THE ENVIRONMENTAL IMPACTS OF MUSSEL (MYTILUS SPP.) AQUACULTURE AT TWO NEWFOUNDLAND SITES

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The environmental impacts of mussel (*Mytilus* spp.) aquaculture at two Newfoundland sites

by

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Abstract

In this thesis I examine the environmental impacts of blue mussel (*Mytilus* spp.) farming at two Newfoundland sites as one component of a larger interdisciplinary study of the environmental sustainability of shellfish aquaculture. Biodeposit production rates of individual mussels were comparable to values measured elsewhere using similar methods. The interaction between spatial and temporal variables was significant in explaining rates of biodeposit production. The impact of mussel farming on the benthos was addressed by examining the macrofaunal community structure at both sites relative to community structure at nearby reference sites. The farms were very different in terms of sediment and benthic macrofaunal community composition. Benthic macrofaunal communities in sediments with mean grain size larger than 500 μm at both farms showed differences in taxa and abundance relative to reference sites. Communities in sediments with mean grain size smaller than 500 μm also differed between farm and reference sites, and all of these stations (farm and reference) had sediments with negative redox values and were dominated by organisms indicative of organic enrichment. I also examine the problem of spatial and temporal scale, and demonstrate that by using statistical inference we have considerable confidence in the descriptions of benthic communities at the farms at each sampling time, but slightly less confidence in describing the communities throughout the year. Beyond the scale of an individual farm, more sampling would be required before any statement could be made with confidence about the species composition of the benthic communities of the entire region.

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1.0 Introduction to the environmental impacts of mussel aquaculture

1.1 General introduction to aquaculture

In the past decade, mussel aquaculture has demonstrated rapid growth and undoubtedly will continue to grow as a resource sector in Newfoundland, Canada (Howlett and Rayner, 2003), largely because of opportunities in expanding markets in developed countries. In Newfoundland, mussel farming has expanded steadily and contributed to the economic development of the province, as well as providing much needed employment in rural areas. Figure 1.1 indicates the aquaculture lease sites (both finfish and shellfish) in the province in 2001. In 1999 and 2000, Newfoundland mussel farming had an export value of $3.8 million and $2.7 million respectively (Government of Newfoundland and Labrador). Coupled with this growth has been an increasing concern about the environmental impacts of aquaculture, which has heightened the need for investigation (Grant et al., 1995; Kaiser et al., 1998; Chamberlain et al., 2001) and prompted the passing of federal legislation requiring environmental impact studies in the licensing process for aquaculture sites.

Most bivalve species that are utilized for aquaculture can be grown by a relatively unskilled workforce with a low level of financial investment. In some areas, the attractiveness of high financial returns and government subsidies has led to the expansion of bivalve farming (Black, 2001). Bivalve species are grown to market size in their natural environment, adding to the marketability of the final product (Kaiser et al., 1998).

Bivalve aquaculture normally has less environmental impact than finfish culture because it relies solely on natural seston for food, thereby eliminating the enhanced sedimentation of particulate organic matter in the form of fish feed (Hatcher et al., 1994; Hargrave et al., 1997; De Grave et al., 1998; Christensen et al., 2003; Crawford et al., 2003). Most studies of bivalve aquaculture have detected modest environmental effects, in contrast to more pronounced effects often found in even small scale finfish culture operations (Kaspar et al., 1985; Baudinet et al., 1990; Hatcher et al., 1994; Grant et al., 1995; DeGrave et al., 1998; Crawford et al., 2003).

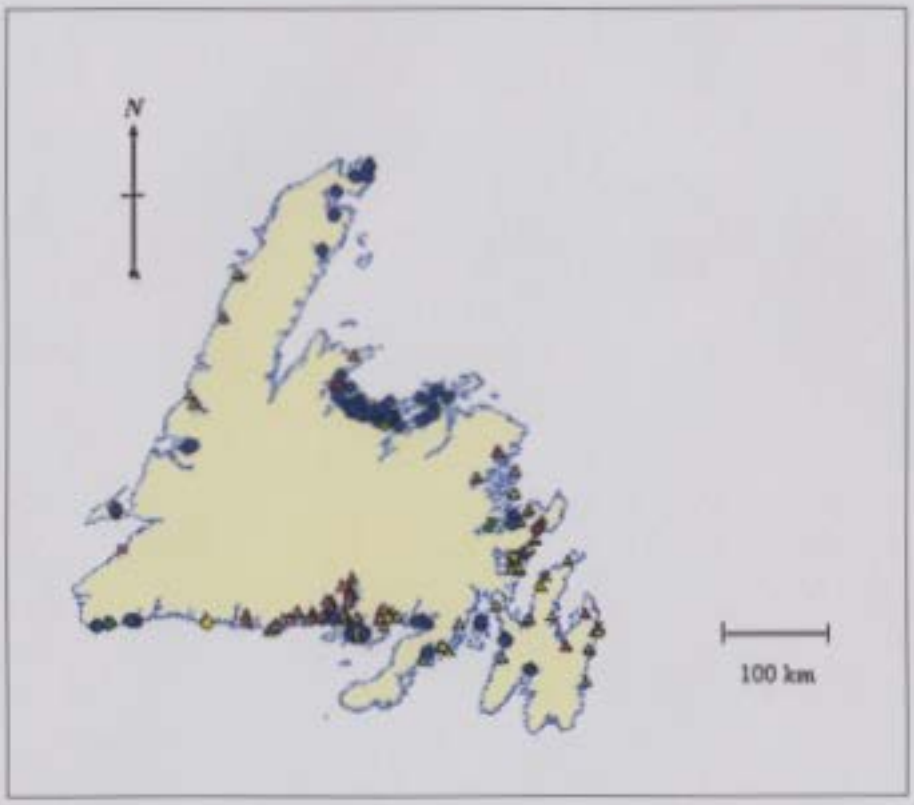

Figure 1.1: Map of distribution of aquaculture leases in 2001 in Newfoundland. Circles indicate bivalve farms (blue = mussel, yellow = oyster). Triangles denote finfish aquaculture sites (yellow =Atlantic Cod, orange = Atlantic Salmon, red = Rainbow trout), while squares denote sea urchins. Map created by the Department of Fisheries and Aquaculture, Government of Newfoundland and Labrador.

Kaspar et al. (1985) have suggested that effects may be reduced even further by using appropriate culture techniques. Environmental effects of aquaculture should be minimized not only to preserve environmental integrity and sustainability, but also to ensure favorable growing conditions for present and future farming operations. Farmers are often the first to notice, and also the first to suffer from, environmental degradation at their sites (Scarratt, 1993). For example, in Japan, bivalve culture in shallow embayments has led to declines in production because of nutrient and organic enrichment (Rosenthal et al., 1987).

1.2 Culture Methods

Bivalve culture has three stages: spat collection (either wild-caught from the farm or from a hatchery), grow-out, and harvesting. Each has potential impacts on the environment. The grow-out stage of shellfish aquaculture occurs in three major forms, benthic culture, intertidal culture and suspended culture. Benthic culture involves culturing bivalves directly on the seabed, or in the sediment in the case of clams (Scarratt, 1993). Intertidal culture takes place within the intertidal zone, where bivalves grow either directly on or just above the substratum. In suspended culture systems, bivalves are either directly attached to ropes (the most common technique for mussels) or held within structures (e.g. pearl and lantern nets for scallops, cages for oysters) suspended in the water column (Black, 2001).

In Newfoundland, as in many parts of the world, mussels are grown using the longline technique, whereby a "longline" supports a suspended array of ropes (Figure 1.2). The longlines are often anchored to the seabed and maintained just below the surface with buoys or floats. Mussels are seeded in socks, which are suspended along the longline for mussel grow-out (Scarratt, 1993).

1.3 Potential effects of mussel aquaculture

The effects of mussel aquaculture are social, economic and environmental, of which the last is the focus of this study. These effects are entirely site specific (Chamberlain et al., 2001), and depend upon the abundance of mussels growing in an area i.e. the scale of production (Kaiser et al., 1998; Chamberlain et al., 2001), the orientation and distribution of mussels within the farm (Chamberlain et al., 2001), food availability (which affects the rate at which feces and pseudofeces are deposited) (Jaramillo et al., 1992; Chamberlain et al., 2001), the nature of the habitat (Kaiser et al., 1998), and how long the site has been used for aquaculture (Chamberlain et al., 2001). Many studies have shown that the environmental effects of mussel cultivation are usually minimal (Baudinet et al., 1990; Buschmann et al., 1996; Chamberlain et al., 2001). Grant et al. (1995) and Crawford et al. (2003) have suggested that because the environmental effects are small, extensive monitoring of shellfish farming on a regular basis is not

necessary. Contrary to this perspective, others have shown that environmental effects can be severe enough to alter the physico-chemical and biological characteristics within the immediate and surrounding areas (Hartstein and Rowden, 2004), including benthic communities (Mattson and Lindén, 1983; Kaspar et al., 1985; Stenton-Dozey et al., 2001).

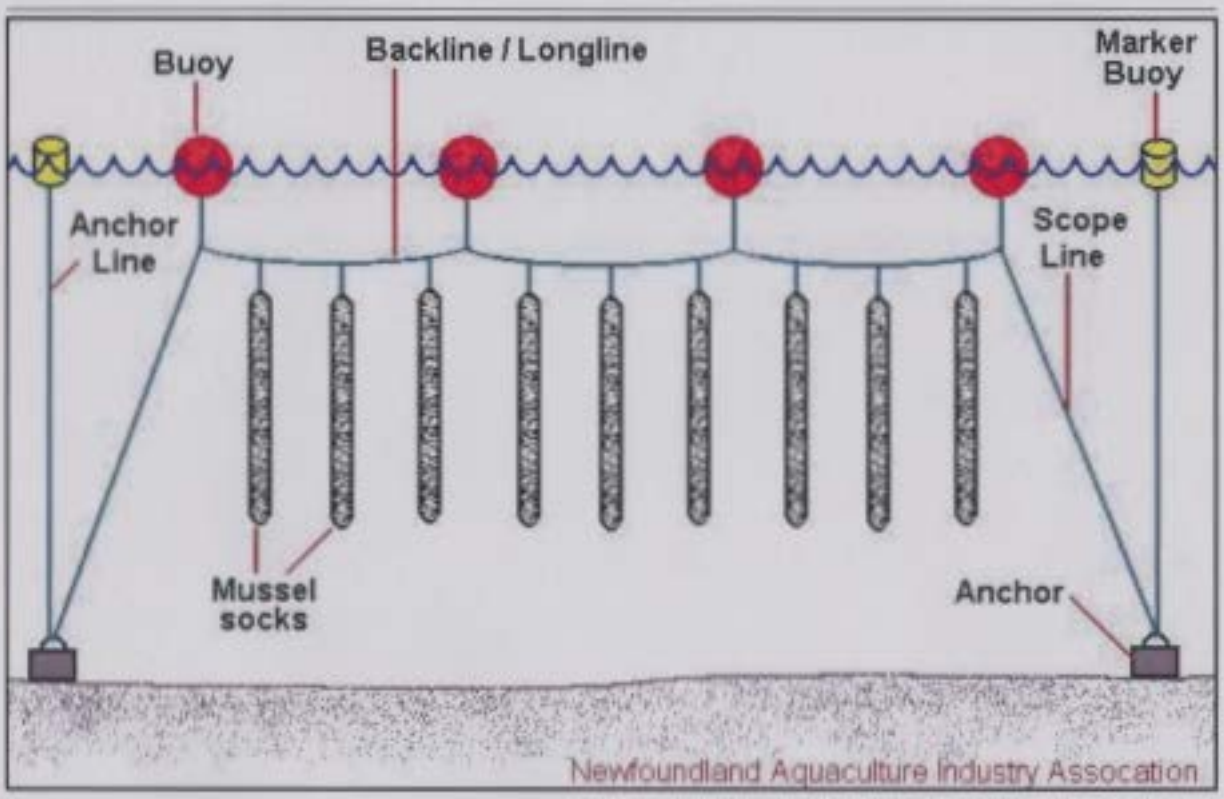

Figure 1.2: Schematic diagram of longline culture. Diagram created by the Newfoundland Aquaculture Industry Association.

1.3.1 Physico-chemical changes in the water column and benthos

Many aspects of bivalve aquaculture have the potential to change the environment both physically and chemically (Kaiser et al., 1998). The most obvious environmental effect is the addition of physical structures, such as the lines and mussels themselves, which can change the hydrodynamics of the water column, potentially slowing currents

and therefore increasing sedimentation. High densities of mussels can provide substrate for the settlement and growth of epibionts. Additionally, ropes and buoys may become detached and litter the environment (Hecht and Heasman, 1999), especially the shoreline. The effects on the benthos of harvesting mussels from long lines are usually minimal because the bivalves are removed without interfering directly with the environment (Kaiser et al., 1998), although some mussels usually fall off the lines onto the bottom during harvesting.

Most studies of environmental impacts of mussel farms have examined the grow-out phase of culture (Kaiser et al., 1998). In some areas, the main effect on the seabed during grow-out is the accumulation of detached mussels below the lines (Christensen et al., 2003) and an increase in attached algae (Grant et al., 1995; Crawford et al., 2003). These shells and algae may increase habitat heterogeneity and create favorable conditions for other taxa, and also act as an additional food source for scavengers. Furthermore, they can increase organic matter input to the sediment (Jaramillo et al., 1992; Black, 2001).

During the grow-out stage, the release of dissolved nutrients (especially ammonia) and particulate matter (feces and pseudofeces) by the mussels can also potentially alter the environment. Farmed bivalves can play a significant role in controlling the amounts and forms of nitrogen as well as the rate of nitrogen cycling within the ecosystem via the consumption and deposition of suspended material (Dame et al., 1991). The biological ramifications of such nitrogen production on phytoplankton and zooplankton are discussed in section 1.3.2.

It is also during the grow-out stage that suspension-feeders, such as mussels, remove particles from the water column, repackage them, and release them as feces or pseudofeces in a process known as biodeposition (Mattson and Lindén, 1983; Kautsky and Evans, 1987; Jaramillo et al., 1992). This removal of particles from the water column can impact both the pelagic and benthic environments. The influence of bivalve culture on the soft bottom environment has been examined by many researchers (Kaspar et al., 1985; Kautsky and Evans, 1987; Chamberlain et al., 2001) who have concluded that impacts vary (Chamberlain et al., 2001; Crawford et al., 2003). Input of organic matter to the sediment is mainly a result of these sedimenting biodeposits (Hatcher et al., 1994; Ragnarsson and Raffaelli, 1999) and can lead to a locally increased deposition of

materials, especially in low energy environments (Kautsky and Evans, 1987; Grant et al., 1995). In contrast, Hartstein and Rowden (2004) found that sites with hard substrates (rocks/gravel) are areas of relatively high-energy and that the benthic community is less likely to change as a result of biodeposition by mussels.

Organically-enriched environments are those with organic carbon levels that are elevated above those observed in the absence of human activity. Organic enrichment is one of the most common disturbances of marine benthic communities (Gray, 1981; Weston, 1990). The effects of natural increases in organic matter are often comparable to organic enrichment from human sources (Simon and Dauer, 1977; Thiel, 1978) and the benthos often reacts similarly regardless of the source of the organic input. In the case of mussel farming the source of organic enrichment is the sedimenting biodeposits from the mussels themselves. The accumulation of biodeposits on the seabed can enrich the sediments organically, thereby increasing the sediment oxygen demand and potentially resulting in an anaerobic environment (Grant et al., 1995). Sediments with very high carbon levels are often associated with hypoxia of the overlying water, an increased sediment oxygen demand (Kaspar et al., 1985), decreased sediment redox potential (Dahlbäck and Gunnarsson, 1981) and increased sulphate reduction.

1.3.2 Biological changes in the water column and benthos

The physical and chemical environmental effects of mussel farming described above can lead to biological changes in the environment. The first level of influence is on the phytoplankton community. Feeding by dense populations of bivalves in the grow-out phase of culture can modify phytoplankton community structure (Norén et al., 1999), reduce seston levels, and increase primary productivity in the system (Navarro et al., 1991; Smaal and Prins, 1993).

Mussel farming can also influence the cycling of nitrogen, which in turn affects the flora and fauna. Once the spring diatom bloom has exhausted available nitrate (Semeneh et al., 1998), phytoplankton biomass can gradually increase again as nitrate in the upper water column is replenished by remineralization and upwelling. Mussels excrete considerable amounts of ammonia (Bayne et al., 1985), stimulating productivity of the algae attached to mussel lines (LaPointe et al., 1981). The effect of this change in

the system's nitrogen budget is greater if nitrogen is limiting (Rodhouse and Roden, 1987), which is typical for most marine environments. If the rate of nitrogen cycling increases, primary production also increases, with the possibility of local toxic and non-toxic blooms (Rodhouse and Roden, 1987). These impacts may become more evident in small inlets, such as those found in this study, as a result of restricted tidal flushing (Dowd, 1997) and limited access to the open sea (Archambault et al., 1999).

This increase in nitrate (at a time when silicate limits diatom blooms) stimulates the growth of flagellates and/or dinoflagellates, which is the normal seasonal sequence for Newfoundland waters (Thompson, personal communication). This increase can also influence the size distribution of the zooplankton community by replacing larger zooplankton species with smaller ones (Uye, 1994). In the sites used in this study, size structure of zooplankton communities is not consistently affected by mussel grazing (Stacey, 2003). Additionally, juvenile and adult zooplankton stages are both susceptible to ingestion by bivalves (Davenport et al., 2000; Green et al., 2003).

Changes in the structure of the zooplankton community may have implications for other pelagic food web components, especially juvenile fish (Stacey, 2003). As the number of bivalves increases, the number of zooplankters decreases, altering the system (Rodhouse and Roden, 1987) by diverting primary production and energy flow from planktonic to benthic food webs (Cloern, 1982; Norén et al., 1999) and thus changing the availability of food resources to other species.

The alteration of macrofaunal community structure by organic enrichment has formed the basis of many benthic environmental studies (Hartstein and Rowden, 2004; Findlay et al., 1995). In areas where bivalve density is high, the settlement of larvae from all benthic species may be reduced because the larvae may be filtered and/or digested by adult bivalves and become bound in the feces and pseudofeces (Baldwin and Newell, 1995). Furthermore, soft-bottom communities are often dependent on production sinking from the water column as a major food source, especially at depths below the photic zone (Sumich, 1992; Pinet, 1998). In mussel farms, feces and pseudofeces account for most of this sinking production. Sedimentation rates may be as much as three times higher in mussel farms than in nearby reference areas that are not influenced by mussel culture (Dahlbäck and Gunnarsson, 1981). Thus, the amount and composition of organic matter

that reaches the seabed is very important to benthic community structure, biomass and metabolism (Mills, 1975). The degree of organic enrichment will determine the magnitude of the change in benthic macrofaunal composition and consequently the extent to which the environment is affected.

In some cases, the composition of the macrofaunal community may simply shift towards species that are more tolerant of hypoxic conditions and finer sediments (Tenore et al., 1982; Kaspar et al., 1985). Such a change has been seen at some bivalve aquaculture sites (Tenore et al., 1982; Mattson and Lindén, 1983; Kaspar et al., 1985). Mattson and Lindén (1983), for example, observed an almost complete disappearance of the original benthic macrofaunal community. Generally, relative to organically-enriched environments, benthic communities with lower detrital input are more species-rich, have a relatively low total abundance to species richness ratio, and include a wide range of higher taxa (Rhoads et al., 1978). Diversity initially increases in response to organic enrichment because an increased supply of nutrients can support not only existing populations but also new individuals (Grant et al., 1995). Kaspar et al. (1985) and Kautsky and Evans (1987), for example, found an increase in species diversity in organically-enriched areas. If bottom water currents are high, biodeposit dispersal may be adequate and thus deoxygenation may be avoided and infauna may survive (Tenore et al., 1982; Rodhouse and Roden, 1987; Black, 2001). In areas where this is not the case, decreased oxygen levels within the sediment bring the redox boundary layer nearer to the sediment surface, and thus infaunal organisms must live closer to the surface (Weston, 1990). As the organic content of the sediment further increases, larger, long-lived, deep burrowers are gradually eliminated and smaller opportunists take over because only a small number of specialist taxa can survive the anoxic conditions that eventually develop in surface sediments. This dominance of opportunistic species reduces species richness but increases the total number of individuals. Weston (1990) also suggested that a decrease in individual biomass could be expected, although an increase in total biomass is also possible as a result of a dense assemblage of these opportunists. These small individuals (for example, *Capitella* spp.) may be very abundant because of their ability to occupy sub-optimal habitats, thereby serving as indicator species of organic enrichment (Pearson and Rosenberg, 1978; Tenore et al., 1982; Mattson and Lindén, 1983; Kaspar et

al., 1985; Weston, 1990; Grant et al., 1995). If organic enrichment increases even further, the biological oxygen demand of the sediment may result in the deoxygenation of the overlying water column, and consequently the elimination of benthic macrofauna (Black, 2001). This situation is more likely to be found in areas of low flow rates, when bottom water renewal is also low and the biological oxygen demand of the sediment is high, putting the benthic macrofaunal community under hypoxic stress. Josefson and Widbom (1988), for example, found that organic enrichment increased mortality of benthic macrofauna. In areas of high impact, extensive bacterial mats often form beneath mussel lines (Dahlbäck and Gunnarsson, 1981; Kaspar et al., 1985).

Weston (1990) has stated that a shift in the prevailing feeding groups (guilds) is also possible. The feeding behaviors of a benthic community can be quantified by means of the infaunal trophic index, and a community may be classified as "normal", "changed" or "degraded". This index is based on whether organisms are suspension or deposit feeders and whether they feed above or below the surface of the sediment. Classic models by Pearson and Rosenberg (1978), for example, predict that subsurface deposit feeders (e.g. *Capitella* spp.) dominate enriched areas.

Alternatively, mussels can also influence nutrient concentrations because they remove large amounts of nutrients from the ecosystem. This can be advantageous in a eutrophic environment, and mussels can help mitigate the nutrient overloading in eutrophic areas (Soto and Mena, 1999). It could be argued, however, that mussels merely transfer the problem of eutrophication from the water column to the seabed.

1.4 AquaNet project ("Environmental requirements for sustainable shellfish aquaculture")

This study is one component of a collaborative project between Memorial University and Fisheries and Oceans Canada as part of AquaNet, one of the National Centres of Excellence funded by the Government of Canada. The larger project examines relationships among important environmental variables central to selection of suitable sites to support sustainable mussel aquaculture in Canadian coastal waters, and has three major themes. The first deals with remote sensing of sea surface temperature, primary production and chlorophyll *a* concentration, the second examines coastal morphometry,

bathymetry and bottom features, and the third deals with simulation models of mussel production, biodeposition and the food web on which mussel production depends (Anderson et al., unpublished report).

1.5 Objectives

The general objective of this project is to determine the effects of farming blue mussels (*Mytilus* spp.) on the benthic macrofauna at selected Newfoundland aquaculture sites. The goal is to establish whether or not the biodeposits produced by the mussels in the grow-out stage of aquaculture affect the benthic macrofaunal assemblages at these sites. Specifically, if there are differences in the rates of biodeposit production at each farm or at different times of the year (Ch.2), are these differences reflected in the species composition of the benthic macrofaunal community (Ch. 3)? The first chapter of this thesis provides a general introduction to aquaculture, with particular reference to Newfoundland, and the potential effects of aquaculture on the environment. Chapter 2 investigates biodeposition at each mussel farm and quantifies experimentally the production rate of biodeposits of individual mussels, to determine whether there is a difference between the two farms and whether there is seasonal variation in the rate of biodeposit production. Chapter 3 examines the effects of mussel biodeposition on benthic macrofaunal community structure at two aquaculture sites by comparing the benthos at farm stations with stations at adjacent reference sites. Chapter 4 exploits a novel theoretical approach to the problem of scale in ecology to determine if the benthic community of inshore waters of Newfoundland can be characterized by extrapolating the data on a larger spatial scale. Finally, Chapter 5 provides a general discussion/summary of the experimental, field and theoretical knowledge acquired during the study.

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2.0 Biodeposit production by *Mytilus* spp. and the potential for environmental impact

2.1 Introduction

2.1.1 General Introduction

In the past decade, bivalve aquaculture has grown rapidly and will undoubtedly continue to grow (Howlett and Rayner, 2004) in many parts of Canada, including Newfoundland. This growth has been coupled with an increased awareness of the environmental impacts of aquaculture, which has heightened the need for investigation of aquaculture practices and consequential effects (Grant et al., 1995; Kaiser et al., 1998; Chamberlain et al., 2001). The grow-out phase of mussel aquaculture is most likely to produce environmental impacts, and for this reason it has received most of the attention (Kaiser et al., 1998).

2.1.2 What is biodeposition?

Mussels (Mytilidae) are bivalve suspension feeders that remove large amounts of suspended particulate matter from the water column. Phytoplankton cells are the principal food source for both larvae and adults. Some of the particles removed from the water column by mussels are ingested, while others may be rejected as pseudofeces. Clearance rates (feeding rates) increase non-linearly with an increase in seston concentration (Bayne et al., 1993). Some of the ingested material is absorbed and used for growth, metabolism, and reproduction, and the remainder is voided as waste in the form of feces (Kautsky and Evans, 1987). Feces and pseudofeces are expelled into the water column and settle to the bottom, a process termed biodeposition (Haven and Morales-Alamo, 1970; Grant et al., 1995; Hartstein and Rowden, 2004). Tenore and Dunstan (1973) reported that biodeposition rates of mussels, oysters, and clams increase logarithmically with an increase in food concentration. Biodeposits can accumulate on the bottom and produce a thick layer of material, depending on how quickly bottom currents disperse them (Chamberlain et al., 2001).

Feces and pseudofeces differ from one another in appearance, in how they are released, and in quantities produced. Although feces are always formed, production of pseudofeces is variable. Increasing proportions of pseudofeces are rejected by the mussel at particle concentrations above 5 mg L^{-1} (Newell et al., 1989). Feces are light to dark brown and are ejected via the exhalant siphon. Pseudofeces, in contrast, are yellowish-brown, flocculent aggregates that are rejected from the mantle cavity (Bayne et al., 1993) and deposited alongside the inhalant siphon (Newell et al., 1989; Navarro and Thompson, 1997).

Particles suspended in the water column settle to the bottom by natural sedimentation (Jaramillo et al., 1992), by aggregating as flocs called "marine snow", or through incorporation into the feces of zooplankton grazers. At a mussel farm, this process is enhanced by biodeposition. It has been estimated that sedimentation rates within a mussel farm can be up to three times higher than in other areas (Dahlbäck and Gunnarson, 1981).

The rate of production of biodeposits can be affected by several factors related to the mussels themselves, as well as to the environment in which they live. These factors, which are discussed in section 2.4, include the size and age of the mussels, the availability of food and the time of year, as well as cycles of temperature, spawning and phytoplankton blooms.

2.1.3 Potential environmental impacts of biodeposits

Suspension-feeding bivalves play a significant role in bentho-pelagic coupling at farm sites (Dame et al., 1980; Kautsky and Evans, 1987; Urrutia et al., 1996) and their biodeposits serve as an important medium for the flow of energy between the two systems (Haven and Morales-Alamo, 1966). Because biodeposits sink rapidly from the water column, they can affect the physico-chemical properties of the benthic environment (see section 1.3).

2.1.4 Measuring biodeposit production rate

The rate of biodeposit production has been measured in several ways, both in the field and in the laboratory. Annular flumes or flux systems (Widdows et al., 1998; Jie et al., 2001), benthic ecosystem tunnels (Smaal and Zurburg, 1997), biodeposition collectors (Valenti and Epifanio, 1981), collector plates (Pouvreau et al., 2000) and sediment traps (Kautsky and Evans., 1987; Hatcher et al., 1994) have all been used to collect biodeposits and to measure rates of biodeposition by individuals of several bivalve species. Collectors and sediment traps tend to overestimate vertical flux of particles in shallow water systems such as mussel farms because of resuspension from the bottom and lateral advection. In the current study, biodeposit production rate was measured for individual mussels held in an experimental laboratory apparatus containing seawater pumped from the farm (Navarro and Thompson, 1997; Iglesias et al., 1998). However, the rate of production of biodeposits could not be extrapolated to the scale of the mussel farm because no reliable data on stocking density was available.

2.1.5 Objectives

The main objective of the present study was to determine biodeposit production by mussels at two farms in coastal Newfoundland at different times of the year. This information will contribute to understanding the potential impacts of biodeposition on the benthos, in particular macrobenthic fauna, and complements the work described in Chapter 3.

2.1.6 Hypotheses

The following hypotheses were examined in the biodeposition experiments:

1. The production rate of biodeposits by mussels does not vary among sites.
2. The rate of production of biodeposits by mussels varies temporally.

2.2 Materials and methods

2.2.1 Site description

Two mussel farms located in northeastern Newfoundland (Figure 2.1), Fortune Harbour (FH) and Charles Arm (CA), were chosen as representative study sites. Blue mussels (predominately *Mytilus edulis*) have been cultured on long lines at each farm site for at least a decade. These sites are typical of the sheltered locations commonly used for aquaculture within the province. Like most mussel farms in Newfoundland, the study sites are small, semi-enclosed inlets with a narrow opening to the open ocean, are ice covered in winter, and receive little anthropogenic input. The sites were chosen for their year-round accessibility as well as the logistic support received from growers at these locations. Previous research had also been undertaken at these locations, providing some useful, site-specific historical information.

Atlantic Ocean Farms (Figure 2.1) is located in FH (49°30 N, 055°15W). The surface area of the farm is 0.87 km^2 with a maximum bottom depth of 35 m. Its axial length is ca. 2.5 km with a width of ca. 400 m over most of its length, although it is less than 100 m wide at its narrowest point. Production of the farm during the time of study was approximately $1.8 \cdot 10^5$ kg yr^{-1} ($2.1 \cdot 10^5$ kg y^{-1} km^{-2}) (J. Wiseman, farm manager, personal communication).

The second farm (Figure 2.1), operated by Black Gold Inc., is located in CA (49°20 N, 055°16W), and has a surface area of 0.59 km^2 and a maximum bottom depth of 20 metres. The axial length of the farm is approximately 3.1 km with a width of 200-500 metres (50 m at its narrowest) (Penney et al., 2001). Production of the farm during the study period was approximately 3.75-4.5$\cdot 10^5$ kg yr^{-1} (6.4-7.7$\cdot 10^5$ kg y^{-1} km^{-2}) (T. Mills, farm owner and operator, personal communication).

Currents at both farms are weak, with a maximum velocity of 3 cm $s^{-1}$and 5-10 cm s^{-1} in the mouths of FH and CA respectively (max. velocity < 2 cm s^{-1} at their heads) (Timko et al., 1999; Coffin, 2001). CA flushes between 1 and 2.75 times per week and the mean tidal range is 0.75m. No flushing data are available for FH.

At both sites, chlorophyll *a* concentrations generally reach ca. 5 $\mu g\ L^{-1}$ during phytoplankton blooms (Clemens et al., 2000; Coffin, 2001). During winter, food quality may be poor, because phytoplankton: detritus ratios are low (Penney et al., 2001).

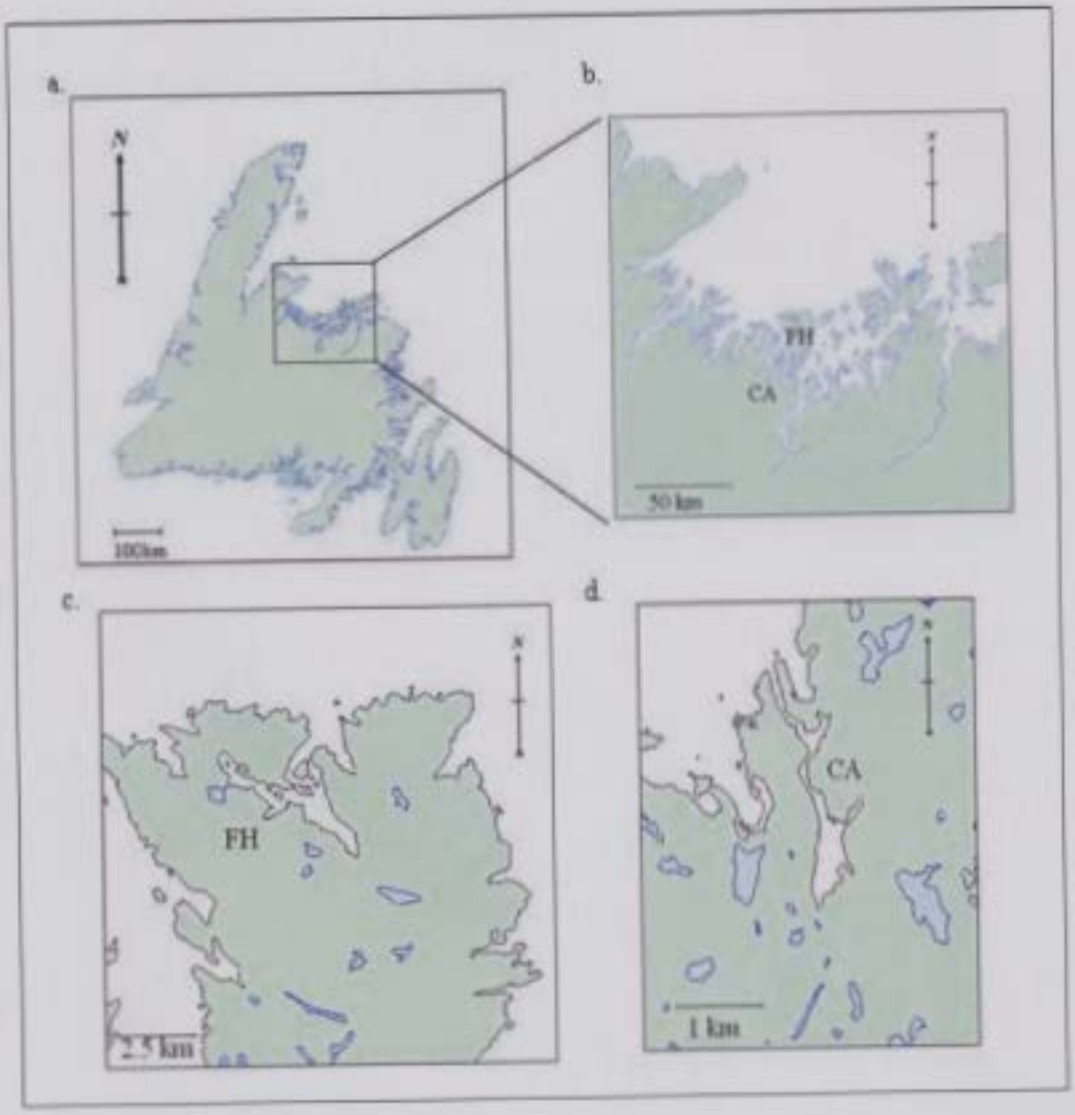

Figure 2.1: a) Map of Newfoundland highlighting Notre Dame Bay b) Notre Dame Bay showing Fortune Harbour (FH) and Charles Arm (CA) c) FH d) CA. Green shading- land; blue shading- freshwater; no shading (white)- seawater.

2.2.2 Sample collection

The rate of production of biodeposits by blue mussels (*Mytilus* spp.) at each farm site was measured using the method of Navarro and Thompson (1997). Several hundred mussels were collected from each farm during four sampling periods (June 26-29, August 21-24 and November 21-24, 2002 in FH; June 30-July 3 and August 27-30, 2002 and March 24-27, 2003 in CA). Both sites were covered by ice during sampling in March. Unfortunately, because of operational requirements of the farms and stocking practices, it was not always possible to sample a large range of mussels having different shell lengths. Size classes were often selected based on harvesting schedules in the farm. Thus, it was not possible to standardise the size of the mussels used in the study. Size standardisation is problematic in seasonal studies of bivalves because soft tissue weight varies for an individual of given shell length at different phases of the growth and gametogenic cycles. For convenience, all samples collected in late June or early July will be referred to as July. From the hundreds of mussels collected at a given site on a given date, 18 were selected haphazardly for use in experiments. For each sampling occasion and site, measurements were spread over three consecutive days, six mussels per day, i.e. 18 replicate measurements in all. For each experimental run, six mussels were held undisturbed for 19-24 hours in unfiltered, ambient running seawater pumped from the farm site to allow them to feed on natural seston. Mussels not used immediately were held in running ambient seawater. Experimental mussels were placed individually in plastic containers and flow rates were controlled using flow restrictors in the plastic tube feeding each chamber to keep the flow between 100-200 mL min^{-1} and avoid particle resuspension (Figure 2.2). Ambient seawater was supplied to the experimental containers from a header tank. Mussels that did not begin to feed almost immediately (within 30 seconds) were replaced. A 0.5 mm mesh screen was placed at the outflow of each container to prevent loss of biodeposits. During each experiment, one control container with no mussels present accounted for sedimentation not attributable to mussels. At the end of each trial, accumulated biodeposits were collected from each mussel and container with Pasteur pipettes, concentrated under gentle vacuum on pre-combusted (450 ^{o}C), weighed glass-fibre filters (Whatman GF/C, 47 mm diameter) and rinsed with distilled

water to remove salts. The filters were then frozen until they could be transported to the laboratory in St. John's to be dried and weighed. Upon completion of each trial, mussels were carefully bagged, labeled and frozen until they could be processed, measured, dried and weighed. Replicate trials were typically completed over a three day period at a given site, based on the assumption that environmental conditions and food supply did not vary significantly within each three day period at each site; this assumption was verified statistically.

Although this method does not provide *in situ* measurements of biodeposits from directly beneath the culture lines, it does allow mussels collected from their respective sites to feed in ambient seawater conditions and eliminates the problem of resuspension of biodeposits within the water column. An alternative method would have been to measure the amount reaching the bottom with sediment trapping, but this approach also has disadvantages. Sediment traps can overestimate biodeposition rate in shallow water, owing to resuspension from the bottom, and there are also differences in trapping efficiency in different hydrographic regimes (Butman, 1986). These problems are exacerbated by herbivore grazing in the water column, bacterial microbial action, and reprocessing of biodeposits by the mussels themselves.

2.2.3 Sample Analysis

In the laboratory, filters were dried at 60 ^{o}C for at least 24 hours and cooled in a desiccator. The filters were then reweighed and the weight of the biodeposits determined by difference. The measured value from the control container was subtracted from the weight of biodeposits after each trial to correct for sedimentation. Typical control chamber values were generally 2-20% of measured biodeposit production. The shell lengths of the mussels were measured (± 0.1mm) with digital calipers, and soft body tissues of each mussel were removed, dried at 90 $^{\circ}$C for 72 hours, and weighed.

2.2.4 Data analysis

2.2.4.1 Relationships among variables

Regression analyses were carried out to determine the relationship between biodeposition rate and dry tissue weight and between biodeposition rate and shell length.

Figure 2.2: Apparatus used to collect biodeposits. The hose in the top left hand corner brings ambient running sea water into the header tank (white bucket), which allows water to enter individual containers holding experimental mussels. The outflow from the bucket, visible at the water surface, provides a constant head.

2.2.4.2 Comparison of biodeposit production rates between sites

Because the sampling regime was not identical at the two sites in winter, it was not possible to analyze the entire data set in a single Analysis of Variance (ANOVA). First, a two-way ANOVA compared biodeposition rates between FH and CA during July and August only. The variation among biodeposition rates for the three consecutive sampling days at each site in each month was not significant ($p > 0.05$) and the 18 values obtained in each experimental series were therefore treated as replicates. In this model, the dependent variable was production rate of biodeposits (log transformed mg h^{-1}), and

the fixed factors were site (FH and CA) and month (July and August). Residuals were examined to ensure that log transformation of the data was appropriate in meeting the assumptions of normality.

2.2.4.3 Comparison of biodeposit production rates within each site

To determine whether there were differences among specific months in production rates of biodeposits, a one-way ANOVA was carried out for each site separately, with production rates of biodeposits as the dependent variable (log transformed; mg h^{-1}) and sampling month as a fixed factor. Where the F-value was significant at $p \leq 0.05$, a post-hoc Tukey test determined which group means were significantly different.

2.2.4.4 Comparison of biodeposit production rates in summer and winter at each site

To determine whether there was a difference in biodeposition rate between summer and winter, 2-sample t-tests were carried out for each site. At both sites July and August data were combined to form the summer data. November data was used for winter in FH and March data was used for winter in CA.

2.3 Results

2.3.1 Sample description

Table 2.1 summarizes the data from the biodeposition experiments for both FH and CA and includes chl *a* data (Rivkin, personal communication). Throughout the sampling year, biodeposits consisted solely of feces, and pseudofeces were not produced by any of the experimental mussels at any time. Biodeposit production rates were highest in July in CA (ca. 2.7 mg h^{-1}) and lowest in CA in March (ca. 0.125 mg h^{-1}).

2.3.2 Relationships among variables

2.3.2.1 Shell length and dry tissue

There was a logarithmic relationship between dry tissue weight (mg) and shell length (mm) for all sampled mussels throughout the sampling period (Figure 2.3; R^2 =

0.84, $p<0.001$). Because the two variables were so highly correlated, shell length was omitted from correlation and regression analyses to prevent colinearity.

2.3.2.2 Relationships between biodeposit production rate and other variables

For each of the six sampling dates, linear regression eqautions were obtained to relate $\log_{10}$ transformed production rate of biodeposits and $\log_{10}$ transformed dry tissue weight (Figure 2.4). The relationship was not significant ($p > 0.05$ in each case), probably because the shell lengths of the mussels did not differ greatly, and there was as a result no need to correct biodeposition rate values for mussel size. Furthermore, as indicated in Table 2.2, there was no correlation between rate of production of biodeposits (mg h^{-1}) and surface temperature ($p=0.165$), dry tissue weight ($p= 0.307$) or chl *a* concentration ($p=0.257$).

Table 2.1: Mean values (± standard deviation) of dry tissue weight (mg), shell length (mm) and biodeposition rate (mg h^{-1}) for mussels used in experiments in July, August and November of 2002 and March 2003 at FH and CA. Chl *a* ($\mu g \cdot L^{-1}$) data is also included (* = not sampled in March 2003).

Site	Date	Surface Temp. (°C)	Dry tissue weight (mg)	Shell length (mm)	Rate of production of biodeposits (mg h^{-1})	Time (h)	Chl *a* conc. ($\mu g \cdot L^{-1}$)
FH	July	15	993.0 ± 497.0	58.1 ± 9.61	0.633 ± 0.311	24	0.140
	Aug	19	596.4 ± 301.5	50.3 ± 11.7	0.868 ± 0.362	21	0.219
	Nov	5	550.4 ± 360.7	47.5 ± 11.4	0.296 ± 0.126	22	0.198
CA	July	18	539.0 ± 137.6	42.1 ± 3.90	2.70 ± 0.829	24	0.0542
	Aug	17	536.0 ± 483.0	44.9 ± 15.1	0.988 ± 0.624	19.5	0.307
	March	-1	1061 ± 319.5	63.5 ± 5.20	0.117 ± 0.0776	20	*

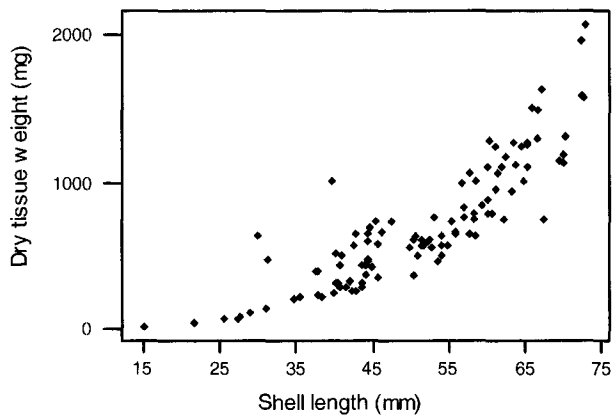

Figure 2.3: Relationship between dry tissue weight (DW, mg) and shell length (SL, mm) for all mussels used in biodeposition experiments. The power function relationship of the regression equation is: $DW = 4.26\ SL^{2.72}$, $R^2=0.84$.

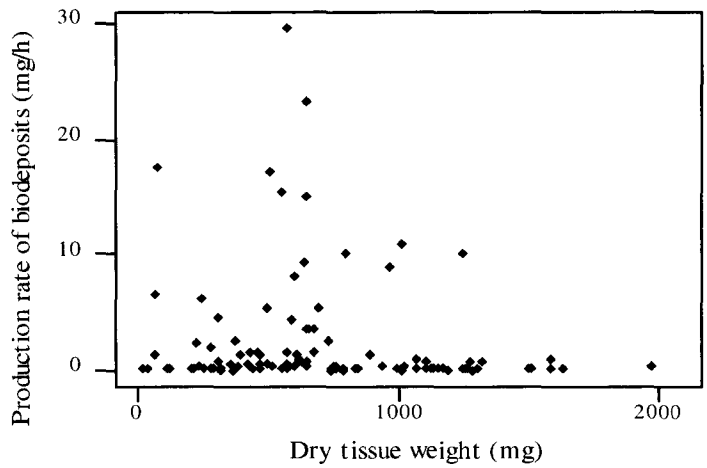

Figure 2.4: Relationship between production rate of biodeposits (mg h^{-1}) and dry tissue weight (mg) for all mussels used in biodeposition experiments, $R^2=0.018$.

Table 2.2: Correlation between biodeposit rate (mg h^{-1}) and surface temperature, dry tissue weight and chl *a* concentration in CA and FH.

Explanatory Variable	Correlation coefficient	p
Surface Temperature	0.647	0.165
Dry Tissue Weight	-0.505	0.307
Chl *a* concentration	-0.627	0.257

2.3.3 Comparison between CA and FH

Unfortunately, technical problems prevented the measurement of rates of production of biodeposits in CA in November 2002 and in FH in March 2003. Thus, production rates of biodeposits at the two farms could only be compared directly in July and August 2002, when both locations were sampled (Figure 2.5).

Results from the two-way ANOVA are presented in Table 2.3. Production rates of biodeposits were $\log_{10}$ transformed to meet the requirements of ANOVA. A significant interaction was found between site and month ($p<0.001$). Thus, site and month, in combination, were both important factors in explaining differences in production rates of biodeposits throughout the sampling period. Mussels were therefore responding differently at each farm at different times of the year. That is, the effects of the two factors, site and month, were not additive. This point is clearly seen in comparing sites in August (Figure 2.5). In FH, mussel biodeposit production showed an increasing trend from July to August, while in CA the opposite was found. Thus, site has an effect on mussel biodeposit production, as does month, although the two cannot be considered independent of one another. Site and month alone were also significant factors ($p < 0.001$ and $p= 0.004$ respectively).

In general, biodeposit production rates were higher in CA than in FH, and higher in the summer months (July and August) at both farms (Figure 2.5). The highest rates of biodeposit production recorded in the study were obtained at CA in the summer (Table 2.3, Figure 2.5).

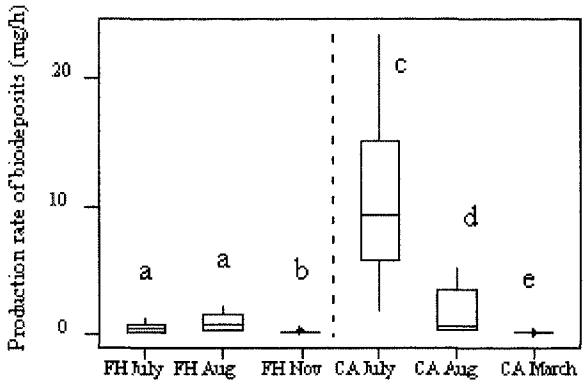

Figure 2.5: Production rates of biodeposits (mg h^{-1}) at each site (FH and CA, separated by a dashed vertical line) at each sampling time (July, August, November 2002, March 2003). The median is represented by the middle horizontal line. The top and bottom horizontal lines indicate the 75th and 25th percentiles respectively. Vertical lines indicate standard deviations. Means with the same letter are not significantly different ($p < 0.05$, pairwise Tukey test). NB: Data for FH (left of dashed vertical line) and CA (right of dashed vertical line) are compared separately.

Table 2.3: Two-way ANOVA. Dependent variable: $\log_{10}$ transformed production rate of biodeposits (mg h^{-1}); fixed factors: site (CA and FH) and month (July and August).

Source	DF	SS	MS	F	p
Month	1	2.514	2.514	9.68	**0.003**
Site	1	10.950	14.950	42.15	**< 0.001**
Interaction	1	6.811	6.811	26.22	**< 0.001**
Error	64	16.626	0.260		
Total	67	36.902			

2.3.4 Comparison of biodeposit production rate in different months within each farm

2.3.4.1 Fortune Harbour

ANOVA indicated that there was a significant difference in production rate of biodeposits with month (Table 2.4). Production rates of biodeposits in July and August at FH were similar, but there was a significant difference among biodeposit production rates in November and both July and August 2002 (pairwise Tukey test, $p< 0.05$) (Figure 2.5).

Table 2.4: One-way ANOVA. Dependent variable: $\log_{10}$ transformed rate of production of biodeposits (mg h^{-1}) at FH; fixed factor: month (July, August and November 2002).

Source	DF	SS	MS	F	p
Month	2	8.508	4.254	17.43	**< 0.001**
Error	48	11.713	0.244		
Total	50	20.221			

2.3.4.2 Charles Arm

ANOVA (Table 2.5) indicated that production rates of biodeposits at CA varied temporally ($p<0.001$). Mean values of production rates of biodeposits for each month were significantly different from each other (pairwise Tukey test, $p<0.05$) and are displayed in Figure 2.5.

Table 2.5: One-way ANOVA of rates of $\log_{10}$ transformed production rates of biodeposits measured in CA on three different occasions (July and August 2002 and March 2003).

Source	DF	SS	MS	F	p
Month	2	78.456	39.228	141.41	**< 0.001**
Error	48	13.315	0.277		
Total	50	91.771			

2.3.4.3 Comparison of biodeposition production rate between summer and winter months at each site

The results of 2-way ANOVA (Table 2.6) further indicated that at both FH and CA there was a significant difference ($p<0.05$) between $\log_{10}$ transformed biodeposition rates in summer (July and August) and winter (November and March). That is, production rates of biodeposits by individual mussels varied between summer and winter differently depending on farm site. Graphing of the data indicated that the decrease in biodeposit production from summer to winter was much smaller in FH than in CA.

Table 2.6: Two-way ANOVA for $\log_{10}$ transformed biodeposit production rates (mg h^{-1}) between summer and winter in FH and CA.

Source	DF	SS	MS	F	p
Site	1	11.017	11.0117	30.15	**< 0.001**
Season	1	3.239	3.239	8.87	**0.004**
Interaction	1	10.501	10.501	28.74	**< 0.001**
Error	68	24.845	0.365		
Total	71	49.601			

2.4 Discussion

The quantity and quality of feces and pseudofeces released by mussels is dependent upon many factors, including food quality, (Tenore and Dunstan, 1973; Valenti and Epifanio, 1981; Bayne et al., 1993) temperature/season (Tsuchiya, 1980; Kautsky and Evans, 1987; Jaramillo et al., 1992), the timing of phytoplankton blooms (Tenore and Dunstan, 1973; Valenti and Epifanio, 1981; Bayne et al., 1993; Navarro and Thompson, 1997) and spawning episodes (Bayne and Widdows, 1978; Newell and Thompson, 1984; Thompson, 1984; Thompson and Newell, 1985).

In cool temperate climates, such as that of Newfoundland, a spring diatom bloom begins around the end of March, when conditions are ideal for rapid and abundant growth (Tian et al., 2003), and finishes in late April when water temperatures are approximately 1 °C (Stead and Thompson, 2003). No spring bloom was observed during this study.

Food supply can affect rates of biodeposit production because blue mussels can adjust clearance rate to increase the efficiency with which material is ingested and assimilated, and can also regulate the amount rejected as pseudofeces (Bayne et al., 1993). Navarro and Winter (1982) demonstrated that clearance rate decreases with an increase in food concentration in *Mytilus chilensis,* but biodeposition rate increases only slightly. Conversely, higher seston concentration and higher food quality may enhance suspension feeding and in turn, biodeposition (Tenore and Dunstan, 1973; Valenti and Epifanio, 1981; Bayne et al., 1993). Navarro and Thompson (1997) found that the main peak in the biodeposition rate in *Modiolus modiolus* correlated with the main peak of chl *a* in the spring phytoplankton bloom (highest food quality and quantity), and that this was the only period when pseudofeces were produced. After the bloom, biodeposition rate decreased considerably.

Unfortunately, samples in this study could not be collected year round and thus the timing and dynamics of the spring bloom are unclear. Ideally, sampling should be done more often to determine whether biodeposition is highest during bloom conditions. Similarly, because of limitations on the sampling team, chl *a* values were not always measured on the same day as production rates of biodeposits. This may be one reason that a significant positive relationship was not found between food level and biodeposit production. Another reason may be that the depth at which chl *a* was sampled was not the same as that from which ambient seawater was pumped into the processing plant.

In some cases biodeposition patterns are linked temporally to water temperature, which generally follows a seasonal cycle in temperate climates (Tsuchiya, 1980; Kautsky and Evans, 1987; Jaramillo et al., 1992). Biodeposition rates of *M. chilensis* and *Choromytilus chorus* closely followed temporal changes in temperature in an estuary in southern Chile, peaking during mid-fall in one year and during summer in the next, with lowest values in winter (Jaramillo et al., 1992). Similarly, Tsuchiya (1980) and Kautsky and Evans (1987) found that biodeposition rates for *M. edulis* were lowest during the coldest months of the year and highest during autumn, when water temperatures were still relatively high. On the other hand, low biodeposition rates in winter may be attributable to low metabolic activity (Chiantore et al., 1998), although Thompson (1984) determined that *M. edulis* has a remarkable ability to maintain relatively high clearance rates at very

low temperatures. Furthermore, maximum biodeposition rates of the horse mussel *M. modiolus* were observed during the spring diatom bloom, when the water temperature was ca. 1 °C, further indicating that biodeposition is food-dependent rather than temperature dependent (Navarro and Thompson, 1997). Similarly, Jaramillo et al. (1992) proposed that the decline in biodeposition rate recorded during winter was probably attributable to the high concentration of inorganic seston in the water column rather than to a temperature decrease.

Another factor that greatly reduces production rate of biodeposits is spawning (Bayne and Widdows, 1978; Thompson and Newell, 1985). Clearance rate of *M. edulis*, and presumably biodeposition rate, is lowest during the spawning period (Thompson, 1984), but mussels resume filtering at the cessation of spawning (Newell and Thompson, 1984).

2.4.1 Comparison of biodeposit production rates

Although it is difficult to compare data from different studies that have utilized different methodologies, values for rates of production of biodeposits from this study are similar to those observed elsewhere when similar methods were used. The maximum value for rate of production of biodeposits was found in CA (ca. 64.6 mg day^{-1}) and is similar to values recorded by Navarro and Thompson (1997) for *M. modiolus* (max. value 40.9 mg day^{-1}, shell lengths 7.5-8.5 cm) in Logy Bay, Newfoundland. After the algal bloom in Logy Bay, biodeposition rates of *M. modiolus* dropped to 4.8 mg day^{-1} (Navarro and Thompson,1997), which is of the same order as values recorded in CA in March under ice cover (2.8 mg day^{-1}) and similar to values recorded in FH in November (7.1 mg day^{-1}). Values reported by Navarro and Winter (1982) for *M. chilensis* (53 mm shell length) were also within the range of the present study, (10-38 mg day^{-1}). Hatcher et al. (1994) found that in Upper South Cove, Nova Scotia, chl *a* concentrations peaked in March under ice cover, but the bloom ended in April when the ice began to break up. In Logy Bay, the spring diatom bloom occurred from April – May (Navarro and Thompson, 1997). Although a spring phytoplankton bloom is common in northern latitudes (Berg and Newell, 1986; MacDonald and Thompson, 1986), Penney et al. (2001) did not observe a single spring phytoplankton bloom in CA between 1989 and 1992. They

concluded that either the bloom is completely absent in the area or else it is very short and thus was missed by their 2 week sampling frequency. In the present study, a spring phytoplankton bloom was also not observed. Presumably, higher levels of chl *a* during a phytoplankton bloom would have resulted in higher production of biodeposits, as was the case with *M. modiolus* in Logy Bay (Navarro and Thompson, 1997), but it is unclear whether a bloom event occurs in Notre Dame Bay during the spring or if the timing is different than in other parts of Newfoundland. The agreement among this study and those of Navarro and Thompson (1997) and Hatcher et al. (1994) is probably because all three studies considered mussels that have similar feeding and digestion habits and live in cold sub-arctic waters with similar food availability.

Pinctada margaritifera, the black pearl oyster (5-18 cm shell height), has very high pseudofeces production rates (6 mg h^{-1} g^{-1}) (Pouvreau et al., 2000). Oysters are known for producing large amounts of pseudofeces, in contrast to the total absence of pseudofeces production by *Mytilus* spp. in this study, probably because food concentrations were not sufficiently high for production of pseudofeces.

Other studies have used sediment traps (Jaramillo et al., 1992; Hatcher et al., 1994) and annular flumes (Widdows et al., 1998; Jie et al., 2001) to measure biodeposition rates. Data obtained by these sampling methods cannot be compared with those in the present study, because flumes and traps measure the mass of biodeposits reaching the bottom, which may or may not be equal to the mass produced. Furthermore, *in situ* methods also integrate biodeposition from many mussels, rather than measuring the biodeposition rate for individuals, making comparisons with this study difficult.

2.4.2 Relationship between food level and biodeposition rate

Suspension feeders such as *M. edulis* filter the water column and ingest large amounts of suspended particulate matter. Thus, an increase in food supply (chl *a*) would be expected to increase fecal production. In seasons where food supply is highest, one would expect a greater production of biodeposits and thus a greater influence on the benthic macrofaunal community that exploits them. This study indicates that during the warmer months biodeposition rates are higher and biodeposition may therefore have a greater influence on the benthic community at this time. Food supply is known to affect

rates of biodeposition (Tenore and Dunstan, 1973; Valenti and Epifanio, 1981; Bayne et al., 1993). Although there was no relationship in the present study between the amount of food (chl *a*) and the rate at which biodeposits were produced, this does not necessarily mean that increased food levels do not result in greater biodeposit production, since there were confounding factors that may have masked such a relationship. Had sampling been carried out during a spring phytoplankton bloom, the rates of biodeposit production may have been significantly higher, as found by Navarro and Thompson (1997) in Logy Bay. Similarly, it is possible that heterotrophic protists dominate the food supply of mussels during the summer. This food source would not be captured by the chl *a* measurements made in this study. Furthermore, rates of production of biodeposits were significantly greater in summer than in winter in this study. This finding is similar to that of Jaramillo et al. (1992) in southern Chile, where biodeposition rate values were highest in summer and mid-fall and lowest during the winter, perhaps because of higher amounts of inorganic seston.

Penney et al. (2001) concluded that mussel culture in semi-enclosed inlets in Newfoundland is limited by food supply rather than by space. Thus, it is unlikely biodeposition by mussels at these two farms would have a significant impact on the benthic environment compared with those parts of the world where food supply is more abundant and mussel cultivation much more intense.

2.4.3 Comparison between farms

At times, rates of production of biodeposits were higher in CA than in FH, but this was not always true and the hypothesis that there is no difference between sites (section 2.1.6) cannot be rejected. The highest rates of production of biodeposits were observed in CA in July. Maximum chl *a* values were recorded in August and were higher in CA than in FH (Rivkin, unpublished data). Similarly, Penney et al. (2001) showed that in most years the maximum value for total particulate matter in CA is higher than in more open coastal areas in Newfoundland, which may explain the higher rates of production of biodeposits observed in CA in this study. Lower rates of production of biodeposits in FH, however, can be explained in part by lower ingestion rates resulting from lower food concentrations at this site. In general, chl *a* concentrations were higher in CA than in FH

(Rivkin, unpublished data). Because production of pseudofeces was not observed at either site, food levels are probably not high enough for production of pseudofecal material.

2.4.4 Temporal trends in biodeposition rate

A temporal effect was observed in the production rate of biodeposits at each location. Values were similar in July and August, but significantly lower in November, reflecting chl *a* concentrations, which were highest in August and lowest in November in FH (Rivkin, unpublished data). However, it is possible that higher rates occur during the spring diatom bloom, if indeed there is one in this area.

In CA, rates of production of biodeposits were significantly lower in March 2003 than in July and August 2002. In March, water temperatures are approximately -1.8 °C, the farm is covered with land-fast ice, and rates of production of biodeposits are low. The second hypothesis of the study (section 2.1.6) was that there is no temporal difference in rates of production of biodeposits measured at FH and CA; this hypothesis can be rejected because biodeposition experiments showed a temporal difference with higher rates in summer than in winter. It is possible, however, in light of other experiments (Navarro and Thompson, 1997), that if measurements had been made in spring during a phytoplankton bloom, maximum rates of production of biodeposits would have been recorded while water temperatures were still below 0° C.

A summary of research conducted by Penney et al. in 1989-1992 in CA concluded that the food supply for *M. edulis* is quantitatively limited in summer but qualitatively limited in the winter (Penney et al., 2001). Food supply in general is more favourable during spring and autumn, which may explain the high biodeposition rates recorded in August in FH and CA in this study and the lower levels in winter. It does not, however, explain the highest levels of biodeposition in CA in July.

2.4.5 Recommendations

In order to further quantify environmental impact and also to compare the two sites, CHN analysis of biodeposits would be useful to determine the amount of carbon entering the system and being utilized as food by benthic organisms. Depending on the

nutritional composition of the deposited material, biodeposits could be nutritionally significant for the benthic macrofaunal community (Navarro and Thompson, 1997). Kautsky and Evans (1987) found that organic carbon and nitrogen were higher in biodeposits than in naturally sedimenting material, and thus biodeposits may be a very good energy source for benthic deposit feeders.

Notwithstanding the likelihood of overestimating deposition rates from sediment traps deployed in shallow water, owing to resuspension, it may be informative to estimate the total flux of deposited material to the benthos by sediment trapping.

More frequent sampling is required to determine the timing of the spring phytoplankton bloom and its impact on the rate of biodeposition and consequently on the benthic environment. Seston POC should be measured in order to determine if heterotrophic protists are consumed by mussels during summer. Most importantly, growers should collect reliable information regarding stocking densities in the farm at any given time to allow calculation of the amount of biodeposits reaching the benthic environment throughout the life of the farm.

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3.0 Impacts of mussel aquaculture on benthic macrofauna and sediment redox profiles

3.1 Introduction

In the past decade, the growth of bivalve aquaculture in parts of Canada, including Newfoundland, has increased the need to understand the potential for environmental impacts of bivalve farming (Grant et al., 1995; Kaiser et al., 1998; Chamberlain et al., 2001). Increased awareness has also prompted the passing of federal legislation requiring environmental impact studies before the licensing of aquaculture sites by Fisheries and Oceans Canada.

Adverse environmental effects of aquaculture have been reported in various parts of the world and a marked impact on the benthic community has often been noted (Kaspar et al., 1985; Hatcher et al., 1994; Grant et al., 1995; Kaiser et al., 1998). However, some studies have found that the environmental effects of bivalve farming are low (Baudinet et al., 1990; Grant et al., 1995; DeGrave et al., 1998) and it has been suggested by Kaiser et al. (1998) that environmental impacts can be minimized using suitable culture techniques. A discussion of potential environmental impacts from mussel (genus *Mytilus*) farms can be found in section 1.3.

The degree of enrichment that results from increased sedimentation of particulate matter associated with cultivated mussels is dependent upon many variables, including stocking density, intensity of production, age of the farm, nature of the habitat, food available for mussels, and biodeposition rate (Chamberlain et al., 2001). The impact on the benthic macrofaunal assemblage is also dependent upon hydrodynamic energy within the farm site (Chamberlain et al., 2001; Hartstein and Rowden, 2004), which will determine settlement and flushing rates of organic particulate matter.

3.1.1 Objectives

The principal objective of this study was to determine whether or not mussel farms influence the benthos under culture lines through eutrophication. Nixon (1995) defines eutrophication as an increase in the flux of organic matter beyond "normal" values. Normal is a relative term and in this case refers to baseline conditions that occur

before the input of organic matter under natural conditions. The present study is significant because it not only compares two different farms in a sub-arctic environment in which they are covered in ice for a portion of the year, but it does so during both summer and winter. Secondary objectives were to compare the benthic communities at two mussel farm sites in Newfoundland, to determine whether there was temporal variation in the benthos and to identify differences in macrofaunal composition within a farm (among stations at each of the two sites and selected reference areas) throughout the sampling period.

3.1.2 Hypotheses

1. There is no difference between the benthic macrofaunal communities at the two farm sites.

2. There is temporal variation in the composition of the benthic macrofaunal communities associated with seasonal changes in redox in the sediments.

3. There is a difference in the macrofaunal community between each farm site and its paired reference site.

3.2 Methods

3.2.1 Site description

Two mussel farms located in Notre Dame Bay, Newfoundland (Figure 2.1) were chosen as study sites. A description of these sites is given in section 2.2.1. The sites, Fortune Harbour (FH) and Charles Arm (CA), were each paired with nearby reference sites (Figure 3.1, Figure 3.2, Table 3.1) where no mussel farming activity had occurred. The farms were relatively close to each other (approximately 20 km apart), and CA was the shallower of the two (Table 3.1). Reference sites with no mussel culture were chosen for each farm based on their similarity to the nearby farms, including such key factors as depth, bathymetry and proximity to the farm sites. Reference sites were used in lieu of baseline data because environmental sampling was not done prior to the establishment of the mussel farms. This is the preferred study design where no data are available before an impact occurs.

3.2.2 Sample collection

Sampling by divers was not possible and thus sampling was undertaken with an Ekman grab (15.24 cm x 15.24 cm x 22.86 cm deep). Labeling of stations is such that FH or CA identifies the farm site (FH = Fortune Harbour, CA = Charles Arm), the number denotes the nth station number in the farm or reference site and F or R denotes whether it is a station located in a farm (F) or reference (R) site. At FH, four farm stations (FH 1F, FH 2F, FH 3F, and FH 4F) were sampled (Table 3.1 and Figure 3.1) and compared with three reference stations (FH 1R, FH 2R, FH 3R). In CA, two farm (CA 1F and CA 2F) and two reference stations (CA 1R and CA 2R) were sampled (Table 3.1, Figure 3.2). All stations were located with GPS on each sampling day.

In order to survey the benthic macrofaunal community, four Ekman grab samples were taken at each of the above mentioned stations. Collected sediment was washed with filtered seawater through a series of sieves with 5 mm, 1 mm and 0.5 mm mesh size to retain macrofauna greater than 0.5 mm. A list of collected macrofauna can be found in Appendix A. Samples whose sediment was retained in the 5 mm mesh were classified as sand, otherwise, they were classified as mud.

In March 2003, a core sample was taken from each grab to determine the redox potential gradient of the sample. This subsample was taken using a section of PVC pipe (2 inch diameter) with a bevelled edge and holes drilled in the side at 2 cm intervals. An Orion platinum electrode redox probe was placed in each hole in succession (while other holes were covered), and a redox potential reading (mV) taken. The probe was calibrated with Zobell's solution prior to each field trip and the readings were corrected to normal hydrogen electrode potential using temperature corrections supplied by the manufacturer.

3.2.3 Sample treatment

Collected macrofauna were fixed in 4% formaldehyde buffered with borax and transferred to 70% ethanol for long-term preservation. Samples were also stained with rose bengal in order to facilitate identification. Preserved specimens were enumerated and identified to the lowest possible taxonomic level under stereo and compound microscopes in order to construct a species-abundance matrix. Various taxonomic guides and keys

were used to identify macrofauna (Gosner, 1978; Gosner, 1979; Pocklington, 1989; Wallace et al., 1989; Ramey, 2001; Harris, 2003; Quijon and Snelgrove, 2005).

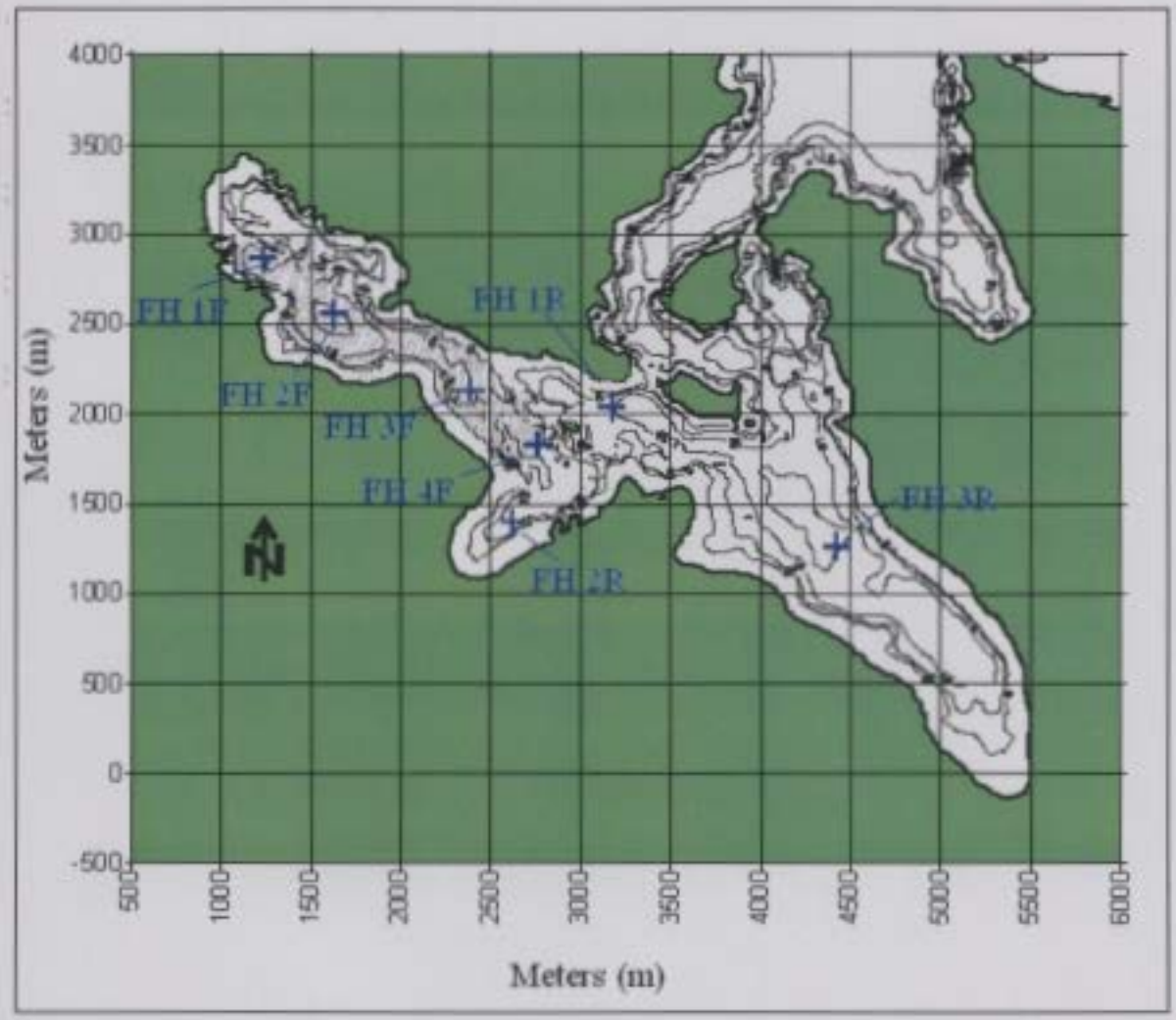

Figure 3.1: Map of FH showing locations of sample stations (+). FH 1F, FH 2F, FH 3F, and FH 4F are farm stations, FH1 R, FH 2R and FH 3R are reference stations. FH 1F and FH 1R are sandy, whereas the other stations have muddy sediments. Horizontal and vertical axes are in metres based on a 'nominal grid origin'. The outer line delineating the inlet indicates the high high water mark (HHW), the inner line the low low water mark (LLW). Depths (m) are relative to chart datum. Shaded areas (grey lines) are locations of mussel lines. Map created by Dr. Jon Chamberlain.

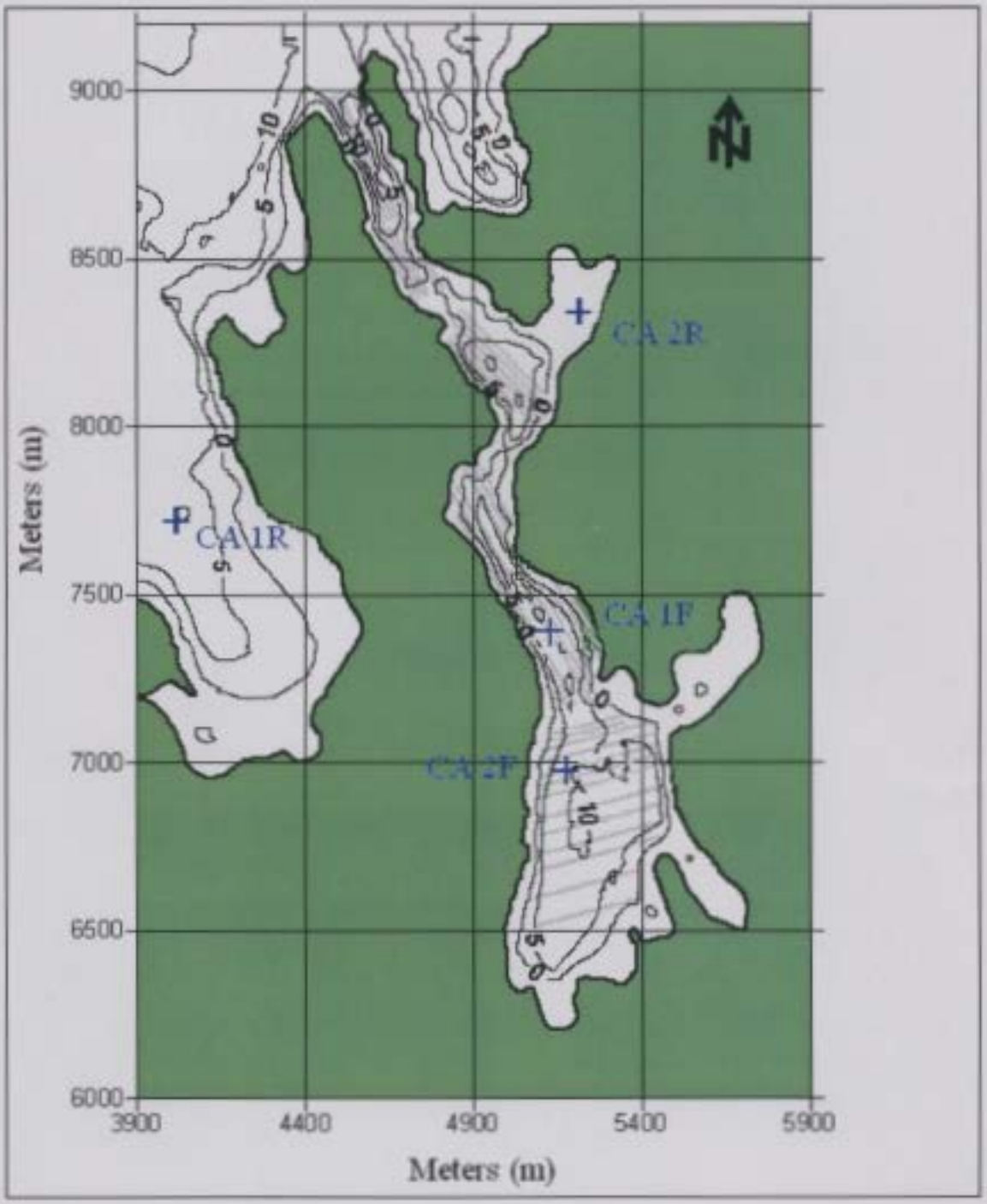

Figure 3.2: Map of CA showing sample stations (+). CA 1R and CA 2R are reference stations, CA 1F and CA 2F stations sampled within the farm. Horizontal and vertical axes are measured in metres based on a 'nominal grid origin'. Outer line on the map (labeled -5) indicates the high high water mark (HHW), the inner line (labeled 0) the low low water mark (LLW). Depths (m) are relative to chart datum. Shaded areas (grey lines) are locations of mussel lines. Map created by Dr. Jon Chamberlain.

Table 3.1: Locations and depths of study sites used for benthic macrofaunal sampling at two Newfoundland mussel farm sites (FARM= farm site, REF = reference area). This table is organized by sediment type.

Site $_{Treatment}$	Station	Sediment Type	Latitude (N)	Longitude (W)	Depth (m)
FH $_{FARM}$	FH 2F	Mud	49° 31.425	055° 16.283	33.5
FH $_{FARM}$	FH 3F	Mud	49° 31.103	055° 15.445	21.4
FH $_{FARM}$	FH 4F	Mud	49° 31.007	055° 15.359	22.8
FH $_{REF}$	FH 2R	Mud	49° 30.814	055° 15.344	21.4
FH $_{REF}$	FH 3R	Mud	49° 30.652	055° 13.791	39.4
FH $_{FARM}$	FH 1F	Sand	49° 31.572	055° 16.600	18.5
FH $_{REF}$	FH 1R	Sand	49° 31.112	055° 15.018	9.7
CA $_{FARM}$	CA 1F	Sand	49° 20.779	055° 16.645	12.4
CA $_{FARM}$	CA 2F	Sand	49° 20.692	055° 16.600	11.5
CA $_{REF}$	CA 2R	Sand	49° 21.281	055° 16.536	4.4
CA $_{REF}$	CA 1R	Sand	49° 20.937	055° 17.513	10.4

3.2.4 Sample analyses

The PRIMER (**P**lymouth **R**outines **I**n **M**ultivariate **E**cological **R**esearch) v5 software package was used to analyse the species abundance matrix. A square root transformation was consistently applied throughout the analysis of the data matrix to compensate for the bias associated with highly abundant species and to ensure that the contributions of less common species to community composition were also taken into account. A presence-absence transformation was also applied to correct for the effect of common species (Warwick and Clarke, 2001), because differences in abundance were generally found, rather than differences in species present.

3.2.4.1 Measures of similarity

Bray-Curtis similarity coefficients (S) were used to quantify similarities among different factors (ie. site, month, station) within the species abundance matrix using the following equation:

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

where S_{jk} is the similarity between the j_{th} and k_{th} samples, y_{ij} is the entry in the i_{th} row and j_{th} column of the data matrix, and y_{ik} is the count for the i_{th} species in the k_{th} sample (Bray and Curtis, 1957). This similarity measure is often used in ecology because of its ability to deal with issues that are not adequately considered by other similarity coefficients. For example, the Bray-Curtis similarity coefficient is able to handle "joint absences", in which similarities are dependent on species that are present in one or both samples, and not on species that are absent from both samples (Clarke and Gorley, 2001). This similarity measure varies from 0 if samples have no species in common to 100 when samples are identical. Furthermore, the inclusion of a third sample has no impact on the similarity of the first two samples. An example of a Bray-Curtis similarity matrix can be found in Appendix B.

3.2.4.2 Univariate analyses

The Shannon-Wiener diversity index (H') was used as a univariate measure of diversity within a sample:

$$H' = -\sum_i p_i \log_e (p_i)$$

where p_i is the proportion of the total counts of the i^{th} species (Warwick and Clarke, 2001). The mean diversity index as well as mean number of species and mean number of individuals were determined for all samples taken at each station on each sampling occasion. This was done by adding together all specimens collected at the station in question and dividing by the number of grabs taken at that station on that sampling occasion.

3.2.4.3 Multivariate analyses

Kruskal's non-metric multidimensional scaling (MDS) plots were the principal means to examine similarity (or dissimilarity) within the data. MDS constructs a

multidimensional plot of the samples based on relative values from the Bray-Curtis similarity matrices. Samples located closer to each other on an MDS plot are more similar to each other than to samples located further away. The stress value is a measure of goodness-of-fit of the regression used in the MDS algorithm and is determined from the following equation:

$$Stress = \sqrt{\frac{\sum_j \sum_k \left(d_{jk} - \hat{d}_{jk}\right)^2}{\sum_j \sum_k d_{jk}^2}}$$

where $\hat{d}_{jk}$ is the distance predicted from the fitted regression line corresponding to dissimilarity σ_{jk} . If $d_{jk} = \hat{d}_{jk}$ for all the n (n-1)/2 distances in this summation, stress is zero. Stress values increase with reducing dimensionality of the ordination and also with an increasing quantity of data. For a 2-dimensional plot, a stress level <0.05 gives an "excellent representation with little possibility of misinterpretation" (Warwick and Clarke, 2001). Stress <0.1 is a "good ordination with no real prospect of misleading interpretation". Stress <0.2 gives a "potentially useful 2-dimensional picture" and a stress level <0.3 "indicates that the points are arbitrarily placed in the 2-dimensional ordination space". In the interpretation of MDS plots, Bray-Curtis similarities were used to generate clusters on the plot based on group average sorting. Generally it was decided that those stations or replicates within a station with Bray-Curtis similarities greater than 30% would be grouped together in a cluster on the MDS plot. For ease of presentation, MDS plots used in this study show means of samples. That is, after similarity analysis was completed on unpooled data, MDS plots were reconstructed using means of the grab samples collected on each sampling occasion in order to show a clearer and more legible picture.

Once the MDS and cluster analyses were performed, the data matrix was examined in light of the multivariate results. Where very pronounced clusters were observed (i.e. high within cluster similarity and distinct clusters), a SIMPER (similarity percentages) routine was used to indicate which species were principally responsible for clusters, or to establish differences among sets of groups that were identified *a priori*

(stations within farms, stations with different sediment compositions, and stations within different sampling periods). A SIMPER output table first records the average similarity of all pairwise coefficients followed by the species that contribute to this level of dissimilarity, to what degree, and by what percentage. SIMPER results can be seen in Appendix C.

An analysis of similarity (ANOSIM) was used to test for significant differences among groups of samples in the species abundance matrix (determined *a priori*); for example, farm stations versus reference stations, specific stations at different sampling times, or the differences among macrofaunal communities at muddy stations and sandy stations. Data were not pooled for this analysis. ANOSIM is a multivariate test that applies a simple non-parametric permutation procedure to the rank Bray-Curtis (dis)similarity matrix underlying the classification of samples (Clarke and Green, 1988). The test statistic (R) that is used for ANOSIM relates the differences between the samples compared to the difference among replicates within the samples (Warwick and Clarke, 2001). R is calculated from:

$$R = (\bar{r}_b - \bar{r}_w)/(M/2)$$

where $\bar{r}_w$ is the average of all rank similarities among replicates *within* samples, $\bar{r}_b$ is the average of rank similarities from all pairs of replicates *between* or *among* samples, and $M = n(n-1)/2$ where n is the total number of samples (Clarke,1993). The null hypothesis (H_0) for a 1-way ANOSIM is that there are no significant differences in community composition among different groups of sites/stations.

3.2.4.4 Infaunal trophic index (ITI)

According to Pearson and Rosenberg (1978), trophic relationships and trophic structure are fundamental to any analysis of community change in relation to organic input to the benthos. The infaunal trophic index (ITI) was developed by Word (1978). It is used to quantify and describe the feeding behaviours of macrofaunal organisms in soft-bottom benthic communities. The index is based on the premise that the benthic community can be divided into four feeding groups, suspension and deposit feeders that feed above or below the mud surface. The feeding groups used in Word's index were described by Cromey et al. (2000), and can be paraphrased as follows:

Group 1: Suspension feeders

Active suspension feeders obtain food from the water column by pumping water and suspended particles through a filtration apparatus. Some species of suspension feeders use bottom currents and highly developed feeding appendages to remove particles. Some passive suspension feeders utilise detrital matter that accumulates near the burrow. Examples of suspension feeders in this study include *Cucumaria frondosa* (holothurian echinoderm), *Dyastylis* spp. (cumacean shrimp), and the polychaete family Serpulidae.

Group 2: Surface detritus feeders

Surface detritus feeders obtain their food from the upper 0.5 cm of the sediment. Behavioral observations are the only means of assigning animals to group 1 or 2 because stomach content analyses do not reveal any major differences between the two groups. There are two groups of surface detritus feeders, motile and stationary. Motile surface detritus feeders move between food sources whereas stationary feeders have modified appendages to probe the surface of the sediment to locate and capture food. Examples of surface detritus feeders in this study are *Nephtys incisa*, *Polydora spp.*, and members of the polychaete family Cirratulidae.

Group 3: Surface deposit feeders

These animals generally feed in the top few centimetres of the sediment and remove particles greater than 1 mm diameter, including encrusted mineral aggregates, deposited particles and biological remains. Animals found in this group can be either mobile or stationary. Examples in this study are *Scoloplos armiger* (mobile) and *Goniada maculata* (stationary).

Group 4: Sub-surface deposit feeders

Animals in group 4 are generally mobile, deep burrowers that feed on deposited organic material. This feeding behaviour is variable and adapted for life in anaerobic sediment. The only organism found in this group was *Capitella* spp.

According to Word (1990), a benthic community can be designated as "degraded" (ITI < 30), "changed" (ITI = 30-60) or "normal" (ITI > 60), depending on its infaunal trophic index. It is important to note that Word (1990) was cautious about using a single ITI value as a descriptor of the benthic community because groupings are based on a continuum and should not be considered discrete. For the purpose of this analysis, the infaunal trophic index was calculated for each station. However, rather than use these values to describe the various locations in terms of Word's terms "normal", "changed" and "degraded", the ITI scores were used to compare stations with a similar sediment composition. The index was computed as:

$$ITI = 100 - \left[33.33 \left(\frac{0n1 + 1n2 + 2n3 + 3n4}{n1 + n2 + n3 + n4} \right) \right]$$

where n_x is the number of individuals in feeding group x. A sample calculation can be found in Appendix D.

An ANOVA was used to compare ITI values of farm stations and reference station with similar sediment types. Additionally, where necessary, stations were nested within farm and reference.

3.3 Results

3.3.1 Characterization of benthic community structure (univariate analyses)

Preliminary analyses of both square root and presence absence transformations of data revealed a difference among stations with different sediment composition within each site (ANOSIM, $p < 0.05$). Subsequently, benthic community macrofaunal composition differences were examined within the different farm and reference stations within the two different observed sediment types (based on grain size).

Univariate analysis of the benthic macrofaunal data from FH and CA indicated that the benthic community composition at the two sites was very different, with more species and total number of individuals at FH than at CA. A 2-sample t-test showed that CA and FH were significantly different ($p< 0.001$) in terms of the mean number of

individuals at each site. The first null hypothesis (section 3.1.2) of no difference between abundances at farm sites was therefore rejected.

3.3.1.1 Fortune Harbour

Sediment at FH was easily classified into two different types according to median grain size as retained in a 5 mm mesh seive. Sediment from stations FH 1F and FH 1R was coarse (> 500 μm), and for this study the sediment at these locations was classified as sand. Sediment from stations FH 2R, FH 3R, FH 2F, FH 3F and FH 4F, in contrast, had a much smaller grain size (< 500 μm) and was classified as mud. Macrofauna in FH were usually found in high numbers (Figures 3.3 and 3.4). Mean numbers of species, individuals and Shannon Weiner diversity indices can be found in Table 3.2. These are mean numbers per grab (area = 0.0232 m^2). To obtain mean numbers per m^2, a multiplication factor of 43 can be used.

At muddy stations (Figures 3.3a, 3.3b, 3.3c), macrofauna were relatively small. The benthic macrofaunal community was usually dominated by *Capitella* sp., a taxon that is commonly found in areas of organic enrichment.

Sandy stations were generally dominated by larger species than those collected at muddy stations, including *Macoma calcarea*, *Cucumaria frondosa* and *Nephtys incisa* (Figures 3.4a, 3.4b, 3.4c). In general, species diversity was greater at sandy stations (Shannon-Wiener diversity index, Table 3.2). Benthic macrofaunal assemblages were significantly different between sediment types at FH (ANOSIM R = 0.601, p=0.001 for presence/absence data).

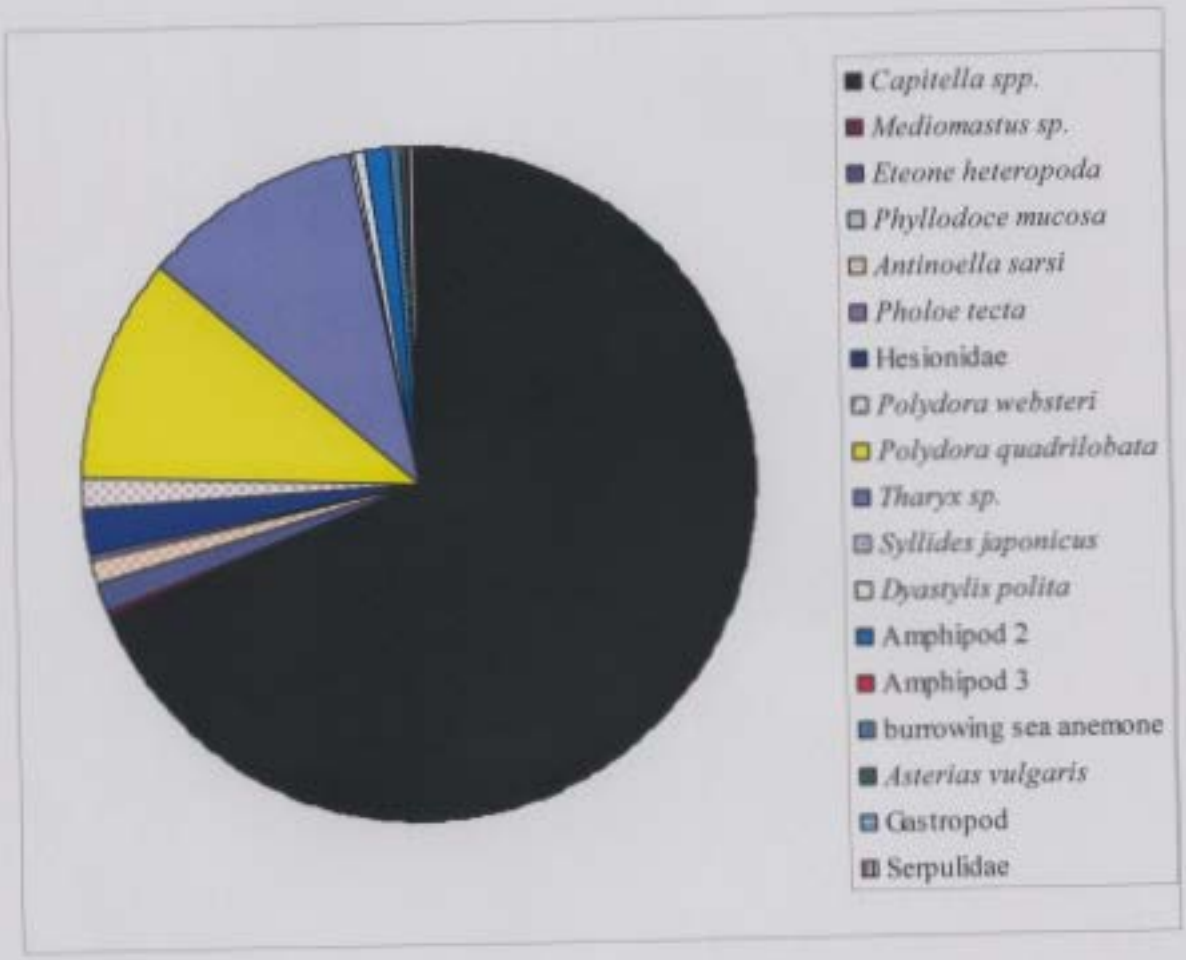

Figure 3.3 a: Macrobenthic species (proportion of total numbers) collected at farm and reference stations with muddy sediments at FH in 2002-2003.

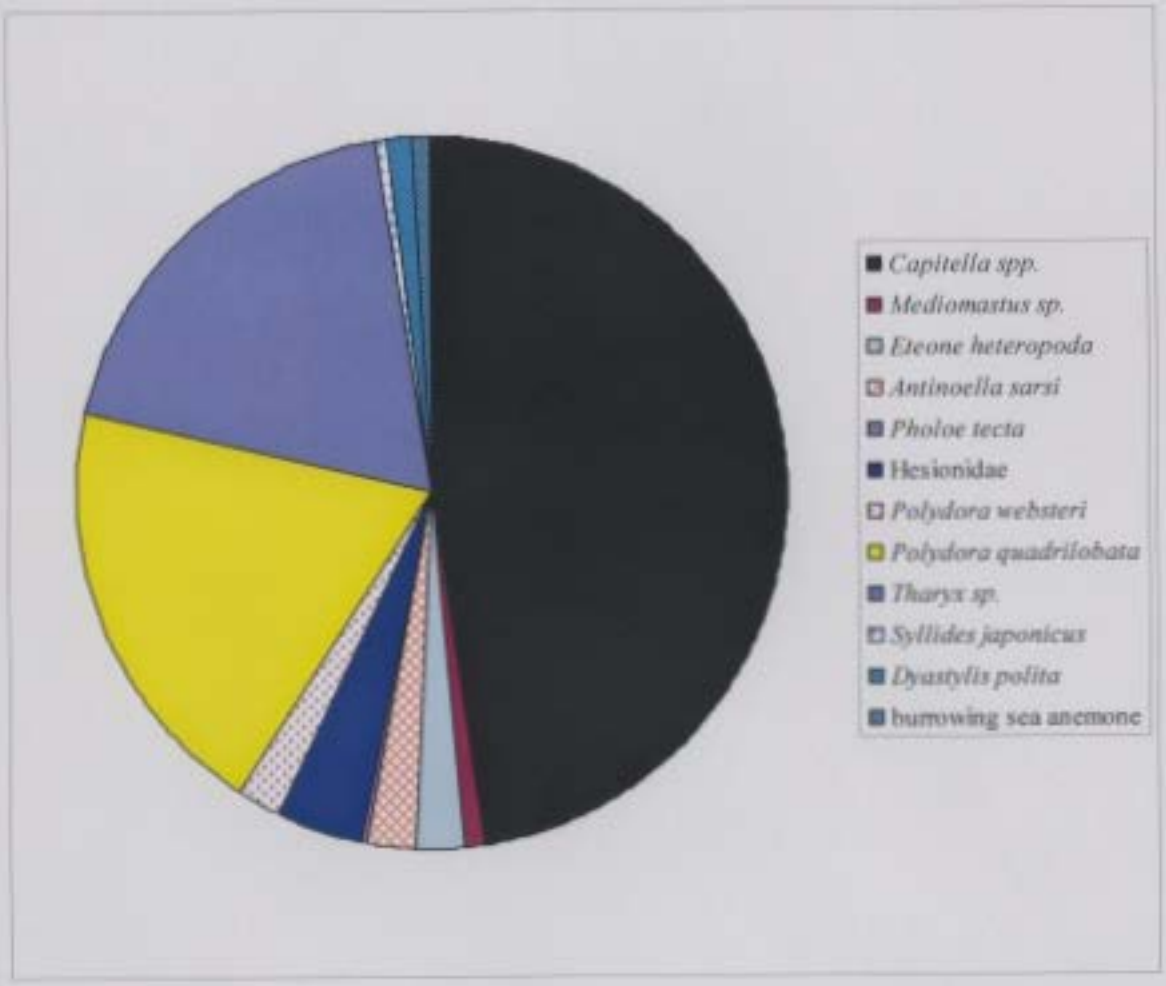

Figure 3.3b: Macrobenthic species (proportion of total numbers) collected at reference stations with muddy sediments at FH in 2002-2003.

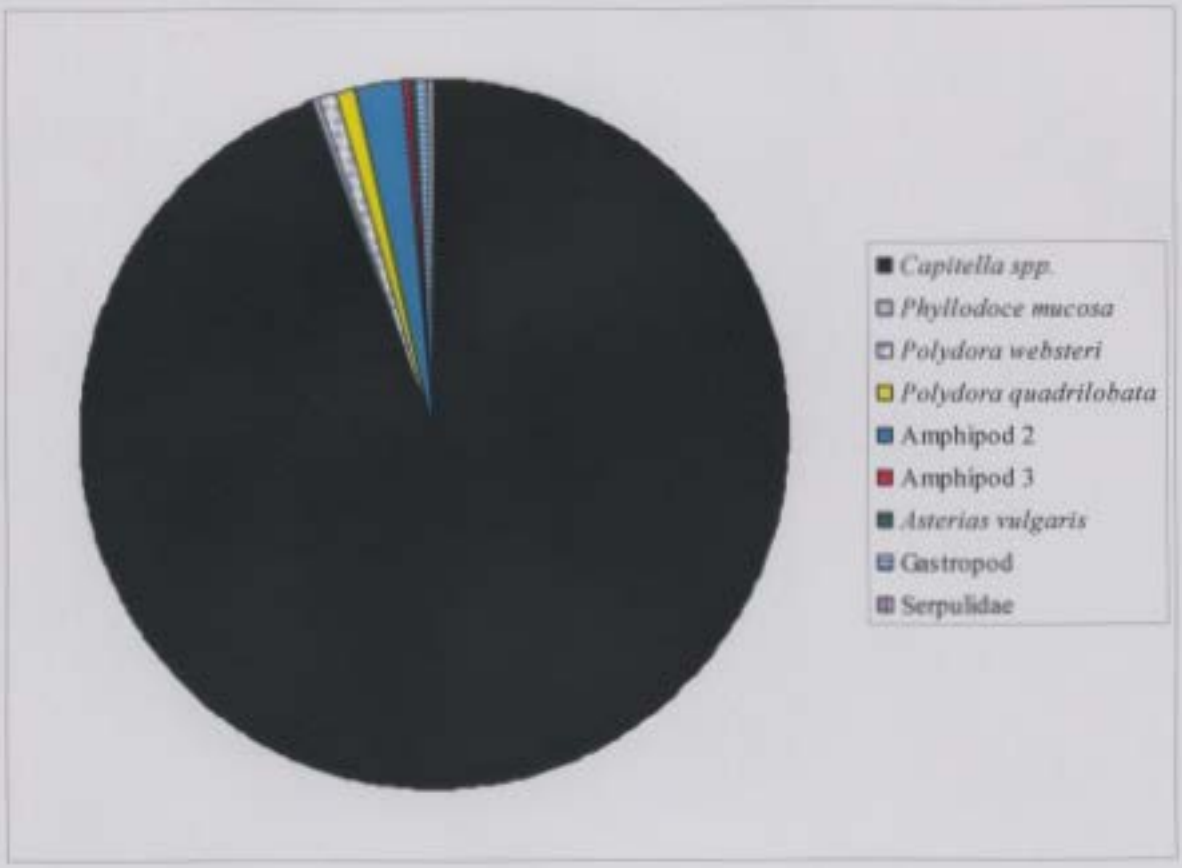

Figure 3.3c: Macrobenthic species (proportion of total numbers) collected at farm stations with muddy sediments at FH in 2002-2003.

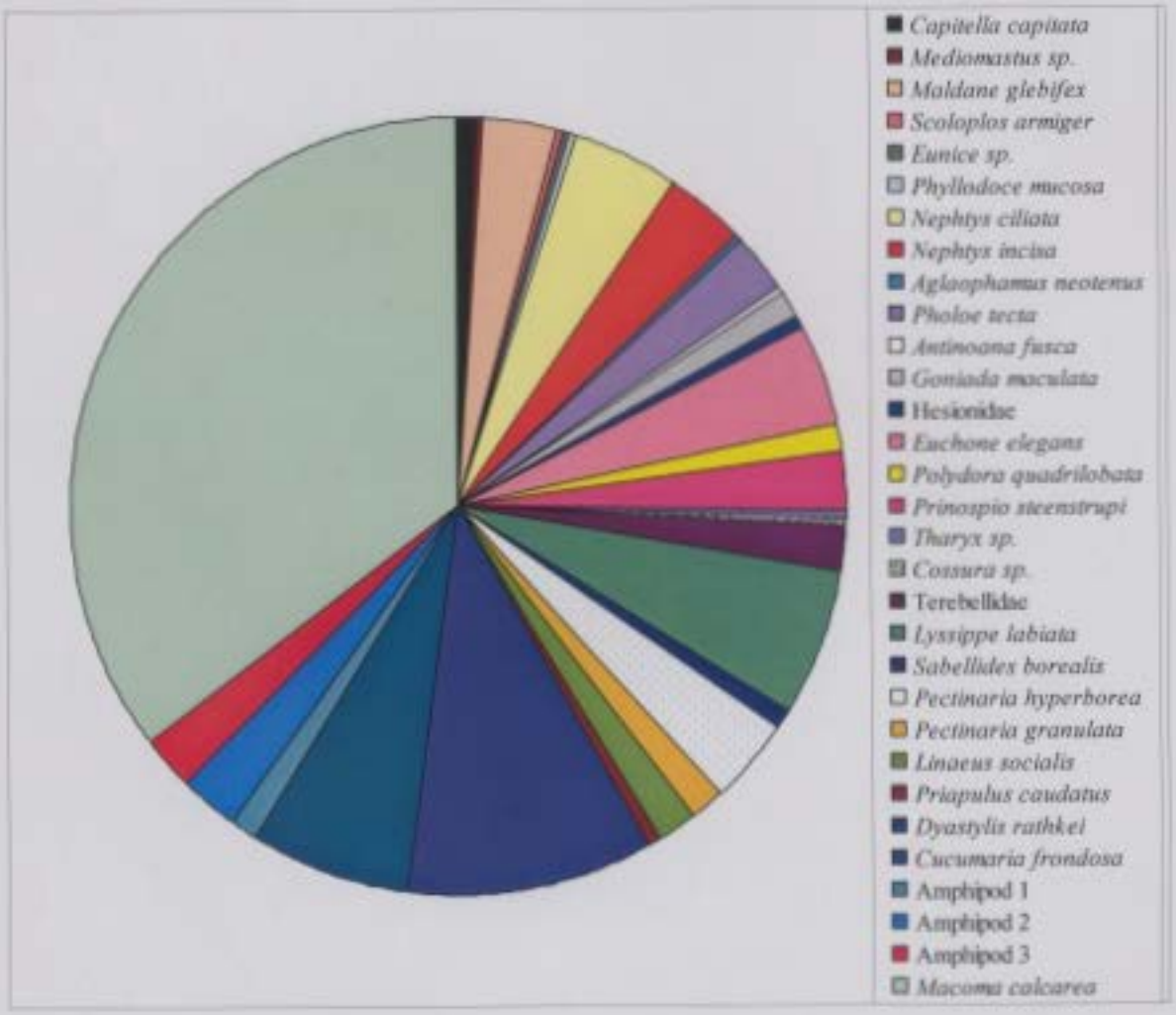

Figure 3.4 a: Macrobenthic species (proportion of total numbers) collected at farm and reference stations with sandy sediments at FH in 2002-2003.

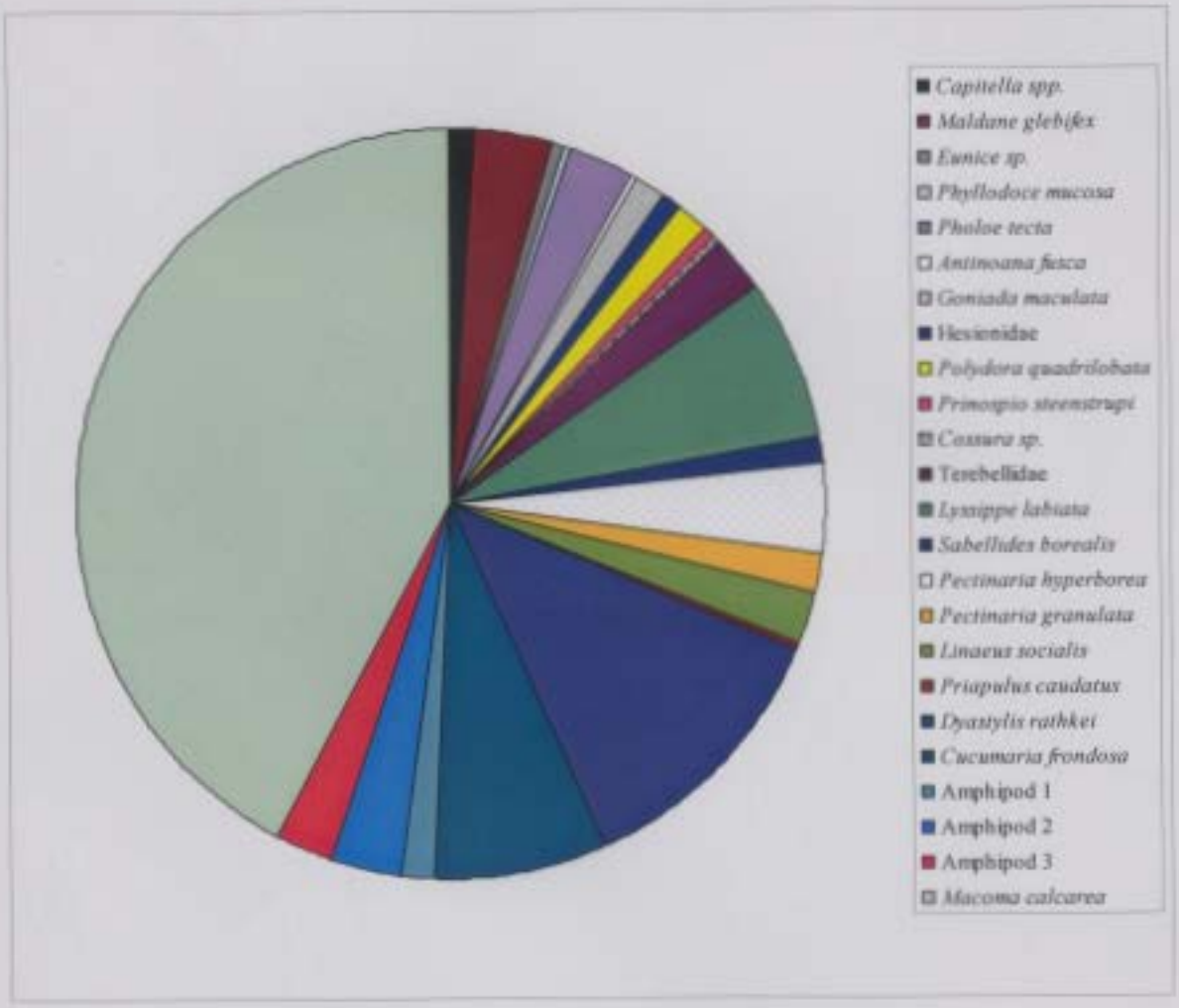

Figure 3.4b: Macrobenthic species (proportion of total numbers) collected at farm stations with sandy sediments at FH in 2002-2003.

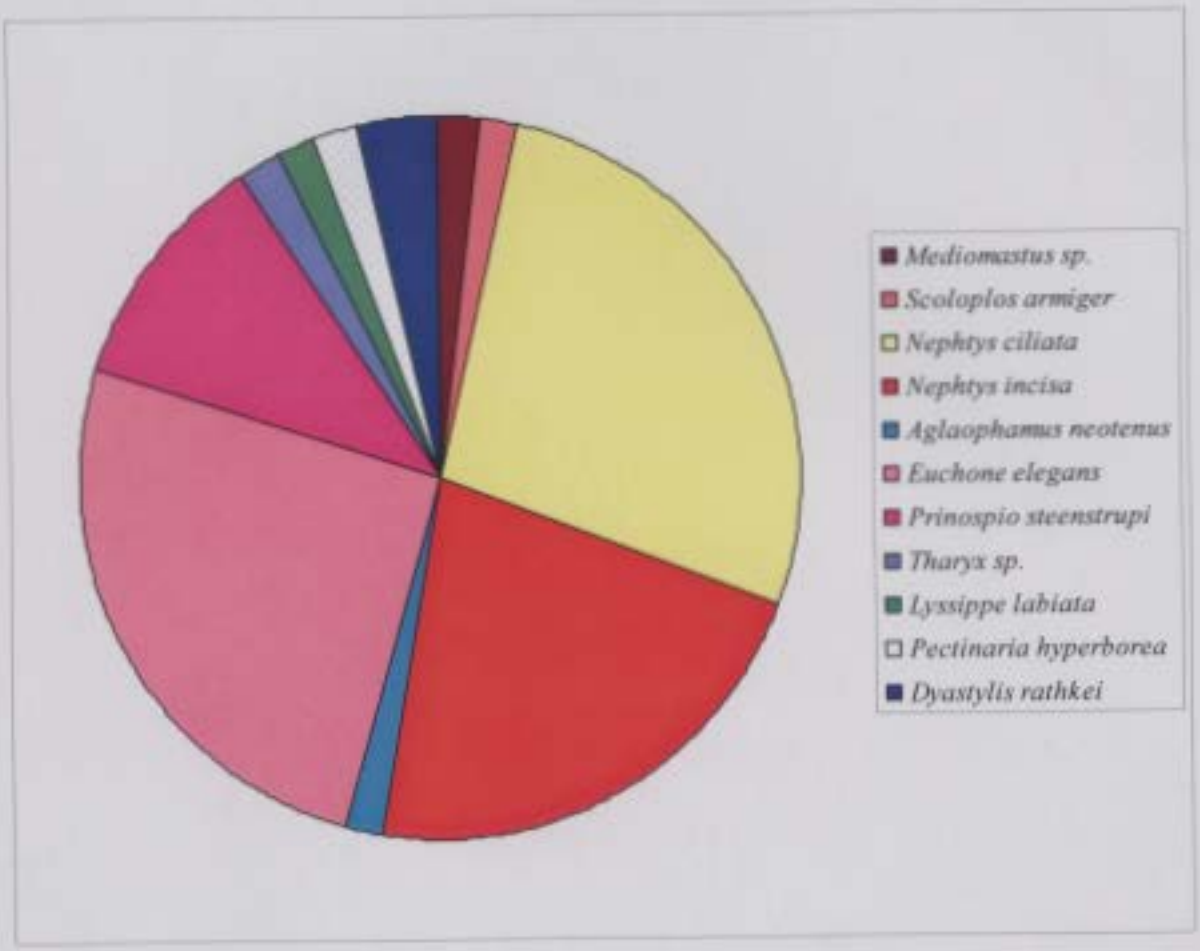

Figure 3.4c: Macrobenthic species (proportion of total numbers) collected at reference stations with sandy sediments at FH in 2002-2003.

Table 3.2: Mean values for univariate measures of diversity for FH samples in different months (n = number of grabs containing macrofauna, mean S = mean number of species, mean N = mean number of individuals, mean H'= mean Shannon-Wiener Diversity Index, SE = standard error, J = July, A=August, N= November, M= March). '-' indicates that no samples were taken. "*" indicates S.E. could not be calculated because n = 1 or n = 0.

Sample	Month	Sediment Type	Farm/ Ref	Total # of grabs	n	Mean S	SE	Mean N	SE	Mean H'	SE
FH 1F	J	Sand	Farm	4	4	5.3	1.3	15.5	3.1	1.3	0.3
	A			4	4	6.3	1.3	14.5	1.9	1.4	0.3
	N			4	4	9.8	1.1	23.6	4.6	1.9	0.2
	M			4	4	6.3	0.8	15.3	3.7	1.5	0.1
FH 1R	J	Sand	Ref	3	3	3.7	0.7	9.3	3.2	1.1	0.1
	A			4	4	1.3	0.3	2.5	0.9	0.1	0.1
	N			4	3	3.7	0.3	5.7	1.2	1.2	0.1
	M			-	-	-	-	-	-	-	-
FH 4F	J	Mud	Farm	4	4	1	0	23.8	2	0	0
	A			4	4	1.3	0.3	9	2.7	0.1	0.1
	N			4	0	0	*	0	*	0	*
	M			4	4	1.5	0.5	7.5	6.5	0.1	0.1
FH 3F	J	Mud	Farm	4	2	1	0	3.5	2.5	0	0
	A			4	4	1.8	0.5	13.3	3.9	0.2	0.1
	N			4	0	0	*	0	*	0	*
	M			4	4	1.5	0.3	18	9.4	0.1	0.1
FH 2F	J	Mud	Farm	4	0	0	*	0	*	0	*
	A			4	0	0	*	0	*	0	*
	N			-	-	-	-	-	-	-	-
	M			4	0	0	*	0	*	0	*
FH 2R	J	Mud	Ref	4	4	2.8	0.3	27.8	2.5	0.5	0.1
	A			4	0	0	*	0	*	0	*
	N			4	1	0.25	*	0.25	*	0	*
	M			4	2	1.5	0.5	3	2	0.3	0.3
FH 3R	J	Mud	Ref	4	4	3.8	0.5	30.5	12.3	0.8	0.2
	A			4	4	1.8	0.3	7.5	9	0.3	0.1
	N			4	2	3	1	25	23	0.8	0.1
	M			4	2	1	0	3	1	0	0

3.3.1.2 Charles Arm

In CA, sediment type was fairly consistent at all sampling locations on all sampling occasions. Sediments were generally coarse (> 500 μm) and were therefore classified as sand.

In general, this site was characterized by larger macrofaunal species than were seen at FH, including *N. ciliata, N. incisa, M. calcarea* and *A. vulgaris* (Figure 3.5). Mean numbers of species, individuals and Shannon Weiner diversity indices are presented in Table 3.3. These are mean numbers per grab (area = 0.0232 m^2). To obtain mean numbers per m^2, a multiplication factor of 43 can be used.

Table 3.3: Mean values for univariate measures of diversity for all CA samples on 3 different occasions (n = number of grabs containing macrofauna, mean S = mean number of species, mean N = mean number of individuals, mean H'= mean Shannon-Wiener Diversity Index, SE = standard error). '*' indicates S.E. could not be calculated because n = 1 or n = 0. '-' indicates that no samples were taken.

Sample	Month	Farm/ Ref	Total # of grabs	n	Mean S	SE	Mean N	SE	Mean H'	SE
CA 1F	J	Farm	4	2	1	0	1	0	0	0
	A		4	0	0	*	0	*	0	*
	M		4	1	1	*	1	*	0	*
CA 2F	J	Farm	4	3	1.3	0.3	1.3	0.3	0.2	0.2
	A		4	1	1	*	1	*	0	*
	M		4	0	0	*	0	*	0	*
CA 2R	J	Ref	4	3	2	0.6	3.7	1.7	0.6	0.3
	A		4	4	1.8	0.3	3.3	0.5	0.5	0.2
	M		4	4	1.8	0.3	3.3	0.5	0.5	0.2
CA 1R	J	Ref	4	3	1.7	0.3	3.3	0.8	0.4	0.2
	A		4	3	1.3	0.3	2.3	1.3	0.2	0.2
	M		-	-	-	-	-	-	-	-

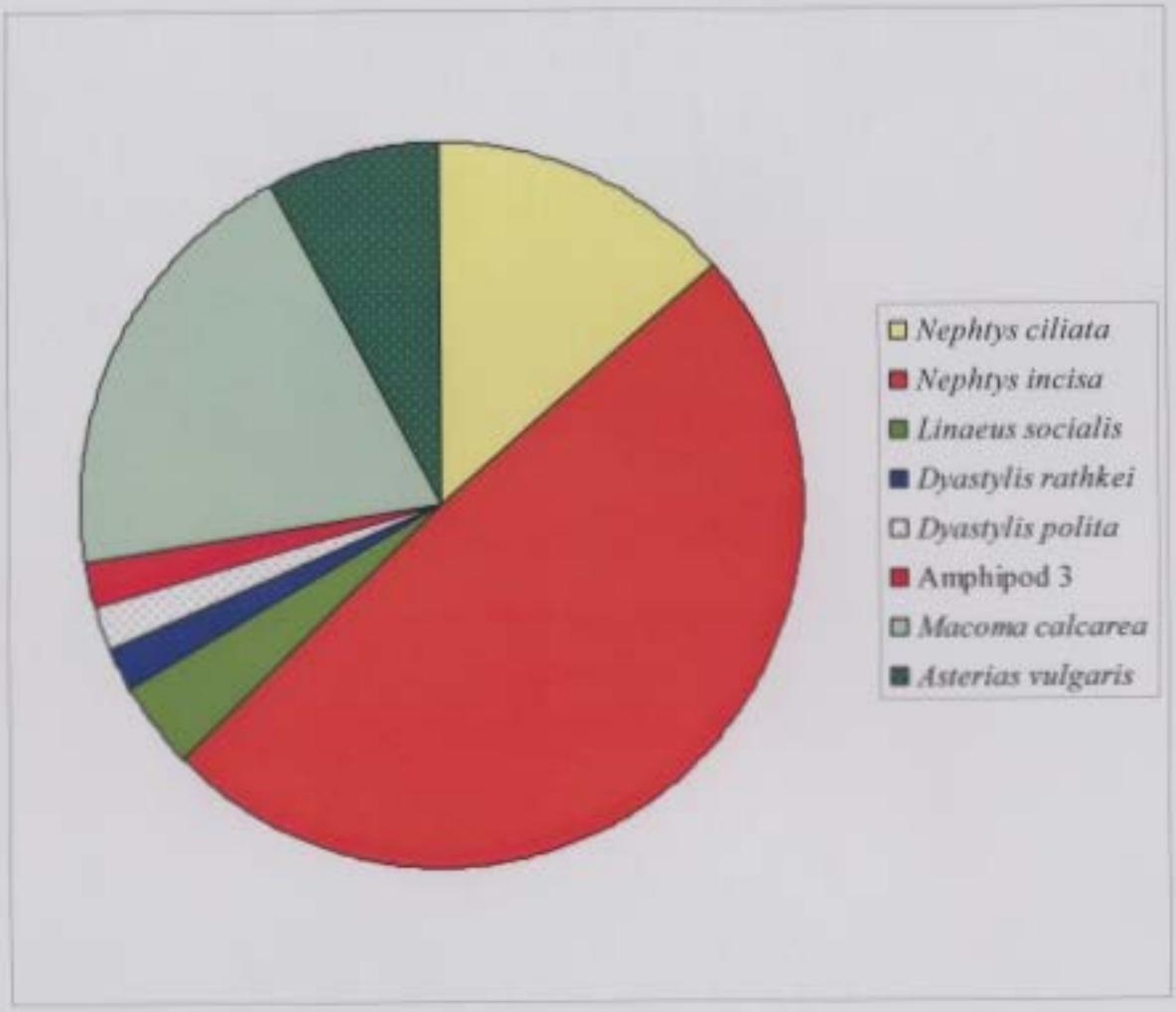

Figure 3.5a: Macrobenthic species (proportion of total numbers) collected at farm and reference stations in sandy sediments at CA in 2002-2003.

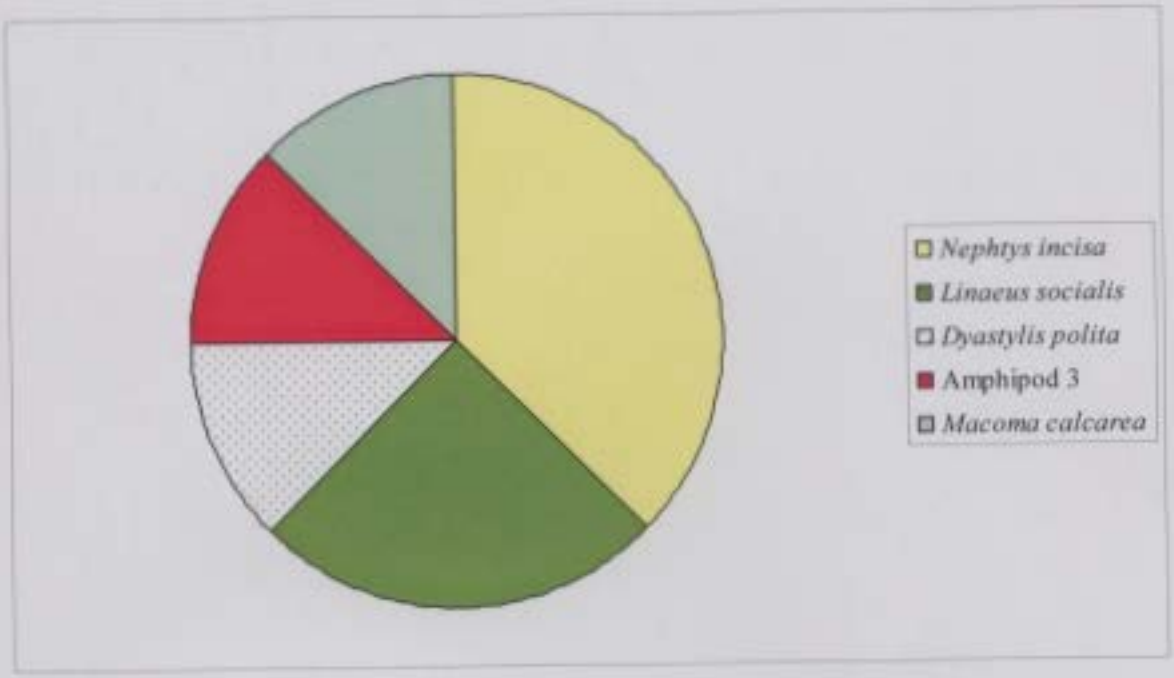

Figure 3.5b: Macrobenthic species (proportion of total numbers) collected at farm stations in sandy sediments at CA in 2002-2003.

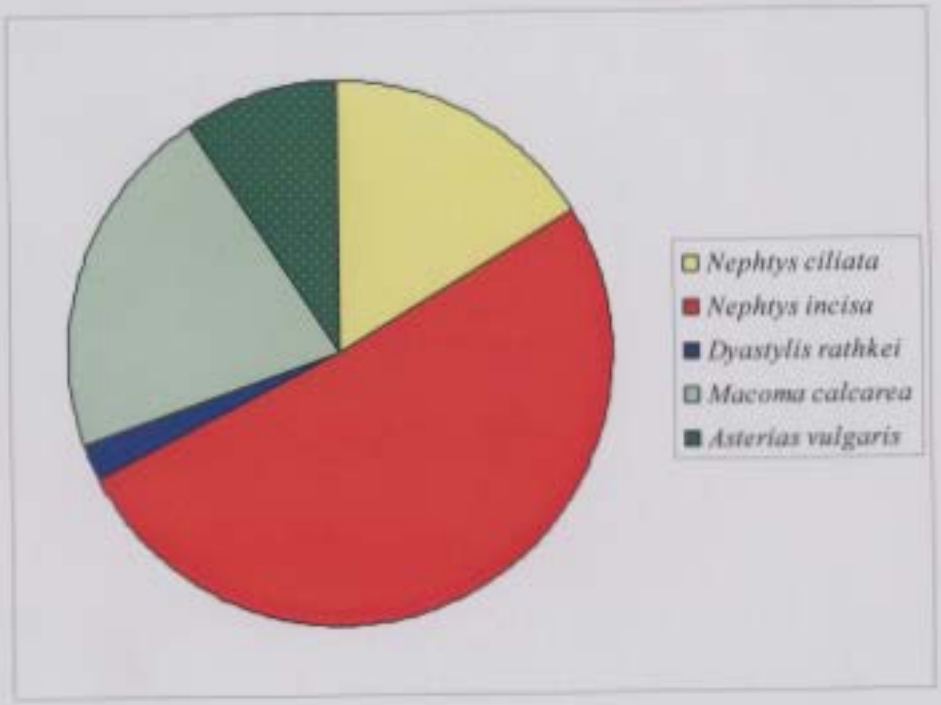

Figure 3.5c: Macrobenthic species (proportion of total numbers) collected at reference stations in sandy sediments at CA in 2002-2003.

3.3.2 Multivariate analyses of benthic community structure

3.3.2.1 Temporal analysis

To determine the effect of mussel culture on the benthic macrofauna at different times of the year (July, August, November 2002 and March 2003), samples collected at each station were compared at each sampling time. ANOSIM was carried out on presence/absence data because differences among replicates were often not a matter of which species were present, but rather of differences in abundance. Nevertheless, the results were very similar to those obtained from square root transformed data. ANOSIM (Table 3.4) indicated that 3 of the 11 stations sampled showed significant differences between macrofaunal communities among sampling occasions, and in these cases the second null hypothesis (no temporal variation in the benthic macrofauna) can be rejected, whereas in the remaining 8 cases it cannot be rejected. Temporal variation was more apparent in muddy stations in FH, both at farm (FH 3F) and reference (FH 3R) stations. The exception is FH 1R, a sandy station which also differed among months. FH 2F was not considered because macrofauna were not obtained at any time.

Table 3.4: ANOSIM results indicating the relative similarities among months at each station for presence-absence macrofaunal data. '*' indicates that ANOSIM could not be carried out because there was no macrofauna in samples.

Station	Sediment	Farm/ Ref	R-value	p-value
FH 1F	Sand	Farm	0.075	0.227
FH 1R	Sand	Ref	0.416	**0.006**
FH 3F	Mud	Farm	0.423	**0.032**
FH 4F	Mud	Farm	0.173	0.2
FH 2F	Mud	Farm	*	*
FH 3R	Mud	Ref	0.75	**0.002**
FH 2R	Mud	Ref	0.313	0.171
CA 1F	Sand	Farm	0	0.1
CA 2F	Sand	Farm	1	0.25
CA 1R	Sand	Ref	0.019	0.7
CA 2R	Sand	Ref	0.123	0.261

3.3.2.2 Spatial analysis

ANOSIM of presence absence data showed that there was a significant difference between sediment types for macrofaunal species presence/ absence data (R=0.791, p=0.001). SIMPER analysis showed 96.8 % dissimilarity between muddy and sandy sediments. Fifty percent of this dissimilarity was attributable to the following taxa: *Capitella spp.* (14.0%), *N. incisa* (12.1%), *M. calcarea* (7.2%), *P. quadrilobata* (6.5%), *N. ciliata* (5.3%) and *L. socialis* (5 %).

Multidimensional scaling plots demonstrated that the benthic communities at the two farms were very different (Figure 3.6). FH 2F contained no macrofauna at any time, and was therefore removed from the similarity matrix to allow for a better determination of similarity among the remaining stations. ANOSIM showed that CA and FH were significantly different for both square root transformed and presence/absence macrofaunal data (R=0.412, p=0.001 and R=0.42, p=0.001 respectively). Throughout the entire sampling period, SIMPER analysis indicated a dissimilarity of 91.3% between FH and CA. Sixty percent of this dissimilarity was attributable to the following taxa: *Capitella* spp. (14.1%), *N. incisa* (13.6%), *M. calcarea* (9.2 %), *P. quadrilobata* (6.3%), *L. socialis* (6.1%), *N. ciliata* (5.4%) and *A. vulgaris* (5.4%).

3.3.2.2.1 Spatial analysis - Fortune Harbour

Initial analysis of both presence absence and square root transformed FH data showed that there was no significant difference between farm (FH 1F, FH 2F, FH 3F, FH 4F) and reference (FH 1R, FH 2R, FH 3R) stations (ANOSIM R=0.101, p = 0.093). There was a significant difference, however, in the composition of benthic macrofaunal communities at stations with different sediment compositions (R=0.825, p = 0.001). MDS indicated that benthic communities at muddy stations in FH were generally more similar to each other than they were to sandy stations and vice versa (Figure 3.7). Thus for the remainder of the analyses stations were divided into 2 groups: those with sandy sediments and those with muddy sediments. SIMPER analysis showed an average dissimilarity of 94.6% between benthic macrofaunal communities in sandy and muddy sediments. Thirty-eight percent of this dissimilarity could be attributable to the following

taxa: *Capitella* spp. (8.2%), *N. ciliata* (6.4%), *N. incisa* (6.4%), *P. hyperborea* (5.7%), *D. rathkei* (5.7%) and *L. labiata* (5.1%). For the sandy stations at FH, there was a significant difference between benthic macrofaunal communities at farm stations (FH 1F) and reference stations (FH 1R) (ANOSIM R=0.917, p = 0.001 for square root transformed, R=0.896, p=0.001 for presence/absence data); this difference is reflected in the MDS plot (Figure 3.8).

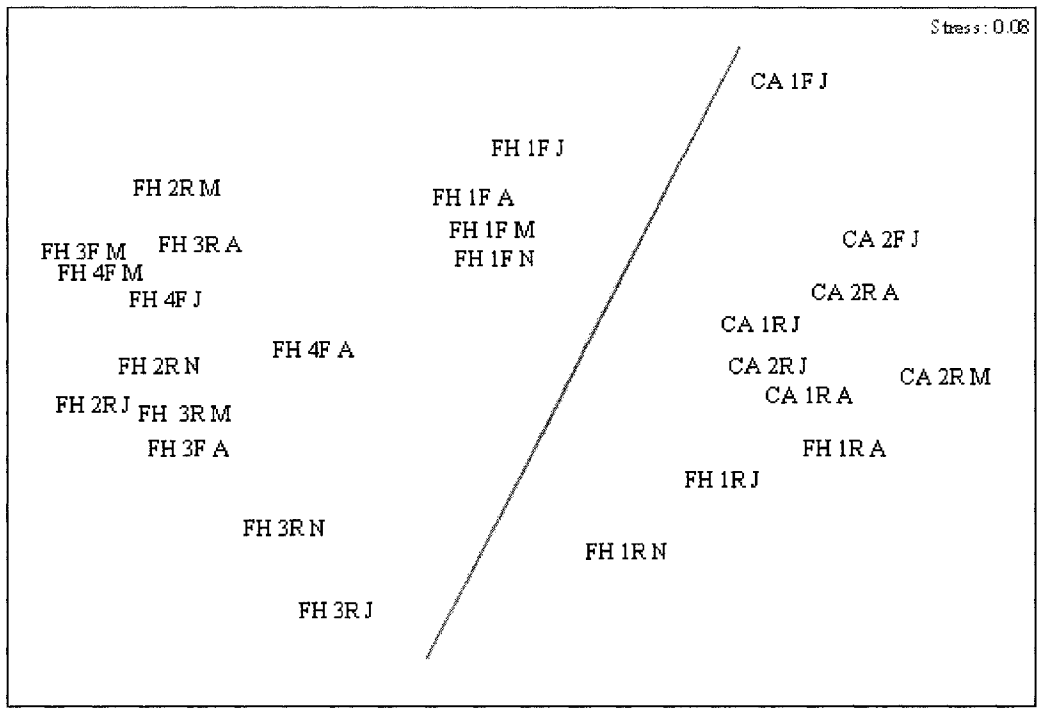

Figure 3.6: MDS of presence/absence macrofaunal data for CA (CA 1F, CA 2F, CA 1R and CA 2R) and FH stations (FH 1F, FH 3F, FH 4F, FH 1R, FH 2R, FH 3R), throughout the sampling period (J=July, A=August, N= November, M=March). FH 3F J, CA 2FA and CA 1F M have been removed from the plot because initial analysis showed that they were outliers. The line represents the division between the two groups determined by cluster analysis.

Thus the third null hypothesis, that FH farm and reference stations are not significantly different, can be rejected for stations with sandy sediments. SIMPER analysis showed an average dissimilarity among farm and reference stations of 95.28 %, of which 51 % was attributable to the following taxa: *C. frondosa* (8.29%), *M. calcarea* (12.3%), *N. ciliata* (5.44%), *N. incisa* (10.14%), Terebellidae (5.12%), *L. labiata* (7.23%), *D. rathkei* (7.22%).

Muddy sediments also showed a significant difference in benthic macrofaunal communities (ANOSIM R=0.109, p= 0.012) among farm (FH 3F, FH 4F) and reference stations (FH 2R, FH 3R) for presence/absence transformed data (Figure 3.9). Thus the third null hypothesis, that farm and reference stations are not significantly different, can also be rejected for stations with muddy sediments. SIMPER analysis showed an average dissimilarity of 55.7%, almost all (69%) being attributable to the following taxa: *P. quadrilobata* (19.6%), *Capitella* spp. (15.1), Hesionidae (11.2%), *P. websteri* (8.48%), *A. sarsi* (7.51%), and *E. heteropoda* (6.85%). Separation of groups on MDS was not as discrete at FH muddy stations as it was at FH sandy stations.

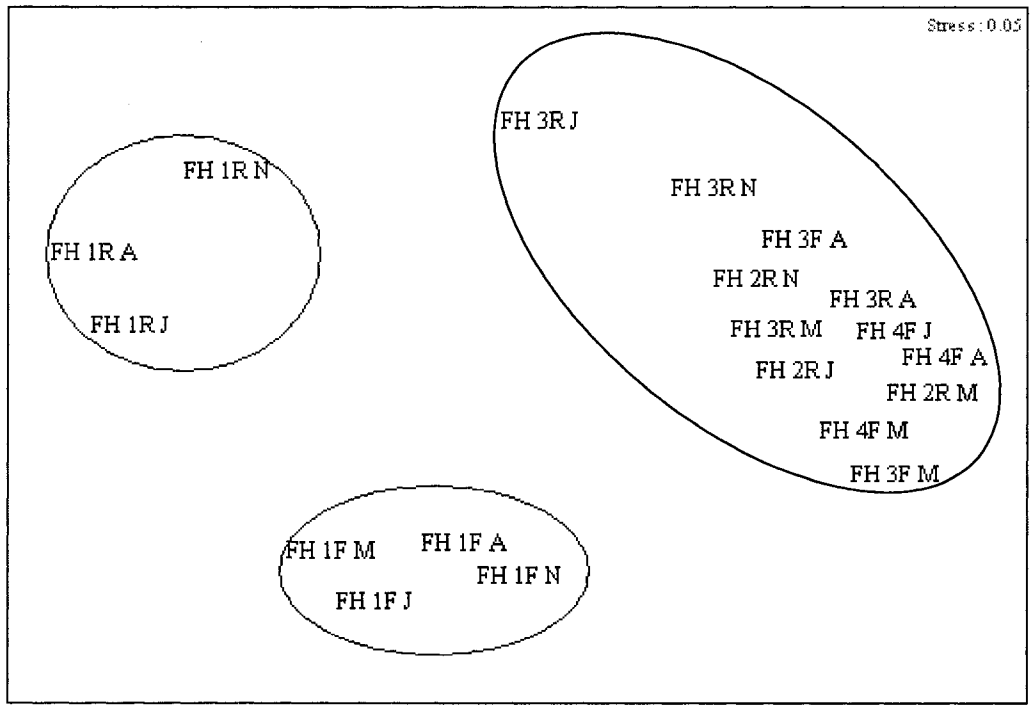

Figure 3.7: MDS plot of presence/absence macrofaunal data for all stations in FH throughout the sampling period (J=July, A=August, N=November, M=March). FH 1R and FH 1F are stations with sandy sediments. FH 3F data are not included (shown to be an outlier). The three groups from the cluster analysis are delineated by ellipses.

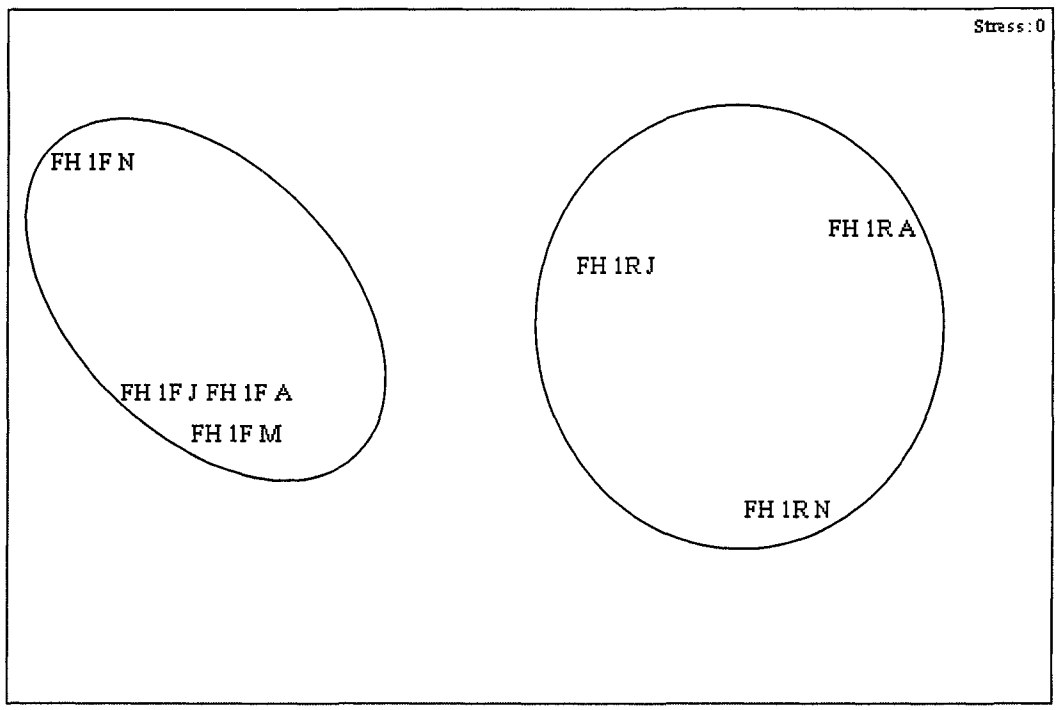

Figure 3.8: MDS plot of presence/absence macrofaunal data for sandy stations (FH 1F and FH 1R), throughout the sampling period (J=July, A=August, N=November 2002, M=March 2003).The two groups from the cluster analysis are delineated by ellipses.

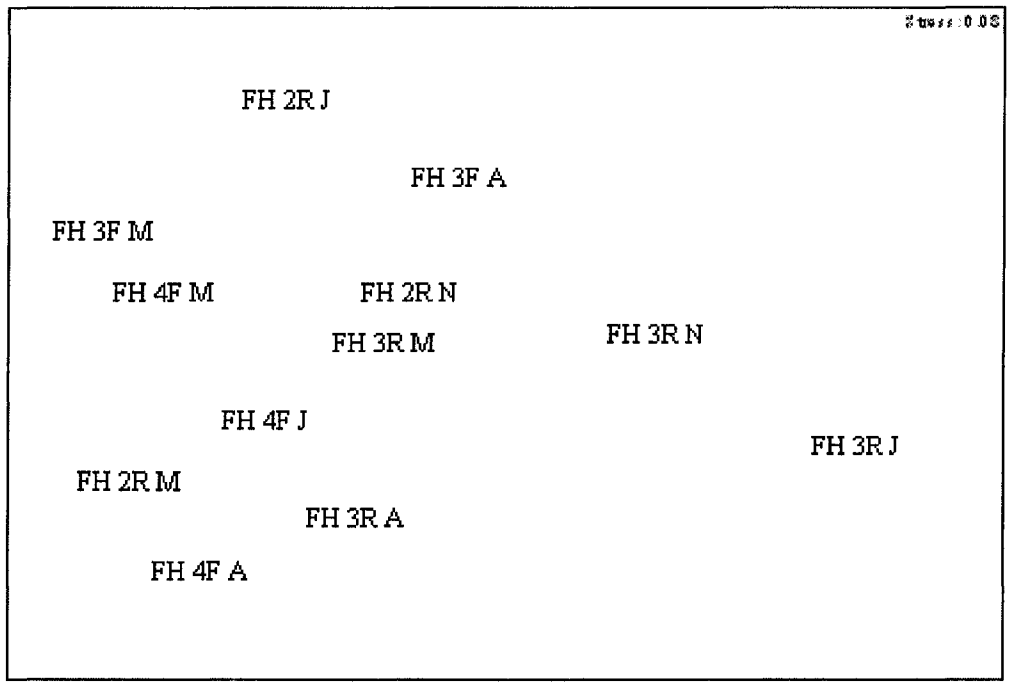

Figure 3.9: MDS plot of presence/absence macrofaunal data for muddy stations (FH 3F, FH 4F, FH 3R, FH 2R), throughout the sampling period (J=July, A=August, N=November 2002, M=March 2003). FH 3F J has been excluded (demonstrated outlier).

3.3.2.2.2 Spatial analysis - Charles Arm

All stations in CA had sandy sediments and thus the data did not need to be separated by sediment type before analysis. The difference between farm and reference stations was significant (ANOSIM R=0.151, p = 0.047) for presence/absence transformed data. Thus, the third null hypothesis, that farm and reference stations at CA are not significantly different, can be rejected. Separation of groups by MDS was not as discrete at CA (Figure 3.10) as at FH sandy stations. SIMPER analysis of the macrofaunal data indicated that there was a 65% dissimilarity between the farm and reference stations, of which 81% was attributable to the following taxa: *L. socialis* (12.8%), *M. calcarea* (20.9%), *N. incisa* (29.6%), *A. vulgaris* (7.6%) and *N. ciliata* (10.4%).

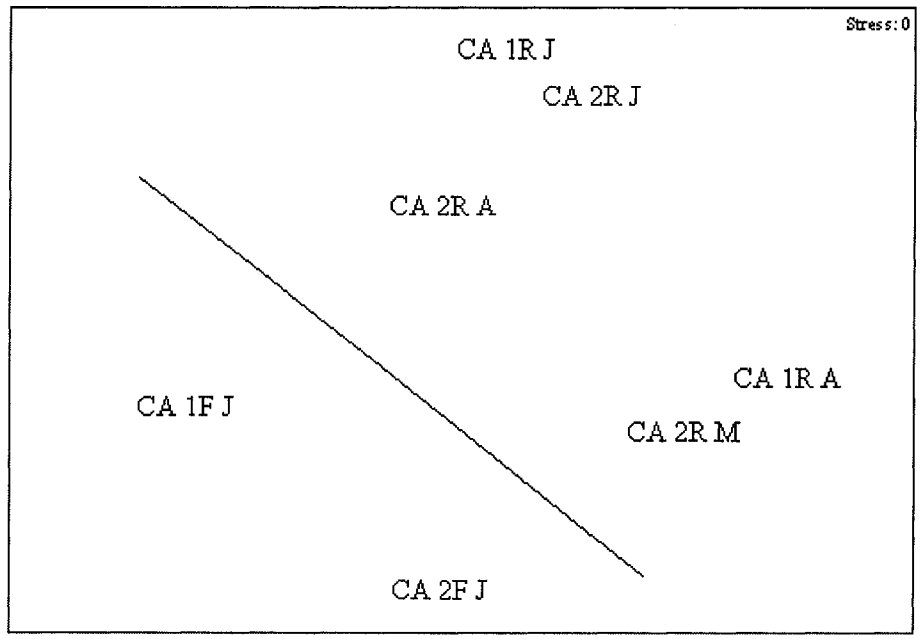

Figure 3.10: MDS plot of presence/absence macrofaunal data for CA stations (CA 1F, CA 2F, CA 1R and CA 2R) throughout the sampling period (J=July, A=August 2002, M=March 2003). CA 2F A and CA 1F M have been excluded (shown to be outliers). The line denotes the division between the two groups from cluster analysis.

3.3.3 Redox profiles

Redox profiles indicated that sediments were hypoxic (Eh = 0mV to -100mV) to anoxic (Eh = < -100 mV) in March 2003, although redox values in CA were less negative than in FH (Figures 3.11, 3.12). All stations sampled for redox in FH were muddy (grain size < 500 μm).

Sediments from FH 2F had the lowest redox values overall, with less than -120 mV at all depths of the profile. Redox potential was also very negative at FH 4F, where only the upper 2 cm of the sediment was more oxygenated than at FH 2F, a station

lacking macrofauna. Similarly, redox potential became very negative just below the surface at FH 3F. At FH 3R and FH 1R, which were both reference stations, redox was greater than -100 mV until the 8-10 cm level below the sediment surface.

For each site (CA and FH), a two-way ANOVA of redox potential values was done to test for differences among farm and reference stations and differences among depths within the sediment core (Tables 3.5, 3.6). In both cases the interaction between the farm/reference effect and sediment depth effect was not significant. In FH, there was a significant difference between redox potential among depths ($p=0.010$) as well as between farm stations and reference stations ($p<0.001$) (Table 3.5). Thus redox potential became more negative deeper into the sediment core, and sediment was more anoxic at the farm stations than at the reference stations (Figure 3.11, Table 3.5).

Table 3.5: ANOVA of values for sediment redox potential from FH farm and reference stations in March 2003.

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Farm/ref	1	8767.3	8767.3	8767.3	62.65	**0.000**
Depth	3	2422.1	2490.2	830.1	5.93	**0.010**
Interaction	3	170.5	170.5	56.8	0.41	0.751
Error	12	1679.3	1679.3	139.9		
Total	19	13039.2				

CA 2R was the only station in either mussel farm that exhibited positive redox values (March 2003). It was also the only reference station sampled in March at this site because of ice conditions. CA 2F, which was a farm station, nonetheless had a sediment redox potential profile that was not very negative; values were greater than -80 mV until the 8 cm depth horizon. CA 1F, however, had very negative redox values relative to other stations in CA, beginning at -100 mV at the surface. This station was comparable with FH 4F and FH 3F in FH in terms of redox potential.

At CA, there was no significant difference in redox potential between sediments from farm and reference stations ($p=0.113$), nor among depths ($p=0.582$) (Table 3.6).

Table 3.6: ANOVA of values for sediment redox potential from CA farm and reference stations in March 2003.

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Depth	6	8197	8197	1366	0.83	0.582
Farm/Ref	1	5737	5737	5737	3.28	0.113
Interaction	6	809	809	135	0.08	0.997
Error	7	12233	12233	1748		
Total	20	26977				

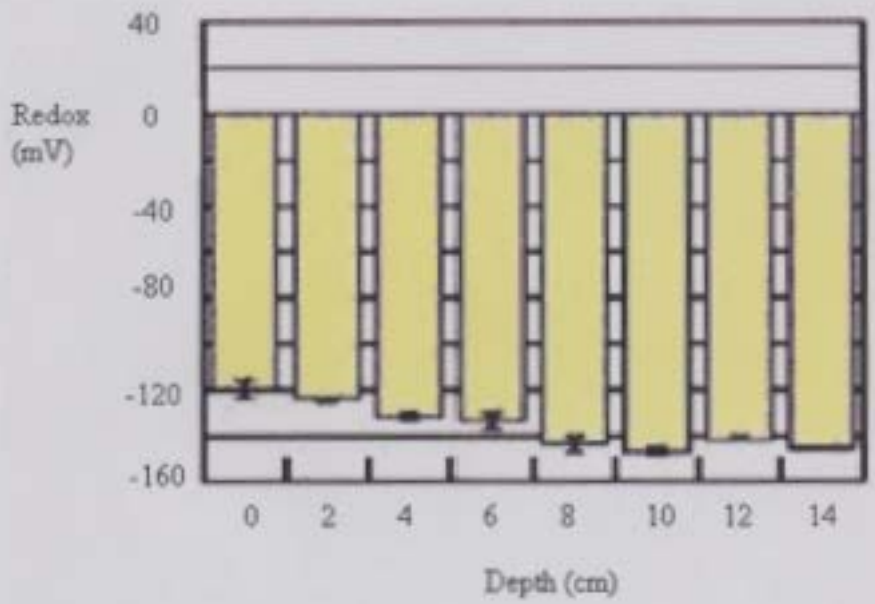

Figure 3.11a: Mean (± S.E.) redox values (mV) from measurements taken at 2 cm intervals in sediment cores from FH 2F, a farm station with muddy sediment, in March 2003.

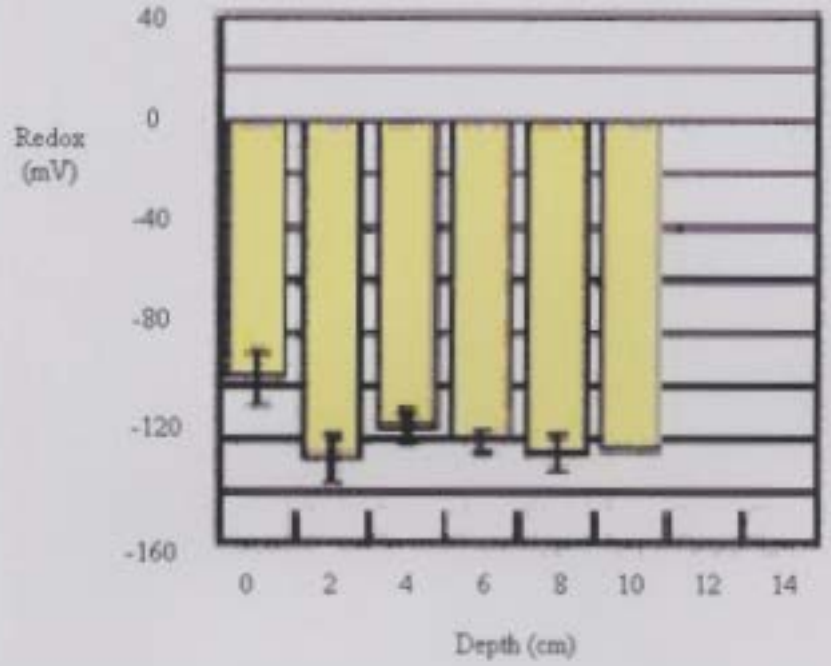

Figure 3.11b: Mean (± S.E.) redox values (mV) from measurements taken at 2 cm intervals in sediment cores from FH 3F, a farm station with muddy sediment, in March 2003.

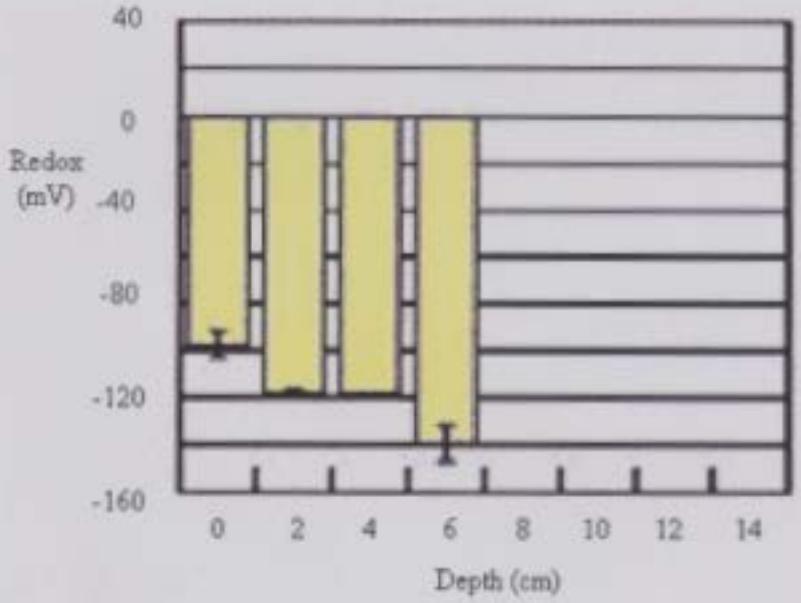

Figure 3.11c: Mean (± S.E.) redox values (mV) from measurements taken at 2 cm intervals in sediment cores from FH 4F, a farm station with muddy sediment, in March 2003.

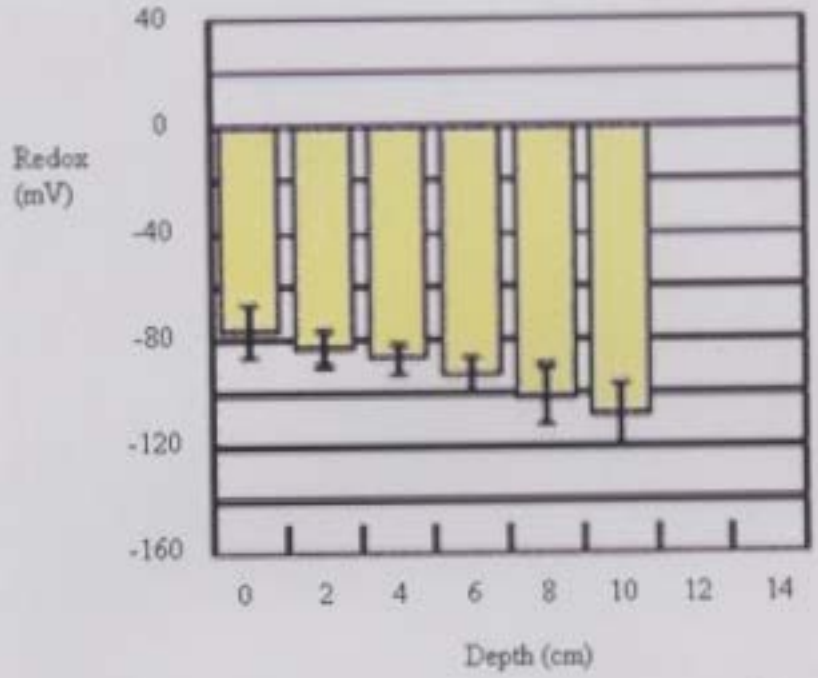

Figure 3.11d: Mean (± S.E.) redox values (mV) from measurements taken at 2 cm intervals in sediment cores from FH 2R, a reference station with muddy sediment, in March 2003.

Figure 3.11e: Mean (± S.E.) redox values (mV) from measurements taken at 2 cm intervals in sediment cores from FH 3R, a reference station with muddy sediment, in March 2003.

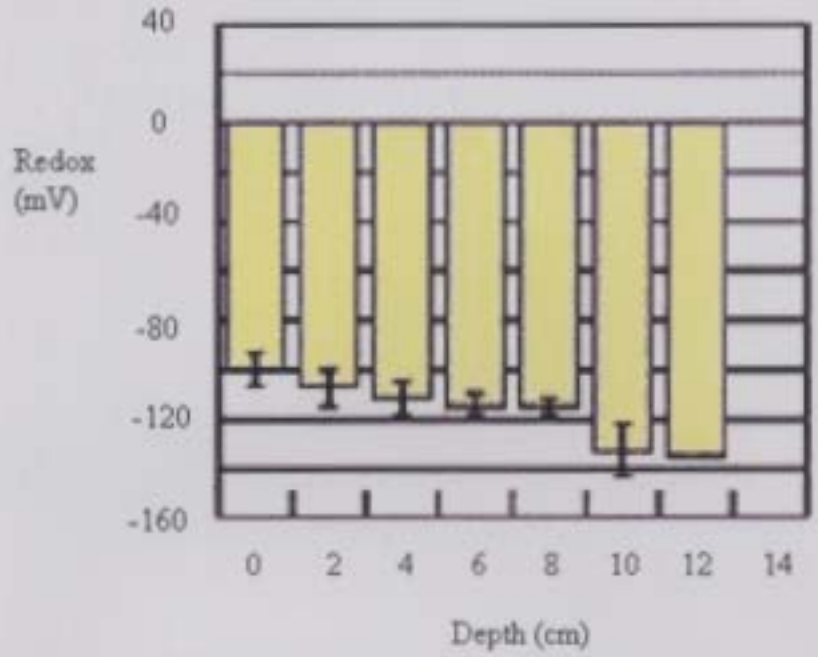

Figure 3.12a: Mean (± S.E.) redox values (mV) from measurements taken at 2 cm intervals in sediment cores from CA 1F, a farm station in CA with sandy sediment, in March 2003.

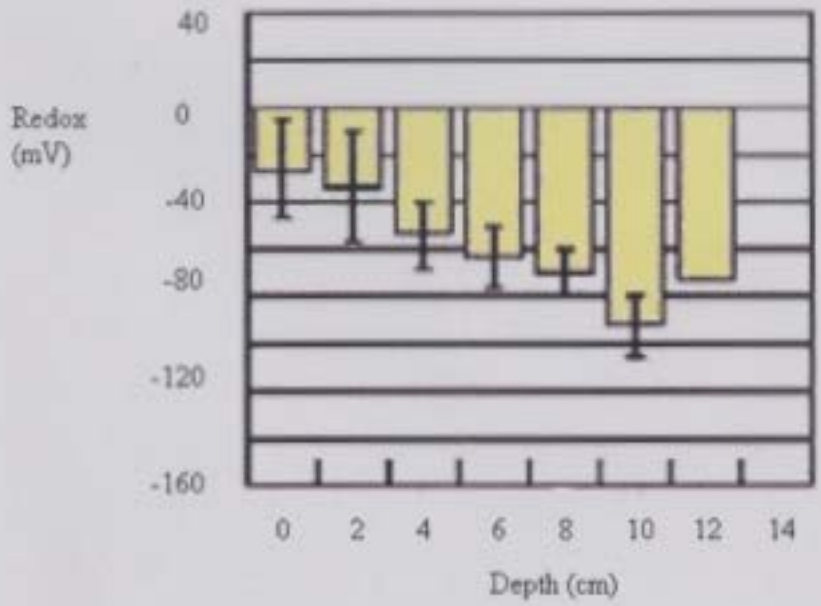

Figure 3.12b: Mean (± S.E.) redox values (mV) from measurements taken at 2 cm intervals in sediment cores from CA 2F, a farm station in CA with sandy sediment, in March 2003.

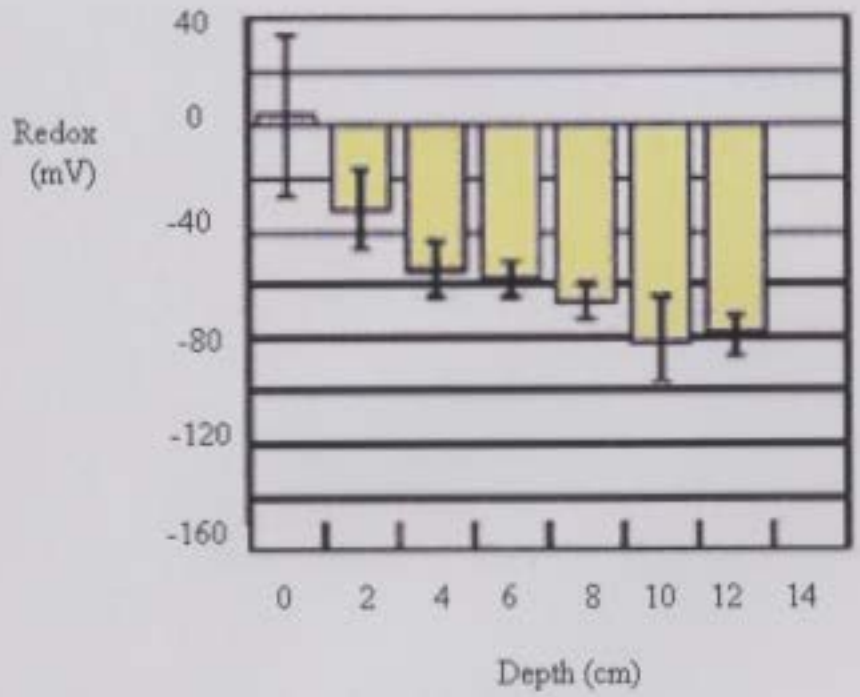

Figure 3.12c: Mean (± S.E.) redox values (mV) from measurements taken at 2 cm intervals in sediment cores from CA 2R, a reference station in CA with sandy sediment, in March 2003.

3.3.4 Infaunal trophic index (ITI)

Table 3.7 shows the assignment of species collected into the four feeding groups described in section 3.2.4.4., following Nickell (2004) and Maurer et al. (1999). For individuals that had not been previously classified by other authors, the feeding apparatus was examined and organisms were assigned to the most appropriate feeding group. Very few organisms could not be classified, and these taxa were omitted from the analysis. A sensitivity analysis showed that omitting these individuals did not affect the outcome of the classification procedure.

Table 3.7: Thirty-five invertebrate taxa collected from FH and CA, Newfoundland, classified according to four trophic groups identified by Word (1990).

Feeding Group1: Suspended detritus feeders
Maldane glebifex
Euchone elegans
Pectinaria hyperborea
Pectinaria granulata
Dyastylis rathkei
Dyastylis polita
Cucumaria frondosa
Serpulidae
Feeding Group 2: Surface detritus feeders
Mediomastus sp.
Nephtys ciliata
Nephtys incisa
Hesionidae
Polydora websteri
Polydora quadrilobata
Prinospio steenstrupi
Tharyx sp.
Cossura sp.
Terebellidae
Lyssippe labiata
Sabellides borealis
Feeding Group 3: Surface deposit feeders
Scoloplos armiger
Eteone heteropoda
Phyllodoce mucosa
Aglaophamus neotenus
Antinoella sarsi
Pholoe tecta
Goniada maculata
Linaeus socialis
Priapulus caudatus
Macoma calcarea
Amphipod 1
Amphipod 2
Amphipod 3
Burrowing sea anemone
Feeding Group 4: Subsurface deposit feeders
Capitella spp.

3.3.4.1 Fortune Harbour

Table 3.8 shows ITI values for both sandy and muddy stations in FH. Figure 13 shows relative abundances of ITI feeding guilds at different stations with different sediment types. Muddy stations were often devoid of macrofauna. Even when macrofauna were present at muddy stations, ITI scores were very low (Table 3.8), especially at farm stations. This pattern suggests that the benthic environment at this location was not optimal for macrofauna. FH 2F, for example, was not included in the ITI analysis because there were no macrofauna and thus an ITI score could not be calculated. ANOVA indicated a significant difference between ITI values at a sandy farm (FH 1F) and a reference station (FH 1R) ($p=0.035$) (Table 3.9). ANOVA also indicated that there was a statistically significant difference in mean ITI scores among farm (FH 2F, FH 3F, FH 4F) and reference stations (FH 2R and FH 3R) in muddy sediments at FH ($p=0.021$, Table 3.10). Differences among stations nested within farm/ref were not statistically significant. At reference stations, macrofauna were present in most samples and ITI values were greater than at farm stations in the muddy sediments of FH. In general, moderate to low ITI scores in muddy FH stations were attributable to large numbers of subsurface deposit feeders such as *Capitella* spp.

Table 3.8: Mean Infaunal Trophic Index (ITI) (organized by sediment type) for replicates of sandy and muddy FH samples in July, August, November 2002 and March 2003. '-' = no sample '*'= no macrofauna present in samples, 'n'=number of grabs containing macrofauna, SE = Standard error of the mean.

Sample	Month	Sediment Type	Farm/ Ref	total # of grabs	n	Mean ITI	SE Mean ITI
FH 1F	J	Sand	Farm	4	4	52.00	8.45
	A			4	4	47.09	2.89
	N			4	4	48.42	3.07
	M			4	4	57.84	3.56
FH 1R	J	Sand	Ref	3	3	90.22	4.90
	A			4	4	66.67	0.00
	N			4	3	63.00	3.86
	M			-	-	-	-
FH 2F	J	Mud	Farm	4	0	*	*
	A			4	0	*	*
	N			-	-	-	-
	M			4	0	*	*
FH 3F	J	Mud	Farm	4	2	33.34	0.00
	A			4	4	3.76	2.46
	N			4	0	*	*
	M			4	4	1.76	1.35
FH 4F	J	Mud	Farm	4	0	*	*
	A			4	4	0.01	0.00
	N			4	0	*	*
	M			4	4	2.34	2.34
FH 2R	J	Mud	Ref	4	4	6.76	1.55
	A			4	0	53.30	29.10
	N			4	2	66.67	8.00
	M			4	2	*	*
FH 3R	J	Mud	Ref	4	4	64.67	0.982
	A			4	4	6.67	2.26
	N			4	2	32.34	1.00
	M			4	2	44.50	22.2

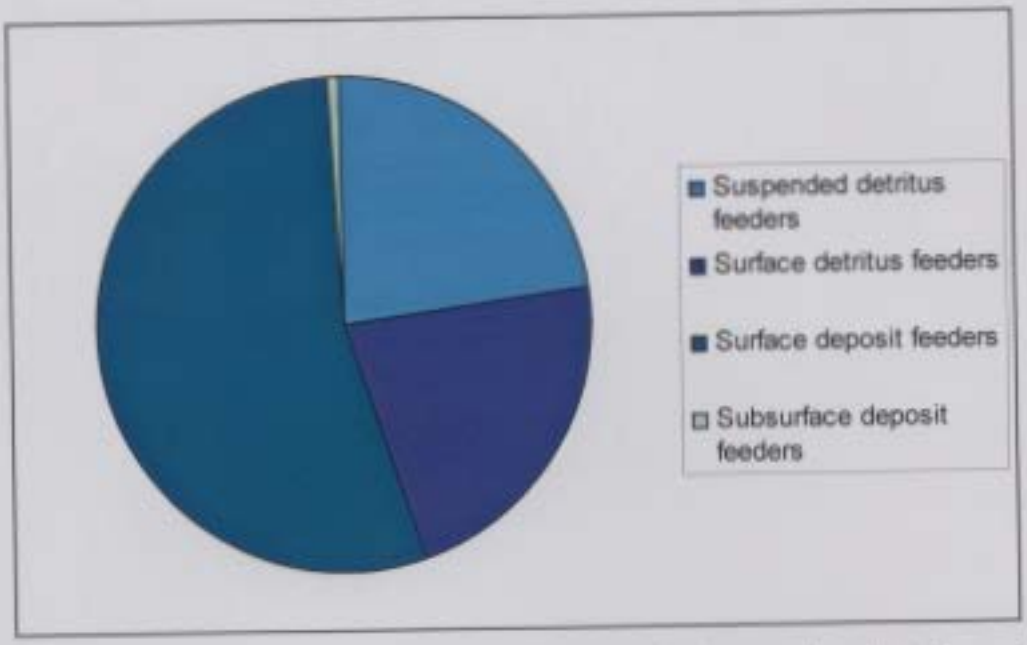

Figure 3.13a: ITI feeding groups of macrobenthic species (proportion of total numbers) collected at farm and reference stations in sandy sediments at FH in 2002-2003.

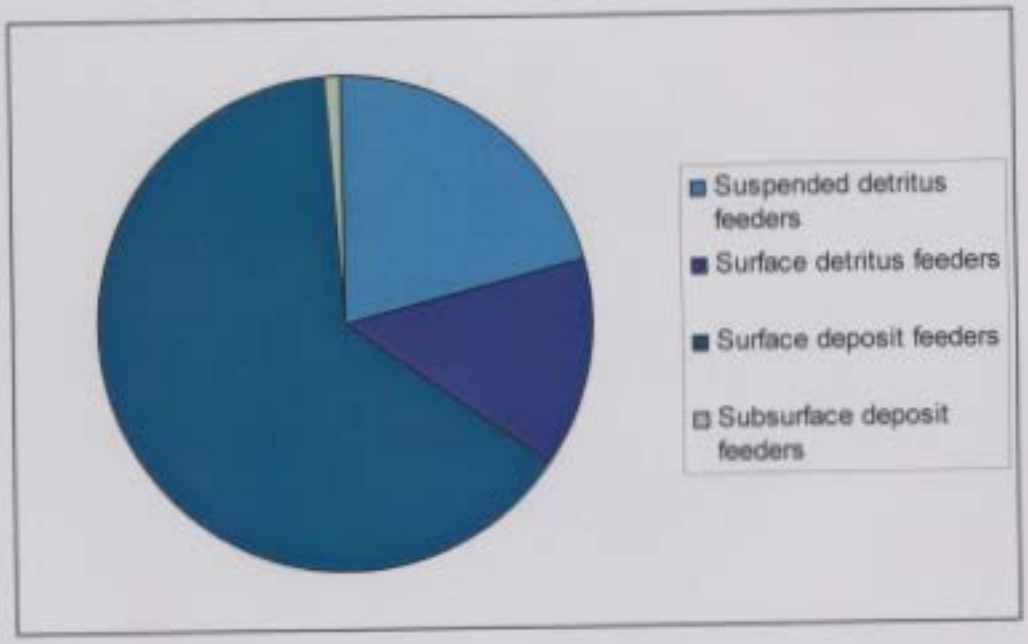

Figure 3.13b: ITI feeding groups of macrobenthic species (proportion of total numbers) collected at farm stations in sandy sediments at FH in 2002-2003.

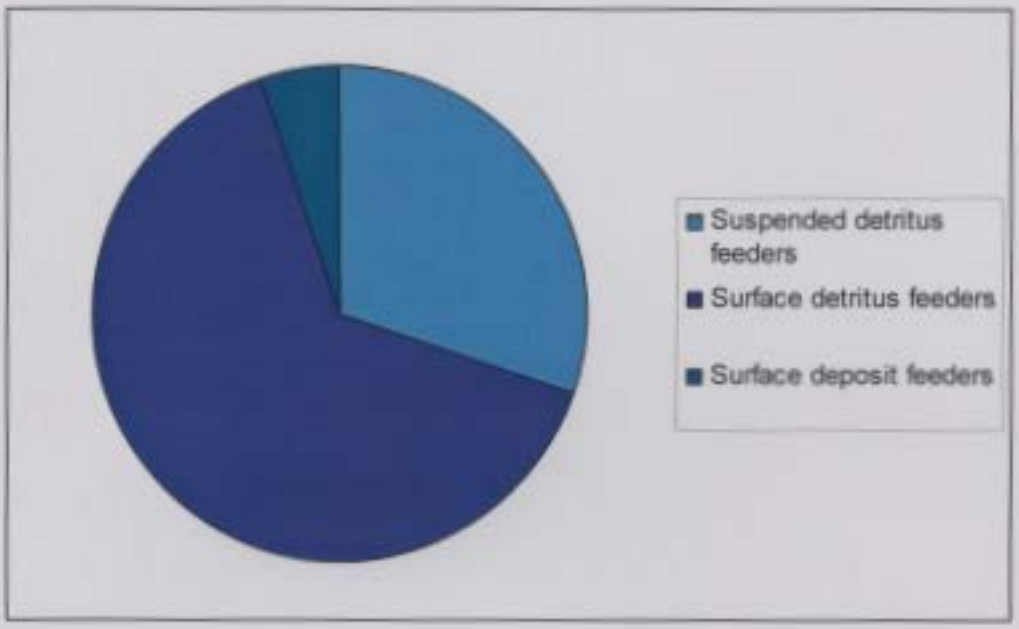

Figure 3.13c: ITI feeding groups of macrobenthic species (proportion of total numbers) collected at reference stations in sandy sediments at FH in 2002-2003.

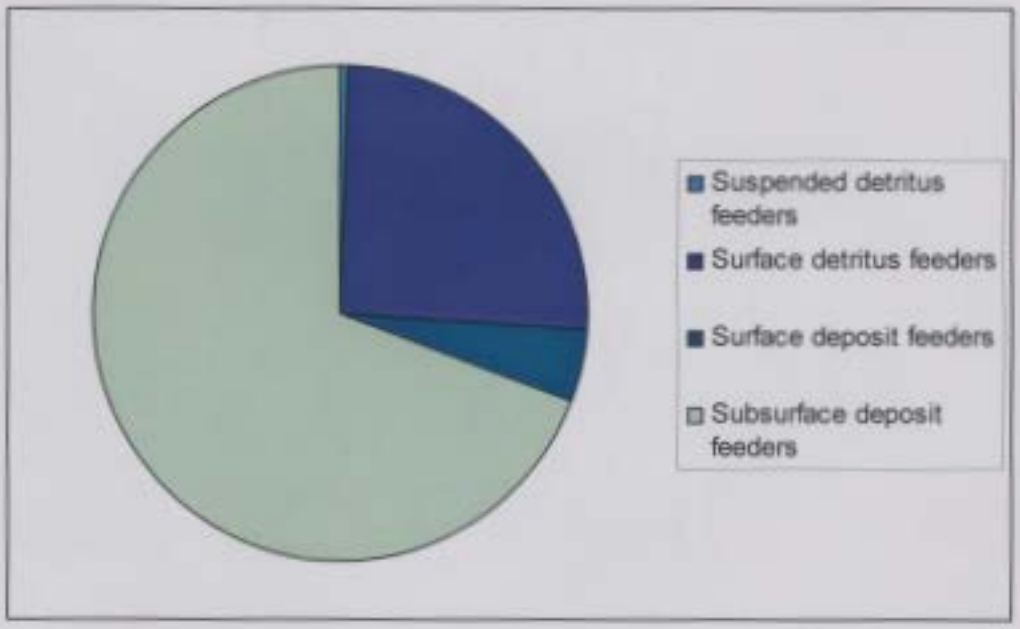

Figure 3.13d: ITI feeding groups of macrobenthic species (proportion of total numbers) collected at farm and reference stations in muddy sediments at FH in 2002-2003.

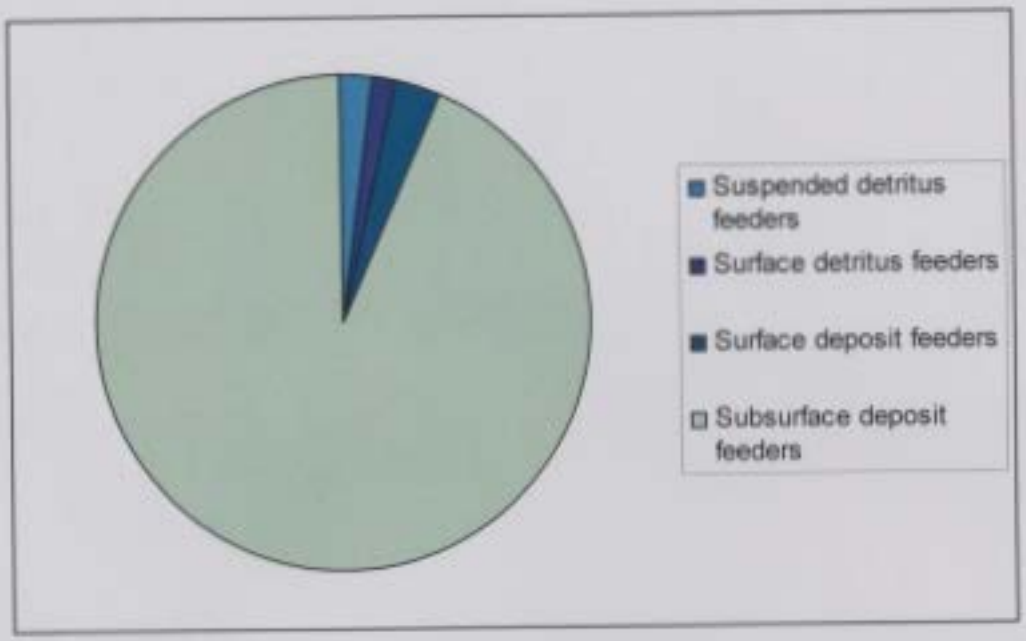

Figure 3.13e: ITI feeding groups of macrobenthic species (proportion of total numbers) collected at farm stations in muddy sediments at FH in 2002-2003.

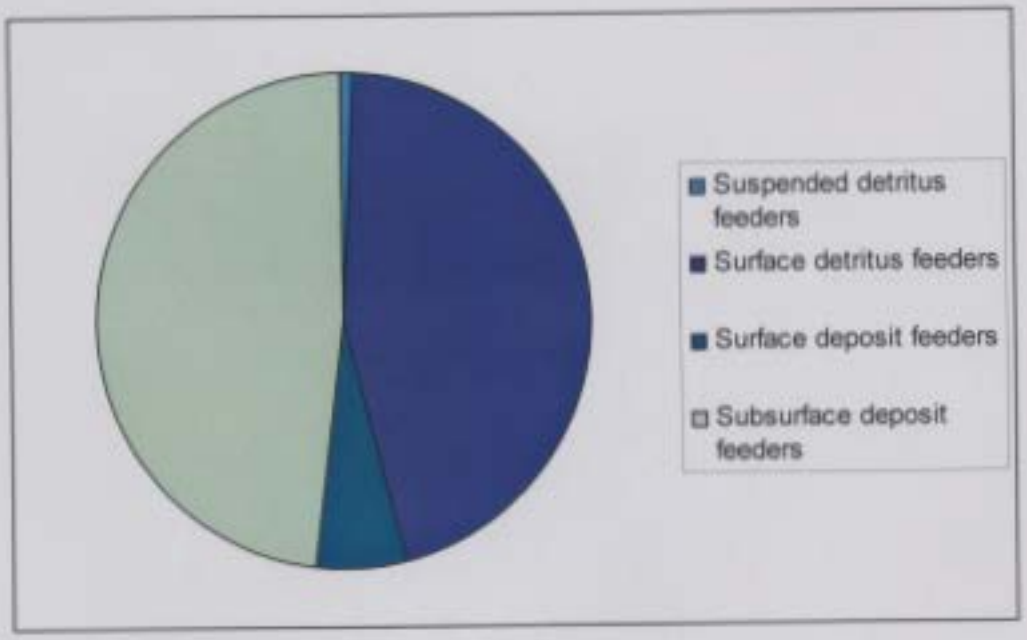

Figure 3.13f: ITI feeding groups of macrobenthic species (proportion of total numbers) collected at reference stations in muddy sediments at FH in 2002-2003.

Table 3.9: ANOVA: ITI scores of sandy sediments (farm *vs* reference) in FH.

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Farm/ reference	1	826.6	826.6	826.6	8.17	**0.035**
Error	5	505.6	505.6	101.1		
Total	6	1332.2				

Table 3.10: ANOVA: ITI scores for muddy sediments (farm *vs* reference and station nested within farm/ref) in FH.

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Farm/ reference	1	3347.6	3347.6	3347.6	7.47	**0.021**
Station (farm/ref)	2	231.4	231.4	115.7	0.26	0.777
Error	10	4483.0	4483.0	448.3		
Total	13	8062.0				

3.3.4.2 Charles Arm

In CA, which is mainly sandy, ITI scores (Table 3.11) were similar to those of sandy sediments in FH (Table 3.8), probably because of the high numbers of surface and subsurface detritus feeders that the two sites had in common. Figure 14 shows relative abundances of ITI feeding guilds at different stations with different sediment types. Although on two sampling occasions there were 2 stations in CA that did not possess macrofauna, ITI values were moderately high elsewhere in CA. These values suggest that the absence of macrofauna is not a result of eutrophication. At times, ITI was lower at farm stations than at reference stations, but mean ITI scores between farm and reference stations in CA were not significantly different (p=0.819, Table 3.12).

Table 3.11: Mean Infaunal Trophic Index (ITI) values for replicates of CA samples in July and August, 2002 and March 2003. '-'= no sample, '*'= no macrofauna in sample, or SE could not be calculated because n=1, 'n'=number of grabs containing macrofauna, SE = Standard error of the mean.

Station	Month	Farm/ Ref	Sediment Type	total # of grabs	n	Mean ITI	SE Mean ITI
CA 2F	J	Farm	Sand	4	2	61.11	5.56
	A			4	0	*	*
	M			4	0	*	*
CA 1F	J	Farm	Sand	4	3	33.33	0
	A			4	0	*	*
	M			4	1	100	*
CA 2R	J	Ref	Sand	4	4	61.89	4.78
	A			4	4	*	*
	M			4	1	66.67	*
CA 1R	J	Ref	Sand	4	3	65.89	7.03
	A			4	3	55.6	11.1
	M			-	-	-	-

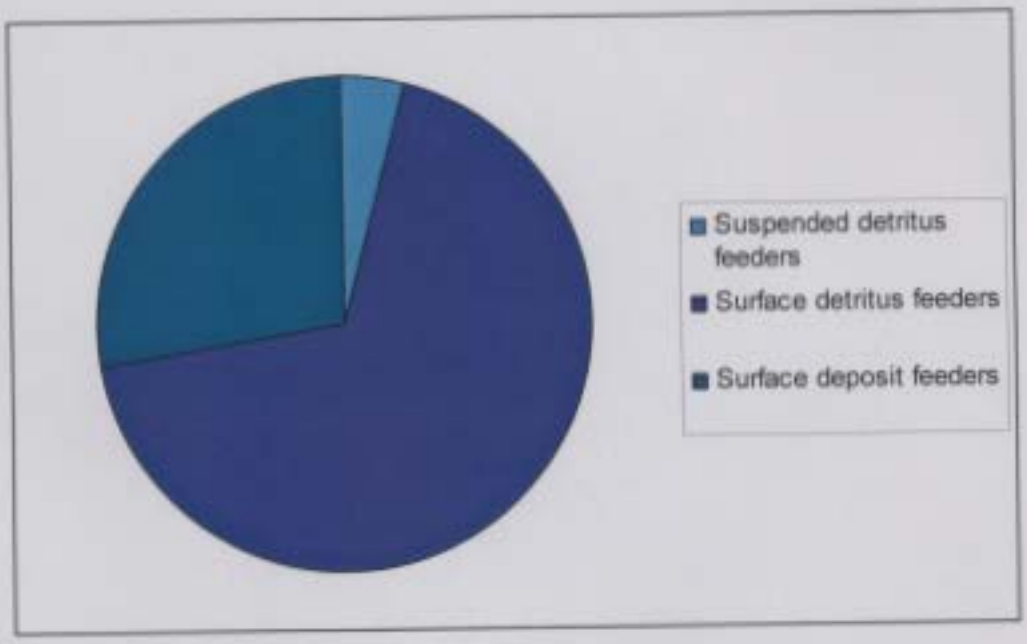

Figure 3.14a: ITI feeding groups of macrobenthic species (proportion of total numbers) collected at farm and reference stations in sandy sediments at CA in 2002-2003.

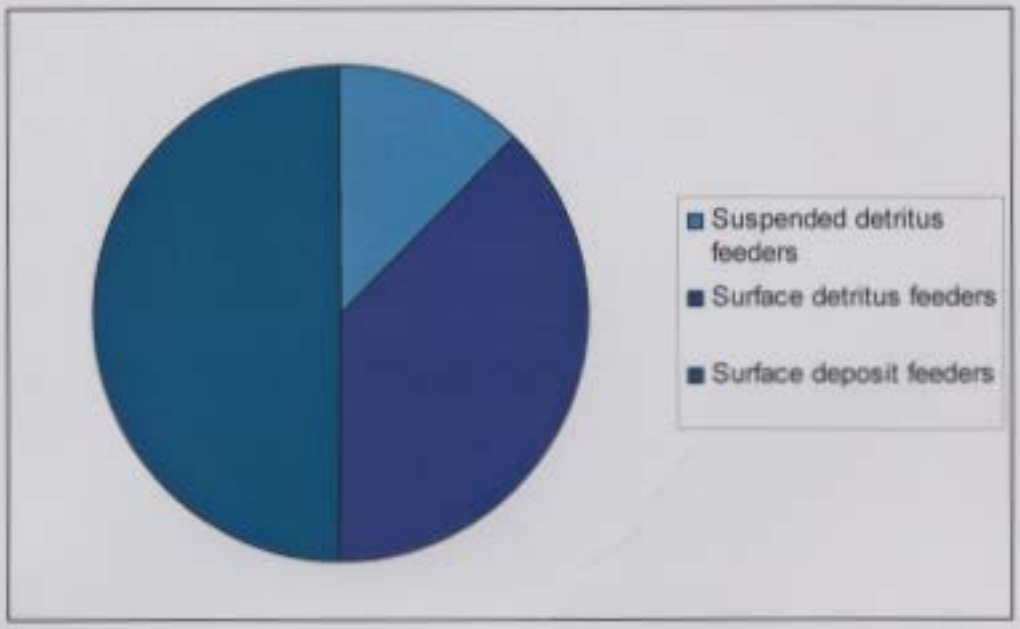

Figure 3.14b: ITI feeding groups of macrobenthic species (proportion of total numbers) collected at farm stations in sandy sediments at CA in 2002-2003.

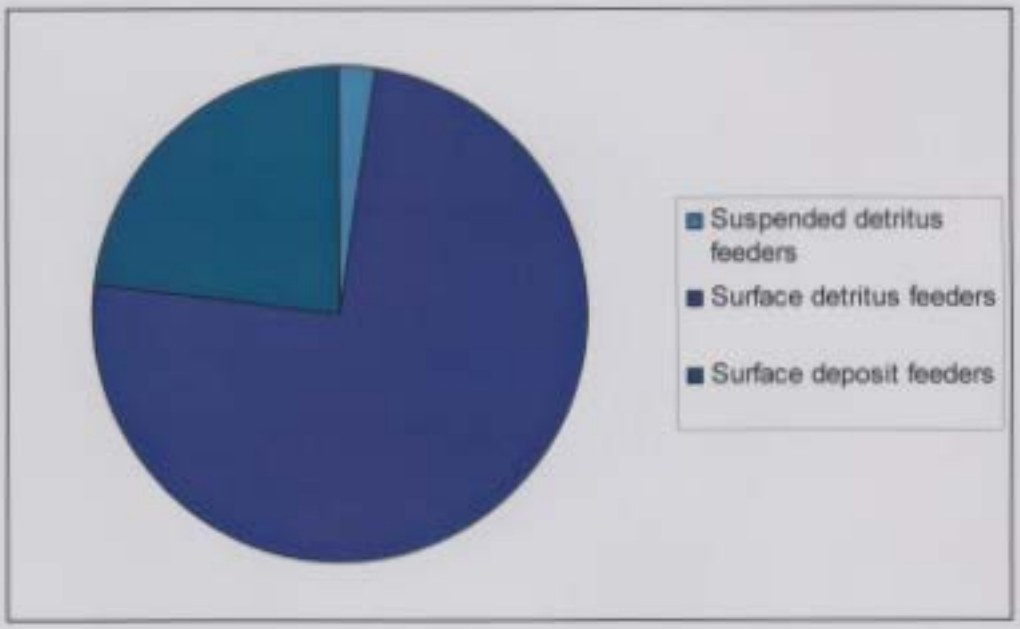

Figure 3.14c: ITI feeding groups of macrobenthic species (proportion of total numbers) collected at reference stations in sandy sediments at CA in 2002-2003.

Table 3.12: ANOVA: ITI scores (farm *vs* reference) in CA.

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Farm/reference	1	22.2	22.2	22.2	0.06	0.819
Error	6	2346	2346	391		
Total	7	2368.2				

3.3.5 Relationship between Eh and ITI

A simple linear regression between the ITI values and the Eh values in the 0-2 centimetre depth range of the sediment profile (Table 3.13) of muddy stations FH 3F, FH 4F and FH 3R indicated a positive relationship between Eh and ITI ($R^2 = 1.00$, $p = 0.006$). The analysis produced the following regression equation: ITI = 30.4 + 0.258 Eh. A similar analysis of CA data could not be done because ITI values could not be calculated for each station and thus there were not enough data points to carry out a regression.

Table 3.13: ANOVA: ITI *vs* Eh in 0-2 cm depth in FH stations FH 3F, FH 4F and FH 3R.

Source	DF	Seq SS	Adj MS	F	p
Regression	1	1201.4	1201.4	10457.93	**0.006**
Residual Error	1	0.1	0.1		
Total	2	1201.5			

3.4 Discussion

3.4.1 Comparison of sites

Although the two farm sites selected for this study are geographically close to one another, they are very different in terms of hydrography, sediment type, organic content, and benthic macrofaunal community composition. CA is much shallower than FH, and CA sediments are therefore likely to be better oxygenated because of wind and wave action. The water column in FH is also consistently more strongly stratified than in CA (Stacey, 2003). Higher sediment temperatures at CA throughout the sampling period (Anderson, unpublished data) further suggest that the water column is well mixed. Furthermore, CA has more bare rock patches where use of an Ekman grab is not possible.

Redox potential profiles of sediments in March 2003 indicate that although oxygen levels are generally low in CA, they are higher than in muddy stations in FH. Unfortunately, there is no data for sediment redox potential at sandy FH stations.

Sandy stations in FH and CA are dominated by very low numbers of large polychaetes (relative to those collected at muddy stations) and other smaller invertebrates. Heterogeneous sediment exhibits a higher infaunal diversity than does homogeneous sediment (Gray, 1974), which may explain the higher number of species found in sandy stations of FH and CA. In order for these stations to support such large organisms year round, there must be an adequate supply of oxygen in the sediment.

Muddy stations in FH, conversely, exhibit some characteristics of organic enrichment. These sediments are dominated by small, opportunistic polychaetes including *Capitella* spp. and *Polydora* spp., which are often used as indicator species for organically polluted sediments (Pearson and Rosenberg, 1978). At least one station in the farm is completely devoid of benthic macrofauna and has sediments with a strong hydrogen sulphide odour, which is not apparent at the CA site.

Different farms have varying degrees of environmental impact that depend on a multitude of factors. Thus aquaculture sites, no matter how close geographically, are not necessarily similar and should be treated individually for management purposes.

3.4.2 Comparisons among stations within farms

The most obvious physical difference among stations is sediment grain size, and thus stations must be discussed with respect to whether they have sandy (>500 μm) or muddy (<500 μm) sediments. Within farms, some stations are more similar in terms of the benthic macrofaunal community than others. Sandy sediments consistently show a similar benthic macrofaunal composition, as do muddy sediments. This information (sections 3.3.1.1 and 3.3.1.2) is further supported by the infaunal trophic indices, which differ between sandy and muddy stations in FH. ITI scores are higher in sandy areas of FH and CA owing to the presence of large numbers of detritus feeders. Conversely, those stations dominated by smaller individuals in much larger abundances (FH 3F, FH 4F, FH 2R, FH 3R) have much lower ITI values because of the dominance of deposit feeders. According to Pearson and Rosenberg (1978), the number of deposit feeders generally increases in response to high organic inputs. Thus, the degree of impact from the mussel farm appears to be greater in areas of muddy sediment. Furthermore, analysis of Eh and ITI values in muddy FH sediments shows a positive relationship, thus helping to demonstrate that that sediment anoxia related to mussel biodeposition is the most likely mechanism by which mussel farming may affect benthic biota.

According to Pearson and Rosenberg (1978), a reduction in oxygen availability is the most serious effect of organic pollution on aquatic organisms. Sediments are generally considered to be hypoxic if redox values fall between -100 and 0 mV, and are anoxic if they are less than -100 mV (Wildish et al., 1999). By this definition, many farm stations at FH and CA are anoxic throughout the sediment profile (especially FH 2F, FH 3F, FH 4F, CA 1F). Other stations are anoxic in deeper sediment layers and hypoxic near the surface (FH 2R and FH 3R), whereas a few stations are hypoxic from the sediment surface downward (CA 2R, CA 2F). Anderson et al. (2005) noted that natural levels of organic matter in coastal Newfoundland sediments are generally high, which explains the low Eh values recorded in this study.

Pearson and Rosenberg (1978) describe a "classic" faunal succession along a gradient of organic enrichment. In their diagrammatic representation, no macrofauna survive when bottom waters are completely anoxic and redox potential values are

negative. Pearson and Rosenberg (1978) describe such a situation as "grossly polluted". This is the case for FH 2F, a farm station that is devoid of benthic macrofauna. Mattson and Lindén (1983) found that anoxic conditions at a mussel farm on the west coast of Sweden led to the almost complete disappearance of the benthic macrofauna.

Other muddy stations in FH (FH 3F, FH 4F, FH 2R, FH 3R) exhibit properties that Pearson and Rosenberg (1978) describe as being characteristic of a "polluted" environment. In this phase of organic enrichment, the changes in the physical and chemical conditions in the sediment gradually eliminate larger burrowing species and favour smaller and faster growing opportunistic species such as *Capitella* spp.

Sandy stations such as those found in FH (FH 1R, FH 1F) and CA (CA 1R, CA 2R, CA 1F , CA 2F) appear to fall between Pearson and Rosenberg's categories of "normal" and "transitory" in terms of the amount of enrichment, based on the species present. That is, these areas can be described as diverse because they are comparatively rich in species and include a wide range of higher taxa, body sizes and functional types.

According to Pearson and Rosenberg (1978), in well-flushed locations there is continuous oxygen renewal and organic matter is transported over a wider area, as opposed to local deposition. This renewal creates more favorable conditions for macrofaunal organisms, compared with the paucity or lack of macrofauna and anoxic sediments associated with stagnant or poorly flushed waters. Thus, FH 3R is likely to be representative of a well flushed area whereas FH 2F (no macrofauna present) is representative of poorly flushed waters as described by Pearson and Rosenberg (1978). On the other hand, FH 3R, a relatively deep reference station, is not devoid of macrofauna. Unlike FH 2F, FH 3R lies in an inlet without a sill, and thus may be better flushed. Furthermore, it lacks the added influence of a mussel farm. FH 2F may be a depositional zone in which biodeposits accumulate from a wider area. This point illustrates the importance of siting aquaculture operations in areas where flushing is adequate to avoid pockets of local eutrophication.

Results from both FH and CA differ from those of Crawford et al. (2003), who found that shallow inshore stations in Tasmania, Australia, are characterized by organic enrichment and macrofaunal communities that are tolerant of low oxygen levels. One possible explanation is that mussel biodeposits in the Australian site may be transported

inshore and concentrated in shallow water, creating anoxic conditions. FH 1F and CA 1F, both with sandy sediments, are the shallowest of the farm stations in FH and CA and neither is dominated by opportunistic polychaetes, nor shows low ITI scores indicative of enriched conditions.

It is also likely that the stocking densities at FH and CA, which are lower than at most culture sites around the world (personal communication, T. Mills, mussel farmer), help to minimize the environmental impact. The higher mussel stocking density at CA compared with FH and the lower surface area of the former site suggest that the benthic community at CA should be more adversely affected than at FH. This is not the case, however, perhaps as a result of higher flushing rates in CA.

3.4.3 Comparison between farms and their respective reference stations

As previously determined, different sediment types, as well as different farm sites, respond differently to the presence of mussel lines, and as a result of organic enrichment the difference between farm and reference stations depends primarily on the characteristics of the sites (ie. sediment type, flushing rates, stocking densities, etc.). With respect to the logical hypotheses of this study, the third hypothesis that the benthic macrofaunal assemblages are different between farm and reference stations is supported for both sandy and muddy sediments. That is, the statistical null hypothesis of no difference between benthic macrofaunal assemblages can be rejected for both sediment types. There is an apparent difference in benthic macrofaunal community composition between each farm and its reference site. In terms of the benthic macrofaunal communities, sandy sediments in both CA and FH differ depending on whether cultured mussels are present or absent. These sandy sediments do not, however, support macrofaunal communities normally associated with highly enriched sediments. Specifically, capitellid polychaetes do not dominate (Bellan, 1967; Bagge, 1969; MacKay et al., 1972; Halcrow et al., 1973). Nevertheless, the sandy stations do support species known to tolerate organic enrichment, and the presence of these species suggests minor effects from mussel culture. Nephtyid polychaetes are characteristically resistant to eutrophication (Bagge, 1969) and are found in high numbers in sandy sediments in CA and FH. Similarly, at sandy farm stations in FH, *P. quadrilobata* is present. This species

also occurs in high numbers in organically enriched sediments in Japan (Kitamore and Funae, 1959). Furthermore, *Goniada maculata* is present only in sandy farm stations in FH and likewise occurs in organically polluted areas in the Baltic Sea (Bagge, 1969) and the NW Mediterranean (Bellan, 1967). Moreover, Pearson and Rosenberg (1978) identified *Goniada* sp., *Pholoe tecta*, and *Pectinaria* sp. as species that characterize the transitory zone along a gradient of organic enrichment. These organisms are also present in sandy farm stations in FH, further suggesting that sandy stations in FH may show minor effects of enrichment. *Capitella* spp. are present in sandy FH sediment, but not in the high numbers that would be expected in an organically enriched area. Interestingly, organisms associated with organic enrichment that are found in these sandy farm stations in FH are much larger than individuals of the same species collected in muddy areas. However, although the presence of these species in FH does point to enriched conditions, in muddy sediments these species are found in areas both with and without cultured mussels, indicating that both farm and reference stations show evidence of organic enrichment.

In muddy stations, which are found only in FH, differences between farm and reference stations are apparent. Redox potential, ITI values and the composition of the benthic macrofaunal community are significantly different between farm and reference stations. Redox values and ITI scores are very negative and low respectively at both farm and reference stations. Benthic macrofaunal assemblages at both farm and reference sites show signs of enrichment, although the sites are significantly different. The most conspicuous organisms that are associated with organic enrichment and dominate both farm and reference stations are capitellid polychaetes. The large numbers of taxa associated with organic enrichment and the low redox potentials and ITI scores in both farm and reference stations indicate an overall enrichment in muddy FH sediments at both farm and reference stations.

Since reference stations in this study also demonstrate naturally enriched conditions, especially in muddy sediments, it appears that although mussel culture may be adding to this enrichment, it is not the only factor that affects the environment. The fact that redox potentials are below zero at each station, including stations without mussel culture, should be considered when monitoring requirements are established for

aquaculture sites in Newfoundland. Specifically, low redox values may not be a result of the presence of a mussel farm, but merely characteristic of coastal sediments in the area. Anderson et al. (2005) found that Eh values recorded in this study were not significantly different from reference sites at other farms in Newfoundland, where redox values may be even lower. The high organic matter values recorded at other reference sites support the conclusion that the presence of cultivated mussels is not the sole factor altering the benthic environment in these areas.

3.4.4 Temporal variation

Three of the eleven stations sampled showed temporal variation throughout the sampling period. This variability was more pronounced in muddy sediments at FH. Even under ice cover in March, there was no increase in abundance of organisms with a high tolerance of hypoxia, nor was there a decrease in organisms with high metabolic rates.

3.4.5 Conclusion

The data show that each site is unique in terms of its physical, chemical and biological characteristics. Depending on these characteristics, mussel farms may or may not have an impact on the local benthic environment. In CA, farm and reference stations are not significantly different in terms of ITI scores and redox potential and the site is not dominated by capitellid polychaetes. Thus the area is unlikely to be experiencing a high level of enrichment as a result of mussel aquaculture. In FH, sandy sediments show a difference among stations with and without mussel culture in terms of ITI scores and benthic macrofaunal communities, although like CA they are not dominated by capitellid polychaetes. These sediments therefore show signs of a small effect of organic enrichment, less than the levels characterizing "grossly polluted" or "polluted" environments (Pearson and Rosenberg, 1978). Muddy sediments in FH show signs of extreme enrichment both inside and outside the farm. The farm, however, is not the only determining factor, as reference stations at this site also exhibit considerable amounts of organic enrichment.

Because of site-specific conditions and effects, environmental data for impact assessment should be obtained before and after a mussel farm is established.

Measurements should include redox potential of sediments, sulphide measurements, sediment organic content, grain size analysis, bathymetry, current measurements, flushing rates, bottom water renewal and hydrography of the area (Anderson et al., 2005). Finally, some measure of baseline faunal characteristics is required, including benthic macrofaunal composition as well as trophic structure of the macrofaunal communities.

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4.0 Effects of Scale

4.1 Introduction

Although the concept of scale has long been recognized in ecology, interest in the problem of scale has increased since the 1980s (Wiens, 1989; Steele, 1991; Levin, 1992; Schneider, 2001). The idea that biological changes can be observed on some temporal and spatial scales but not others has directed attention towards the issue of scale in quantitative ecology (Levin, 1992; Schneider 1994, 2001; Schneider et al., 1997). Although marine ecological processes often occur on very large temporal and spatial scales (decades and large ecosystems), we can often only work on small scales (short time scales, small areas) (Schneider, 2001). For the purpose of this chapter, scale is defined as "the resolution within the range of a measured quantity" and can be applied to the space, mass and time components of any measurement (Schneider, 1994). Scale-dependent patterns can be defined as a "change in some measure of pattern with change in either the resolution or range of measurement" (Schneider, 1994). In these processes, the ratio of one rate to another varies with either the resolution or the range of measurements (Schneider, 1994).

In chapter 3 the effects of mussel aquaculture on the benthic community were quantified by comparing benthic macrofaunal communities directly beneath mussel lines with those distant from cultured mussels. According to Schneider (1994), the purpose of any survey is to obtain an accurate estimate of quantity. The scope of this survey, like any other, was determined by several factors, most importantly the extent of impact and the constraints of resources (Schneider, 1994), both logistic and scientific.

In this study, the environmental impact of the farm is investigated from a small spatial scale (the area of the Ekman grab used to collect each sediment sample) to a larger scale (the combined surface area of the mussel farms and their reference areas, i.e. the total number of Ekman grab samples that could have been collected). The temporal scale is also part of the analysis, the smallest scale being the time taken to collect one macrofaunal sample, the largest time scale the entire sampling period of the project.

From the point of view of the aquaculture industry, it would be useful to know the degree to which the data from the present study can be generalized to other sites.

However, mere extrapolation of the data to larger spatial and temporal scales is not scientifically acceptable without appropriate analysis. The purpose of the theoretical treatment in this chapter was to determine whether the collected data and subsequent analysis could be discussed with confidence, and then extrapolated to a larger area of the island of Newfoundland and surrounding coastal areas with a reasonable amount of scientific judgment.

4.2 Materials and Methods

4.2.1 Definition of terms

In comprehending this exercise in scale, it is important that the basic terms and procedures used are clearly understood. Replicate grab samples were taken at a series of stations (see Chapter 3) on several sampling occasions (July, August, November 2002 and March 2003) throughout the year at two mussel farm sites (FH and CA) and at paired reference sites. "Station" refers to the specific areas sampled within each farm and reference site (see Chapter 3). "Replicate sample" refers to the multiple grabs taken at each station on each sampling occasion to ensure accuracy within the sampling design. Finally, the term "survey" defines the entire collection of samples, from the first grab of sediment collected in July 2002 to the last grab in March 2003.

The scope of a measurement is a dimensionless number and can be defined as the ratio of the range to the resolution, or the ratio of the magnitude to the precision of the measurement (Schneider, 1994). Scope diagrams are used to display the temporal and spatial scopes of the data and are useful for evaluating research programs in terms of the phenomenon being studied (Schneider, 1994).

4.2.2 Description of Sites and Samples

The surface areas of FH (49° 31 W, 055° 16 N) and CA (49° 20 W, 055° 16 N) were 0.87 km^2 and 0.59 km^2 respectively. In CA, the combined surface area of the reference arms was approximately 1.3 times that of the farm itself (i.e. 0.77 km^2), whereas at FH the combined surface area of the reference arms was approximately twice

that of the farm (i.e. 1.7 km^2) (Table 4.1). The surface areas of the reference stations were estimated from accurate maps of the study areas.

The benthic habitat at each site was not entirely sediment. Rocky and gravel bottoms were also encountered in places, as described in Chapter 3. The area of sediment available for sampling was therefore less than the total surface area of each site and was determined from nautical charts of the area as well as an initial survey of the areas and communication with the owners of the farms (Table 4.1). For example, only 75% of the farm area in FH contained enough sediment to allow collection of benthic macrofauna with the Ekman grab (potential sampling area = $0.75 \cdot 0.87\ km^2 = 0.65\ km^2$). The reference area for FH was ca. 80% sandy or muddy sediment (potential area for sampling $1.74\ km^2 \cdot 0.80 = 1.39\ km^2$). In CA, only 50% of both farm and reference areas was sandy or muddy sediment (potential area for sampling $0.59\ km^2 \cdot 0.50 = 0.30\ km^2$ and $0.79\ km^2 \cdot 0.50 = 0.40\ km^2$ for farm and reference stations respectively).

Table 4.1: Description of sample sites including potential sampling area (depositional zones).

Site	Farm/Ref	Surface Area (km^2)	Potential Sampling Area (km^2)
FH	Farm	0.87	0.65
FH	Ref	1.7	1.4
CA	Farm	0.59	0.30
CA	Ref	0.77	0.40

Benthic samples were collected as described in section 3.2.2., with 4 replicate grabs at each station on each date. The Ekman grab (area = 0.0232 m^2) was deployed from a small boat or through a hole in the ice. Approximately 20 minutes were required to sample each station. Sampling of each site (CA or FH) was carried out over a period of one week on each occasion from July 2002 to March 2003. Given that the probability of the grab hitting exactly the same point on repeated deployments was extremely small, it was assumed that the total area covered by collecting four samples at each station was ca. 0.0929 m^2 (rather than 0.023 m^2, the area of the grab if 4 samples were collected directly

on top of each other). For the purposes of this exercise in scaling, only the surface area of the grab is of interest because macrofauna were found primarily at the surface or within the upper few centimetres of the sediment.

4.3 Scope diagram

Table 4.2 summarises the data used to construct the scope diagram. The following scales were defined as 'levels' for this study: sampling unit (grab), replicate samples (4 times at each station), site (CA and FH), which is divided into stations (farm and reference stations within each site at each sampling date), and survey, which is the entire set of sediment samples (July 2002 – March 2003).

Column 2 in Table 4.2 is the replication in the survey. Replication refers to the sampling effort at each level and is distinct from the number of grabs made (represented by 'units'); it is thus not cumulative. The first level of replication is the replicate samples of the grabs at each station at each sampling time (4 grabs at each station at each sampling date). The level of station has a replication of 11:

CA reference site (2 stations) + CA farm site (2 stations) + FH reference site (3 stations) + FH farm site (4 stations) =11 stations.

At the level of survey, replication is theoretically 4 because sampling was nominally carried out on 4 occasions but actually undertaken 3 times at CA (July, August 2002, March 2003) and 4 times at FH (July, August, November 2002 and March 2003).

The third column in Table 4.2 is labeled 'units', obtained by multiplying the replication at a given level by the level of replicate sample to determine the number of grabs taken at each level. For example, there are 11 stations, and at each station the sediment collection was repeated 4 times, thus there are 44 units at the station level. At the level of site, CA has 16 units, (4 stations · 4 sediment grabs at each station). At the level of survey, the number of units is the product of the number of stations, the number of replicate samples and the number of sampling occasions for each site. In CA, sampling was carried out three times (July, August 2002 and March 2003), resulting in 48 units at

the level of survey (16 units · 3 sampling times). FH has 28 units at the level of site (7 stations · 4 grabs at each station) and at the level of survey there are 112 units (28 units · 4 sampling times (July, August, November 2002 and March 2003)). Thus in total there are 160 sampling units (112+48 = 160) in the survey. Owing to logistical difficulties, sampling was incomplete, reducing the number of units from the theoretical value (Table 4.2). Thus, with the exception of November, when only stations in FH were sampled (except FH 2F), the number of units is actually 24 (4 replicates · 6 FH stations). In March, because of ice conditions, the number of units was 36 (4 replicates · 9 stations (6 FH + 3 CA)). Furthermore, there were actually 44 units at the level of site in CA, 103 at the level of site in FH and 147 at the level of survey (103+44).

Values for A_o, the spatial support (or the total area sampled at each level) are presented as the surface area of the Ekman grab (0.0232 m^2) multiplied by the number of units at each level. For example, at the level of replicate sample, A_o was 0.0928 m^2 (0.0232 m^2 · 4 units). At the level of station, A_o was 1.021 m^2 (0.0232 m^2 · 44 units). In terms of each site, A_o was theoretically 0.371 m^2 (0.0232 m^2 · 16 units) and 0.650 m^2 (0.0232 m^2 · 28 units) for CA and FH respectively. The term theoretical is used because the original sampling design had to be modified as a result of unforeseen circumstances. At the level of survey, A_o was theoretically 1.111 m^2 (actually 1.021 m^2) for CA and 2.602 m^2 (actually 2.38 m^2) for FH. Therefore, A_o for the entire survey was theoretically 3.712 m^2 (i.e. 160 units · 0.0232 m^2) but actually 147 · 0.0232 m^2 = 3.410 m^2.

The term A is defined as the spatial extent, or the total area to which the study is being extrapolated. Since the exact area that 4 grabs covered was unknown, it was estimated that at the level of the grab, 'A' was ca. 0.1 m^2. The bottom at the sites was not entirely covered in sediment, and this was reflected in the calculations of spatial extent (A). For example, as previously shown, A for the farm area in CA was 0.50 · 0.59 km^2 = 0.30 km^2. At the level of survey, A is the potential amount of sediment that could possibly have been sampled, thus:

$A = FH_{farm}(0.65\ km^2) + FH_{ref}(1.4\ km^2) + CA_{farm}(0.30\ km^2) + CA_{ref}(0.39\ km^2)$
$= 2.74\ km^2$.

The temporal support (T_o) at each level is the time required for the jaws of the Ekman grab to close after the messenger hits the trigger. Thus, for one grab T_o was 0.5

seconds, for 4 grabs, 2 seconds, etc. T, on the other hand, is the temporal extent, or the total amount of time that it took from the time the first grab was deployed until the last deployment at each level. Thus, at the level of the grab, T was 300 seconds and at the level of sample replication, T was 1200 seconds (300 seconds · 4 units). Since it took 2 days to complete the collection of sediment samples in CA, T was 2 days ($1.7 \cdot 10^5$ sec) at this level. The first sediment sample for the survey was collected in July 2002, the last in March 2003, thus T for the survey was ca. 10 months ($2.6 \cdot 10^7$ sec).

The spatial scope of each level is defined as the ratio of the spatial extent to the support in the set (A/A_o). That is, for the level of replicate sample, the spatial scope was 1.08 m^2 i.e. 0.1 m^2 (the approximate area that is covered by 4 grabs) divided by the actual area covered by a grab that is deployed 4 times (0.0232 $m^2 \cdot 4 = 0.0928$ m^2); thus spatial scope is 0.1/0.0928=1.08). Similarly, at the level of site (CA reference area), the spatial scope was $(3.92 \cdot 10^5$ m^2 / 0.186 $m^2) = 2.11 \cdot 10^6$ in July and August, but only $9.2 \cdot 10^{-2}$ in March, because sampling was incomplete owing to ice conditions. The spatial scope of the survey can be defined as the area of the frame (which in this case is the farm) relative to the area of the unit (in this case the area of the grab). Thus, the spatial scope of the survey was $(2.7 \cdot 10^6$ m^2 / 0.0232 $m^2) = 1.2 \cdot 10^8$. That is, 120 million samples could have been taken for this survey.

The temporal scope at each level is the ratio of temporal extent to support in the set (T/T_o). For example, it took approximately 5 minutes (300 sec) to obtain one sediment sample, but only 0.5 seconds for the grab to close once the messenger hit the trigger. Thus, the temporal scope at the level of the grab was (300 sec /0.5sec) = 600. More generally, the temporal scope of the survey was $(2.6 \cdot 10^7$ sec /88 sec) $= 2.9 \cdot 10^5$.

Scope diagrams normally have two logarithmic axes, one displaying the temporal scope and the other the spatial scope. The scope is the distance between two points on a logarithmic scale (Schneider, 1994). As can be seen in Figure 4.1, the relationship between the measured (n) and inferred magnification factor (MF) components is fairly linear. The curved line in the middle represents the data collected from the overall survey during the entire sampling year. The magnification factor (spatial scope/ total number of units) represents the total area that each collected sample actually represents. Thus, each sample that was collected in this survey can be multiplied by $(1.2 \cdot 10^8$ / 147 units) = 8.1 ·

10^5 to determine the area that it actually represents in the entire survey. The line that connects NL to the survey represents the idea that, at least in theory, mussels could be cultivated anywhere along the NL coastline, thus increasing the number of potential sampling sites to the perimeter of the shoreline.

Table 4.2: Values used to create a scope diagram for the benthic surveys of FH and CA farm and reference sites in Notre Dame Bay, Newfoundland. See text (section 4.3) for description of values (A_o, T_o, A and T). For units, both theoretical and recorded values (in parentheses) are presented. Boldface indicates main levels that were calculated by summation of other levels.

Level	Replication	Units	A_o (m^2)	T_o(s)	A(m^2)	T(s)	A/Ao	T/To
Grab		1	$2.3 \cdot 10^{-2}$	0.5		$3.0 \cdot 10^2$		$6.0 \cdot 10^2$
Repl. Sample	4	4	$9.3 \cdot 10^{-2}$	2	$1.0 \cdot 10^{-1}$	$1.2 \cdot 10^3$	1.1	$6.0 \cdot 10^2$
Station	11	44	1.0	22	$2.7 \cdot 10^6$	$4.3 \cdot 10^5$	$2.7 \cdot 10^6$	$2.0 \cdot 10^4$
CA	4	16	$3.7 \cdot 10^{-1}$	8	$6.9 \cdot 10^5$	$1.7 \cdot 10^5$	$1.9 \cdot 10^6$	$2.2 \cdot 10^4$
CA Farm	2	8	$1.9 \cdot 10^{-1}$	4	$3.0 \cdot 10^5$	$8.6 \cdot 10^4$	$1.6 \cdot 10^6$	$2.2 \cdot 10^4$
CA Ref	2	8	$1.9 \cdot 10^{-1}$	4	$3. 9 \cdot 10^5$	$8.6 \cdot 10^4$	$2.1 \cdot 10^6$	$2.2 \cdot 10^4$
FH	7	28	$6.5 \cdot 10^{-1}$	14	$2.0 \cdot 10^6$	$2.6 \cdot 10^5$	$3.1 \cdot 10^6$	$1.9 \cdot 10^4$
FH Farm	4	16	$3.7 \cdot 10^{-1}$	8	$6.5 \cdot 10^5$	$1.3 \cdot 10^5$	$1.8 \cdot 10^6$	$1.6 \cdot 10^4$
FH Ref	3	12	$2.8 \cdot 10^{-1}$	6	$1.4 \cdot 10^6$	$1.3 \cdot 10^5$	$5.0 \cdot 10^6$	$2.2 \cdot 10^4$
Survey	4	160 (147)	3.7	88	$2.7 \cdot 10^6$	$2.6 \cdot 10^7$	$6.2 \cdot 10^6$	$2.9 \cdot 10^5$
CA	3	48 (44)	1.1	32	$6.9 \cdot 10^5$	$2.6 \cdot 10^7$	$7.9 \cdot 10^5$	$8.1 \cdot 10^5$
FH	4	112 (103)	2.6	56	$2.0 \cdot 10^6$	$2.6 \cdot 10^7$	$7.4 \cdot 10^6$	$4.6 \cdot 10^5$

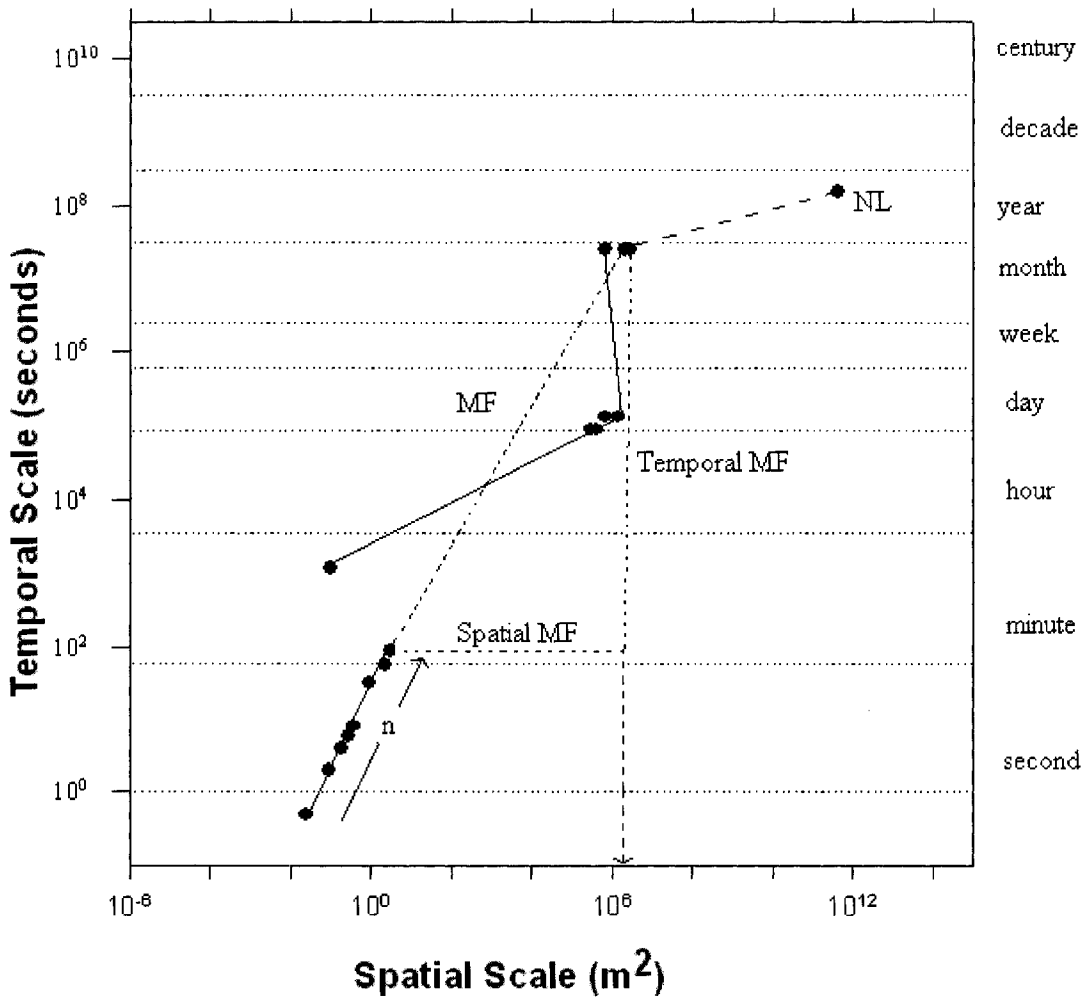

Figure 4.1: Scope diagram of the benthic survey completed from July 2002 to March 2003 in FH and CA, Notre Dame Bay, Newfoundland and extrapolated to the perimeter of Newfoundland. n= samples collected, MF = Magnification Factor. Points associated with 'n' represent sampling that was actually carried out while points associated with 'MF' and connected by a solid line represent extrapolated data related to the number of samples that could have been sampled with unlimited resources. Arrow intersecting with x axis represents spatial scale at which the model deviates from linearity. See Table 4.2 for data points.

4.4 Discussion

According to Schneider et al. (1997), scope diagrams are useful for illustrating sampling effort and highlight the magnitude of the gap between what has been done in field studies and what can be done using models. Scope diagrams therefore have application in planning models, surveys, and experimental designs. In the present study, the scope diagram tells us that using statistical inference we have considerable confidence in describing the benthic community (FH and CA) at each sampling time (because of the linear relationship at the smaller spatial and temporal scale; Figure 4.1), but slightly less confidence in describing the community throughout the year (because of the breakdown of the linear relationship with an increase in spatial and temporal scale; Figure 4.1). Extrapolation beyond 10^6 m^2, however, is less certain, because the linear relationship between the temporal and spatial scales no longer holds. Thus more sampling would be required before any statement could be made with confidence concerning the composition of the benthic community at a scale beyond that of an individual farm. A variety of methods have been suggested to bridge the gap among field surveys, experiments and larger scale questions (Schneider et al., 1997), most of which involve alternating between small-scale data and larger-scale models (Rastetter et al., 1992; Wiens, et al., 1993; Root and Schneider, 1995). Alternatively, extrapolation to a larger area may be possible by carrying out an embedded experiment (Eberhardt and Thomas, 1991), which can extend spatially and temporally limited experiments to larger scale conclusions. In this approach, an experimental set up is placed at areas of high or low density values of an explanatory variable that has been identified by a larger-scale survey (Schneider et al., 1997). This increases the sensitivity of detecting effects relative to random placement (Schneider et al., 1997) and allows for results to be related to larger scale estimates of density variation (Legendre et al., 1997), as it did for Schneider (1978) in an investigation of effects on avian predators.

Without any single "correct" scale at which a population or community can be investigated, environmental biologists are obliged to use a multiscale approach (Levin, 1992), in which there is a shift from one range and resolution to another within a study (Schneider, 1994). One of the main uses for spatial-temporal plots is to compare

monitoring programs or experiments. This benthic survey has been described in the context of scaling analysis so that the data can be subsequently compared with other studies in Newfoundland and Labrador or in areas of the world with similar environmental conditions. In the discipline of environmental science, this scaling analysis can assist scientists and governing bodies in making sound decisions concerning site selection for aquaculture or other proposed water use, because it sets limits on how far data can be extrapolated in making policies or assessing impacts with a reasonable amount of judgment and confidence.

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5.0 Summary

5.1 General Conclusions

Bivalve aquaculture has the potential to cause a variety of environmental impacts, especially on the benthos. Each stage of culture can impact the environment in different ways, but the grow-out phase has the largest potential for doing so. The accumulation of biodeposits from large numbers of mussels can increase the oxygen demand in the sediment and generate anaerobic environments (Black, 2001). The degree of organic enrichment caused by sinking biodeposits will determine the magnitude of the impact or change to the benthic system, which is what makes the link between the rate at which biodeposits are eliminated and changes in benthic macrofauna so important.

Results of this study suggest that mussel farms in Newfoundland can be very different from one another in terms of sediment and benthic community composition. In Notre Dame Bay, the physical characteristics of these environments may contribute to such differences. It is important for farmers and managers to realize that physical differences between sites lead to differences in potential environmental impacts at the farm. Different areas within each farm may also display different levels of environmental impact. Sediments, and therefore benthic macrofaunal communities, are affected differently between farms. In general, FH sediments exhibit a much higher species diversity and abundance than those of CA, and sandy sediments in FH have a higher species diversity than muddy sediments. Generally, all sediments in both FH and CA are influenced by the farm in that the benthic macrofaunal communities are different in areas with and without mussels. Sandy sediments contain organisms that are tolerant of hypoxia, although the species that inhabit them are not those that normally occur in severely anoxic sediments. In the present study muddy sediments were found only at FH, and although there was a significant difference in benthic macrofaunal communities between farm and reference stations, both exhibit classic features of organic enrichment. Sediments at FH stations both with and without mussels growing on long lines have extremely low oxygen levels and are dominated by polychaetes or are devoid of macrofauna, suggesting organic enrichment.

The differences in benthic communities observed between farm and reference stations, coupled with the low levels of oxygen found in all sediments surveyed, indicates that there are factors other than the presence of mussels that affect the benthic environment in FH and CA. These factors may be natural and should be considered when determining the placement of mussel farms and the subsequent monitoring protocol. Additionally, appropriate allowances should be made when setting guidelines for suitable redox values in Newfoundland sediments during environmental assessments of aquaculture sites.

As stated in Chapter 1, the degree of impact caused by a mussel farm can be influenced by several factors, including the scale of production, the orientation and distribution of mussel lines within the farm, the age of the farm, the nature of the habitat and the rate at which feces and pseudofeces are deposited (Jaramillo et al., 1992; Kaiser et al., 1998; Chamberlain et al., 2001). However, it is unlikely that production is sufficiently high to produce severe environmental impacts in areas such as FH and CA, and low stock densities are helping to minimize the environmental footprint. The two farms have been established for at least a decade, and any serious impact should therefore have been evident by now. Currents are probably strong enough in regions with sandy sediment to flush biodeposits from the area, thereby decreasing their long-term impact. In areas of low flushing, as in some parts of FH (FH 2F, an azoic zone), the presence of a sill creates a depositional area and causes local organic enrichment. All these factors can result in a mussel farm that is potentially detrimental to the surrounding environment.

When considering monitoring requirements in Newfoundland, it is important to consider that no two sites are the same, regardless of their proximity to each other, and therefore sites should be treated individually. Sufficient environmental data should be collected at each proposed farm site in the shallow inlets of coastal Newfoundland for adequate determination of the potential environmental impact..

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Appendix A: List of collected macrofauna

Table A1: Macrofauna found in this benthic study. Missing entries (*) result from an inability to assign the individuals concerned to a lower taxonomic level.

Phylum	Class	Family	Genus	Species
Annelida	Polychaeta	Capitellidae	*Capitella*	*
			Mediomastus	*
		Phyllodocidae	*Phyllodoce*	*mucosa*
			Eteone	*heteropoda*
		Nephtyidae	*Nephtys*	*incisa*
			Nephtys	*ciliata*
			Aglaophamus	*neotenus*
		Pectinaridae	*Pectinaria*	*granulata*
			Pectinaria	*hyperborea*
		Spionidae	*Prinospio*	*steenstrupi*
			Polydora	*websteri*
			Polydora	*quadrilobata*
		Ampharetidae	*Lyssippe*	*labiata*
			Sabellides	*borealis*
		Pholoidae	*Pholoe*	*tecta*
		Polynoidea	*Antinoana*	*fusca*
			Antinoella	*sarsi*
			Syllides	*japonicus*
		Cirratulidae	*Tharyx*	*
		Goniadidae	*Goniada*	*maculata*
		Sabellidae	*Euchone*	*elegans*
		Orbeniidae	*Scoloplos*	*armiger*
		Eunicidae	*Eunice*	*
		Maldanidae	*Maldane*	*glebifex*
		Cossuridae	*Cossura*	*
		Hesionidae	*	*
		Terebellidae	*	*
		Surpulidae	*	*
Crustacea	Malacostraca	*	*Dyastylis*	*rathkei*
	Malacostraca		*Dyastylis*	*polita*
	Malacostraca	*	*	*
Echinodermata	Holothuroidea		*Cucumaria*	*frondosa*
	Asteriodea		*Asterias*	*vulgaris*
Mollusca	Bivalvia		*Macoma*	*calcarea*
	Gastropoda	*	*	*
Nemertea	Anopla		*Linaeus*	*socialis*
Priapulida			*Priapulus*	*caudatus*
Cnidaria	Anthozoa	*	*	*

Appendix B: Sample Bray-Curtis similarity matrix

The following equation was used by PRIMER to to generate the Bray-Curtis similarity matrix below:

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

where S_{jk} is the similarity between the j_{th} and k_{th} samples, and y_{ij} is the entry in the i_{th} row and j_{th} column of the data matrix and y_{ik} is the count for the i_{th} species in the k_{th} sample.

	FH 1F	FH 2F	FH 3F	FH 4F	FH 2R	FH 1R	FH 3R
FH 1F	0	0	**0**	0	0	0	0
FH 2F	0	0	0	0	0	0	0
FH 3F	17.83	0	0	0	0	0	0
FH 4F	4.38	0	71.33	0	0	0	0
FH 2R	9.78	0	63.34	**66.58**	0	0	0
FH 1R	11.18	0	0	0	0	0	0
FH 3R	10.48	0	34.34	32.80	34.57	7.44	0

Thus, from the above matrix, we can see that there is a 66.58% similarity between FH 4F and FH 2R or a 0% similarity between FH 3F and FH 1F. This high similarity value would locate these two stations fairly close together on an MDS plot.

Appendix C: SIMPER output showing the most abundant and least abundant/absent species and their contributions to the dissimilarity between CA and FH farm and reference stations with different sediment types.

Location (Sediment)	Dis-similarity (%)	Contributing Species	% Contribution	Abundant Species 1	Abundant Species 2	Species Absent 1	Species Absent 2
CA farm (1) *vs* ref (2) (sand)	65	*L. socialis*	12.8	*N. incisa*	*N. incisa*	*A. vulgaris*	Amphipod 3
		M. calcarea	20.9	*L. socialis*	*N. ciliata*	*N. ciliata*	*L. socialis*
		N. incisa	29.6	*M. calcarea*	*M. calcarea*	*D. rathkei*	*D. polita*
FH farm and ref (sand (1) *vs* mud (2))	94.6	*Capitella* spp.	8.2	*M. calcarea*	*Capitella spp.*	*E. heteropoda*	*M. calcarea*
		N. ciliata	6.4	*D. rathkei*	*P. quadrilobata*	*A. sarsi*	*D. rathkei*
		N. incisa	6.4	*L. labiata*	*Tharyx spp.*	*P. websteri*	*L. labiata*
FH farm (1) *vs* ref (2) (sand)	95.28	*C. frondosa*	8.29	*M. calcarea*	*N. incisa*	*N. ciliata*	*M. glebifex*
		M. calcarea	12.3	*D. rathkei*	*N. ciliata*	*N. insica*	*C. frondosa*
		N. incisa	10.14	*C. frondosa*	*E. elegans*	*E. elegans*	*M calcarea*
FH farm (1) *vs* ref (2) (mud)	55.7	*P. quadrilobata*	19.6	*Capitella* spp.	*Capitella* spp.	*E. heteropoda*	*Amphipod 1*
		Capitella spp.	15.1	Amphipod 2	*P. quadrilobata*	*Hesionidae*	*Amphipod 2*
		Hesionidae	11.12	*P. websteri*	*Tharyx sp.*	*Tharyx* sp.	Gastropod

Appendix D: Sample calculation of Infaunal Trophic Index (ITI)

ITI for the pooled data for Charles Arm (presented in Table 3.8) was found using the following equation:

$$ITI = 100 - \left[33.33 \left(\frac{0n1 + 1n2 + 2n3 + 3n4}{n1 + n2 + n3 + n4} \right) \right]$$

where n_x is the number of individuals in feeding group x.

In this site, the pooled data showed the following division of organisms into feeding groups based on classification used by Maurer (1999) and Nickell (2004):

n_1 (Surface detritus feeders) =2
n_2 (Suspension feeders) =32
n_3 (Surface deposit feeders) =*13*
n_4 (Sub-surface deposit feeders*)* =*0*

Thus,

$$ITI = 100 - \left[33\frac{1}{3} \left(\frac{0(2) + 1(32) + 2(13) + 3(0)}{2 + 32 + 13 + 0} \right) \right]$$

$$ITI = 58.87$$