

Species	*Dosinia exoleta*	*Venerupis pullastra*
Individual	Palp Length (mm)	
1	11	6
2	12	5
3	10	7
4	11	6
5	11	6
6	9	3
7	10	7
8	8	8
9	12	5
10	10	6
Mean	10.4mm	5.9mm

3.4. Discussion

It is clear that the huge gill systems, specialising in the removal of fine particulates from the water column, have been damaged by the presence of barite. Standard barite is very abrasive and appears to account for the damage, and in some cases, removal of large parts of the gill structure. Fine barite is responsible for altering the state of the individual gill filaments, but the whole structure remains intact. The findings in chapter two showed that although the bivalves increased their filtration rates in the presence of barite they still died off. The damage to the natural conditions of the gill tissue, where particle sorting occurs, along with an increased filtration rate, will result in an energy deficit within the individuals. Filtration, particle sorting and pseudofaeces production are all highly energetic processes and the combination of these factors appears to be responsible for the deaths of the bivalve species.

The particle shape and texture of natural sediment differs from both standard barite and fine barite. Sediment grains are rougher and rounder than both standard barite and fine barite, which are quite smooth with sharp edges. Fine barite and standard barite both affected the algal uptake rate by the suspension feeding bivalves, shown in chapter two, but with different consequences on the life spans, with the larger grained standard barite having a lethal effect. Chapter two demonstrated that the bivalve species were able to cope with the influx of the smaller, fine barite particles, surviving for the full 28-days, with exception of *Venerupis senegalensis* which only had a 60% survival rate. Natural sediment caused no adverse reaction towards the filtration rates of the four bivalve species, showing that particle shape and texture are responsible for the results achieved. It was possible that throughout the drilling process, the drilling action would soften and round the sharp edges of the barite particles. However, examination of used drilling mud indicated that this was not the case, with the particles remaining sharp and angled. As the shape is not altered by the drilling process, the laboratory exposure experiments and gill studies using freshly milled barite can be related to possible responses from suspension feeders around offshore drilling platforms.

It is normal for a bivalve mollusc to encounter fluctuations in the quantity and quality of suspended particulate matter to which they are exposed within their natural environment (Navarro *et.al.*, 2004). It, therefore, is essential for a bivalve to be able to adapt its filtration abilities to accommodate a change in suspended silt and clay levels in the surrounding environment (Cranford and Gordon, 1992). Pseudofaeces are rejected particles encased in mucous, produced by the gills and labial palps, and do not pass through the gut (Urrutia *et.al.*, 2001). Bivalves have the ability to separate unwanted particles (barite) from wanted particles (algae). The probability of particles being trapped and ejected as pseudofaeces depends upon the characteristics of the particles themselves, i.e. size and shape (Jørgensen, 1996). Bivalves feeding on mixed suspensions of algae and silt have the ability to sort the particles on their gill structure for preferential ingestion of the food particles (Jørgensen, 1996). There was a constant production of pseudofaeces when the bivalves were in the presence of both standard and fine barite. Analyses of the pseudofaeces showed that algal cells were present in high numbers together with the barite. This suggested that the gills were unable to separate the organic algal cells from the unwanted inorganic barite particles.

The control gills of *Modiolus modiolus* and *Dosinia exoleta,* shown by the SEM, reveal full-bodied filaments unlike the standard and fine barite exposed gills, which had a very different appearance. Cilia on the gill filaments are responsible for the sorting of particulate matter. The control bivalves all followed the same filtration pattern through the 28-day test period, showing that the gills were healthy and functioning properly. The sediment-exposed bivalves again all followed a similar filtration pattern. This indicates the cilia were functioning properly and were able to separate the unwanted sediment particles from the required algal cells. The gill tissues of *D. exoleta,* taken from individuals living in the presence of fine barite did not suffer the same amount of damage as the standard barite exposed gills. Although they looked sparse and thin they still maintained the straight appearance of the control gills. Pseudofaeces were produced by all four bivalve species when in the presence of fine barite however, the animals survived the duration of the experiment suggesting that they were still able to utilise the algal cells. The gill structures of the standard barite exposed *M. modiolus* and *D. exoleta* had a ‘crinkled’ appearance. This evidence suggests that the damage to the gills from the standard barite is likely to be responsible for the high mortality rate within all four bivalve species.

The results displayed in chapter two show that *Venerupis senegalensis* and *Dosinia exoleta* reacted differently to the presence of both standard and fine barite. The two bivalve species used were of a similar size and both contain the same specialised gill structure. The labial palps of similar sized *V. senegalensis* and *D. exoleta* were 6mm and 10mm in length respectively, see section 3.3.3. In general, in areas of highly turbid waters, bivalves contain palps of a larger size than the same species in areas of a reduced turbidity (Thiesen, 1977). In eulamellibranchs, particles are initially captured on the gill surface and then passed onto the labial palps, the main site for particle sorting. *D. exoleta* withstood the presence of both standard and fine barite better than

V. senegalensis. The difference within the filtration of the algae suspension and the survival rates of *D. exoleta* and *V. senegalensis* suggest that the palp size is responsible for the difference in the results. The results indicate that *D. exoleta* has a greater ability to separate particles, and therefore expel the barite particles more easily, than *V. senegalensis*, allowing a longer survival period.

As mentioned in chapter two, the results from the main laboratory experiments show that generally the bivalves increased their filtration rate when in the presence of both the standard and fine barite. The production of pseudofaeces is an energetic process and the purpose of the increased filtration rate could be to compensate for this energy loss. The increased filtration rate would also have resulted in an increased influx of barite particles, and since the gills were damaged the bivalves could not take advantage of the algae cells. The 'creased' form of the individual filaments could hinder the expulsion of the standard barite allowing it to damage the gill, in some cases, causing quite extreme harm to a whole demibranch. The damaged gills prevented the separation of the algal cells from the barite particles. The denuded gills probably lacked cilia required for the sorting of particulates although the evidence from the SEM work is inconclusive.

Chapter Four:

Field Observation on the Effects of Barite Deposition on Benthic Community Structure

4.1. Introduction

The results of the laboratory experiments indicated a range of responses of suspension feeding bivalves to barite in the water column. These responses ranged from sublethal effects, in which a change to the gills of the test organism was clearly indicated, to lethal effects in which the bivalves did not survive the duration of the experiments.

It can be disputed whether a whole community will have the same response as an individual in the laboratory and there is much evidence to show that laboratory based observations do not always match field observations (Kingston, 1987). Unless an effect is found at community level, it can be argued that a response at an individual level may be ecologically insignificant (Gray *et.al.* 1980). Field experiments within the natural environment are the only way to measure changes in community directly. Most benthic organisms do not have the ability move so they must either, tolerate pollution, adapt or be killed (Gray and Elliott, 2009).

Although there is evidence of localised effects of water-based drilling mud cuttings discharged to the seabed (Neff *et.al.* 1989), there have been no such studies carried out in the North Sea. Unlike the impact of oil-based mud cuttings discharge, where change in community structure has been largely attributed to the presence of hydrocarbons (Davies *et.al.* 1984; Daan and Mulder, 1996; Olsgard and Gray 1995), any difference in community structure, where water based mud is used, is more likely to come from physical effects or the presence (at least in the early stages of drilling) of non-hydrocarbon contaminants.

The present study has focused on suspension feeding bivalves since it was anticipated that this trophic group would likely be the most sensitive to suspended particles. Most infaunal communities comprise a large number of species (Currie and Isaacs. 2005) and, although suspension feeders are the most likely to be initially affected by drilling mud discharge, once settled onto the seabed, deposit feeders will be exposed. Physiochemical variables, including sediment type and water movements, produce conditions which compose an essential niche, which allow benthic organisms to inhabit an area (Gray and Elliott, 2009). Human influences will alter these niches and in turn will affect the benthic communities present. There is often a close relationship between sediment type and composition of benthic community present (Gray and Elliott, 2009).

The presence of drilling mud residues on bottom surfaces may alter sediment granulometry and reduce the passing of oxygenated water into the sediment (Cantelmo *et.al.* 1979), which may also affect interstitial fauna. The dispersal of fly-ash, a very fine powder produced by the burning of pulverized coal in coal-fired power stations, was found to increase the fineness of seabed substrate (Bamber, 1984). In-turn this increased the instability of the sediment, reducing the porosity and permeability, and thus reduced the suitability for infaunal species (Bamber, 1980). Due to the dependence on organic content and structure of the sediment, deposit-feeders (Bamber, 1984) and tube builders (Gray and Elliot, 2009) will be the first affected by a change in sediment type and structure.

In an attempt to provide some initial indications of the influence of barite on benthic macrofaunal communities, field experiments were conducted to determine the impact of a single dosing of barite on communities of two sediment types representative of North Sea conditions.

4.2. Materials and Methods

Exposure experiments were carried out in two separate locations in the West Coast of Scotland chosen to represent as near as possible seabed conditions in the oil development areas of the northern and central North Sea. The fate of drilling discharges will depend on the platform location and will be determined by tides and currents, sediment type present and depth of the seabed.

The North Sea is situated on the continental shelf on Northwest Europe. It has a surface area of about 750, 000 km^3, volume of about 94, 000 km^3 (North Sea Task Force, 1993) and a mean depth of 90 m (Ducrotoy *et.al.*, 2000). It can be separated into three main regions, the northern, central and southern North Sea (figure 4.1).

Figure 4.1: Edited Map of North Sea

(North Sea Task Force, 1993)

There are mainly oil platforms in the northern and central North Sea and gas platform in the southern North Sea. The northern and central North Sea waters are influenced by an expansion of the north-easterly flowing Atlantic Current (North Sea Task Force, 1993). In the largest part of the northern and central North Sea, the near-surface currents are weak, and this region is thermally stratified in the summer months (Basford and Eleftheriou, 1988). The movement of bottom water at depths generally greater than 70 metres is related to seasonal fluctuations. Drilling wastes entering the northern and central North Sea will be distributed but then may lie on the seabed until seasonal fluctuations re-suspend them.

In contrast, within the shallow areas of the North Sea, mainly the Southern region, intensive sediment transport occurs frequently, owing to strong currents and tides. The physically powerful currents of the English Channel mainly influence sediment transport in the Southern North Sea. Atlantic water enters from the channel with a mean transport of 0.1 x $10^6 m^3/s$ and on occasions this flow can be reversed by winds. This flow through the channel moves erratically towards the Skagerrak, as does the water bordering the continental shelf. These strong currents maintain a well-mixed bottom throughout the year (North Sea Task Force, 1993) and will allow immediate distribution of drilling waste material on discharge.

4.2.1. Field Experiments

Two experimental sites (figure 4.2) were chosen; Ardmucknish bay, Loch Linne, 56°29.519’N 005°25.217’W, an area with a relatively high-energy homogenous sandy bottom (representative of southern North Sea) and the Sound of Shuna, Loch Linne, 56°35.754’N 005°22.208’W, an area with a heterogeneous muddy sand bottom (representative of northern North Sea).

Figure 4.2: Locations of the Experimental Sites

Twelve quadrats, constructed of PVC with dimensions of 0.5m x 0.5m x 0.1m with a prong of 0.25m in each corner, were placed into the seabed at each location, as seen in figures 4.3 and 4.4. They were positioned at a distance of 3m apart in an area 11m by 7.5m.

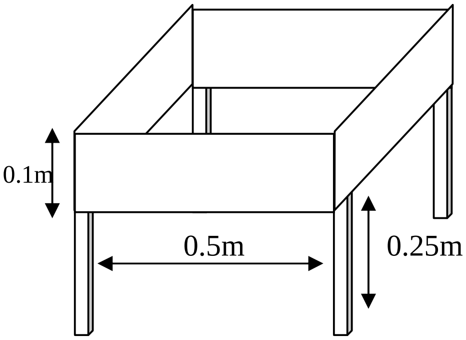


Figure 4.3: Quadrat

Figure 4.4: Control quadrat in Ardmucknish Bay

An initial single macrofaunal core and one single chemical core were taken adjacent to each quadrat. The macrofaunal cores, made of plastic and with an internal diameter of 15cm, were taken to a depth of at least 15cm. The chemical cores, made of metal, had an internal diameter of 5cm and were taken to a depth of at least 6cm. The macrofaunal samples were sieved through a 1mm mesh size and the fauna fixed with a 10% formaldehyde solution. The chemical cores were frozen whole. The pre-treatment cores allowed the identification of the natural fauna of the area and the natural barite levels within the sediment. After the initial cores were collected, treatments were randomly assigned to the twelve quadrats (Figure 4.5). Four quadrats were subjected to a barite treatment, four were subjected to a sediment treatment and four were left unaltered to act as controls.

A 4mm thick layer of barite was evenly spread over the sediment surface within four quadrats. Before dispersal, the barite was repeatedly washed in seawater, allowing removal of ultra fine particles, which could spread easily under water and contaminate the other quadrats. A 4mm layer of sediment, that approximately matched local sediment size, was used to evenly cover four quadrats. This sediment was sieved before use to ensure the removal of all macrofauna. A higher (4mm) layer was used instead of the lower (2mm) continuous dose previously used (chapter two) in an attempt to elicit a measurable response over the short time scale of the experiment.

The barite and sediment treatments were mixed into slurries with seawater. They were placed into a sandwich bags and fastened tight with cable ties. The slurries were distributed into the assigned quadrats by squeezing them out of holes cut into the corners of the sandwich bags, allowing an even spread of each treatment. The treatments were left to settle out for an hour before a core was taken from each quadrat for possible future chemical analysis.

Control 3	**Control 1**	**Barite 4**
Sediment 4	**Barite 1**	**Sediment 2**
Control 4	**Sediment 3**	**Sediment 1**
Barite 2	**Barite 3**	**Control 2**

Figure 4.5: Treatment layout

The quadrats remained in Ardmucknish Bay for six months, from July 2007 until January 2008, and in the Sound of Shuna for three months, from April 2008 to July 2008. The experiments were run over different lengths of the year due to time constraints. Upon relocation, a physical observation of the quadrats was recorded. Post treatment cores were collected from both locations at the end of the experimental period only. Three chemical and three macrofaunal cores were taken from inside each quadrat. The chemical cores were frozen whole and the macrofaunal cores were sieved and the retrieved macrofauna fixed in a 10 % formaldehyde solution.

4.2.2. Sample Preparation

4.2.2.1. Macrofauna Analysis

The sieved macrofaunal samples were put through a 1mm sieve, washed clean of formaldehyde and then stored in 70% ethanol. The samples were then sorted and specimens separated out into phyla and later identified to species level where possible. The initial 12 macrofauna cores collected from outside each quadrat were merged together into four groups. The samples were randomly selected and placed into four groups each containing three macrofauna cores. The two areas samples from, Ardmucknish Bay and Sound of Shuna, were both homogenous unsloped sites, and therefore it was deemed suitable to group together the initial cores as the benthic fauna was expected not to differ in regards to fauna present. The three post treatment cores collected from within each quadrat were grouped together. In total there were four initial samples, four controls, four sediment samples and four barite samples, each containing three macrofaunal cores.

4.2.2.2. Chemical Analysis

- Sodium Fusion (barium analysis)

Three sections were taken from the frozen sediment samples. The surface layer of sediment was cut into three layers each at a different depth within the first 6cm of core and each section placed into a crucible and oven dried at 70°C for 12 hours until all water had dried to a constant weight. Samples (0.2g) of the dried sediment were weighed into platinum crucibles. Sodium carbonate (1.2g) was added and mixed in with the weighed samples. The mixture was placed in a furnace to fuse at 800°c for 30 minutes. Once cooled, the newly fused sample was dissolved in nitric acid. The platinum crucibles were placed in beakers and 5 ml of nitric acid (50%) added. After 30 minutes the solution was filtered into a volumetric flask. This was repeated a further three times to ensure the fused sample was completely dissolved. The volumetric flask was then filled up to 100 ml with distilled water to provide a 10% nitric acid solution.

- Elemental Analysis

The samples from Ardmucknish Bay were analysed by SEPA, Riccarton Research Park, Edinburgh. The samples from Sound of Shuna were analysed at Fisheries Research Services, Marine Laboratory, Victoria Road, Aberdeen. The samples were analysed using the Inductively Coupled Mass Spectrometer (ICPMS), Perkin Elmer, Model 6100 DRC plus. The ICPMS is a type of mass spectrometry that is highly sensitive and capable of the determination of a range of metals at very low concentrations. ICPMS is based on the coupling together of an inductively coupled plasma as a method of producing ions (ionization) with a mass spectrometer as a method of separating and detecting the ions. The analytical procedures used by SEPA and the Fisheries Research Services can be seen in appendices A and B respectively.

4.2.3. Statistical Analysis

A range of univariate faunal parameters were calculated on both the initial fauna and post-treatment fauna. Total species (S), total individuals (N), Shannon Weiner (H') and Pielou eveness (J') were initially calculated and then t-tests were performed.

A range of multivariate techniques were then applied to the faunal data using the statistical packages PRIMER (Plymouth Routines in Multivariate Ecological Research) and MVSP (Multi Variate Statistical Package). ANOSIM provides a method to test statistically whether there is a significant difference between the faunal composition of two or more groups of samples. If ANOSIM produces a level of significance below 5% then there is a significant difference between the two samples. ANOSIM produces an R-statistic and if the probability of obtaining the R-value by chance is 5% then this signifies a significant difference. It is important to determine the level of similarity between any pair of biological samples, in terms of the communities they contain. Two samples are only deemed completely similar if they contain exactly the same species present in the same abundance.

The similarity measure used was Bray-Curtis. Bray-Curtis similarity is a measure of how similar data sets are to each other. The Bray-Curtis similarity is affected by both taxa present and their relative abundance (Clarke and Warwick, 1994).

Two ordination analyses, MDS and DCA, were used to examine the data. Multi-dimensional scaling (MDS) explores the similarities or dissimilarities (distances) of data and composes a 'map' showing the results. Samples are deemed more similar the closer they are plotted on the 'map.' An ordination plots the samples on a 'map' with their similarity represented by the closeness of the samples. Detrended Correspondence Analysis (DCA) is usually used to find the main gradients in species rich but usually sparse data matrices. When running standard ordination on data, when presented as a graph, an arch is produced against two axes. DCA reduces the 'horse shoe' arch (Hill and Gauch, 1980).

The multivariate statistical analysis was performed initially on the discrete data then it was $\log_{10}$ transformed. Presence/absence was also used to help analyse the data sets. Similarities calculated on continuous data can often be over dominated by a small number of highly abundant species, so they may not reflect the overall community structure. Logging the data reduces the importance of species present in high abundance and allows rarer species to contribute to the similarity. Rare species often make up roughly 70% of the total number of species and therefore rareness is a fundamental feature of marine benthic communities and studies of their biodiversity (Gray and Elliott, 2009).

4.3. Ardmucknish Bay Results

4.3.1. *In-situ Observations*

In Ardmucknish Bay, upon return (6 months post treatment) it was noticed that the quadrat legs were exposed for eleven of the quadrats indicating a change in the level of the sand surface of approximately 5cm (figure 4.7). This level change seemed to be exacerbated by a physical scouring effect caused by the presence of the quadrat. There was no evidence of barite on the sediment surface in the treated quadrats. One of the quadrats was broken in half but still remained in the correct position. Although the natural physical scouring that took place in Admucknish Bay is reflective of conditions in the southern North Sea, the localised erosion of substratum around the edges of the quadrats will have to be taken into consideration in interpreting the results of the experiment.

Figure 4.6: Initial quadrat

Figure 4.7: Quadrat post six months

4.3.2. Benthic Community of Ardmucknish Bay

The benthic community of Ardmucknish Bay was typical of a highly energetic sandy bottomed area. The initial macrofauna can be seen on pages 101 and 102 with the post-treatment macrofauna displayed on pages 103 and 104. The community of Ardmucknish Bay was dominated by polychaetes, fast burrowers that have the ability to adapt to an area that has a constantly changing seabed surface. The polychaete species, before and after exposure, contained a mixture of predators, deposit feeders and omnivores. The second largest populated class within Ardmucknish Bay was found to be bivalvia. The bivalves contained a ratio of 2:1 of suspension to deposit feeders in both the initial and post-treatment samples. The community of Ardmucknish Bay resembles both the *Tellina* and *Venus* assemblages. It has been noted that different assemblages may contain similar morphological species with the same environmental preferences (Gray and Elliott, 2009).

4.3.3. Univariate Faunal Parameters

Diversity indices were calculated for the initial and post treatment macrofauna. Each value is derived from three cores. The initial data is composed from three random cores taken from the 12 initial cores collected from outside each quadrat. The control, sediment and barite results were derived from the combined data from each of the three macrofaunal cores collected from inside each quadrat. Table 4.1 shows the calculated diversity indices for each treatment. There is very little difference between total species and total individuals of all four treatments. The Shannon Weiner diversity index determines the combination of species richness and evenness within a sample. The results from Ardmucknish Bay range from 2.0 to 2.9 showing that the samples contain a similar distribution between numbers of indiviudals. Pielou’s evenness gives results ranging from 0-1, with results close to one indicating a relatively high level of evenness in the distribution of abundances among species within a sample. The results displayed in table eight ranges from 0.8 to 1.0.

Table 4.1: Univariate statistical parameters for Armucknish Bay data (S = Total Species, N = Total Individuals, H’ = Shannon Wiener, J’ = Pielou’s evenness)

Treatment	S	N	H'	J'
Initial 1	15	30	2.35	0.8677
Initial 2	10	23	1.968	0.8545
Initial 3	19	47	2.522	0.8565
Initial 4	16	31	2.531	0.9127
Control 1	12	24	2.219	0.8928
Control 2	13	31	2.318	0.9037
Control 3	12	27	2.365	0.9517
Control 4	17	35	2.624	0.9262
Sediment 1	21	33	2.859	0.939
Sediment 2	15	43	2.493	0.9208
Sediment 3	18	34	2.688	0.93
Sediment 4	16	26	2.651	0.9563
Barite 1	15	34	2.344	0.8657
Barite 2	13	30	2.212	0.8623
Barite 3	16	27	2.673	0.964
Barite 4	11	27	2.021	0.8429

A one-way ANOVA followed by a multicomparison Tukey test was run to determine if a significant difference was present between the treatments (initial, control, sediment, barite) within each of the univariate indices. The p-values produced (0.259, 0.731, 0.151, 0.101 for S, N, H’, J’ respectively) show that there is no significant difference within the total species, total individuals or how evenly distributed the community is between the different treatments.

Ardmucknish Bay Pre-treatment macrofauna

Species	**C1**	**C2**	**C3**	**C4**	**S1**	**S2**	**S3**	**S4**	**B1**	**B2**	**B3**	**B4**	**FT***
Abra alba (Wood W., 1802)	0	0	0	1	0	0	0	0	0	0	1	0	D
Arctica islandica (Linnaeus, 1767)	0	0	1	0	0	0	1	0	0	0	1	0	S
Chamelea gallina (Linnaeus, 1758)	1	0	0	0	0	0	0	1	0	0	3	0	S
Corbula gibba (Olivi, 1792)	0	2	0	0	0	0	1	0	0	0	0	0	S
Lucinoma borealis (Linnaeus, 1767)	1	1	0	0	2	0	0	0	0	0	1	0	D
Tellina fabula (Gmelin, 1791)	3	2	2	2	1	8	2	1	3	4	5	4	D
Thracia papyracea (Poli, 1791)	1	0	0	1	0	1	0	1	0	0	0	0	S
Thracia villosiuscula (MacGillivray, 1827)	1	0	0	0	2	1	0	1	0	0	2	1	S
Thyasira flexuosa (Montagu, 1803)	1	3	1	1	1	1	1	2	2	0	1	2	S/D
Ampelisca brevicornis (Costa, 1853)	0	0	0	0	0	1	0	0	0	0	2	2	S
Ampelisca macrocephala (Liljeborg, 1852)	0	0	0	0	0	0	0	0	2	0	0	0	S
Harpinia antennaria (Meinert, 1890)	0	0	0	0	0	1	0	0	0	0	0	0	D
Perioculodes longimanus (Bate & Westwood, 1868)	0	0	0	0	0	0	0	0	0	0	1	0	S
Chaetozone setosa (Malmgren, 1867)	0	0	0	2	0	1	0	1	1	1	0	0	D
Glycera convoluta (Keferstein, 1862)	0	0	0	0	0	0	0	0	1	0	0	0	P

Ardmucknish Bay pre-treatment macrofauna (cont.)

Species	**C1**	**C2**	**C3**	**C4**	**S1**	**S2**	**S3**	**S4**	**B1**	**B2**	**B3**	**B4**	**FT***
Goniada maculata (Örsted, 1843)	0	0	0	0	1	1	0	0	0	1	0	0	D
Magelona papillicornis (F.Müller, 1858)	0	0	0	0	0	0	0	1	0	0	0	1	S/D
Nephtys caeca (Fabricius, 1780)	0	0	0	0	1	1	0	0	0	0	1	0	O/P
Nephtys hombergii (Savigny in Lamarck, 1818)	0	0	0	0	0	0	0	0	0	0	0	1	O/P/SC
Notomastus latericeus (Sars, 1851)	0	0	0	0	0	0	1	0	0	0	1	0	D
Oligochaete sp 1	0	0	0	0	0	0	1	0	0	0	0	0	D
Owenia fusiformis (Delle Chiaje, 1844)	0	0	0	0	1	0	0	0	0	0	0	0	D
Poecilochaetus serpens (Allen, 1904)	0	0	0	0	1	1	0	0	0	0	0	1	S/D
Scolelepis cantabra (Rioja, 1918)	0	1	2	2	0	0	0	1	0	0	0	0	D
Scoloplos (Scoloplos) armiger (Müller, 1776)	0	1	0	0	0	0	0	0	0	0	0	0	D
Spiophanes bombyx (Claparède, 1870)	1	0	0	1	0	0	1	1	0	0	1	1	S/D
Sthenelais limicola (Ehlers, 1864)	0	0	0	1	0	0	0	0	0	0	0	0	O/P
Tharyx marioni (Saint-Joseph, 1894)	0	1	0	0	0	0	0	0	0	0	0	0	D
Nemertine sp 1	0	1	0	0	0	0	0	0	0	0	0	0	P

*Feeding Type (FT): D = deposit feeder; S = suspension feeder; P = predator; O = omnivore; SC = scavanger

Ardmucknish Bay Post Treatment Macrofauna

Species	**C1**	**C2**	**C3**	**C4**	**S1**	**S2**	**S3**	**S4**	**B1**	**B2**	**B3**	**B4**	**FT***
Ampharetidae sp.1.	0	0	0	0	0	0	0	1	0	0	0	0	D
Aricidea sp.1.	4	7	4	1	4	7	3	4	10	10	2	8	D
Capitellidae sp.1.	0	0	0	0	1	0	0	0	0	0	0	0	D
Chaetozone setosa (Malmgren, 1867)	1	0	0	4	1	1	1	0	1	1	1	2	D
Diplocirrus glaucus (Malmgren, 1867)	0	0	0	0	1	0	0	0	0	0	0	0	D
Eteone longa (Fabricius, 1780)	0	0	1	0	0	0	0	0	0	0	0	0	D/P
Exogone sp.1. (Örsted, 1845)	0	0	0	0	1	1	4	2	0	0	0	0	D/S
Glycera convoluta (Keferstein, 1862)	0	2	0	1	0	1	1	0	0	0	0	1	P
Glycera sp (no head)	0	0	0	0	1	0	0	0	0	0	0	0	P
Goniada maculata (Örsted, 1843)	0	0	0	3	0	0	0	0	0	0	0	0	D
Gyptis capensis (Day, 1963)	0	0	0	0	0	0	1	1	0	0	0	0	O/P/SC
Magelona papillicornis (F.Müller, 1858)	1	0	3	4	1	5	0	1	0	0	3	1	S/D
Nephtys hombergii (Savigny in Lamarck, 1818)	0	1	0	2	0	0	0	0	0	1	2	0	O/P/SC
Nephtys longesetosa (Örsted, 1842)	5	2	2	2	2	5	1	3	4	1	1	0	O/P
Notomastus latericeus (Sars, 1851)	0	0	0	1	0	0	0	1	0	0	0	0	D
Owenia fusiformis (Delle Chiaje, 1844)	0	0	3	0	0	0	0	0	0	0	0	0	D
Phyllodoce (Anaitides) groenlandica (Oersted, 1842)	0	0	0	0	1	1	0	0	0	0	0	0	O/P
Phyllodoce (Anaitides) maculata (Linnaeus, 1767)	0	0	0	0	1	0	0	0	0	0	0	0	O/P
Poecilochaetus serpens (Allen, 1904)	0	0	1	0	3	3	4	0	0	1	0	1	S/D
Polychaete sp.1.	0	0	0	0	1	0	0	0	0	0	0	0	
Polynoidae sp.1.	0	1	0	0	0	0	0	0	0	0	0	0	O/P/SC
Prionospio cirrifera (Wirén, 1883)	0	0	0	0	0	0	0	0	1	1	0	1	D
Prionospio malmgreni (Claparède, 1869)	0	1	0	1	0	0	0	1	0	1	1	0	D
Pygospio elegans (Claparède, 1863)	0	0	0	0	1	0	1	0	0	1	0	0	D/S
Scoloplos (Scoloplos) armiger (Müller, 1776)	0	0	0	1	0	0	0	1	0	0	0	0	D
Spionidae sp.1.	0	0	0	0	0	0	0	0	0	0	0	1	D/S
Spiophanes bombyx (Claparède, 1870)	0	0	0	0	1	3	1	0	0	0	0	0	S/D
Spiophanes kroyeri (Grube, 1860)	0	0	0	0	0	0	0	0	0	0	0	0	D
Sthenelais limicola (Ehlers, 1864)	0	0	0	1	0	0	0	2	0	0	0	0	O/P
Syllidae sp.1.	0	0	0	0	0	0	0	0	0	2	0	0	O/P/SC

Ardmucknish Bay post treatment macrofauna (cont.)

Species	C1	C2	C3	C4	S1	S2	S3	S4	B1	B2	B3	B4	FT*
Tharyx marioni (Saint-Joseph, 1894)	0	0	0	0	1	0	1	0	0	0	1	0	D
Trichobranchus glacialis (Malmgren, 1866)	0	0	1	0	0	0	0	0	0	0	0	0	D
Tubificoides benedii (Udekem, 1855)	0	0	0	0	1	0	0	0	0	0	0	0	D
Phoronis sp.1.	0	0	0	0	0	0	0	0	0	0	0	0	S
Ampelisca brevicornis (Costa, 1853)	0	0	1	0	0	0	0	0	1	0	0	0	S
Aoridae sp.1.	0	0	0	0	0	0	1	0	0	0	0	0	D/S
Apherusa bispinosa (Bate, 1857)	0	0	0	1	0	0	0	0	0	0	0	0	D/S
Bathyporeia sp.1. (Lindstrom, 1855)	1	0	0	0	0	0	0	0	0	0	0	0	D
Harpinia antennaria (Meinert, 1890)	0	0	0	0	0	0	0	0	0	0	1	0	D
Leucothoe lilljeborgi (Boeck, 1861)	0	0	0	0	0	0	0	0	0	0	1	0	D
Megaluropus agilis (Hoeck, 1889)	0	0	0	0	0	0	0	0	0	0	1	0	S
Perioculodes longimanus (Bate & Westwood, 1868)	0	0	0	0	0	0	0	0	1	0	0	0	S
Urothoe elegans (Bate, 1857)	0	0	0	0	1	0	0	0	0	0	0	0	D
Cylichna cylindracea (Pennant, 1777)	0	0	0	0	0	0	0	2	0	0	0	0	O/P/SC
Abra alba (Wood W., 1802)	1	0	0	0	0	2	0	1	4	0	1	2	D
Arctica islandica (Linnaeus, 1767)	1	0	0	0	0	0	1	0	0	0	0	0	S
Chamelea gallina (Linnaeus, 1758)	0	2	3	2	2	0	0	2	2	2	2	2	S
Corbula gibba (Olivi, 1792)	1	0	0	1	0	0	1	1	1	3	3	0	S
Crenella decussata (Montagu, 1808)	0	0	2	0	0	0	0	0	0	0	0	0	S
Gari juvenille	0	0	0	0	1	0	0	0	0	0	0	0	S
Lucinoma borealis (Linnaeus, 1767)	0	1	2	0	0	2	2	0	1	0	0	1	D
Nucula turgida (Gould, 1846)	0	0	0	0	0	0	0	0	1	0	0	0	D
Tellimya ferruginosa (Montagu, 1808)	0	2	0	0	0	2	0	0	3	0	2	0	
Tellina fabula (Gmelin, 1791)	5	6	4	6	5	6	3	2	2	3	3	7	D
Thracia papyracea (Poli, 1791)	1	1	0	0	0	0	1	0	0	0	0	0	S
Thracia villosiuscula (MacGillivray, 1827)	1	3	0	1	0	3	5	1	1	0	2	0	S
Thyasira flexuosa (Montagu, 1803)	2	2	0	3	2	1	2	0	1	3	0	0	S/D

*Feeding Type (FT): D = deposit feeder; S = suspension feeder; P = predator; O = omnivore; SC = scavanger

4.3.4. Multivariate Analysis

4.3.4.1. All Macrofauna Data

Figure 4.8 displays the ‘map’ produced by the multi-dimensional scale analysis. The distance between each sample shows the similarity or dissimilarity of each of them. The stress value is quite low (stress 0.18) so the plot gives a good representation of the similarity between samples (Clarke and Warwick, 1994). The pre-treatment faunal samples are all located on the left side of the plot and form a group together. The post treatment samples are all scattered towards the right hand side of the plot. The control and sediment samples are generally all intertwined with each other. Three post barite samples form a group at the bottom right corner of the plot. Three of the samples treated with barite appear to form a discrete cluster towards the bottom right of the ordination, (the fourth sample is an outlier to the top of the plot). Although subsequent analysis of the sediment indicated that all the barite had been washed away, the clear separation of the barite treated samples suggests its presence may have had some influence on faunal composition at the end of the trial period.

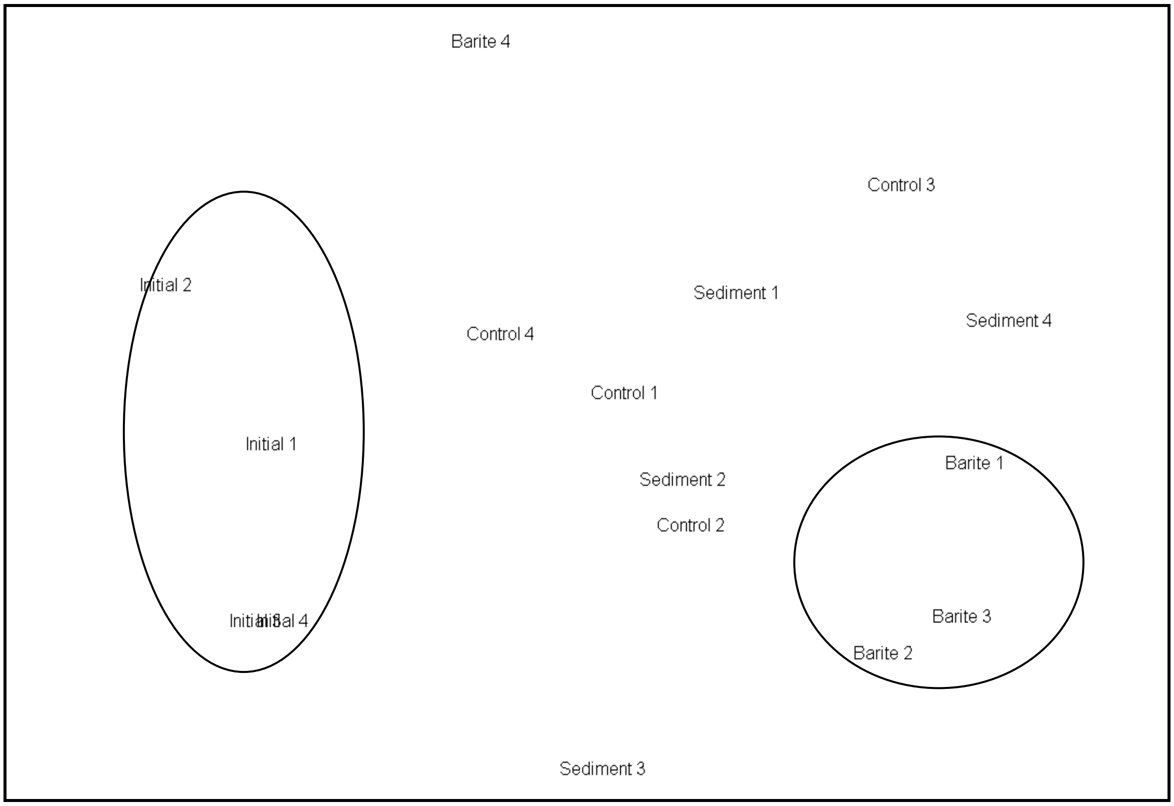


Figure 4.8: MDS plot on Ardmucknish Bay Untransformed Data (stress 0.18)

Figure 4.9 displays the 'map' produced in the $\log_{10}$ transformed data. As mentioned previously, on page 87, logging the data reduces the importance of the species present in high abundance allowing the rarer species to have a greater contribution to the distribution of the data. Figure 4.9 shows a similar pattern to the untransformed data in figure 4.8. The pre-treatment macrofaunal samples are grouped together on the left hand side of the plot. The post-treatment samples, apart from barite 4, are all distributed on the right hand side of the plot and are quite closely grouped together and are placed closer to the initial samples. Similar to the untransformed data, barite 4 is plotted alone on the map. It cannot be explained why barite 4 was plotted away from the other treatments when the data was both untransformed and transformed. The experimental plot at Ardmucknish Bay was a homogeneous flat area and barite 4 was treated in exactly the same manor as the other treatments. Also, the scouring effect was not noted to be any different from the other quadrat areas.

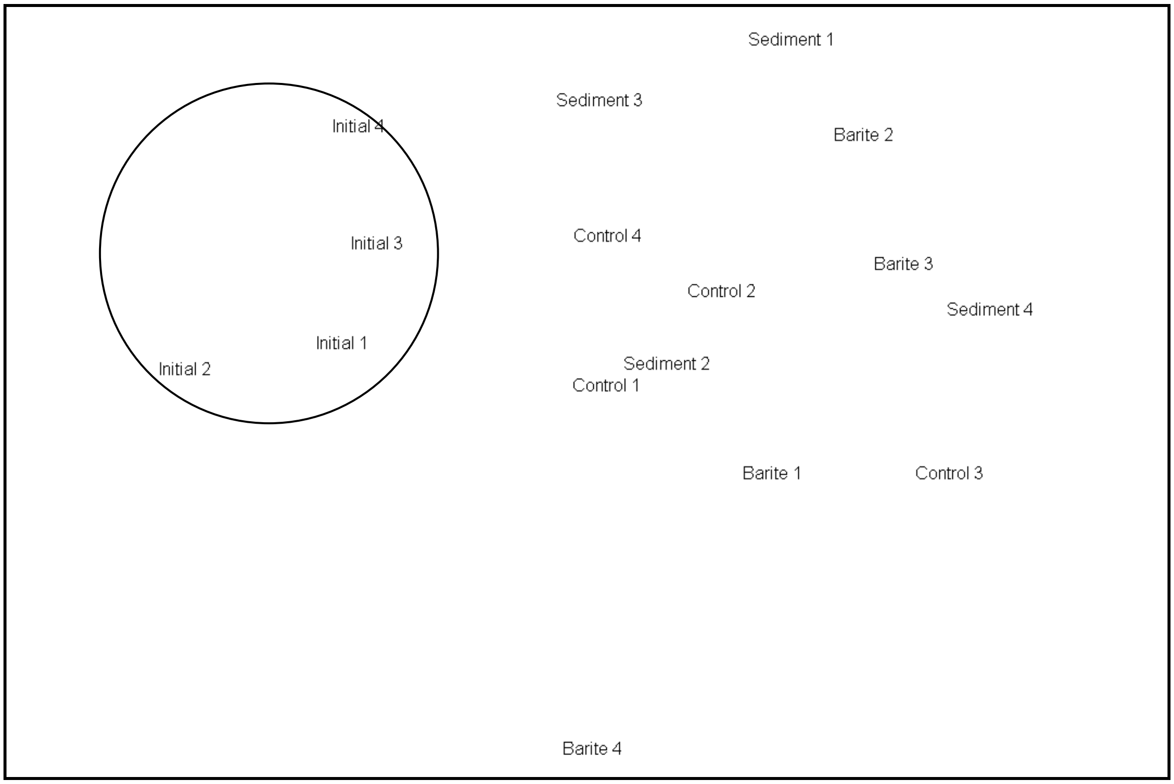


Figure 4.9: MDS plot on Ardmucknish Bay Log_{10} transformed data (Stress 0.18).

The results of the Detrended Correspondence Analysis on the Ardmucknish Bay continuous data is presented in figure 4.10. The similarities of the different treatments are displayed on a plot along two axes. Again, the four pre-treatment fauna samples form a group on the left hand side of the plot. The left to right distribution of the data points (Axis I) suggests a temporal trend in the data, the vertical distribution (Axis II) provides a weak separation of the three treatments. The DCA and MDS plots both display similar results with the general separation of the initial macrofaunal samples from the post treatment samples.

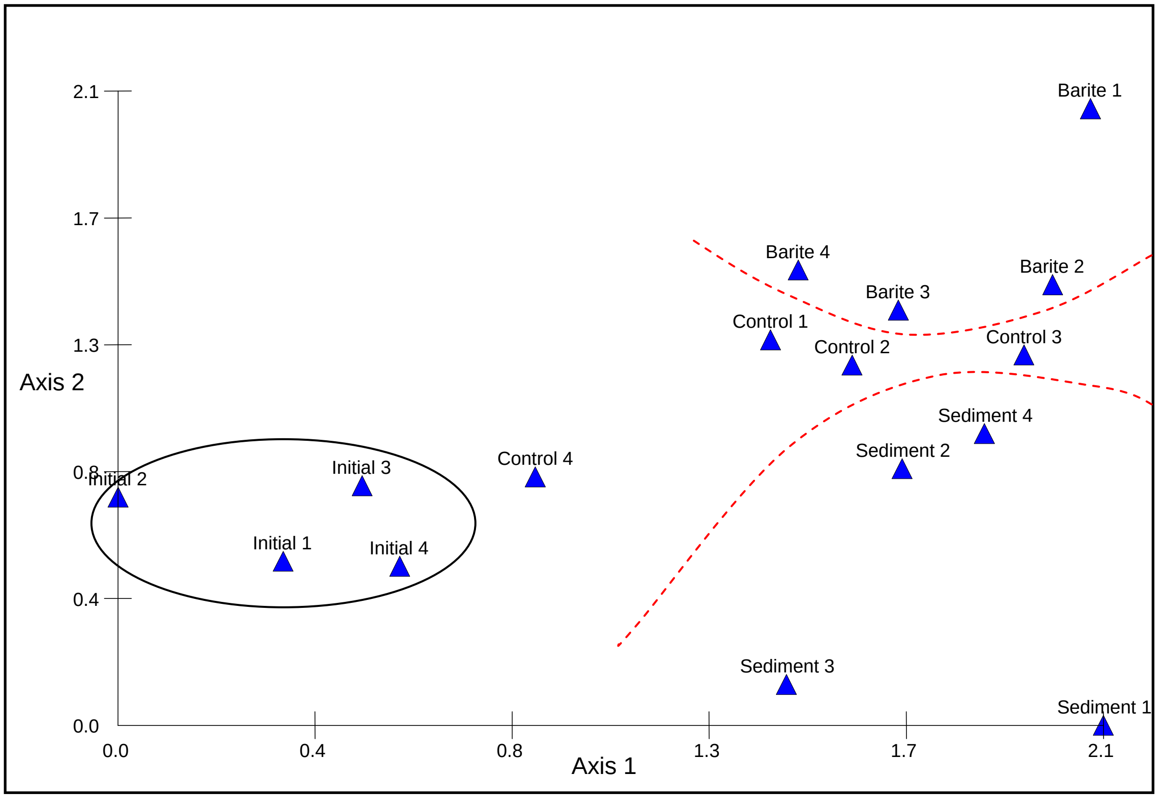


Figure 4.10: DCA Case Scores on Ardmucknish Bay Untransformed Data. Eigenvalues for axis 1 and axis 2 are 0.316 and 0.257 respectively.

The DCA run on $\log_{10}$ transformed data, figure 4.11, shows a different pattern than the untranformed data. The initial samples are still plotted on the left hand side but with the post treatment samples mapped close. The results displayed in the plots indicate that the difference between the pre-treatment and post-treatment samples is caused by the species present in high abundances, although ANOSIM did not support this statistically. The axes scores were not ranked because the only variable between the samples being studied was the introduction of barite. Any natural environmental variables would affect all treatments equally.

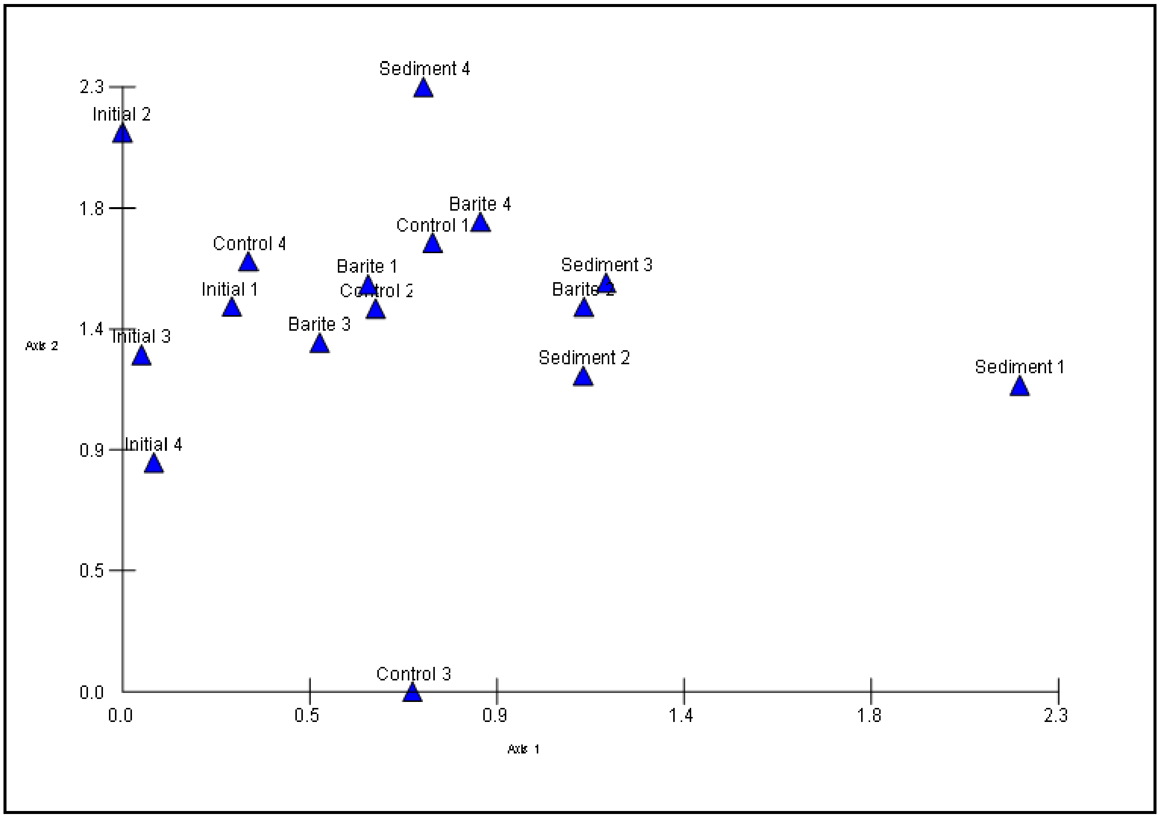


Figure 4.11: DCA Case Scores on Ardmucknish Bay Log$_{10}$ transformed data. Eigenvalues for axis 1 and axis 2 are 0.347 and 0.281 respectively.

Table 4.2 shows the level of similarity between the different samples from Ardmucknish Bay. A comparison between the initial fauna and the fauna of the three post treatments shows a level of significance of 2.9% within the continuous and log$_{10}$ data. This reveals that there is a significant difference between the fauna composition of the pre-treatment and post-treatment samples. These results coincide with the position of the initial fauna samples in the MDS plot. A comparison between the three post treatment samples (control, sediment and barite) all show a result higher than the significant level of 5%, indicating that the samples contain a similar macrofaunal structure. The logged data and presence/absence data do not differ from the continuous data with regards to the samples' significance.

Table 4.2: ANOSIM results for Ardmucknish Bay

Treatment	Continuous Data		Log_{10} Data		Presence/Absence	
	Sig. Level	R-statistic	Sig. Level	R-statistic	Sig. Level	R-statistic
I vs. C	2.9 %	0.69	2.9 %	0.58	11.4 %	0.32
I vs. S	2.9 %	0.82	2.9 %	0.7	2.9 %	0.51
I vs. B	2.9 %	0.98	2.9 %	0.73	2.9 %	0.52
C vs. S	57.1 %	-0.03	45.7 %	0.0	37.1 %	0.03
C vs. B	48.6 %	0.17	65.7 %	-0.06	85.7 %	-0.14
S vs. B	8.6 %	0.4	8.6 %	0.28	14.3 %	0.15

4.3.4.2. Post-treatment Data

More detailed analysis of the post treatment samples was carried out excluding the pre-treatment fauna, to determine if there is any trend in the data that may have been masked by temporal variations and allow a more in depth analysis of the post treatment samples. Table 4.3 displays results from the analysis of similarity run between the post six month treatments from Ardmucknish Bay. None of the significant levels between treatments falls below 5% indicating that the samples are not significantly different from one another.

Table 4.3: ANOSIM details for Ardmucknish Bay post treatment samples

Treatment	Continuous Data		Log_{10} Data		Presence/Absence	
	Sig. Level	R-statistic	Sig. Level	R-statistic	Sig. Level	R-statistic
C vs. S	57.1 %	-0.02	45.7 %	0.0	37.1 %	0.03
C vs. B	20.0 %	-0.17	34.3 %	0.07	65.7 %	-0.05
S vs. B	5.7 %	0.40	5.7 %	0.44	8.6 %	0.31

The MDS plot (figure 4.12) shows separate groupings of the barite and sediment treatments although no significant difference was found, with a value of 5.7%. The stress value of figure 10 is quite low (stress 0.18) suggesting the plot gives a good representation of the similarity between samples (Clarke and Warwick, 1994). The DCA (figure 4.13) run on the continuous data shows two groups, each containing three samples of sediment and barite, but these are not as distinct as within the MDS plot.

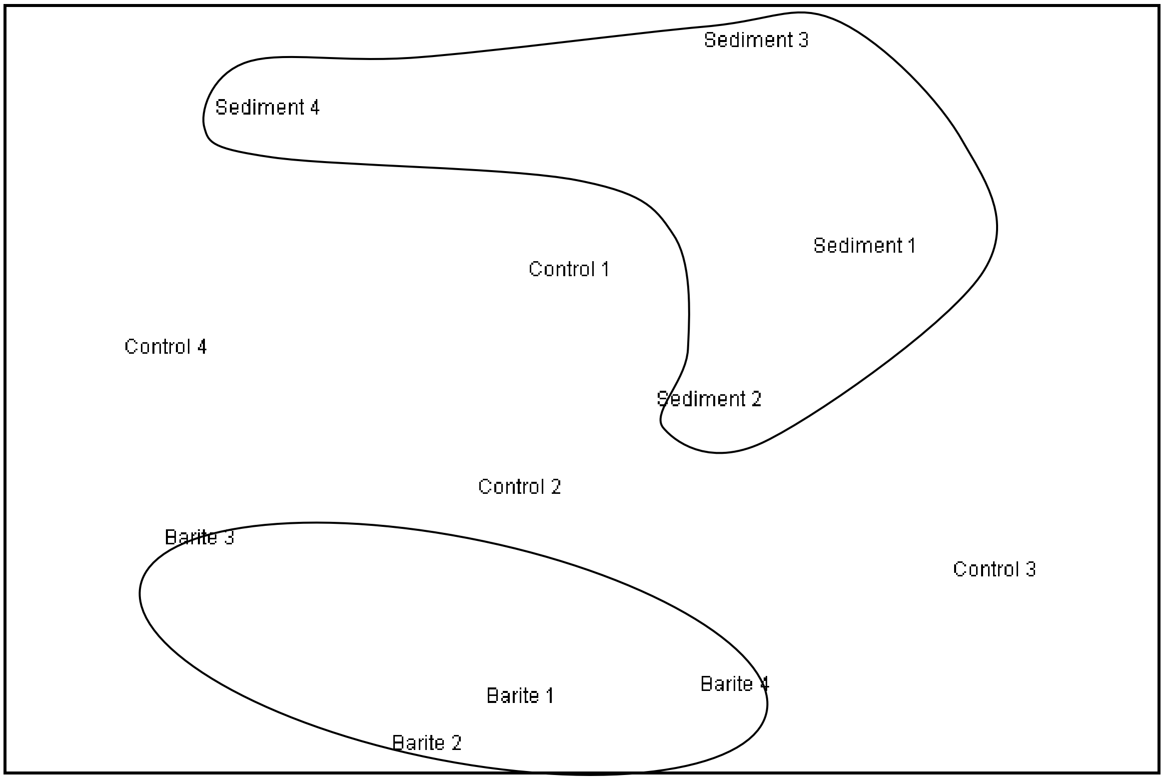


Figure 4.12: MDS plot on Ardmucknish Bay untransformed data (Stress 0.18).

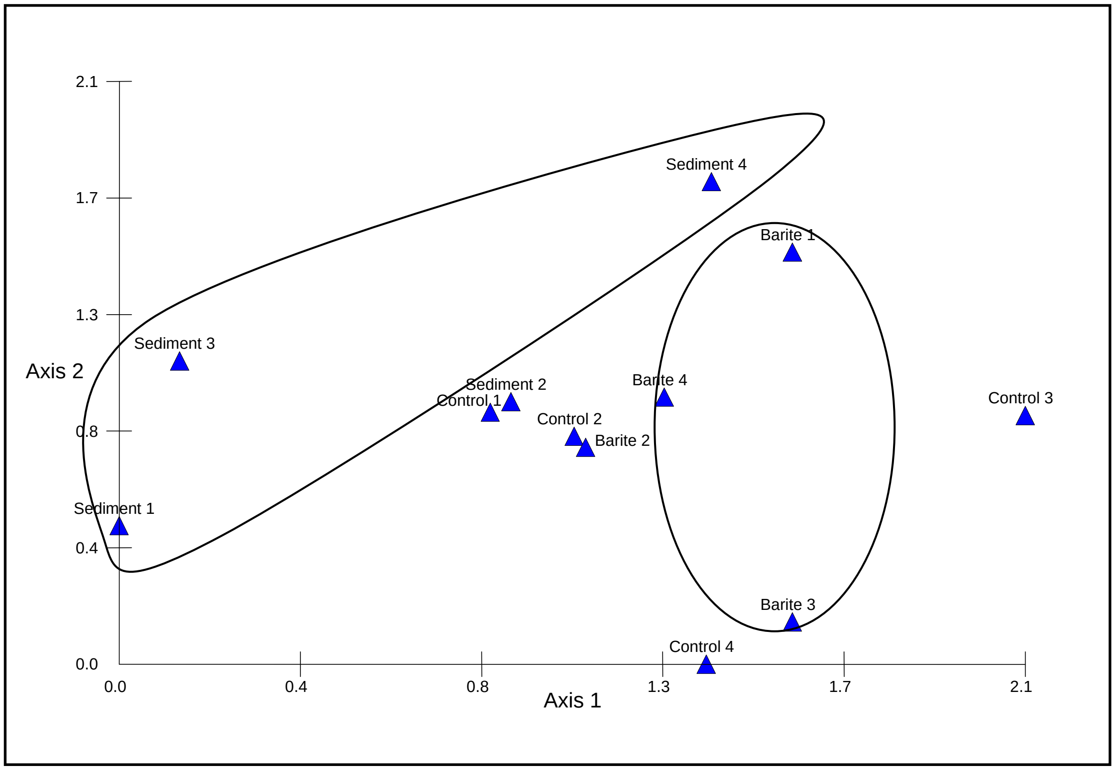


Figure 4.13: DCA on Ardmucknish Bay untransformed data. Eigenvalues for axis 1 and axis 2 are 0.325 and 0.218 respectively.

Figures 4.14 and 4.15 show Ardmucknish Bay data $\log_{10}$ transformed. Figure 82 displays a MDS plot and shows three groupings, separately containing control, sediment and barite treatments. Control 3 and sediment 4 are plotted separately in the 'map'. These results resemble the untransformed MDS shown above in figure 4.12. The DCA plot, figure 4.15, shows the sediment samples 1, 2 and 3 grouped together, the same group displayed in figure 4.14. Unlike the MDS, the DCA plot shows the remaining samples distributed on the top right hand side with no real groupings. Control 3 is plotted away from the other samples in both figures 4.14 and 4.15. Control 3 is also plotted away from the other samples in the untransformed plots. Control 3 contains a few species, either in low numbers or as lone individuals, in comparison to the other control samples. Some of the species present only in control 3 are *Owenia fusiformis, Poechilochaetus serpens, Eteone longa* and *Crenella decussata*.

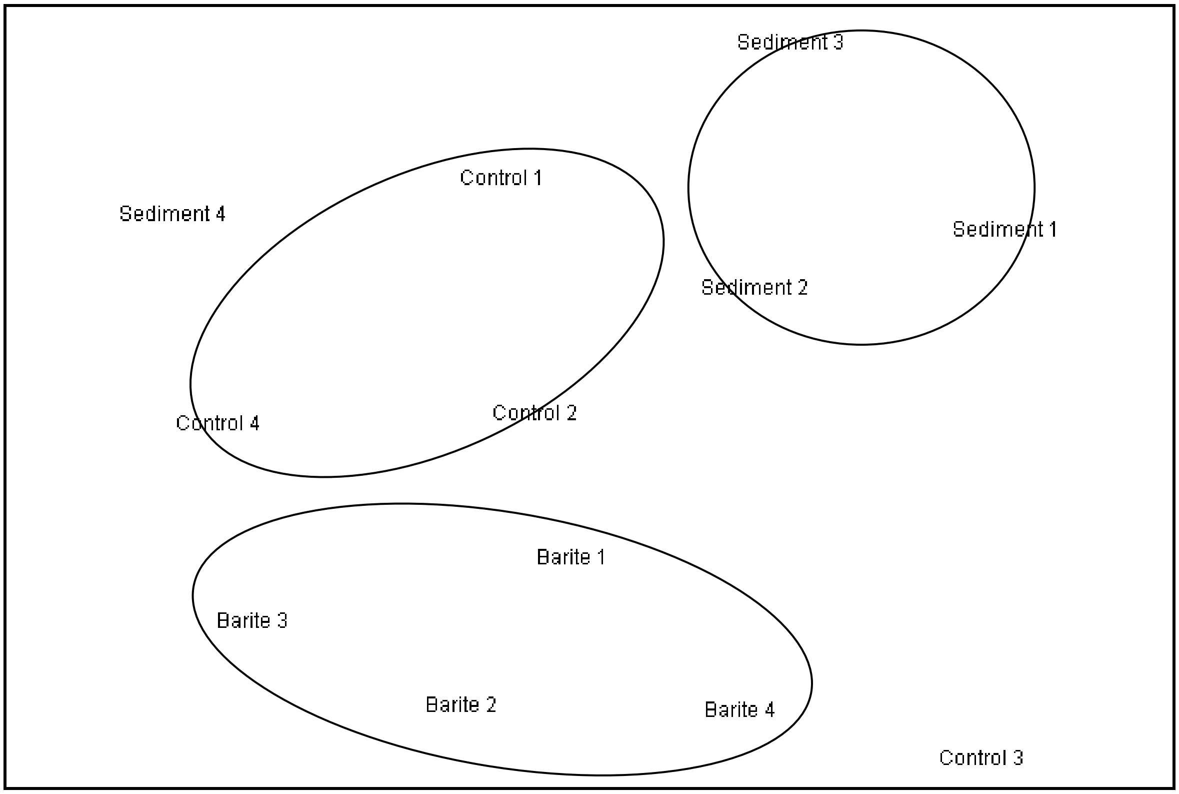


Figure 4.14: MDS plot on Armucknish bay post treatment $\log_{10}$ data (Stress 0.16).

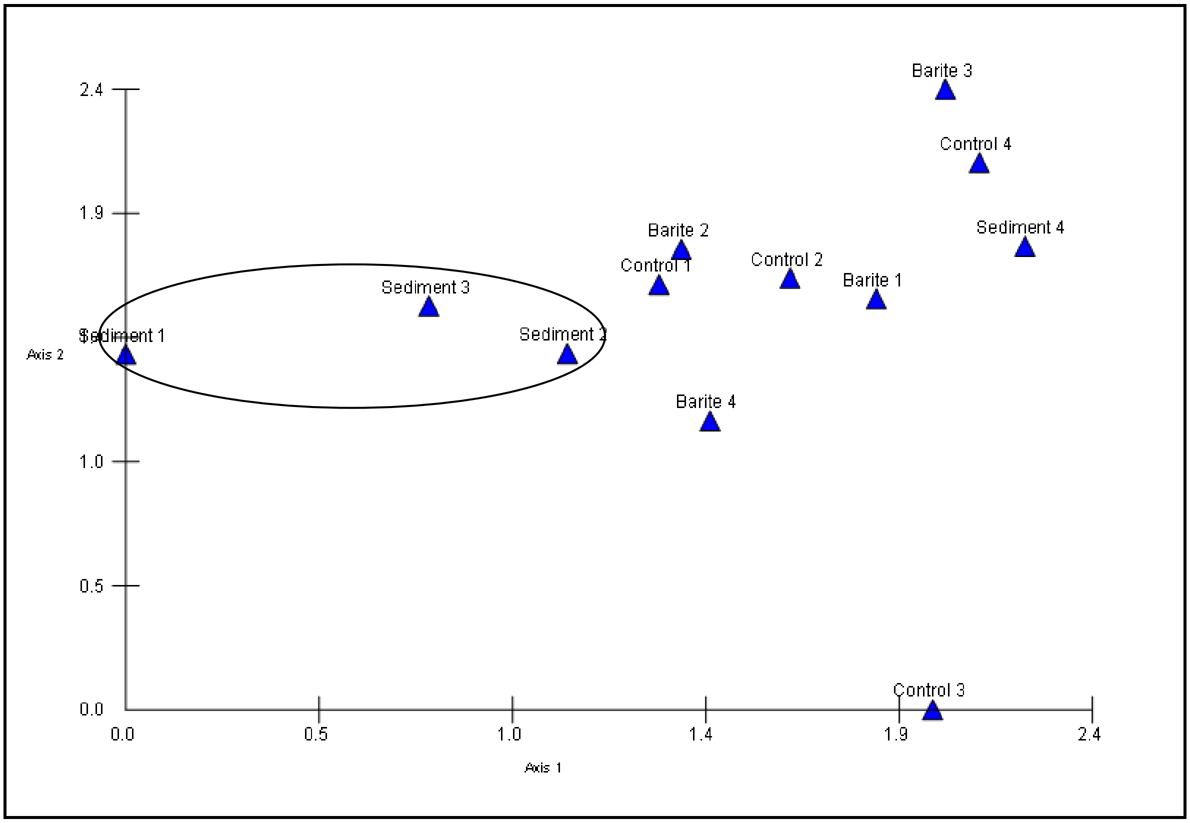


Figure 4.15: DCA on Ardmucknish Bay post treatment $\log_{10}$ data. Eigenvalues for axis 1 and axis 2 are 0.378 and 0.318 respectively.

The ANOSIM results indicate that there is no significant difference between the macrofauna within the control, sediment and barite treatments when the data are untransformed, $\log_{10}$ transformed or within the presence/absence data. Although no significant difference was found, the MDS plot did indicate that there were groupings between the treatments. Further analysis, table 4.5, determined that annelids were responsible for the groupings seen in the previous plots. ANOSIM run on Mollusca, table 4.4, showed that this group did not contribute significantly to the results gained.

Table 4.4: ANOSIM on Ardmucknish Bay post treatment Mollusca.

	Continuous data		Log_{10} Data	
Treatment	Sig. Level	R-statistic	Sig. Level	R-statistic
C,S	100 %	-2.29	100 %	-0.37
C,B	37.1 %	-0.08	42.9 %	-0.60
S,B	65.7 %	-0.10	68.9 %	-0.16

Table 4.5: ANOSIM on Ardmucknish Bay post treatment Annelids

	Continuous data		Log_{10} Data	
Treatment	Sig. Level	R-statistic	Sig. Level	R-statistic
C,S	20 %	0.16	14.3 %	0.29
C,B	42.9 %	0.01	40.0 %	0.05
S,B	5.7 %	0.5	2.9 %	0.54

Table 4.5 shows the results from the ANOSIM run on the Annelid data from the three treatments. A significant difference was found between the sediment and barite treatments when the data was $\log_{10}$ transformed. Figure 4.16 shows the annelid untransformed data and figure 4.17 shows the annelid data $\log_{10}$ transformed. The stress values are low (stress 0.14 and 0.16 respectively) showing that the MDS plots give a good representation of the similarity between samples (Clarke and Warwick, 1994). In both plots there are two separate groupings, one being the sediment treatment and the other being the barite treatment. Each control sample is distributed separately over each plot.

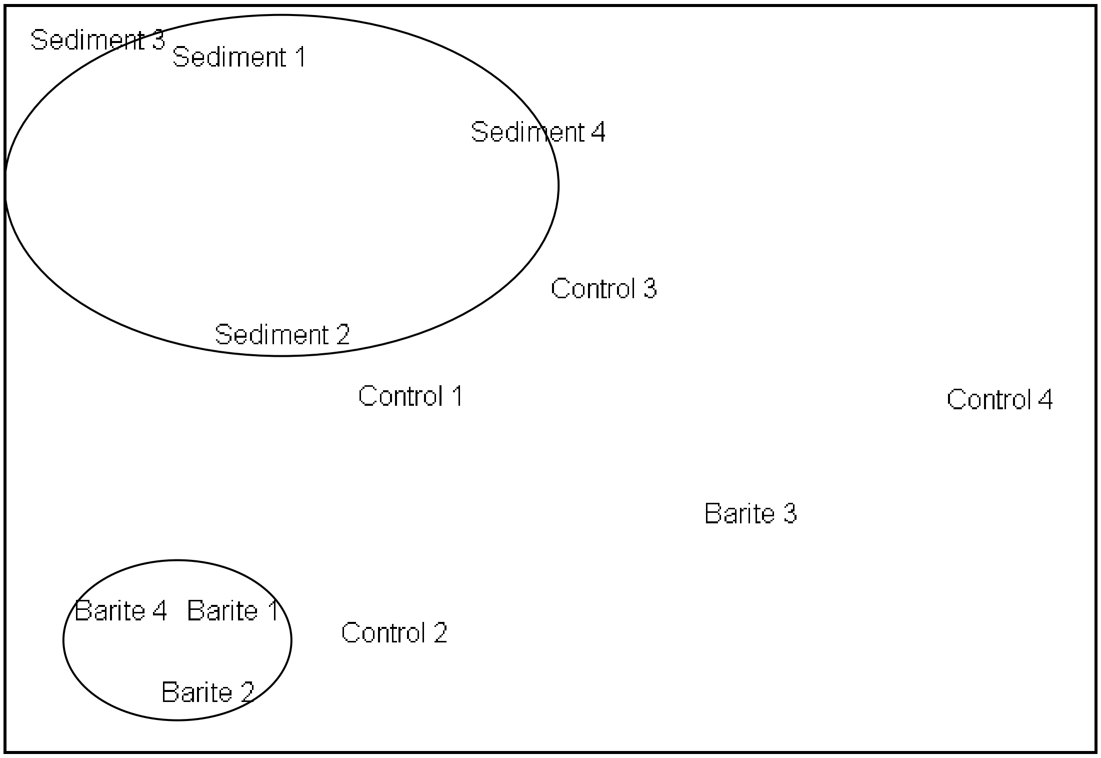


Figure 4.16: MDS on Ardmucknish Bay post treatment annelid untransformed data (stress 0.14).

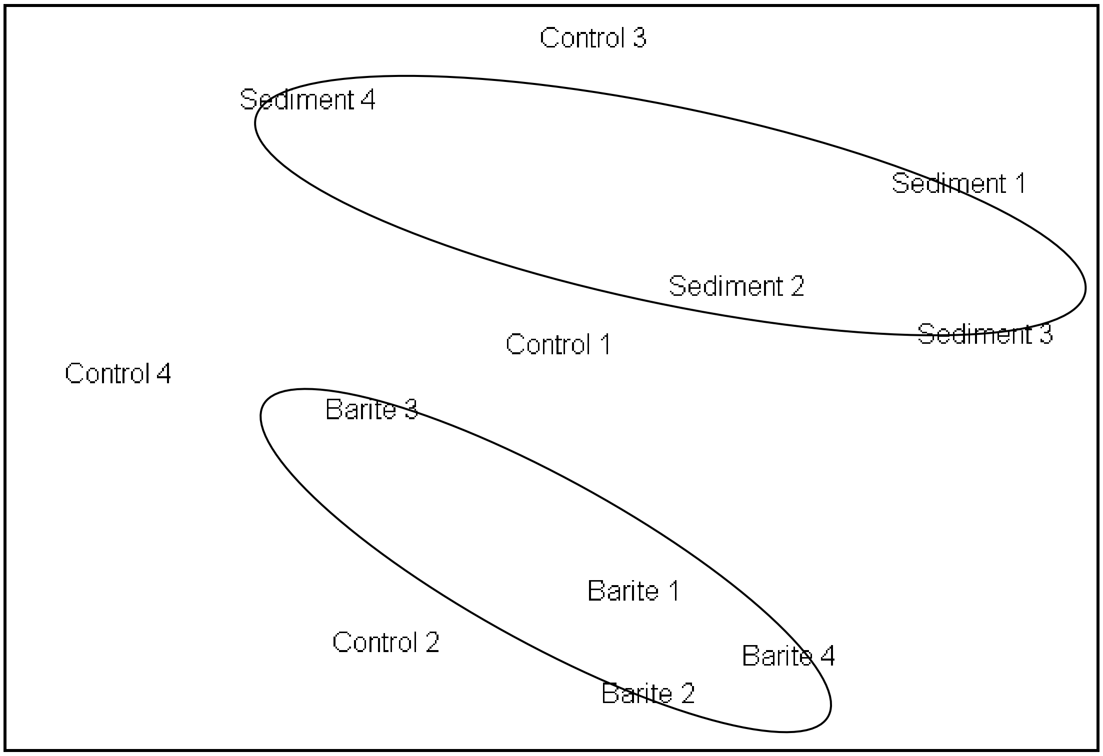


Figure 4.17: MDS plot on Ardmucknish Bay post treatment annelid $\log_{10}$ transformed data (Stress 0.16).

A Levene test, which tests the homogeneity of variances, was run on each of the individual species identified in all Ardmucknish Bay post treatment samples. This test indicates whether a parametric (analysis of variance) or non parametric (Kruskal-Wallis) test should be performed on the data. These tests could not be completed on species that were present in only one treatment or present only as one individual, which would not really affect the data analysis, as any species present as a lone individual can not really be used to analyse the introduction of a foreign substance onto the seabed.

Only one species was found to have a statistically significant difference regarding numbers present between treatments. Analysis of variance followed by the *post hoc* Tukey test showed that *Poechilochaetus serpens* was present in significantly different numbers between treatments with a p-value of 0.033. The polychaete *Poechilochaetus serpens* was present in higher numbers within the sediment treatment in comparison to both the control and barite treatments. SIMPER was run to identify the species primarily responsible for dissimilarity between the post treatment samples. SIMPER (Similarity Percentage) is used to assess which taxa are primarily responsible for an observed difference between groups of samples. The SIMPER results are displayed below and show the average dissimilarity between the treatments and the species that primarily contribute to the difference.

Table 4.6 shows the species that are responsible for the level of dissimilarity, 53.99%, between the control and sediment treatments. The four main contributors of dissimilarity between the control and sediment treatments were all annelids. Apart from *Magelona papillicornis,* the other three species, *Poechilochaetus serpens, Exogene sp, and Aricidae sp,* average abundance increased in the sediment treatment in comparison to the controls. This was probably caused by an increase in organic matter, but because the sediment was not analysed for organic content this cannot be determined for definite.

Groups: Control and Sediment

Average dissimilarity = 53.99%

Table 4.6: SIMPER dissimilarity results for control vs. sediment treatments.

Species	Control Average abundance	Sediment Average abundance	Contribution to dissimilarity %	Cumulative %
Poechilochaetus serpens	0.25	2.50	6.78	6.78
Exogene sp.	0.00	2.00	6.02	12.80
Magelona papillicornis	2.00	1.75	5.68	18.48
Aricidae sp.	4.00	4.40	5.57	24.05

The four main species responsible for the level of dissimilarity, 53.77%, between the control and barite treatments are shown in table 4.7. The main species responsible for the dissimilarity is the polychaete *Aricidae* which average abundance increases from the control to barite treatments. The bivalve *Tellina fabula* is the second main contributor to the level of dissimilarity, with its average abundance decreasing within the barite treatments.

Groups: Control and Barite

Average dissimilarity = 53.77%

Table 4.7: SIMPER results for control vs. barite treatments.

Species	Control Average abundance	Barite Average abundance	Contribution to dissimilarity %	Cumulative %
Aricidae sp.	4.00	7.50	14.46	14.46
Tellina fabula	5.25	3.75	7.44	21.89
Nephtys longosetosa	2.75	1.50	6.57	28.47
Magelona papillicornis	2.00	1.00	5.43	33.89

The highest dissimilarity found was between the sediment and barite treatments, at 58.1%. The polychaete *Aricidae* was the highest contributor to dissimilarity between the barite treatment and both the control and sediment treatments, with its abundance highest in the barite treatments, although, when ANOVA was run the numbers present were not found to be significantly different. The results in table 4.6 show that *Poechilochaetus serpens* was the main contributor for the difference between the control and sediment treatments. It was the second highest contributor for dissimilarity between the sediment and barite treatments (table 4.8). These findings from SIMPER coincide with the results gained from performing the ANOVA. The average abundance of the bivalve *Tellina fabula* decreased in the barite treatments as also seen in table 4.7 comparing the control and barite treatments.

Groups: Sediment and Barite

Average dissimilarity = 58.10%

Table 4.8: SIMPER results for sediment vs. barite treatments.

Species	Sediment Average abundance	Barite Average abundance	Contribution to dissimilarity %	Cumulative %
Aricidae sp.	4.40	7.50	11.64	11.64
Poechilochaetus serpens	2.50	0.50	5.97	17.60
Exogene sp.	2.00	0.00	5.56	23.16
Tellina fabula	4.00	3.75	5.43	28.59

4.3.5. Barium Analysis

Treatment	Depth (cm)	Barium (ug/l)
Control 1	0-2	2128
Control 2	0-2	2031
Control 3	0-2	2647
Sediment 1	0-2	2351
Sediment 2	0-2	1728
Sediment 3	0-2	1891
Barite 1	0-2	3333
Barite 2	0-2	1630
Barite 3	0-2	5535
Barite 4	0-2	2444
Barite 1	2-4	1216
Barite 2	2-4	990

Table 4.9: Barium Levels in Ardmucknish Bay

The levels of barium remaining in the sediment after the six month experimental period were calculated. The barium levels, detected on the ICPMS, of the three treatments are shown in table 4.9. A one-way ANOVA run on the barium levels (from 0-2cm) showed no significant difference between treatments with a p-value of 0.447. The levels of barium present were of an expected background level indicating that the barite introduced onto the seabed had been dispersed and therefore the benthic results gained were determined by natural environmental variables rather than the introduction of barite.

4.4. Sound of Shuna Results

4.4.1. In-situ Observations

In the Sound of Shuna there was no evidence of sediment erosion, and barite was still visible in the four barite treatment quadrats.

4.4.2. Benthic Community of the Sound of Shuna

The benthic community of the Sound of Shuna resembles the *Abra* assemblage grading into the *Amphiura* assemblage (Gray and Elliott, 2009). The community is characterized by soft bottom dwelling species like *Thyasira, Amphiura* and *Nephtys.* The initial and post treatment samples, shown on pages 121 to 126, were both dominated by polychaetes of mixed feeding types including deposit and suspension feeders, omnivores and predators. The second most dominant group for the initial samples were bivalves followed by the malacostraceans. The post treatment samples differed however, with the malacostraceans being the second dominant group followed by the bivalves.

4.4.3. Univariate Faunal Parameters

Diversity indices were calculated for the initial and post treatment macrofauna and are shown in table 4.10. Each value (I = initial, C = control, S = sediment, B = barite) was derived from three macrofaunal cores. As mentioned in earlier, the initial data is composed from three random cores taken from the 12 initial cores collected from outside each quadrat. The control, sediment and barite results were derived from the combined data from each of the three macrofaunal cores collected from inside each quadrat. The pre-treatment samples contained fewer individuals and species than the post treatment macrofauna samples. Each post treatment sample contains similar macrofaunal abundance in regards to the numbers present. The results from the Shannon-Wiener showed that the initial samples 2-4 contained lower values than the remaining samples, indicating a minor difference in regards to the distribution of the numbers of individuals. The Pielou's evenness results ranged from 0.8 to 1.0 showing that the samples from the Sound of Shuna contained species of even distribution.

Table 4.10: Univariate statistical parameters for Sound of Shuna data (S = Total Species, N = Total Individuals, H’ = Shannon Weiner, J’ = Pielou’s evenness.

Treatment	S	N	H'	J'
Initial 1	19	31	2.726	0.9259
Initial 2	13	21	2.442	0.9519
Initial 3	7	14	1.567	0.8053
Initial 4	13	22	2.374	0.9256
Control 1	34	92	3.172	0.8994
Control 2	39	65	3.441	0.9392
Control 3	39	94	3.069	0.8376
Control 4	41	133	2.807	0.7558
Sediment 1	31	79	3	0.8735
Sediment 2	27	81	2.936	0.8908
Sediment 3	33	101	3.094	0.8848
Sediment 4	38	135	3.041	0.836
Barite 1	40	100	3.33	0.9028
Barite 2	30	90	3.926	0.8604
Barite 3	22	73	2.631	0.8511
Barite 4	36	102	3.156	0.8806

A one-way ANOVA was run on each of the diversity indices followed by a Tukey test to determine if there was any statistically significant difference between the treatments. A significant difference was found between the initial faunal samples and all the post treatment samples when the ANOVA was run on total species (p=0.000), total individuals (p=0.000) and the Shannon-Wiener indice (p=0.017). This indicated that the initial macrofaunal samples and post treatment macrofaunal samples contained a different species abundance. The initial and post treatment samples contained species of similar distribution as shown by a p-value result of 0.644, when ANOVA was run on Pielou’s evenness.

Sound of Shuna Pre-treatment Macrofauna

Species	**C1**	**C2**	**C3**	**C4**	**S1**	**S2**	**S3**	**S4**	**B1**	**B2**	**B3**	**B4**	**FT***
Ampelisca tenuicornis (Liljeborg, 1855)	0	0	0	0	1	0	0	0	1	0	0	0	S
Corophium sp.1.	0	0	0	0	0	0	0	0	0	1	0	0	
Harpinia antennaria (Meinert, 1890)	0	0	0	0	0	0	1	1	0	1	0	0	D
Amphiura filiformis (O.F. Müller, 1776)	0	1	0	2	3	1	3	2	3	2	2	2	S
Corbula gibba (Olivi, 1792)	3	0	0	2	0	0	0	0	0	0	0	0	S
Chaetoderma nitidulum (Loven, 1844)	0	0	0	0	0	0	0	0	0	2	0	0	O/P/SC
Dosinia lupinus (Linnaeus, 1758)	0	0	0	0	0	0	0	0	3	0	0	0	S
Kurtiella bidentata (Montagu, 1803)	2	0	1	1	2	2	0	2	1	0	0	1	S
Phaxas pellucidus (Pennant, 1777)	1	0	0	0	0	0	0	0	0	0	0	1	S
Thracia papyracea (Poli, 1791)	0	0	0	0	0	1	0	0	0	0	0	0	S
Thracia villosiuscula (MacGillivray, 1827)	0	0	0	0	0	0	0	0	0	0	1	0	S
Thyasira flexuosa (Montagu, 1803)	2	0	0	0	0	1	0	0	0	0	1	0	S/D
Golfingia	0	0	0	0	0	0	2	1	0	0	0	1	P
Nemertine sp 1	0	0	0	0	0	0	0	0	0	1	0	0	P
Nemertine sp 2	0	0	1	0	0	0	0	0	0	0	1	0	P
Cerianthus lloydi (Gosse, 1859)	0	0	1	0	0	0	0	0	0	0	0	0	P
Halcampa chrysanthellum (Peach in Johnston, 1847)	0	1	0	0	0	0	0	0	1	0	0	0	O/P/SC
Chaetozone setosa (Malmgren, 1867)	0	0	0	0	0	0	0	0	0	0	1	0	D
Diplocirrus glaucus (Malmgren, 1867)	0	0	0	0	1	0	0	0	0	0	0	0	D
Harmothoe sp	0	0	0	1	0	0	0	0	0	0	0	0	

Sound of Shuna pre-treatment macrofauna (cont.)

Species	**C1**	**C2**	**C3**	**C4**	**S1**	**S2**	**S3**	**S4**	**B1**	**B2**	**B3**	**B4**	**FT***
Heteromastus filiformis (Claparède, 1864)	0	0	0	0	0	0	1	0	0	0	0	0	D
Lumbrineris gracilis (Ehlers, 1868)	0	0	0	0	0	0	0	0	0	1	0	0	O/P/SC
Lumbrineris latreilli (Audouin & Milne Edwards, 1834)	0	0	0	0	1	0	0	0	0	0	1	0	O/P/SC
Maldonid sp 1	0	0	0	0	0	0	0	0	0	1	0	0	
Melinna palmata (Grube, 1870)	0	0	1	0	0	0	0	0	0	0	0	0	S/D
Nephtys hombergii (Savigny in Lamarck, 1818)	2	0	1	0	0	0	1	0	0	1	0	0	O/P/SC
Notomastus latericeus (Sars, 1851)	1	0	1	1	0	0	0	0	1	0	0	0	D
Pholoe minuta (Fabricius, 1780)	1	1	0	0	0	0	0	0	0	0	0	0	O/P/SC
Phyllodoce (Anaitides) maculata (Linnaeus, 1767)	0	0	0	1	0	0	0	0	0	0	0	0	O/P
Praxillella affinis (M. Sars in G.O. Sars, 1872)	0	2	0	0	0	1	0	0	0	1	0	0	D
Rhodine gracilior (Tauber, 1879)	0	0	0	1	0	0	0	0	0	0	0	0	D
Spiophanes kroyeri (Grube, 1860)	0	1	0	0	0	0	2	0	1	0	0	0	D
Tubificoides benedii (Udekem, 1855)	0	0	0	0	0	0	0	0	0	1	0	0	D

*Feeding Type (FT): D = deposit feeder; S = suspension feeder; P = predator; O = omnivore; SC= scavenger.

Sound of Shuna Post-treatment Macrofauna

Species	**C1**	**C2**	**C3**	**C4**	**S1**	**S2**	**S3**	**S4**	**B1**	**B2**	**B3**	**B4**	**FT***
Amage adspersa (Grube, 1863)	0	0	1	1	0	0	2	0	0	0	0	0	D
Ampharete acutifrons (Grube, 1860)	0	0	0	0	1	0	1	0	1	0	0	0	D
Amphicteis gunneri (M.sars, 1835)	1	0	0	0	0	1	0	0	0	0	0	0	D
Arabella iricolor (Montagu, 1804)	0	1	0	0	0	0	0	0	0	0	0	1	D
Aricidea jeffreysi (McIntosh, 1879)	0	0	0	2	0	0	0	0	0	0	0	0	D
Aricidea sp.2.	0	0	1	1	0	0	0	1	0	0	0	0	D
Chaetozone caputesocis (Saint-Joseph, 1894)	7	0	1	2	1	2	3	2	5	3	0	1	S/D
Chaetozone setosa (Malmgren, 1867)	2	2	1	3	5	5	0	2	1	1	0	6	D
Diplocirrus glaucus (Malmgren, 1867)	1	1	1	0	0	0	1	0	0	1	0	0	D
Dorvillid sp 1	0	1	0	1	0	0	0	0	0	0	0	0	P
Enteroptnuest sp 1	0	0	0	1	0	0	0	0	0	0	0	0	S
Eteone flava (Fabricius, 1780)	0	0	0	0	0	0	0	1	0	0	0	0	P/D
Exogone (Parexogone) hebes (Webster & Benedict, 1884)	0	1	0	1	1	0	0	0	1	2	0	2	O/P/SC
Glossobalanus marginatus	0	1	0	0	0	0	0	1	0	0	0	0	D
Glycera alba (O.F.Müller, 1776)	1	0	0	0	0	0	0	0	0	0	0	0	O/P/SC
Glycera rouxi (Audouin & Milne Edwards, 1833)	0	0	0	0	0	0	0	0	2	0	0	0	O/P/SC
Gyptis capensis (Day, 1963)	0	0	0	1	1	0	0	0	0	0	0	1	O/P/SC
Heteromastus filiformis (Claparède, 1864)	0	1	1	0	0	0	1	0	1	0	0	0	D
Lumbrineris gracilis (Ehlers, 1868)	0	0	0	0	1	0	0	0	0	0	0	1	O/P/SC
Lumbrineris latreilli (Audouin & Milne Edwards, 1834)	1	2	1	0	1	1	1	3	2	2	2	0	O/P/SC
Magelona alleni (Wilson, 1958)	0	0	0	2	0	0	0	0	0	0	0	0	S/D
Magelona papillicornis (F.Müller, 1858)	5	1	6	4	4	2	3	11	4	3	0	9	S/D
Melinna palmata (Grube, 1870)	0	2	1	0	0	1	0	1	2	3	2	0	S/D
Myriochele heeri (Malmgren, 1867)	1	0	0	1	0	0	0	0	0	2	0	0	S/D
Nephtys caeca (Fabricius, 1780)	0	0	1	1	1	0	5	1	0	3	4	1	O/P
Nephtys hombergii (Savigny in Lamarck, 1818)	1	1	0	0	2	1	0	1	0	2	1	1	O/P/SC
Nephtys longesetosa (Örsted, 1842)	0	1	1	0	0	2	0	0	0	0	0	0	O/P

Sound of Shuna post-treatment macrofauna (cont.)

Species	**C1**	**C2**	**C3**	**C4**	**S1**	**S2**	**S3**	**S4**	**B1**	**B2**	**B3**	**B4**	**FT***
Notomastus latericeus (Sars, 1851)	0	2	0	0	1	3	0	0	1	1	0	1	D
Oligochaete sp 1	1	0	2	4	0	0	0	1	0	0	0	2	D
Oligochaete sp.2.	0	0	0	0	0	0	1	0	0	0	0	0	D
Ophelina aulogaster (Rathke, 1843)	0	2	0	0	0	0	0	0	0	0	0	0	D
Owenia fusiformis (Delle Chiaje, 1844)	0	0	5	0	0	0	0	1	1	1	0	0	D
Paraonis gracilis (Tauber, 1879)	4	3	0	1	1	5	3	2	0	0	1	1	D
Pectinaria (Amphictene) auricoma (O.F. Müller, 1776)	0	0	0	1	1	0	0	0	0	0	0	0	D
Petaloproctus sp.1.	0	1	0	0	1	0	0	0	2	0	0	0	D
Pholoe minuta (Fabricius, 1780)	4	0	0	1	0	1	0	0	1	1	0	0	O/P/SC
Phyllodoce (Anaitides) maculata (Linnaeus, 1767)	0	0	1	0	0	0	0	0	0	0	0	0	O/P
Phyllodoce sp.1.	0	0	1	0	0	0	0	0	2	0	1	0	O/P
Pista cristata (Müller, 1776)	0	0	0	1	0	0	0	0	0	0	0	0	D
Polycirrus medusa (Brube, 1850)	0	0	0	0	0	0	0	0	1	0	0	0	D
Polydora haplura (Claparède, 1869)	4	1	2	0	0	0	2	2	2	0	0	2	S/D
Praxillella affinis (M. Sars in G.O. Sars, 1872)	2	1	2	7	8	1	1	2	8	0	0	2	D
Prionospio malmgreni (Claparède, 1869)	4	0	1	0	1	0	2	1	0	0	0	0	D
Prionospio sp.1.	1	0	21	41	6	17	8	21	8	11	18	14	D
Rhodine gracilior (Tauber, 1879)	3	1	5	6	1	2	5	2	2	1	0	1	D
Sabellid sp.1.	1	0	0	1	0	0	1	1	1	0	0	0	S
Scalibregma inflatum (Rathke, 1843)	2	1	0	1	2	5	6	1	0	1	0	0	D
Scoloplos (Scoloplos) armiger (Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	1	D
Spio filicornis (Müller, 1776)	14	0	1	0	0	0	2	5	1	0	0	0	S/D
Spiophanes bombyx (Claparède, 1870)	1	0	1	1	0	0	0	0	0	0	0	0	S/D
Spiophanes kroyeri (Grube, 1860)	5	0	0	1	1	1	1	4	6	4	3	2	D
Sthenelais limicola (Ehlers, 1864)	1	1	1	1	1	0	1	0	3	1	3	4	O/P
Streblospio sp.1. (Webster, 1879)	2	3	12	17	0	1	3	9	14	20	6	5	S/D
Terebellidae sp.1. (Mamlgren, 18650	1	0	0	0	0	0	0	0	0	1	0	0	D

Sound of Shuna post-treatment macrofauna (cont.)

Species	**C1**	**C2**	**C3**	**C4**	**S1**	**S2**	**S3**	**S4**	**B1**	**B2**	**B3**	**B4**	**FT***
Terebellidae stroemi (Sars, 1835)	0	0	0	0	0	0	1	1	0	0	0	0	D
Tharyx marioni (Saint-Joseph, 1894)	0	1	0	0	1	5	1	4	2	0	1	4	D
Trichobranchus glacialis (Malmgren, 1866)	0	0	0	0	0	0	0	0	0	0	1	0	D
Tubificoides benedii (Udekem, 1855)	1	0	0	0	11	6	20	17	2	0	0	0	D
Ampelisca tenuicornis (Liljeborg, 1855)	0	0	0	0	0	0	0	0	2	0	0	1	S
Araphura brevimanus (Lilljeborg, 1864)	0	0	1	0	0	0	0	0	0	1	0	1	S/D
Carcinus maenas (Linnaeus, 1758)	0	1	0	0	0	0	0	0	0	0	0	0	O/P/SC
Eudorella truncatula (Bate, 1856)	0	0	0	0	0	0	1	0	0	0	0	1	S/D
Gammaropsis (Liljeborg, 1855)	0	1	0	0	0	0	0	0	0	0	0	0	S/D
Harpinia antennaria (Meinert, 1890)	0	1	1	1	0	1	4	1	0	3	1	3	D
Iphinoe serrata (Norman, 1867)	0	0	0	0	0	0	0	1	0	1	0	1	S/D
Leptocheirus pectinatus (Norman, 1869)	0	0	0	0	0	0	0	0	0	0	0	1	S
Leucothoe lilljeborgi (Boeck, 1861)	0	0	0	0	0	0	0	0	0	0	0	2	D
Melita palmata (Montagu, 1804)	0	1	0	0	0	0	0	0	1	0	0	1	P/S
Munida rugosa (Fabricius, 1775)	0	0	1	0	0	0	0	0	0	0	1	0	O/P/SC
Pagurus bernhardus (Linnaeus, 1758)	1	0	0	0	0	0	0	0	1	1	0	0	O/P/SC
Pandalidae sp.1.	0	0	0	0	0	0	0	0	1	0	0	0	O/P/SC
Pandalina brevirostris (Rathke, 1843)	0	0	0	1	0	0	0	0	0	0	0	0	O/P/SC
Synchelidium haplocheles (Grube, 1864)	0	0	0	1	0	0	0	0	0	1	0	0	D
Typhlotanais microcheles (G.O. Sars, 1882)	0	0	0	0	0	0	0	1	0	0	0	1	
Abra nitida (O.F. Müller, 1776)	3	2	1	10	3	2	5	10	4	0	8	12	D
Arctica islandica (Linnaeus, 1767)	0	0	0	1	0	0	0	0	0	0	0	0	S
Chaetoderma nitidulum (Loven, 1844)	0	2	1	1	0	0	0	0	0	0	0	0	O/P/SC
Chamelea gallina (Linnaeus, 1758)	0	0	2	0	2	1	1	0	1	2	1	0	S
Corbula gibba (Olivi, 1792)	0	2	1	1	0	0	3	1	0	3	5	0	S
Cylichna cylindracea (Pennant, 1777)	0	1	0	0	0	0	0	0	0	0	0	0	O/P/SC
Dosinia lupinus (Linnaeus, 1758)	0	1	0	0	0	0	0	0	0	0	0	0	S
Kurtiella bidentata (Montagu, 1803)	8	9	1	0	9	3	0	10	4	5	6	6	S

Sound of Shuna post-treatment macrofauna (cont.)

Species	C1	C2	C3	C4	S1	S2	S3	S4	B1	B2	B3	B4	FT*
Mya truncata (Linnaeus, 1758)	0	0	0	1	0	0	0	0	0	0	0	0	S
Myrtea spinifera (Montagu, 1803)	0	0	0	0	0	0	0	0	1	0	1	1	S
Mysia undata (Pennant, 1777)	1	0	0	0	0	0	0	1	0	0	0	0	S
Nucula turgida (Gould, 1846)	0	0	0	0	1	0	1	0	1	0	0	0	D
Phaxas pellucidus (Pennant, 1777)	0	0	1	0	0	0	0	1	0	0	1	1	S
Thracia papyracea (Poli, 1791)	0	0	1	0	0	0	0	0	0	0	0	0	S
Thyasira flexuosa (Montagu, 1803)	0	3	4	0	7	3	3	0	2	2	0	0	S/D
Amphiura filiformis (O.F. Müller, 1776)	3	4	4	6	1	4	5	8	3	7	5	4	S
Ophiocten affinis (Lütken, 1858)	1	0	0	0	0	0	0	0	0	0	0	0	O/P/SC
Ophiua albida (Forbes, 1839)	0	1	0	0	0	0	0	0	0	0	0	0	O/P/SC
Trachythyone elongata (Düben & Koren, 1846)	0	0	0	0	0	0	0	0	1	0	0	0	S/D
Anthozoa sp.1.	0	0	0	0	0	0	0	1	0	0	0	0	O/P/SC
Cerianthus lloydi (Gosse, 1859)	0	0	1	1	0	0	0	0	0	0	0	0	P
Halcampa chrysanthellum (Peach in Johnston, 1847)	1	2	1	1	0	0	0	0	1	0	0	0	O/P/SC
Nemertine sp 1	0	1	2	1	1	2	0	0	1	0	0	0	P
Nemertine sp 2	0	0	0	1	0	0	0	1	0	0	0	0	P
Golfingia	3	1	0	1	0	3	3	1	0	0	0	4	P
Phoronis sp.1.	0	0	0	0	1	0	0	0	0	0	1	0	S

4.4.4. Multivariate Analysis

4.4.4.1. All Macrofauna Data

The MDS plot, shown in figure 4.18, shows the pre-treatment macrofauna samples clearly separated from the post treatment samples. This suggests that the pre-treatment and post treatments samples contained a different macrofaunal composition. This finding coincides with the ANOVA results comparing the pre-treatment and post treatment samples. The stress value is low (0.09) giving confidence that the MDS plot is an accurate representation of the relationship between the samples (Clarke and Warwick, 1994). Log transforming the data, to reduce the influence of the species of high abundance, shows the same pattern as the untransformed data. Figure 4.19 shows the DCA plot on $\log_{10}$ transformed data. The initial samples are situated on the left hand side of the plot with the post treatment samples closely plotted on the right hand side.

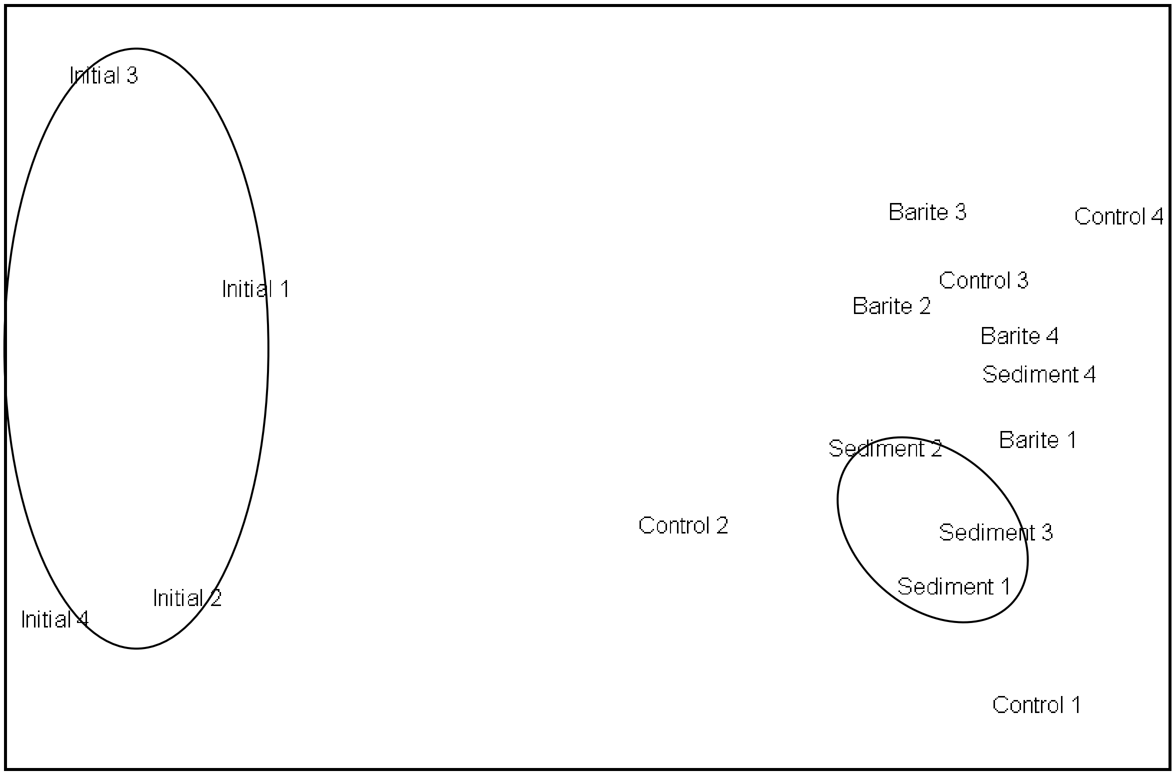


Figure 4.18: MDS Plot on Sound of Shuna Untransformed Data (Stress 0.09).

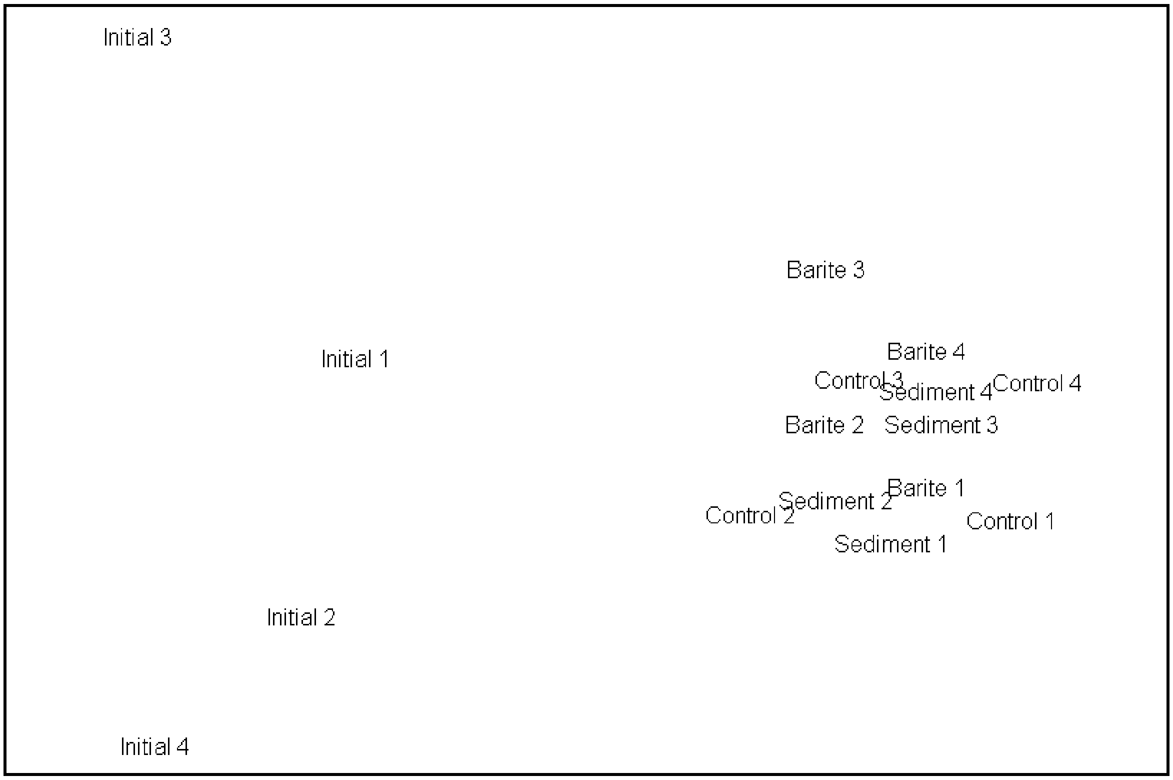


Figure 4.19: MDS Plot on Sound of Shuna $\log_{10}$ Transformed Data (stress 0.09).

The Detrended Correspondence Analysis on the untransformed data of the Sound of Shuna (figure 4.20) also shows the pre-treatment macrofaunal samples grouped away from the post treatment samples with sample 1,2 and 3 forming a relatively tight cluster and 4 positioned at zero along axis one. Within the post treatment samples the barite and sediment samples were grouped separately with the control samples scattered around them. The DCA run on the $\log_{10}$ transformed data of the Sound of Shuna (Figure 4.21) reflects the findings that the initial samples are plotted away from the post-treatment samples. The post-treatment samples are positioned on the right-hand side of the DCA plot, with the sediment and barite samples making two separate groups. These two groups are located close to each other with the control samples scattered around.

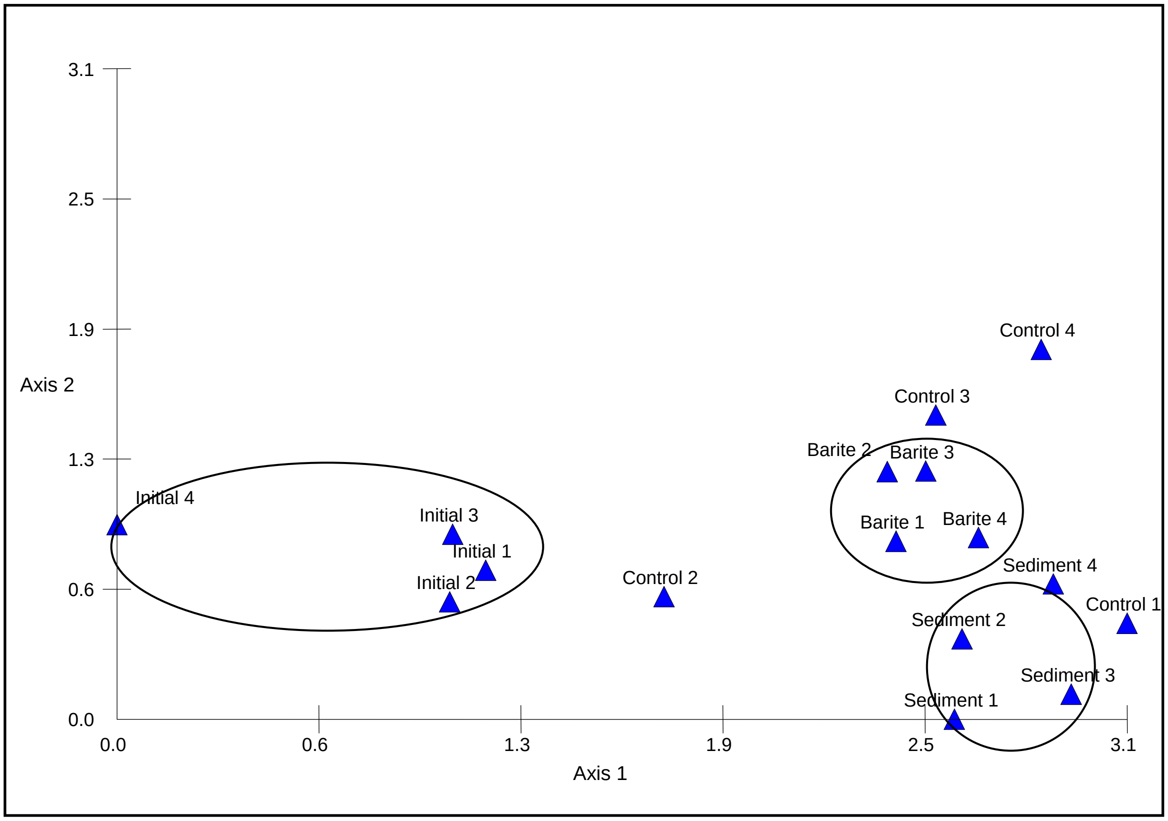


Figure 4.20: DCA Case Scores on Sound of Shuna untransformed Data. Eigenvalues for axis 1 and axis 2 are 0.316 and 0.201 respectively.

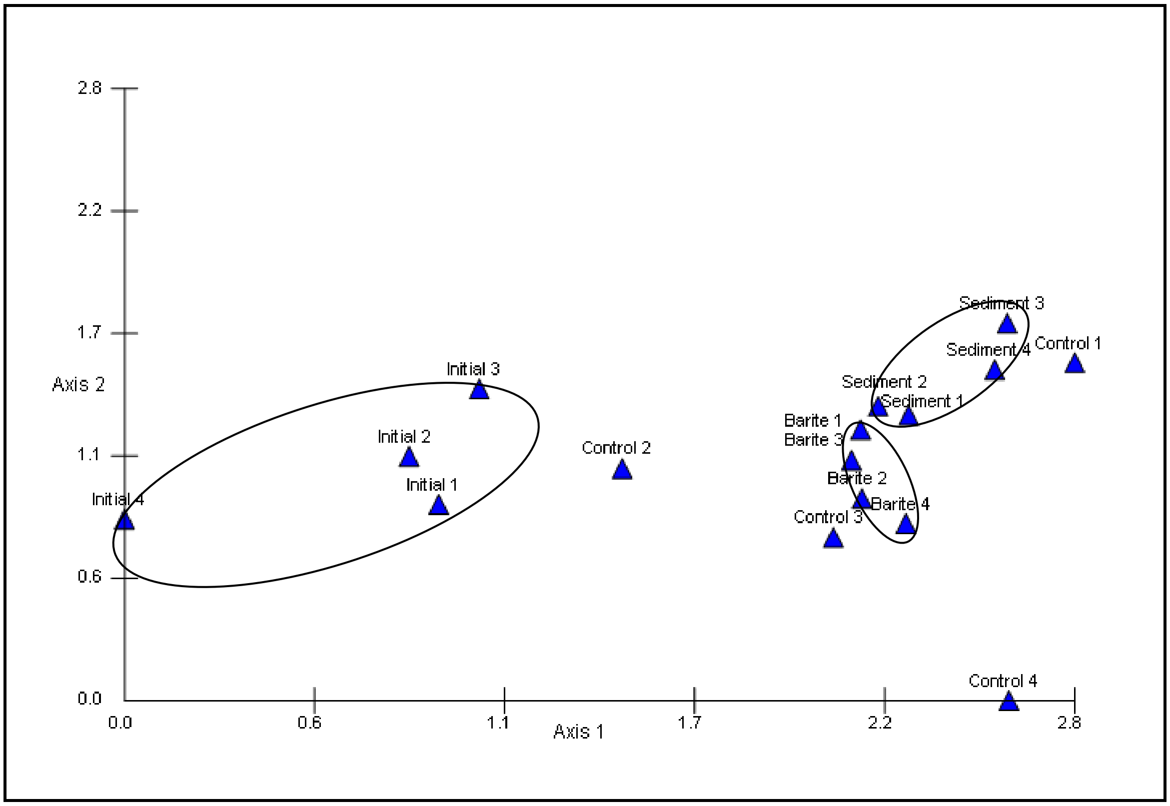


Figure 4.21: DCA case scores on Sound of Shuna $\log_{10}$ transformed data. Eigenvalues for axis 1 and axis 2 are 0277 and 0.196 respectively.

The data produced by the ANOSIM on all the data of the Sound of Shuna also shows that the pre-treatment fauna was significantly different from the post treatment macofauna, with results showing a 2.9% similarity for both the continuous and $\log_{10}$ data (table 4.11). ANOSIM on the post treatment continuous data showed a significant difference between the sediment and barite samples. When the data was $\log_{10}$ transformed a 2.9% result was produced between the control and barite results. The significant value of 2.9% was produced due to the number of replicates used. This value would differ if more replicates had been used but time constraints disallowed this.

Table 4.11: ANOSIM results for Sound of Shuna

	Continuous Data		Log_{10} Data		Presence/absence	
Treatment	Sig. Level	R-statistic	Sig. Level	R-statistic	Sig. Level	R-statistic
I vs. C	2.9 %	0.87	2.9 %	0.87	2.9 %	0.65
I vs. S	2.9 %	1.0	2.9 %	0.82	2.9 %	0.51
I vs. B	2.9 %	0.98	2.9 %	0.85	2.9 %	0.59
C vs. S	22.9 %	0.16	17.1 %	0.2	5.7 %	0.28
C vs. B	25.7 %	0.13	2.9 %	0.24	22.9 %	0.14
S vs. B	2.9%	0.55	5.7 %	0.44	28.6 %	0.10

4.4.4.2. Post Treatment Data

As with the Ardmucknish Bay results, a more detailed analysis on the post treatment samples was carried out excluding the pre-treatment fauna, to try and gain a more in depth analysis into the data. The MDS plot run on the post-treatment untransformed data (figure 4.22) shows two distinct groupings of the barite and sediment treatments, reflecting the result of a 2.9% significant difference, shown in table 18. The stress value of 0.16 is quite low indicating that the MDS plot gives a good representation of the sample relationships (Clarke and Warwick, 1994). DCA (figure 4.23) was also carried out only on the post treatment untransformed data. Figures 4.22 and 4.23 show that the sediment and barite treatments are grouped together with the control samples dispersed over the plots. Control 2 is plotted away from the other control samples. The control samples were not interfered with, with no substance added onto the surface, indicating that this is caused by natural variation with the benthic fauna.

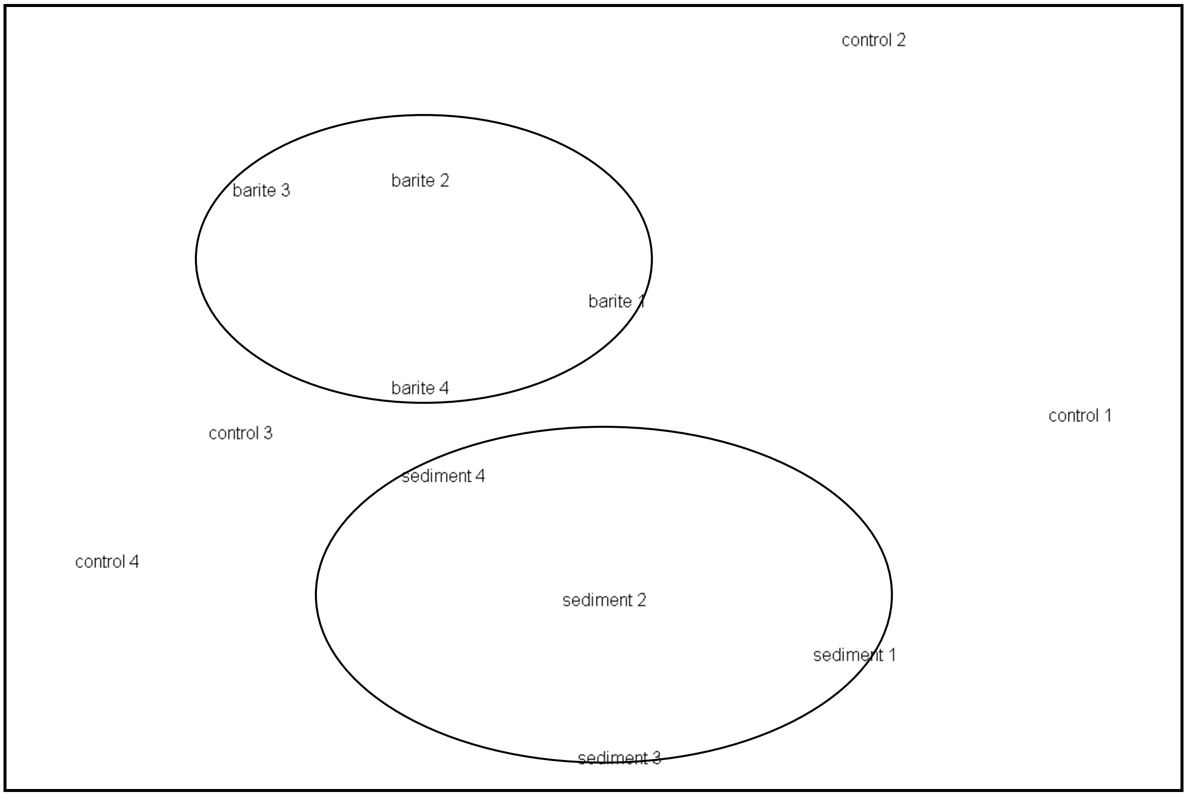


Figure 4.22: MDS Plot on Sound of Shuna Untransformed Data (Stress 0.16)

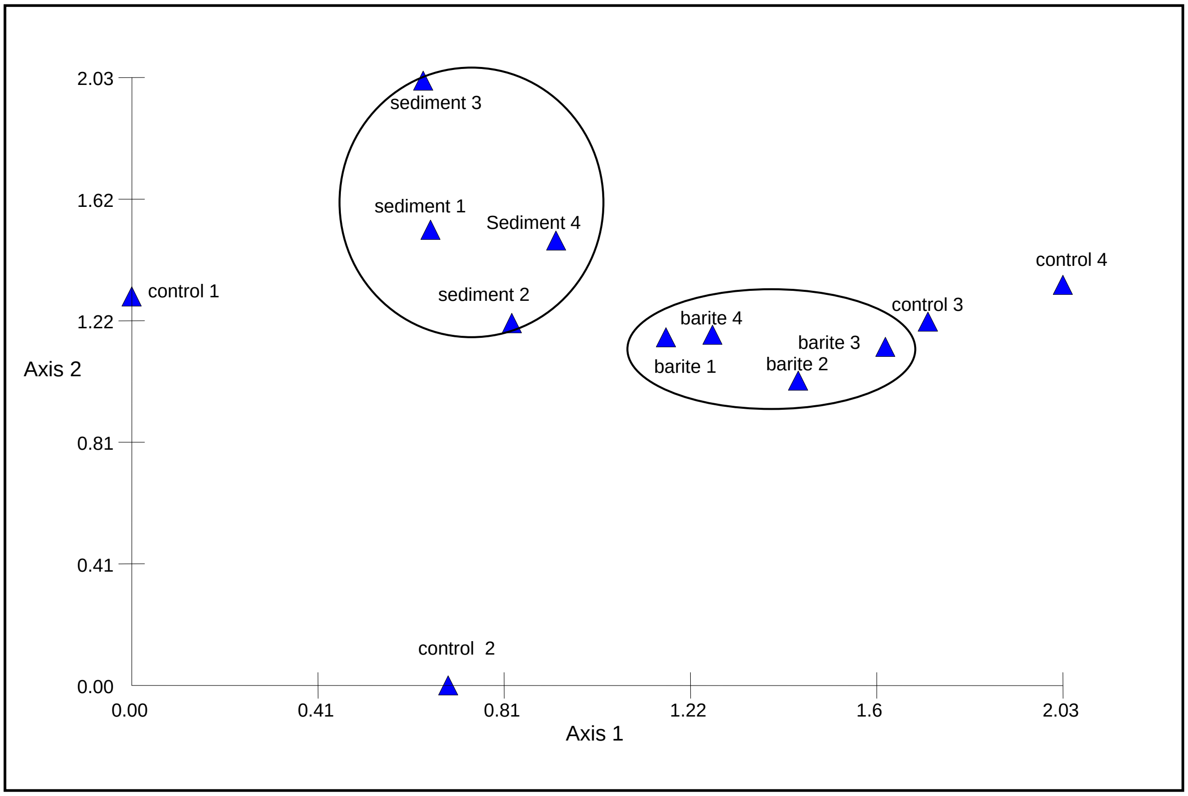


Figure 4.23: DCA Case Scores on Sound of Shuna Untransformed Data. Eigenvalues for axis 1 and axis 2 are 0.253 and 0.199 respectively.

Table 4.12 shows the ANOSIM results for the post treatment data. There is a significant difference (2.9%) between the sediment and barite treatments on the continuous untransformed data. A significant difference was also found between the control and barite treatments on the data once logged. This shows that it was the rarer species contributing to the difference between the control and barite treatments.

Table 4.12: ANOSIM results for Sound of Shuna post treatments

	Continuous Data		Log_{10} Data		Presence/absence	
Treatment	Sig. Level	R-statistic	Sig. Level	R-statistic	Sig. Level	R-statistic
C vs. S	22.9 %	0.15	17.1 %	0.19	5.7 %	0.29
C vs. B	25.7 %	0.16	2.9 %	0.24	22.9 %	0.15
S vs. B	2.9 %	0.55	5.7 %	0.45	34.3 %	0.08

Since $\log_{10}$ transformation of the data produced a different result when running the ANOSIM, MDS and DCA were repeated on the transformed data. The MDS produced on the logged data is shown below in figure 4.24. The MDS produced on the logged data is not that dissimilar from the untransformed data plot. The stress value (0.17) is quite low indicating that the MDS plot gives a good representation of the sample relationships (Clarke and Warwick, 1994). The sediment treatments were still grouped together and the controls were distributed over the whole plot. The DCA on the $\log_{10}$ transformed data, figure 4.25, shows the sediment treatments grouped together and barite 1, 2 and 3 grouped together. In figure 4.25, the sediment treatments are plotted closer to the control samples than the barite treatments are. This reflects the ANOSIM results, that there is a 2.9% significant difference between the control and barite treatments when the data is $\log_{10}$ transformed. The transformed data, for both the MDS and DCA plots, are not as definite as the untransformed data, but do still provide similar patterns within the results.

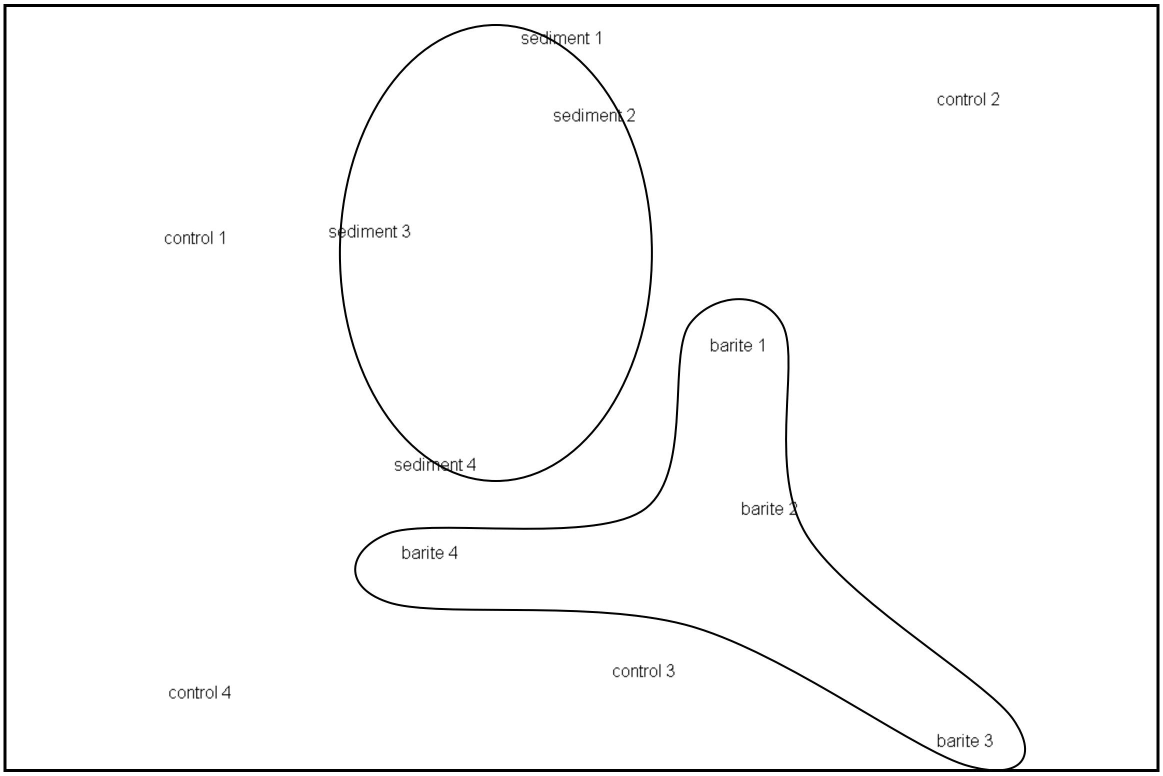


Figure 4.24: MDS Plot on Sound of Shuna Log$_{10}$ Transformed Data (Stress 0.17)

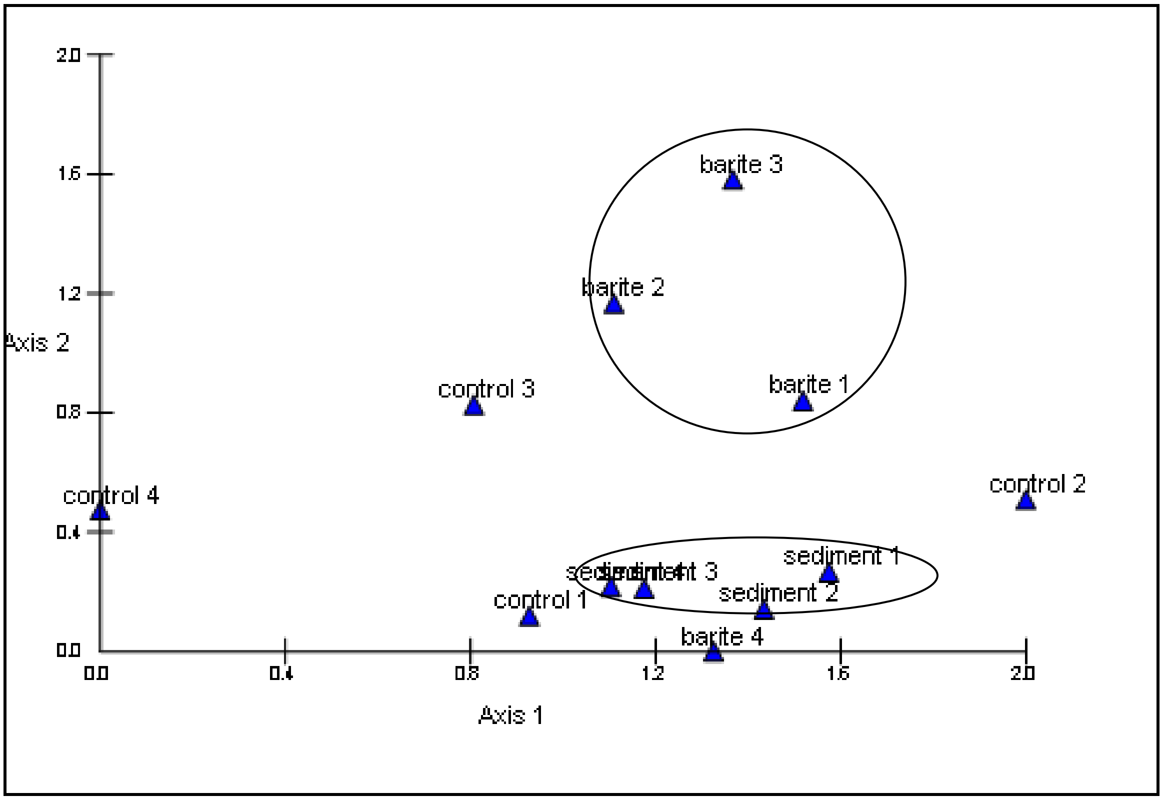


Figure 4.25: DCA Case Scores on Sound of Shuna post treatment log$_{10}$ Transformed Data. Eigenvalues for axis 1 and axis 2 are 0.241 and 0.172 respectively.

The ANOSIM results indicate that there was a significant difference between the macrofauna within the sediment and barite treatments when the data was untransformed and between the control and barite samples when the data was $\log_{10}$ transformed. To determine possible causes the macrofauna was separated into four groups, Annelida, Crustacea, Mollusca and others. Multivariate analysis was run on each group. The results indicate that the annelids were responsible for the outcomes from the Sound of Shuna. When an ANOSIM was run only on the annelids (table 4.13), a 2.9% significant difference between the sediment and barite treatments was discovered, for both the continuous and logged data. None of the other groups, Mollusca (table 4.14), Crustacea (table 4.15) and the others (table 4.16) showed any difference between treatments for either the continuous or logged data.

Table 4.13: ANOSIM results on Sound of Shuna post treatment annelids.

	Continuous data		Log_{10} Data	
Treatment	Sig. Level	R-statistic	Sig. Level	R-statistic
C,S	17.1%	0.18	17.1%	0.24
C,B	28.6%	0.06	5.7%	0.23
S,B	2.9%	0.60	2.9%	0.52

Table 4.14: ANOSIM results on Sound of Shuna post treatment Mollsuca.

	Continuous data		Log_{10} Data	
Treatment	Sig. Level	R-statistic	Sig. Level	R-statistic
C,S	94.3 %	-0.25	88.6 %	-0.17
C,B	77.1 %	-0.14	71.4 %	-0.15
S,B	88.6 %	-0.18	80.0 %	-0.15

Table 4.15: ANOSIM results on Sound of Shuna post treatment Crustacea.

	Continuous data		Log_{10} Data	
Treatment	Sig. Level	R-statistic	Sig. Level	R-statistic
C,S	65.7 %	-0.06	100 %	-0.07
C,B	77.1 %	-0.19	82.9 %	-0.22
S,B	51.4 %	-0.03	40.0 %	-0.03

Table 4.16: ANOSIM results on Sound of Shuna post treatment others.

	Continuous data		Log_{10} Data	
Treatment	Sig. Level	R-statistic	Sig. Level	R-statistic
C,S	62.9 %	-0.05	11.4 %	-0.23
C,B	14.3 %	-0.22	5.7 %	-0.42
S,B	31.4 %	-0.13	17.1 %	-0.13

The MDS plot produced on the Annelida untransformed data is shown below in figure 4.26. The sediment treatments are all plotted together. The stress value of 0.16 is quite low showing that the plot gives a good representation of the sample relationships (Clarke and Warwick, 1994). Three of the barite treatments are mapped together. The four control treatments are scattered over the MDS plot similar to their positions in figures 4.22 and 4.23. The MDS was repeated on the $\log_{10}$ transformed Annelida data and is displayed in figure 4.27. Within this plot, the four barite treatments are grouped together and the four sediment treatments are grouped together, with the control samples scattered over the plot. These findings agree with the ANOSIM results that the annelids are responsible for the significant difference between the sediment and barite treatments.

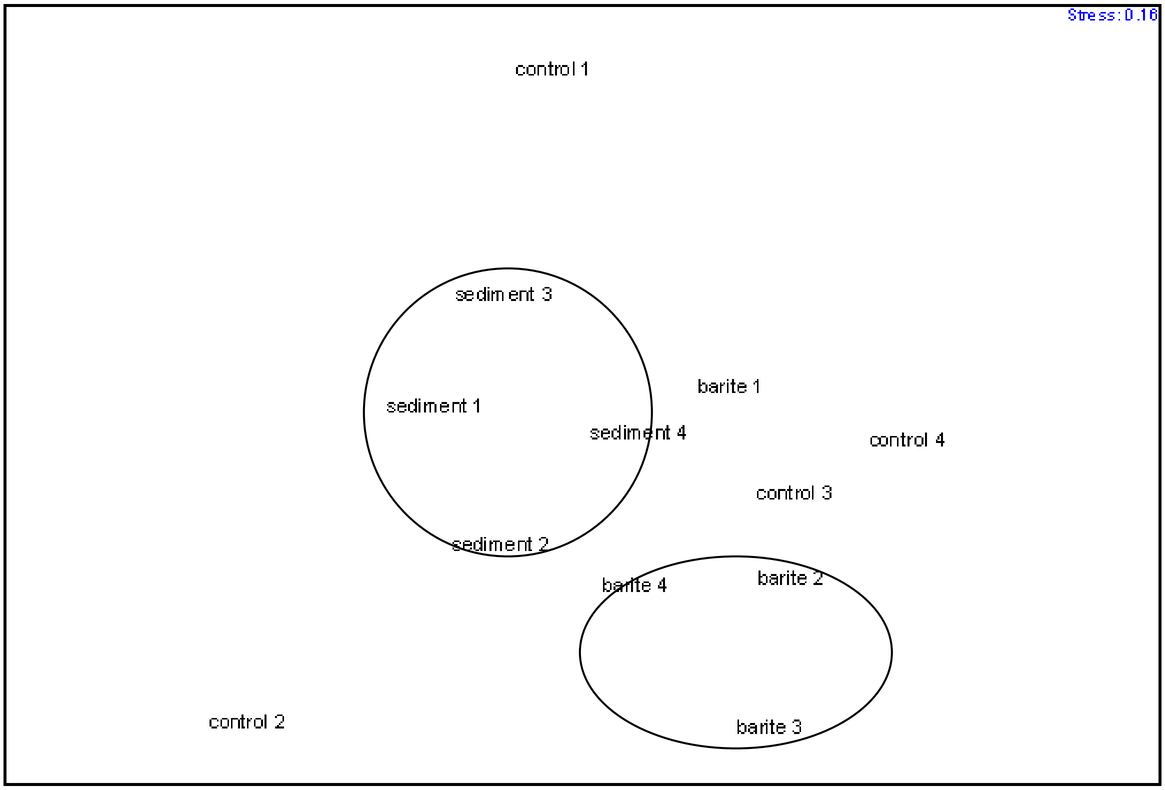


Figure 4.26: MDS Plot on Sound of Shuna Annelida Untransformed Data (Stress 0.16).

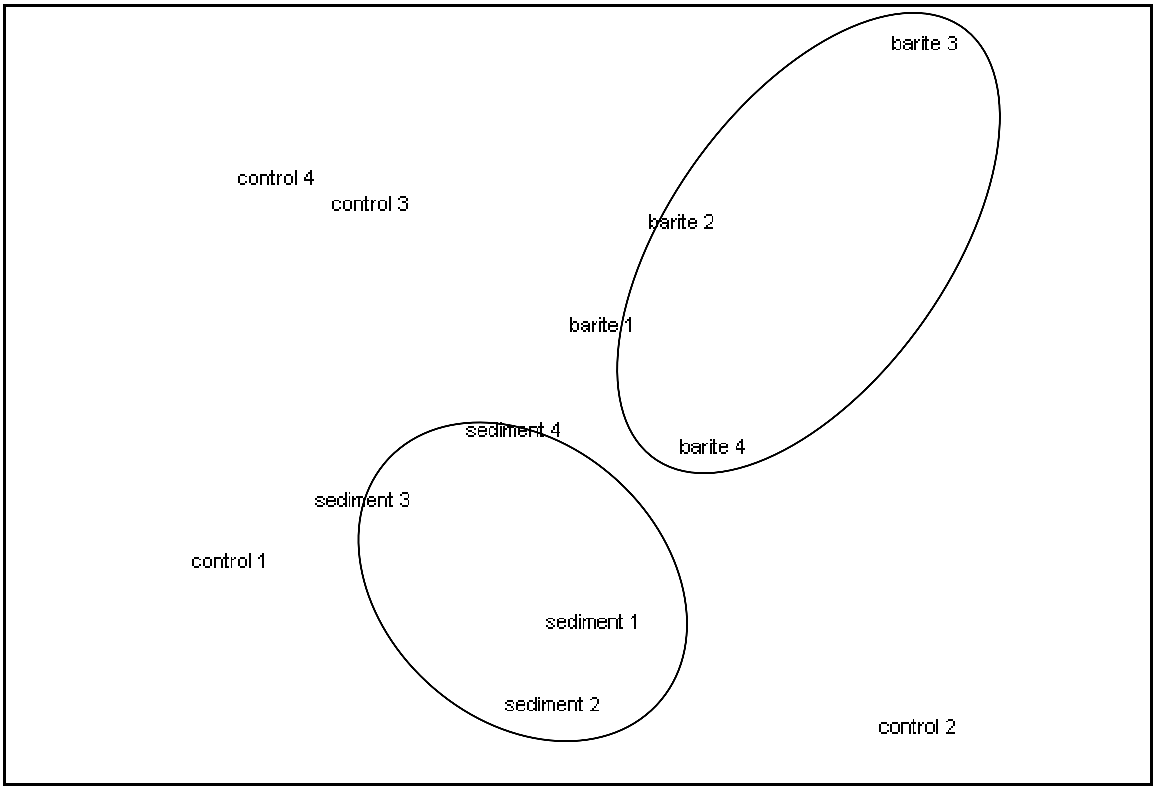


Figure 4.27: MDS on Sound of Shuna Post Treatment Annelida $\log_{10}$ Transformed Data (stress 0.14).

The annelids appear to be the reason behind the results gained so far for the Sound but this does not mean that other species were not affected by the different treatments. A Levene test, which tests the homogeneity of variances, was run on each of the 102 individual species. This determines whether to do a parametric (analysis of variance) or non parametric (Kruskal-wallis) test on the data. These tests could not be completed on species that were present in only one treatment or present only as one individual. This does not really affect the data analysis as any species present as a lone individual cannot really be used to analyse the introduction of a foreign substance onto the seabed.

The ANOVA and Kruskal-wallis tests found that there were four species that showed a statistically significant difference in numbers present between treatments. The four species were *Halcampa chrysanthellum* (p-value 0.004), *Scalibregma inflatum* (p-value 0.038), *Sthenelais limicola* (p-value 0.008) and *Tubificoides benedeni* (p-value 0.015). The sea anemone *Halcampa chrysanthellum* was present with highest numbers in the control samples. Present in highest numbers within the sediment treatments were the polychaete worm *Scalibregma inflatum* and the oligochaete *Tubificoides benedeni.* The scale worm *Sthenelais limicola* was most abundant within the barite treatment quadrats. SIMPER (as described previously in section 4.1.3.2) was run on all the post treatment data.

The results from SIMPER state that the highest average dissimilarity of 59.18% was between the control and sediment treatments (table 4.17). This differs from the ANOSIM results which found a significant difference between the continuous data of the sediment and barite treatments. *Tubificoides benedeni* was the second highest contributor to the dissimilarity between the control and sediment treatments. This oligochaete species was also the main contributor to dissimilarity between the sediment and barite treatments (table 4.19). The sediment treatment was the similarity factor between these two results. One-way ANOVA found that the oligochaete *T. benedeni* was present in significantly different numbers between treatments. Analysis into the raw data showed that *T. benedeni* was present in higher numbers within the sediment treatment than both the control and barite treatments. SIMPER shows that *Prionospio sp.1* was the main causative species for the dissimilarity levels between both the control and sediment treatments (table 17) and control and barite treatments (table 4.18), with the control being the common connection, although *Prionospio sp.1* was not present in significantly different numbers. The sum of *Prionospio sp.1* in the three treatments was 63, 52 and 51 for the control, sediment and barite treatments respectively.

The bivalves *Kurtiella bidentata* and *Abra nitida* were also found to be responsible for the average dissimilarity between samples. *Kurtiella bidentata* was the fourth species responsible for the dissimilarity between both the control and sediment samples and the control and barite samples. In both cases the average abundance increased with the addition of the sediment and barite particulates. *Abra nitida* was the third species responsible for the dissimilarity between the control and barite treatments and the fourth species responsible for the dissimilarity between the sediment and barite treatments. *Abra nitida* also increased its average abundance in the barite treatments.

Groups: Control and Sediment

Average dissimilarity = 59.18%

Table 4.17: SIMPER dissimilarity results for control vs. sediment treatments.

Species	Control Average abundance	Sediment Average abundance	Contribution to dissimilarity %	Cumulative %
Prionospio sp.1.	15.75	13.00	12.94	12.94
Tubificoides benedeni	0.25	13.50	11.41	24.35
Streblospio sp.1.	8.50	3.25	5.85	30.20
Kurtiella bidentata	4.50	5.50	5.85	34.22

Groups: Control and Barite

Average dissimilarity = 58.15%

Table 4.18: SIMPER dissimilarity results for control vs. barite treatments.

Species	Control Average abundance	Barite Average abundance	Contribution to dissimilarity %	Cumulative %
Prionospio sp.1.	15.75	12.75	13.68	13.68
Streblospio sp.1.	8.50	11.25	7.17	20.85
Abra nitida	4.00	6.00	4.57	25.52
Kurtiella bidentata	4.50	5.25	3.68	29.20

Groups: Sediment and Barite

Average dissimilarity = 55.45%

Table 4.19: SIMPER dissimilarity results for sediment vs. barite treatments.

Species	Sediment Average abundance	Barite Average abundance	Contribution to dissimilarity %	Cumulative %
Tubificoides benedeni	13.50	0.50	12.13	12.13
Streblospio sp.1.	3.25	11.25	8.71	20.83
Prionospio sp.1.	13.00	12.75	5.83	26.66
Abra nitida	5.00	6.00	4.43	31.09

4.4.5. Barium Analysis

Barium levels, three months after distribution, are shown in table 4.20. All treatments contained four samples for analysis apart from the barite 0-2cm which contained eight. This higher level of samples was due to the appearance of barite after the three month exposure period and wanting to gain an accurate measure of the levels remaining on the sediment surface. The results, displayed in table 4.20, show that barite remained on the sediment surface for three months. The barium levels in the top two centimetres in the barite quadrats are highly elevated in comparison to both the control and sediment quadrats. Running a one-way ANOVA, followed by a Tukey test, found a significant difference (p=0.001) within the levels of barium between the barite and both the control and sediment treatments. The levels were also elevated at the depth of 2-4cm. At the depth of 4-6cm, the barium levels had returned to similar levels as the control and sediment samples. A significant difference (p=0.001) was also found between different depths of the barite treatments, with barite (0-2cm) containing significantly different levels than both the barite (2-4cm) and barite (4-6cm).

Table 4.20: Barium Levels in the Sound of Shuna

Treatment	Depth in Sediment (cm)	Barium Levels (mg/kg)
Control	0-2	319
Control	0-2	1233
Control	0-2	220
Control	0-2	524
Sediment	0-2	257
Sediment	0-2	860
Sediment	0-2	303
Sediment	0-2	580
Barite	0-2	51491
Barite	0-2	47425
Barite	0-2	34454
Barite	0-2	33234
Barite	0-2	6387
Barite	0-2	6428
Barite	0-2	34747
Barite	0-2	35383
Barite	2-4	1203
Barite	2-4	1675
Barite	2-4	1389
Barite	2-4	3965
Barite	4-6	578
Barite	4-6	578
Barite	4-6	503
Barite	4-6	1678

4.5. Discussion

The purpose of this experiment was to give an indication of the effects barite may have towards whole benthic communities. Sedimentary fauna are universally an important constituent of the marine environment, particularly the macrofauna, by sustaining the higher trophic levels, including the larger mobile crustaceans and the fishes and birds. Biological benthic communities are responsible for modifying the physical structure of the sediment of the seabed for example tube builders respire aerobically and actively pump oxygen into the surrounding sediment (Gray and Elliott, 2009). Human influences within the water column will have effect the natural processes of the marine environment.

There were fewer species present in Ardmucknish Bay in comparison to the Sound of Shuna, a result of the difference in sediment characteristics. Ardmucknish Bay has a sandy bottom and Sound of Shuna has a muddy bottom representative of the sediment characteristics of the southern and northern North Sea respectively. Both the macrofauna abundance and diversity are known to increase from the southern to the northern North Sea (Basford and Eleftheriou, 1988; ICES, 2000). In general, the harsher the environment, the more homogeneous the sediment, the lower the diversity in living forms present, explaining why fewer species were found in Ardmucknish Bay (~60 species) than the Sound of Shuna (100 species).

The results indicate that barite did not appear to alter the macrofauna present within Ardmucknish Bay. A change detected within the macrofauna was found between the initial fauna and the fauna collected after the six month period, but since no barite was detected upon return, and the sediment was homogenous, this is probably the result of seasonal changes and natural variation within an area of high water movements. The sandy bottom sediments of Ardmucknish Bay are reflective of the bottom type of the southern North Sea. Sandy bottoms are found in areas where intensive sediment movement occurs normally caused by strong currents. Sediment stability is a major factor determining the types of animals present (Gray and Elliott, 2009). Macrofauna present within areas of sandy bottoms are either deep burrowers, which will not be disturbed by the surface sediment movement, or are designed for constant movement by being quick burrowers or living on the surface. The quadrats placed out in Ardmucknish Bay were at a depth of around ten metres. This shallow depth coinciding with energetic waters was responsible for enhanced scouring effects, resulting in the top layer of sediment to be swept away, exposing the legs of the quadrats.

The southern North Sea does not have cutting piles accumulating on the seabed, due to the strong currents and tides maintaining a well-mixed bottom through out the year (UKOOA, 1999; Neff, 2005). In Ardmucknish Bay elevated levels of barium were not detected within the sediment after the six month exposure period indicating that the material deposited had been dispersed over duration of the experiment. The surface layer of sediment is always the first to be re-suspended in areas of high water movement and the barite would have been incorporated into this and re-distributed.

The difference between the macrofauna of the pre-treatment samples and the post treatment samples are likely to be simply a reflection of natural fluctuation caused by the instability of the substratum and the time of year the samples were collected. The initial samples were collected in July and the post treatment samples were collected in January, by which time there had been a substantial change in the bottom profile of the sea bed around the quadrats. Fauna diversity also changes constantly through out the year due to natural seasonal fluctuations and so a significant difference in faunal composition might be expected during the change from the spring to summer months. Seasonal cycles are a major influence on species composition. Annual changes in temperature, light and primary production are likely to manipulate species abundance (Gray and Elliott, 2009). The DCA ordination did indicate a weak alignment of the different post treatment samples on Axis II, suggesting either an incipient or residual effect, although an ANOSIM did not confirm this as statistically significant.

A statistical difference was only found between the annelids of the post six months sediment and barite treatments for the $\log_{10}$ transformed data. Further analysis showed the polychaete *Poechilochaetus serpens* was the only species present in statistically different numbers within treatments. It was present in higher numbers within the sediment treatment in comparison to both the control and barite samples. Running the SIMPER allowed an examination into the main species responsible for the levels of dissimilarity between the control, sediment and barite treatments. The polychaete *Aricidae* was the main species responsible for dissimilarity between the control and barite treatments and the sediment and barite treatments, with its average abundance increasing in the barite treatments. The only bivalve responsible for the difference between treatments was the deposit feeder, *Tellina fabula,* with its average abundance decreasing within the barite samples. Although a difference was found between the average abundance of species with the three treatments this cannot be related to the introduction of barite. As mentioned previously, the barite had been washed away and could not be traced within the sediment upon collection. It cannot be determined when the barite was washed away, if it was immediately after dispersal, gradually or just before collection.

Similar to the results found in Ardmucknish Bay, the initial fauna of the Sound of Shuna, collected in April, significantly differed from the post-treatment fauna taken three months later in July. Seasonal changes occur in within benthic assemblages of soft sediments with the benthos in shallower regions being subjected to greater fluctuations. Through-out spring, there is an increase in the particulate organic matter, which the benthos rely on as a main food source. The main recruitment period is also in spring, generally between May and July, with larvae settling on the sediment due to being photo-negative (Gray and Elliot, 2009). Although seasonal influences may have an effect on sampling the sediment and related benthos it should not affect the data gained from the Sound of Shuna. Any change in macrofauna abundance due to seasonal changes will affect all treatments equally, including the control samples.

Unlike the results of Ardmucknish Bay, the one off distribution of barite was responsible for a significant difference within the macrofauna in the Sound of Shuna. In areas of mud bottoms, such as the Sound of Shuna, there is a larger diversity of macrofauna than in regions of sandy bottoms due to the weaker water movements and the increase in organic content present. The results from the MDS and DCA plots show distinct separate groupings of the sediment and barite treatments. ANOSIM showed that there was a significant difference between the macrofauna present within the sediment and barite treatments. The ANOSIM analysis found a significant difference between the control and barite treatments when the data were $\log_{10}$ transformed. This shows that the presence of rarer species was responsible for the difference between the control and barite treatments. As previously mentioned, rare species often comprise 70% of the total number of species within benthic communities (Gray and Elliott, 2009). They are a fundamental asset of marine benthic assemblages and therefore any impact towards the rarer species may follow through to community level.

Bamber (1984) describes how the release of fly-ash, a fine powder produced by coal-fired power stations, reduced the abundance of the infauna in and around the dumping zone. An inverse gradient was noted between the number of individuals and species and the fly ash content. The decrease in abundance was related to an increase in the presence of silt. Deposit feeders were the main feeding guild affected due to the dependence they have on the organic content and physical structure of the sediment (Bamber, 1984). When further analyses were carried out it was discovered that the annelids were responsible for the difference found between the different treatments. The polychaete *Scaligbregma inflatum* and the oligochaete *Tubificoides benedeni* were both present in significantly large numbers within the sediment treatments in comparison to the controls and barite treatments. In the experiment the sediment distributed onto the seabed was sieved to the same particle size as barite but did not have its organic content removed. It was acting as a natural control against the barite regarding to particle size and natural smothering. Both these species are deposit feeders and may have been taking advantage of the higher organics present. The polychaete worm *S.inflatum* burrows deep into sand and mud and feeds off the detritus present in and on the sediment. The oligochaete *T.benedeni*, known as a 'sludge-worm' is normally found living in areas enriched by organic matter. Only one species, *Sthenelais limicola*, was found to be present within the barite treatments in statistically higher numbers than the control and sediment treatments. The scale worm *S. limicola* lives in a tight secreted tube burrowed into the sediment and is considered to be an omnivore/predator. Although barite was visibly still present after the three month exposure period it would be likely that the scale worm was not exposed to it. The tight burrows they live in protect them from the external sediments and they do not utilize the sediment as a food source unlike deposit feeders.

Although no bivalve species were statistically responsible for the difference found, when SIMPER was run, *Kurtiella bidentata* and *Abra nitida* were found to be partly responsible for the levels of dissimilarity between the three treatments. *Kurtiella bidentata* had a larger average abundance within both the sediment and barite treatments in comparison to the control treatment. The bivalve *Abra nitida* had a larger abundance in the barite treatment than both the control and sediment treatments. The reason the abundance of these two bivalve species increased with the addition of barite onto the sediment surface may be due to their feeding types. *Kurtiella bidentata* is a suspension feeder and *Abra nitida* is a suspension/deposit feeder and therefore would not have been in contact with the sediment surface. Elevated levels of barium were detected within the top four centimetres of sediment, with an average of 31,193 mg/kg detected in the top two centimetres of the barite quadrats, in comparison to an average of 574 mg/kg and 500 mg/kg in the control and sediment samples respectively. The Sound of Shuna had a muddy sand bottom with a reduced current regime, chosen to represent conditions found in the northern North Sea.

This experiment was undertaken to try and gain some knowledge of what may occur to North Sea benthic populations throughout the drilling process. These results are indicative of what may occur to macrofauna in the North Sea but they do not necessarily establish possible full scale effects. A one off dose of barite did not appear to alter the macrofauna in Ardmucknish Bay. There was however an effect detected within the macrofauna of the Sound of Shuna where barium levels were detected in high amounts (6387 to 41,491 mg/kg) within the top two centimetres of sediment, three months after distribution.

The results should be viewed in the knowledge that one dose of barite is not reflective of the drilling process in the North Sea where benthic communities will be continually vulnerable to barite over a period of time, and may reflect the minimal impact.

Chapter Five:

General Discussion

The main focus of this project was to determine the effects of low level inputs of drilling mud components (barite) on suspension feeding bivalves that are found with the North Sea. Most research within the oil sector has focused on the oil coated drill cuttings and the associated drilling mud. As of the 1st January, 1997, the regulations affecting drilling operations in the North Sea stipulated that the amount of oil discharged attached to cuttings must not exceed 10g/kg respectively. Since it was uneconomic to clean cuttings to such low levels of oil contamination, disposal of oil-based mud (OBM) was effectively banned. Initially synthetic-based mud (SBM) was introduced to replace the OBM. However, it was found that the biodegradation rate and environmental impact of the SBM showed was little improvement over the use of OBM (UKOOA 1999b). Government regulations stipulated a reduction in the discharge of SBM to zero by the end of 2000 (Neff, 2005).

Focus and concern has therefore now been directed onto the increased use of the more environmentally friendly water-based drilling mud. The fine particulates associated with drilling mud and cuttings have been detected within the benthic boundary layer up about 8km from a site of active drilling (Cranford *et.al.,* 1999; Muschenheim and Milligan, 1996). Barite has also been identified 65 km downstream from an exploratory drill location (Neff *et.al.,* 1989). The presence of barite within the benthic boundary layer could have adverse effects towards marine communities, especially non-motile suspension feeding bivalves.

Suspension feeding bivalves are responsible for the removal of organic and inorganic particulates from the water column. Bivalve molluscs are ecologically important for both humans and the higher trophic levels of marine environment, especially the mobile crustaceans, fishes and birds (Gray and Elliott, 2009). Bivalves are also an important food source and help to re-circulate nutrients by removing particles (mainly phytoplankton) from the water column and excreting them as either faeces or pseudofaeces (Beninger *et.al.,* 1997; Vaughn & Hakenkamp, 2001).

Bivalves are sessile animals and are dependant on their surrounding waters for a constant food supply. Filtration by sedentary suspension feeding bivalves is therefore mainly a non-selective process, and is constantly exposed to fluctuations of suspended matter that may vary in quantity and quality (Navarro and Velasco, 2003). Filtration by suspension feeding bivalves can be influenced by a number of factors including particle size and concentration, gill morphology and size, current regime and water temperature (Vaughn & Hakenkamp, 2001). A change in suspended solid levels within the water column can have undesirable effects towards marine organisms by damaging the gills, altering the filtration rates and changing their natural behaviour (Cheung and Shin, 2005). Bivalves survive in areas with high turbidity by adapting their feeding behaviour. In areas with high sediment loads, bivalves increase their filtration rates to compensate for the high level of inorganic particles in the surrounding water column. Related to an increased filtration rate is an increase in the production of pseudofaeces (Bayne *et.al.,* 1993; Foster-Smith, 1975). The increased filtration rates and pseudofaeces production are energetic processes and may result in reduced health if not compensated for. Energy loss, in the shape of mucus, depends on the rate pseudofaeces are produced and on the organic content of suspended matter. The loss of energy connected with mucus that is rejected as pseudofaeces is compensated for by an increase in the organic content that results from the chosen rejection of inorganic matter (Urrutia *et.al.,* 2001).

Bivalves are mainly sedentary and therefore must either tolerate pollution, adapt or be killed (Gray and Elliott, 2009). The main test experiments in the present study investigated the effects chronic low level inputs of standard barite and fine barite had towards the filtration of an algal suspension by four bivalve species. The test bivalves, exposed to the different particulate matter (fine barite and standard barite), generally had a higher filtration rate than the control organisms. These results reflect the findings that the filtration rate of bivalves increases with an increase in seston concentration (Bayne *et.al.,* 1993; Navarro and Velasco, 2003) of low organic content (Urrutia *et.al.,* 2001). *Venerupis senegalensis* filtered the largest concentration of the algae numbers suspension when exposed to the highest daily level (2.0mm) of standard barite. The horse mussel *Modiolus modiolus* also filtered the highest quantities of algae when exposed to the 2mm dose of barite and the lowest quantities of algae when exposed to the 0.5mm standard barite dosage. *Dosinia exoleta* did not take up the algal suspension at the same levels as *V. senegalensis* and *M. modiolus. Dosinia exoleta*'s uptake of the algal suspension was highest when in the presence of the 1.0mm dose followed by the 0.5mm dose. *Chlamys varia* did not survive for long enough for the effect barite had on the uptake of algae to be determined.

It has been noted that combined with a bivalves' ability to increase its filtration rate is the capability to increase pseudofaeces production (Bayne *et.al.*, 1993; Foster-Smith, 1975). Foster-Smith (1975) noted that three bivalves, *Mytilus edulis, Cerastoderma edule* and *Venerupis pullastra*, have the ability to restrict the volume of ingested material, whilst raising their filtration rate, as the volume of suspended material increases. The green-lipped mussel, *Perna viridis*, survived in high suspended sediment loads, 1000 mg/l for 14 days and 1200 mg/l for 96 hours, with 87% and 100% survival rates respectively (Cheung and Shin, 2005). Survival was attributed to the high efficiency of particle rejection. This technique by suspension feeding bivalves is not only a mechanism for animals living in areas of high turbidity, allowing them to separate the organic from the inorganics; but it is also related to tidal availability of natural suspended particles. For example, during high tide, when the volume of suspended particles increased, *Venerupis corrugatus* raised its filtration rate and increased sorting ability and pseudofaeces production (Stenton-Dozey and Brown, 1994).

Suspension feeding bivalves regulate their feeding to maximise net energy gains in response to the quality and quantity of suspended matter in the surrounding environment. The basic feeding behaviour is controlled by the bivalves' ability to regulate their valve gape and siphon activity in response to environmental conditions (Jørgensen, 1996). Optimal conditions of the surrounding environment stimulate filtration at maximum capacity with an open valve gape and fully extended mantle edges. In contrast, in suboptimal conditions, including low or high concentrations of suspended particles, lack of oxygen and foul water, the valve gape and mantle edges become reduced (Jørgensen, 1996; Riisgård, 2004). As mentioned previously in chapter two, the valve opening behaviour was not determined due to barite in the seawater obscuring the view of the bivalves. From earlier research on valve opening behaviour (Englund and Heino, 1994; Jørgensen, 1996; Hawkins *et.al.*, 1998; Riisgård, 2004) the gape of the four bivalves when exposed to different barite concentrations can be estimated in relation LT_{50}. LT_{50} showed that the middle 1mm barite daily depth equivalent was the most lethal for the bivalves, excluding *Dosinia exoleta*.

In the present study, there were no mortalities when the four suspension feeding bivalves were exposed to suspended natural sediment. Suspended fine barite (2mm daily level) only affected the survival of *Venerupis senegalensis*, with a survival rate of 60% at 28-days. Standard barite, at all three doses (0.5mm, 1.0mm and 2.0mm), was responsible for 100% mortalities in all four test bivalve species. When the pseudofaeces were analysed under the microscope, together with the standard barite, the test algae, *Tetraselmis chui,* could be seen in substantial quantities. Although the algae were filtered out of the suspension, the bivalves were not utilising it as expected. The presence of barite seemed to hinder the consumption of the algal cells. Bivalves are inclined to reject large or dense particles before smaller or lighter material (Foster-Smith, 1975). The reason for this could be that organic material is not as dense as inorganic (Navarro and Velasco, 2003). As mentioned, in chapter one, barite is a dense mineral with a specific gravity of 4.5. Since bivalves have the ability to reject particles based on load (Foster-Smith, 1975), they should be able to discard the standard barite and utilise the algae. Larger suspended particles enter bivalves, via the water current, into the mantle cavity. Particles of a finer nature can get straight through to the inner side of the demibranchs (Owen, 1974; Cheung and Shin, 2005).

The presence of standard barite appeared to cause physical damage to the gill tissues. Damage was shown by *in-vivo* observations on both the blue mussel *Mytilus edulis* and the horse mussel *Modiolus modiolus,* in some instances showing complete removal of parts of gill tissue. *Post-mortem* microscopy studies of the gills also showed alteration to the individual gill filaments of *M. modiolus* and *D. exoleta.* The filaments taken from these bivalves exposed to standard barite lacked the ‘fullness’ and the straight appearance of the control gills. The test gill filaments had a ‘corrugated’ appearance and lacked inter-lamellar junctions. Damage to the gill from barite has also been observed in the suspension feeding bivalve, *Cerastoderma edule,* and the deposit-feeding bivalve, *Macoma balthica* (Barlow and Kingston, 2001). China clay waste, mainly composed of quartz sand, was recorded to be responsible for cases of gill-damage in trout, *Salmo trutta*, from china-clay polluted reaches of the River Fal (Herbert *et.al.,* 1961), although its effects on suspension feeding species was not reported.

Suspension feeding bivalves rely on their gills and labial palps to sort between the particulates removed from suspension (Drent *et.al.,* 2004). Foster-Smith (1975) observed that particles do not readily fall off the gills of *Mytilus edulis, Cerastoderma edule* and *Venerupis pullastra*. Particles are filtered out from the water column, passed onto the gills, and cilia are then responsible for the transportation of the particulates to the labial palps. High-suspended solid levels were responsible for depletion in the cilia present on the inner and outer demibranchs of the green-lipped mussel *Perna viridis.* An increase in the loss of cilia was observed when particle size increased (Cheung and Shin, 2005). The increased filtration rates, triggered by the presence of barite, lead to an increase in pseudofaeces production to allow the bivalves to separate the organic algae cells from the inorganic barite particles. The damaged gill filaments with large parts of demibranch being removed, hinders the separation of the organic from unwanted cells and prevents the bivalves from utilising the algae cells. The loss of energy, through mucus secretion for pseudofaeces production, will not have been compensated for and therefore the bivalves will have been living with an energy loss on a daily basis.

Throughout the control experiments, in chapter two, the four suspension feeding bivalves followed a similar filtration pattern over the 28 days. Although the filtration pattern was similar, a statistical difference was found between the bivalve species. The main significant difference was between *Chlamys varia* and *Modiolus modiolus* and also between *Venerupis senegalensis* and *Dosinia exoleta.* The difference between the filtration rates of the four bivalves will result from a difference in animal size and gill morphologies.

Modiolus modiolus was the largest of the four bivalves used, with an average length of 12cm, and *Chlamys varia* was the smallest of the species used, with an average length of 5cm. Generally, *C. varia* had a higher filtration rate than *M. modiolus* in the control experiments. Species of a smaller size will require a higher filtration rate, than bivalves of a larger nature, to gain the same number of algal cells out of suspension. *M. modiolus* and *C. varia* are both classed as fillibranchs, where the gill filaments remain mainly independent from each other. Although they contain the same gill morphology, *M. modiolus* and *C. varia* had different reactions towards the standard barite. *M. modiolus* survived for 20 days, 21 days and 18 days when exposed to 0.5mm, 1.0mm and 2.0mm barite levels respectively. *C. varia* survived for 1 day and 3 days when exposed to the 1.0mm and 2.0mm daily depth equivalent of barite respectively. The difference in the results is possibly related to the size difference of these two species and therefore the difference in gill size. Gill size establishes the intake rate (Drent *et.al.,* 2004) and is the initial site of particle capture (Beninger *et.al.,* 1997). The larger size of the *M. modiolus* gills allows better separation of foreign particles in relation to *C. varia*, which contains a smaller gill surface area. Bivalve specimens of a smaller size are more sensitive than larger ones when in the presence of high concentrations of suspended material (Theisen, 1997).

The specimens of *Dosinia exoleta* and *Venerupis senegalensis* used with the experiments in chapter two were of similar size, around 6cm. The difference between these two species is not size related but is caused by the type of bivalve. Both, *V. senegalensis* and *D. exoleta* are eulamellibranchs and have specialised gill structures that contain permanent tissue connections. As previously discussed in chapter three, eulamallibranchs not only rely on their gills, but also on the labial palps, for particle separation and sorting (Yonge and Thompson, 1976). In most eulamellibranchs, the captured particles are transported along to the particle food groove and onto the labial palps the main site for particle sorting (Beninger *et.al.*, 1997). Generally, bivalves with a larger palp size have a greater sorting ability of particulate matter. The labial palps of similar sized *D. exoleta* and *V. senegalensis* were measured and were 10mm and 6mm in length respectively. In general, throughout the control experiments *V. senegalensis* had a higher filtration rate than *D. exoleta*. Due to the inability to sort particles at the same level, *V. senegalensis* will have to filter at a higher level to gain the same number of algal cells as *D. exoleta.*

These two species of bivalves survived for different lengths of time when exposed to the standard barite. *D. exoleta* lived for 23 days, 21 days and 10 days when in the presence of the 0.5mm, 1.0mm and 2.0mm daily depth equivalents of barite respectively. *V. senegalensis* stayed alive for 19 days, 6 days and 11 days when exposed to the different depths of barite, 0.5mm, 1.0mm and 2.0mm respectively. Bivalve species living in highly turbid waters contain palps of a larger size than the same species living in areas of low turbidity (Theisen, 1977). An example of this is the genus *Venerupis,* where this species living in turbid waters has a larger palp size than *Venerupis* living in less turbid waters (Ansell, 1961). *D. exoleta* better withstood the presence of standard barite within the water column than *V. senegalensis.* This related with a larger palp size suggests that they had a greater ability to separate the unwanted barite particles from the wanted algal cells in suspension.

Studies on the impact of suspended material on benthic communities have been carried out in the past. A study of the impact of china clay discharge (Probert, 1975) reported that release of china clay waste lead to an increase in sediment loading which in turn resulted in the decrease of benthos present. Benthic communities inundated by china clay waste had significantly fewer individuals and number of species than control communities. Similarly Bamber (1984) found that dumping of fly-ash resulted in impoverishment of benthic fauna off the Northumberland coast. In this study, field trials were carried out in order to establish whether deposition of barite at the levels used in the experiments had a measurable impact on the composition and structure of benthic communities similar to those found in the central and southern North Sea. Time and logistical constraints dictated that the trials were limited in their scope providing only a general indication of the type and magnitude of impact that might be expected in the North Sea under operational conditions.

It is more probable that standard barite will cause an alteration to benthic communities living in areas of low water movement than in high energy environments as shown by the studies in the Sound of Shuna and Ardmucknish Bay. It is well known that in the southern North Sea, barite becomes re-suspended by strong currents and is dispersed over a wide area reducing any possible threat of barite towards the marine environment. Barite, distributed onto the seabed in Ardmuchknish Bay, could not be traced after six months having been completely dispersed by a change in sediment profile caused by the strong current regime.

In the northern North Sea, where water movement at the sea bed is a lot weaker, the barite will settle onto the mud/gravel bottom. In the Sound of Shuna, which contains a muddy/gravely bottom, barite was still present on the sediment surface three months after distribution. Multivariate statistical analysis of the data obtained from the experiment indicated that there was a significant difference in the faunal communities between quadrats treated with barite and those that were not suggesting that the presence of the barite was the main causal factor for the observed effects. The differences in community structure were most probably the result of changes in the abundance of some of the less common polychaete species which significantly differed between the control and barite treatments. For example, the scale worm, *Sthenelais limicola*, was found to be present in significantly higher numbers within the barite treatments whilst the abundance of the oligochaete *Tubificoides benedeni* was significantly lower. *T. benedeni* is a direct deposit feeder, feeding below the sediment surface, and *S. limicola* a predator/omnivore probably feeding on the sediment surface, possibly feeding on the increased numbers of moribund and dead individuals affected by the treatment.

Fly ash, the waste material from coal fired power stations, was dumped into localized areas of the North Sea up until 1992-93, when licences allowing the dumping of it were removed. Similar to barite, fly-ash is of a small particle size containing no organic matter, and once enters the water column is readily dispersed until the bulk settles out on the seabed. Bamber (1984) found that dumping of fly-ash resulted in impoverishment of benthic fauna. The number of individuals and species displayed an inverse gradient to that of fly ash content. The presence of fly-ash increases the fineness of the sediment (Bamber, 1980) and leads to a decrease in species numbers and diversity (Bamber, 1984). In areas with raised fly-ash content on the seabed surface, deposit feeders were the worst affected. Of the macrofauna, deposit feeders were found to be inhibited the greatest owing to their dependence on the organic content and physical structure of the sediment (Bamber, 1984). In contrast, Howell and Shelton (1970) showed that the presence of china clay waste, on the bottoms of St Austell and Mevagissey Bays, was responsible for replacement of suspension feeders with a rich community of deposit-feeders. In this present study, no change in the status of suspension feeding species was observed in the barite treated quadrats. A possible explanation for Bamber's (1984) observation is that fly ash, being a complex mixture of minerals, may have an undetermined toxic effect on the fauna and deposit feeders, which ingest the material, and may be more critically affected than suspension feeders which use the sediment primarily for support. Conversely, Howell and Shelton's (1970) observations could indicate that china clay waste is chemically inert and that the suspension feeders have been excluded by the presence of suspended material. In the field experiments carried out in this study, barite was applied in a single dose at the beginning of the experiment minimising impact on the suspension feeding members of the benthic community, however, impact on the deposit feeders may indicate that barite has a detrimental effect on some sediment feeding species.

An important difference between the dumping of fly ash and china clay waste is the amount disposed of, and the area over which material is deposited. Large scale disposal of these materials are likely to result in substantial areas of the sea bed being covered in relatively thick layers of disposed material. From the central point of discharge, significant elevated levels of fly-ash covered 43km^2 of the seabed off the Northumberland coast. If an even distribution of fly-ash was dumped over the spoiling ground then an annual thickness of 25cm could be found on the seabed. However, the centre of the dumping ground will contain the highest level of fly-ash on the seabed with an annual thickness of 3m (Bamber, 1984). In comparison, the quantity of barite discharged from water based mud offshore drilling operations is minute, most of which has been spread thinly (< 0.5mm) over a very wide area.

The oil industry is strongly regulated in regards to their offshore processes by regulatory bodies including the OSPAR convention, 1992. Although this research focuses on the effects of the offshore oil industry it must be compared to other physical human impacts towards the marine environment in the North Sea. Human impacts in the sea have lead to concerns about the long term impacts towards food chains and the ecology of the marine environment in the North Sea. In addition to fly ash and china clay waste dumping (both of which have now ceased), dredging is also responsible for damage to the seabed. Vast quantities of seabed can be removed by dredging and dumped elsewhere. Areas containing a muddy sand bottom can take 870 days for annelids, crustaceans and molluscs to make a combined recovery following dredging. Annelids alone, the majority of which are fast burrowers and can survive in harsher environments, are estimated to fully recover in 1210 days (Gray and Elliott, 2009). The local benthos of Chesapeake Bay took up to 18 months to recover following the dumping of dredged natural sedimentary material (Pfitzenmeyer, 1970). In addition, over-fishing has lead to a decline in stocks especially within the white fish populations like cod. The landings of shellfish amount to roughly 250,000 tonnes per year^{-1}. Shellfish are collected by the use of trawls and dredges, with an estimated 30-40% of the total biomass of the North Sea caught each year (Ducrotoy *et.al.*, 2000). Trawling fleets can cause great damage to large areas in a short period of time. It has been estimated that the whole North Sea seabed has been trawled twice yearly, with many areas being trawled 10-16 times in recent years (Gray and Elliott, 2009).

Although the production of oil and gas is expected to decline by 5% by 2013 (Oil and Gas UK, 2008), it has been predicted that there are still approximately 25 billion barrels of oil and gas remaining to be discovered within the North Sea (UKOOA, 2007). The majority of this remaining oil and gas will be drilled using water-based drilling mud. Although it is common practice to re-use drilling mud a portion will always be released into the water column during the initial stages of drilling, when no riser is in use. The barite induced damage towards suspension feeding bivalves, at the levels used (2.0mm, 1.0mm and 0.5mm), will roughly be found at distances 100m to 500m from offshore drilling operations within the North Sea (Barlow and Kingston, 2001).

It may be less detrimental to the marine environment if the oil industry to were to replace the coarser standard barite with fine barite. Fine barite had the least impact on the four suspension feeding bivalves studied here. Although suspensions of finer particle may be dispersed over greater distances those of coarser particles, they will also be more dilute and therefore can be expected to have less impact on the marine environment. The results of the present study has shown that, although chemically inert, suspended barite can have a detrimental effect on suspension feeding bivalves causing demonstrable damage to the gill filtration system and, after prolonged exposure, mortality. When the suspended barite levels used in this study are translated to field conditions *(i.e.* distances from the point of discharge) it is clear that any effects will be very local to a particular installation (well within the statutory exclusion zone of 500m). This level of impact should be viewed in the context of other users of the North Sea.

The objective of this study was to determine the impact of the main weighting agent used in water based drilling mud (barite) on suspension feeding bivalves. Whilst the results of the work provide some clear indications of the response of these organisms to a range of concentrations of suspended material and the physical damage done to the filtration mechanisms, the work has thrown up several questions regarding the nature of these responses and how organisms at other trophic levels might react. Bivalves were chosen for this initial study because it was assumed they would be the most vulnerable to excessive quantities of suspended foreign material; similar studies are needed on deposit feeding organisms. Whilst the experimental programme of the work provided consistent, repeatable results of the filtration rates of the bivalves under various levels of barite exposure, time and resources did not allow any more than a qualitative approach to determining the full nature of the observed physical damage to the ctenidia. A possible future study could focus on establishing a statistical basis for these observations using larger numbers of fenestrated bivalves and a more comprehensive programme of SEM work. The filtration work also posed some new questions regarding the role of pseudofaeces in protecting the filtration mechanism of the animals, whilst leading to a diversion of food materials away from the animal’s mouth possibly resulting in death by starvation. A future study might include a detailed analysis of the digestive tract of individuals subjected to various concentrations of suspended barite and studies on feeding cycles during exposure using valve ‘gape’ as an indicator of activity.

5.1. Conclusions

- Suspended barite, as used in drilling fluids, has been shown to adversely affect suspension feeding bivalves at concentrations expected in the near vicinity of offshore drilling operations in the North Sea.
- In contrast to the hypothesis, that the presence of suspended material will inhibit filtration, an increase in suspended matter has been shown to increase the overall filtration rate, but damage to the gill structure results when the suspension includes barite.
- Physical damage to the gills appears to result from the presence of barite particles within the suspended material with larger grained standard barite having a more detrimental effect than fine barite.
- Damage to the gills, within this study, manifests itself as vertical displacement of the inter-lamellar junctions, destruction of the inter-lamellar junctions, degradation of the cilia and destruction of sections of demibranch.
- The combination of reduced gill functionality, extra energy consumption from the production of excess pseudofaeces and the possible interference with food reaching the mouth is the most likely reason for the early mortality observed in animals exposed to suspended barite.
- Benthic community structure is unlikely to be adversely affected in areas of high current activity where a single dose barite covering is quickly dispersed (as in the southern North Sea). In low energy environments measurable changes in benthic community structure takes place after a single dose of barite, largely as the result of changes in the polychaete component of the fauna.
- Field studies showed that a one off dose of barite could result in a significant difference between the abundance of deposit feeding polychaetes.
- Although the overall bivalve community did not significantly change with the introduction of a one off dose barite, two species of deposit feeding bivalves *(Tellina fabula*, and *Abra nitida*) were statistically responsible dissimilarity between treatments.
- Using published ranges of barite contamination around offshore oil installations, the results of this work suggest that the impact of discharged barite could extend to a distance of at least 500m, with depths of 0.5mm barite on the sediment surface during active drilling.

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Appendix A

Barium Analysis Protocol

Determination of Barium from Sodium Carbonate Fusions supplied in a nitric acid solution (10% v/v).

Provided by David Bolland, SEPA, Riccarton Research Park, Edinburgh.

Instrumentation

Inductively coupled mass Spectrometer (ICPMS), Perkin Elmer, Model 6100 DRC plus, supplied by PE-LAS UK

Operating in standard mode, without DRC gas.

Autosampler Perkin Elmer AS93plus

ICPMS controlling software, ELAN V3.0

Operating principle

An argon icp is supported from an RF generator… The plasma is sampled by a platinum tipped sampler cone and skimmer cone. The ion beam traverses the interface between sampler cone and skimmer cone, and is focused in a Series II lens (® Perkin Elmer). The lens potential of the Series II lens (ion lens) is calibrated against ion intensity at three isotope masses. The optimum potential at each isotope mass is fitted to a straight line equation. This enables a potential to be applied at the ion lens to achieve the optimum ion transmission for each isotope; lens potential is scanned with a period matched to the atomic mass unit (amu) cycle of the MS detector.

With the DRC model a cell gas can be introduced into the front end of the MS, between the ion lens and the MS quadrapoles. No DRC gas was in operation and the DRC cell operating parameters were all set at their default, according the specification recommended by Perkin Elmer.

The mass spectrometer quadrapoles are mass calibrated for absolute mass and resolution. Aspirate a solution default tuning solution which gives a general purpose calibration of the spectrometer at the default resolution of 0.700amu ± 20amu. For the barium determination, and other samples of treated sewage outfall and trade effluent use a tuning solution specific for the operating laboratory. This includes more points than the default tune and enables the resolution to increases above the default at key masses where the element is known to require a particularly extended chemical calibration range or is the element is particularly sensitive to the ICPMS technique. The resolution increased for chromium (^{52}Cr), cobalt (^{59}Co), zinc (^{65}Zn), and lead (^{208}Pb). The resolution profile is generally calibrated to give the default setting of 0.700amu, but is distorted to a greater resolution at each of the specified elements.

Chemical Calibration

Stock Standard Solution for Calibration.

Multi-element standard of 18 elements, including Ba, preserved in 3.5% nitric acid, IV Standards (2008CAL-2), www.inorganicventures.com, supplied by Esslab, Essex, UK.

Working Calibration Solutions.

For chemical calibration of the ICPMS prepare a set of 4 standard solutions (200ug/l, 100ug/l, 25ug/l, 10ug/l) and a calibration blank. Make a calibration drift check with a calibration solution of 100ug/l.

Prepare the calibration solutions as follows. Dilute an aliquot of stock standard solution up to an original volume of 100ml (the original volume of standard represents the volume of untreated sample). Dilute the aliquot of stock standard to an acid concentration equivalent to that of dilution water. On top of the original volume add 4ml of dilution water and 0.5ml of working solution of internal standard.

Stock Internal Standard Solutions

Indium, 1000mg/l, preserved in nitric acid (5%v/v), Alfa Aesar, supplied by VWR, UK.

Rhodium, 1000mg/l, preserved in nitric acid (5%v/v), Alfa Aesar, supplied by VWR, UK.

Triton X-100, Sigma Aldrich, supplied by Sigma Aldrich.

Working Solution of Internal Standard

In (2mg/l), Rh (2mg/l), Triton X-100 (2% v/v).

ICPMS Chemical Calibration

Chemical Calibration of the ICPMS by the analyte to internal standard ratio, $^{137}Ba^+/^{115}In^+$.

Quality Control

Stock Standard Solution for Independent Control.

Barium, 1000mg/l, preserved in nitric acid (5%v/v), Alfa Aesar, supplied by VWR, UK.

Proficiency Tests

Aquacheck Group 4 (typical value 200ug/l to 250ug/l, preserved in 2% nitric acid), supplied by …..UK

RTC WP, (typical value 200ug/l to 250ug/l, preserved in 2% nitric acid), supplied by RTC, USA

Preparation of Samples

Reagents

Nitric acid (sp:1.18), grade trace analysis; supplied by ROMIL, Cambridge, UK.

Hydrochloric acid (sp:1.42), grade "Aristar"; supplied by BDH, Poole, Dorset

Deionised water is dispensed from a Millipore, Milli-Q Gradient A10, feed from a 60l reservoir containing water from Milli-Q Elix; supplied by Millipore, UK.

Equipment

Prepare the dilution in a 50ml centrifuge tube, polypropylene by Elkay (Cat No. 2093-NATX), supplied by Elkay, UK

Autosampler tubes, 15ml centrifuge tube, polypropylene, with caps of polyethylene, by Elkay (Cat No.2086-500); supplied by Elkay, UK.

Manual pipettors variable 10ml to 1ml (Thermo) and 1ml (Eppendorf Reference), both supplied by Fisher Scientific UK. Using disposable polyethylene tips, 10ml by Thermo Finntip, and 1ml by Elkay (Cat No. 18).

The internal standard is spiked individually into each autosampler tube with a repeating stepper pipette, Model 4500, Finnpipette by Thermo; supplied by Fisher Scientific, UK. Matching tips, Finntips, by Thermo; supplied by Fisher Scientific, UK.

Prepare dilution water

Make up to 400ml with deionised water, nitric acid (sp:1.18) 8ml, Hydrochloric acid (sp:1.42) 8ml.

Prepare sample

Make in a 50ml centrifuge tube a dilution of each sample by adding 1ml of sample to 20ml of dilution water. This will reduce both the analyte concentration and the matrix concentration. In a 15ml autosampler tube, spike with 50µl of working solution of internal standard (In, 2mg/l, Rh, 2mg/l, and Triton X-100, 2%) the sample dilution, volume 10ml.

ICPMS Operating Conditions

ICP Peristaltic Pump Speed & Timing

PARAMETER	FlUSH	READ DELAY	WASH
Time (s)	45	30	120
Speed (rpm)	-24	-10	-12

ICP Operating Conditions

Plasma forward power: 1250W

Nebuliser Gas Flow: 1.00 l min^{-1}.

MS Operating Conditions

Series II ion lens, Auto lens function switched on. Plot auto lens function at ^{59}Co, ^{115}In, and ^{208}Pb. Optimum ion lens potential of 9.0V at ^{115}In.

DRC conditions Default

MS vacuum, with plasma off, 2.8 X 10^{-6} Torr.

MS vacuum, with plasma on, 9.1 X 10^{-6} Torr.

Detector, Pulse stage potential 1500V, Analog stage potential -1800V

Detector Scanning conditions,

Daily Performance Conditions

PARAMETER	target value	Actual Value
$^{9}Be^{+}$	≥ 90cps	670cps
$^{115}In^{+}$	≥ 8kcps	24kcps
$^{238}U^{+}$	≥ 8kcps	29kcps
$^{140}Ce^{16}O^{+}/^{140}Ce^{+}$	≥ 0.025 and ≤ 0.035	0.030
$^{137}Ba^{++}/^{137}Ba^{+}$	≥ 0.010 and ≤ 0.030	0.014

Appendix B

Barium Analysis Protocol

Procedure for Determining Ba in 10% Nitric Acid Digests of Na-Fusion Extracted Sediment Samples

Provided by Craig Robinson, Fisheries Research Services, Marine Laboratory, Aberdeen.

Principal

Internal standards are added and the sample solutions diluted before being introduced to the ICP-MS by an autosampler and aspirated into a plasma via a cross-flow nebuliser and Scott double-pass spray chamber. The mass spectrometry of ions generated by an inductively coupled plasma is quantitatively determined for each sample by external calibration curve using the intensity (counts per second) ratio of analyte/internal standard in the unknown sample compared to the regression equation of the intensity ratio (analyte/internal standard) generated for the known concentration matrix-matched calibration standards.

Health and Safety

- Disposable nitrile gloves, safety glasses, waterproof shoes/boots, and clean, Howie-style, lab coats must be warn. Legs must be covered.
- Lone working is not permitted when handling acids.
- Concentrated acids must be handled in the fume cupboard with the extraction system switched on and the sash as low as is practical.
- When preparing dilutions of acids, always **add acid to water, not water to acid**.

Contamination prevention

- Disposable, acid-washed, polypropylene plasticware is used for liquid handling.
- Plasticware is soaked for 24±4 hrs in 10% v/v HNO3, rinsed 3x with ultrapure water, dried in a Class 100 laminar flow cabinet and stored in zip-locked bags.
- All sample and standard solutions are handled in a Class 100 laminar flow cabinet
- Stock standard and CRM solutions are poured from their containers into acid-washed plasticware before being pipetted.

Equipment

- PC controlled ICP-MS Elan 6100DRC+ (EN0504; Perkin-Elmer SCIEX, Thornhill, Canada) fitted with the standard Elan spray chamber, cross-flow nebuliser and Gilson 312 peristaltic pump and operated in standard mode (no gas in the reaction cell).
- AS-90/91 Autosampler (EN0512; Perkin-Elmer SCIEX, Thornhill, Canada).
- UHQ II water deioniser (EN0947 ; Elga, High Wycombe, UK))
- Class 100 laminar flow cabinet (Big Neat Ltd, Hampshire, UK)
- 10-15 ml polypropylene test tubes (acid washed)
- 50 ml polypropylene centrifuge tubes (acid washed)
- 25 ml polypropylene vials (acid washed)
- 10-100 µl and 100-1000 µl calibrated variable pipette and transparent tips
- 10 ml calibrated electronic pipette and transparent tips

Reagents

1. Ultra-pure water, 18.2 MΩ.cm
2. Stock standard solutions: Multielement 2A (10 mg/l), Rhodium (10 mg/l); Ge (1000 mg/l). Claritas PPT ICPMS-grade, obtained from SpexCertiprep Ltd., Middlesex, UK)
3. Triton X-100 (Sigma-Aldrich, Dorset, UK)
4. Aristar-grade (or better) nitric and hydrochloric acids (VWR International, Leicestershire, UK);
5. Prepare 10% HNO3 solution in 50 ml centrifuge tube:
    - 45 ml ultra-pure water
    - 5 ml cHNO3
6. Prepare diluent solution in 1000 ml bottle:
    - 960 ml ultra-pure water
    - 20 ml HNO3
    - 20 ml HCl
7. Prep internal standard (IS) mix (1mg/l Rh, 20 mg/l Ge, 2% v/v Triton-X) in 25 ml centrifuge tube:
    - 1000 ul stock Rh std (10 mg/l)
    - 200 ul stock Ge std (1000 mg/l)
    - 200 ul Triton X
    - 8.60 ml diluent solution
8. Prepare working calibration standards in 50 ml centrifuge tubes:
    - **Standard blank**: Using a calibrated pipette, add 500 µl IS mix, 2.5 ml 10% HNO3 and 47 ml diluent solution.
    - **5 µg/l working standard**: Using a calibrated pipette, add 500 µl IS mix, 25 µl multi-element 2A standard (10 mg/l), 2.5 ml 10% HNO3 and 46.98 ml diluent solution.
    - **10 µg/l working standard**: Using a calibrated pipette, add 500 µl IS mix, 50 µl multi-element 2A standard (10 mg/l), 2.5 ml 10% HNO3 and 46.95 ml diluent solution

- **50 µg/l working standard**: Using a calibrated pipette, add 500 µl IS mix, 250 µl multi-element 2A standard (10 mg/l), 2.5 ml 10% HNO3 and 46.75 ml diluent solution.
- **100 µg/l working standard**: Using a calibrated pipette, add 500 µl IS mix, 500 µl multi-element 2A standard (10 mg/l), 2.5 ml 10% HNO3 and 46.5 ml diluent solution
- **200 µg/l working standard**: Using a calibrated pipette, add 500 µl IS mix, 1 ml multi-element 2A standard (10 mg/l), 2.5 ml 10% HNO3 and 46 ml diluent solution
- **400 µg/l working standard**: Using a calibrated pipette, add 500 µl IS mix, 2 ml multi-element 2A standard (10 mg/l), 2.5 ml 10% HNO3 and 45 ml diluent solution

9. Prepare Quality Control sample in 10 ml autosampler tube:
    - 100 ul mix
    - 0.5 ml 10% HNO3
    - 9.4 ml SLRS-4 freshwater CRM (obtained from NRC Canada)
10. Prepare 20-fold dilutions of samples (currently in 10% HNO3) for analysis in 10 ml autosampler tubes:
    - 0.5 ml sample
    - 100 ul mix
    - 9.4 ml diluent solution
11. Prepare samples with expected high concentrations in 10 ml autosampler tubes using a second dilution factor (200-fold dilution):
    - 0.05 ml sample
    - 0.45 ml 10% HNO3
    - 100 ul mix
    - 9.4 ml diluent solution

Quality control

Samples are analysed randomly, with one procedural blank and one quality control sample (SLRS-4 freshwater Certified Reference Material) analysed with every 18 sediment digests. A new calibration curve is generated every 20 analyses.

Quantification

The signal intensity (counts per second) is measured for Rh at *m/z* 103 and for Ba at *m/z* 137 and 138. The intensity ratio of Ba^+/Rh^+ is obtained for the unknown solution and compared with the regression curve obtained for the Ba^+/Rh^+ ratio of solutions of known Ba concentration in order to obtain the Ba concentration of the unknown solution; the concentration obtained for the diluted sample digest is corrected for the dilution factor to obtain the concentration in the undiluted digests. The concentration determined for the QC sample is compared to that expected. Samples with digest concentrations greater than 8000 µg/l (20x diluted) or 80 mg/l (200x diluted) are off scale and should be further diluted and reanalysed. Digest concentrations should then be blank subtracted and corrected for dry sample mass.