MONITORING BIOREMEDIATION AFTER OIL SPILLS, OLD AND NEW, USING MARSH FORAMINIFERA AS INDICATORS

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Monitoring bioremediation after oil spills, old and new, using marsh foraminifera as indicators

Roxanne A. McMillan

Abstract: Salt marshes of Nova Scotia are highly susceptible to marine oil spills. Removal of oil by natural processes is slow in low-energy environments, allowing oil to remain unaltered for many years. Marsh foraminifera, microfossils sensitive to various environmental stresses, can indicate oil pollution and are useful to monitor bioremediation. Petpeswick Inlet, Nova Scotia, a 14 month-old experimental spill site, and Black Duck Cove, Chedabucto Bay, Nova Scotia, an over 30 year-old spill site, have similar foraminiferal assemblages. At Petpeswick Inlet are 18 plots treated with one of the following: a controlled plot with nutrients (not oiled), a controlled plot without nutrients (not oiled), a plot with natural attenuation (oiled), a plot with nutrient enrichment (oiled), a plot with nutrient enrichment and cut plants (oiled) and a plot with nutrient enrichment and agricultural disking (oiled). Results continue to show deformation in the species *Miliammina fusca* in oil plots with the exception of the plot with nutrient enrichment and agricultural disking. There is no change in the control plots. Cores examined from Black Duck Cove indicate that the oil spill collapsed the foraminiferal population. After the spill *Miliammina fusca* is the dominant species with deformation peaks present in the oil layer and for several years after. The two areas provide present-day and post-spill scenarios that show how foraminifera may be used to detect the duration needed for bioremediation within a marsh environment.

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d) Percentage of total *Miliammina fusca* averaged from Treatment IE, IIE, and IIIE- Nutrient enrichment and cut plants (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

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CHAPTER 1: INTRODUCTION

1.1 Objective and General Statement

Foraminifera are commonly used to indicate and monitor various environmental conditions and stresses. One such stress is the anthropogenic release of oil within a marsh area. This purpose of this project is two fold: first, to monitor the effects of oil on foraminifera by looking at a 14-month-old controlled oil spill within a coastal salt marsh (a continuation project from last year (Sabean, 2001)); second, to determine if foraminifera may be used to indicate the occurrence of an over 30-year-old oil spill, and the amount of remediation that has transpired since the oil spill.

In June 2001, Fisheries and Oceans Canada (DFO) and the United States Environmental Protections Agency (USEPA) began a project called "Evaluation of Salt Marsh Oil Spill Counter Measures". The project included a controlled oil spill along Conrod's Beach at the mouth of Petpeswick Inlet, Nova Scotia (44°42'N; 63°11'W) (Sabean, 2001). Although the project was conducted between May and October 2000, additional samples were collected in July and August 2001 for the purpose of viewing the continued response of foraminifera to the oil.

The oil tanker *Arrow* was grounded within Chedabucto Bay on February 4, 1970 releasing 11.4 million liters of crude oil (Owens et al., 1994). Black Duck Cove, Canso, Nova Scotia is located within Chedabucto Bay and is the focus area of this study. Oil was deposited over 300km of the shoreline during storm wave conditions, but was not totally cleaned naturally because it resides above the normal occurrence of wave action (Buckley et al., 1974). Although the *Arrow* spill was documented, there have been a limited number of studies on the long-term effects of the oil, and none that have specifically studied the effect of oil on foraminifera.

1.2 Project Importance

Since marshes in Atlantic Canada are susceptible to marine oil spills it is important to monitor the mitigation and the spill response with different restoration techniques. The restoration is important for accommodating the variety of flora and fauna that inhabit the salt marshes. When oil is stranded over an extended period, much like the oil on the beaches of Black Duck Cove, it allows the impact of the oil to continue over an extended period of time, depending on the amount of the natural degradation (Lee and Levy, 1991). This project determines the ability to detect past oil spills and again monitor long-term recovery. The use of benthic foraminifera may provide an inexpensive, rapid and accurate account of recovery from the effects of oil spills.

1.3 Study Area

1.3.1 Physical Environment

Marshes are areas of high fluctuations in terms of temperature, salinity, and water depth (Phleger and Bradshaw, 1966). Generally, the sediments deposited in the salt marshes are finer than all other intertidal environments due to the vegetation acting as a trapping mechanism (Basan and Frey, 1985). The physical environment is controlled by the tides that flood the marsh area twice daily, although both marshes, Petpeswick Inlet and Black Duck Cove, are protected, limiting the amount of weathering that can occur (see Figure 1.1). Marshes are divided into two zones, high and low (Ranwell, 1972). However, others have suggested a third zone called the middle/intertidal marsh zone (Lane et al., 1987). This zonation is marked by differences in flora present. Vegetation seen in various marsh zones include *Spartina alterniflora , Spartina patens, Aster subulatus, Solidago sempervirens, Junicus Gerardo*, as well as other sedges. A complete overview of Atlantic Canada's salt marsh vegetation is contained in Scott et al. (1988).

Salinity and temperature within a marsh vary according to the season. Because foraminifera are sensitive microorganisms, each species has a different tolerance to salinity, thus a change in salinity or temperature may change the dominant species.

1.3.2 Petpeswick Inlet

Conrod's Beach is located in the Eastern Shore, Nova Scotia, 2km from the mouth of Petpeswick Inlet (see Figure 1.2). Sandy, cobble, and boulder beaches separate the marsh from the mud and sand flats (Department of Fisheries and Oceans Canada, 2000). This estuarine system extends approximately 11km inland (Sabean, 2001).

The salinity levels of tidal pools and tidal creeks recorded in this experiment ranged between 24 and 39 ‰ The dominant vegetation of the marsh zone used in the experiment is *Spartina alterniflora*, just below the boundary with *Spartina patens.*

1.3.3 Black Duck Cove

Black Duck Cove is located in Canso County, Nova Scotia (see Figure 1.3). It is in an open-coast environment with bedrock shorelines and no rivers to transport fine-grained sediments to the shore zone (Owens et al., 1994) (see Figure 1.4). The beach consists of boulders, brought in by storms and nearby drumlins while the near shore sediments are cobble and sand grading offshore to silt and clay (Buckley et al. 1974). The boulder beach contains oil residue in the form of a thick asphalt layer, as well as within the voids between rocks. There are also sections that consist of low tide sand and mud

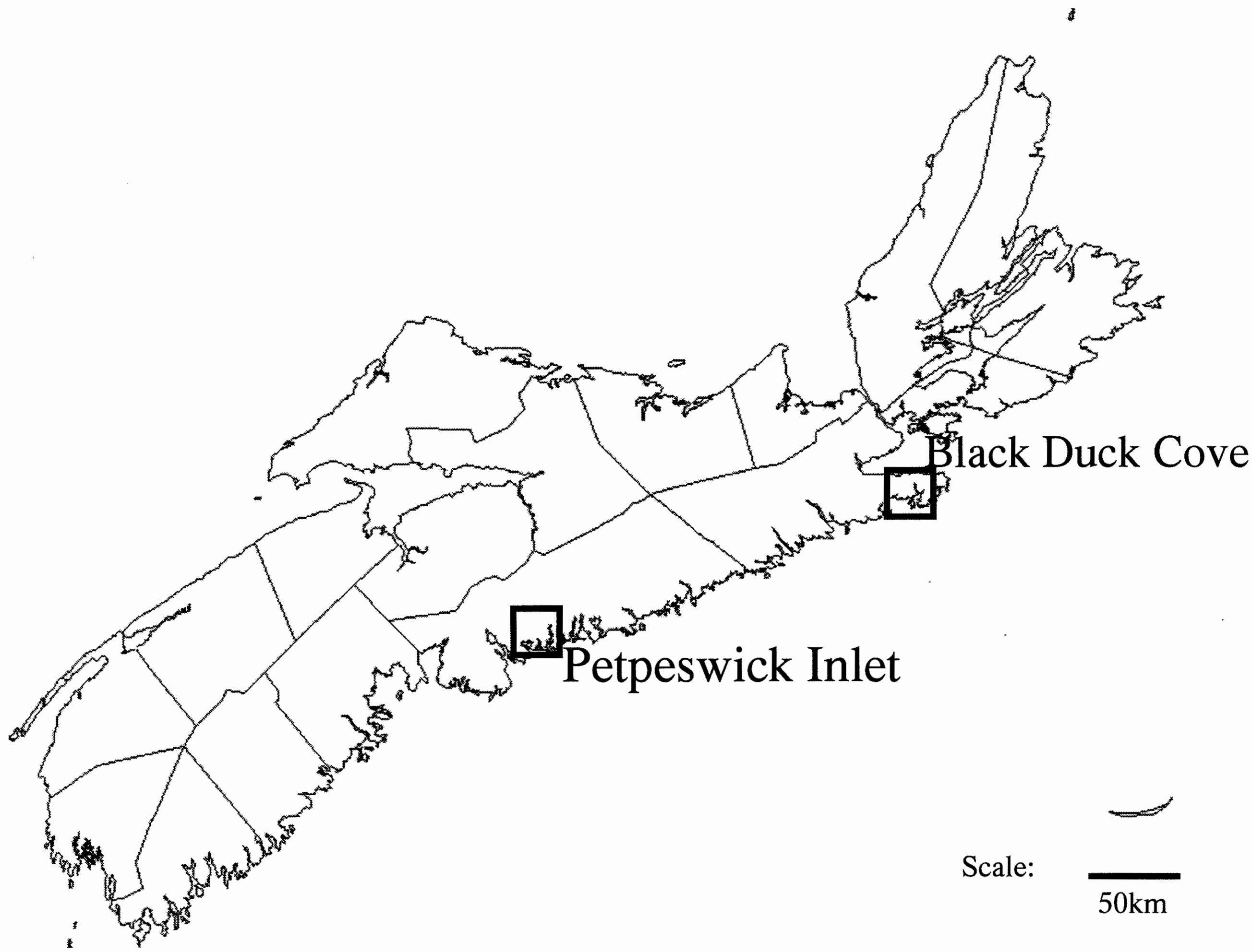


Figure 1.1 Map of Nova Scotia with both site locations

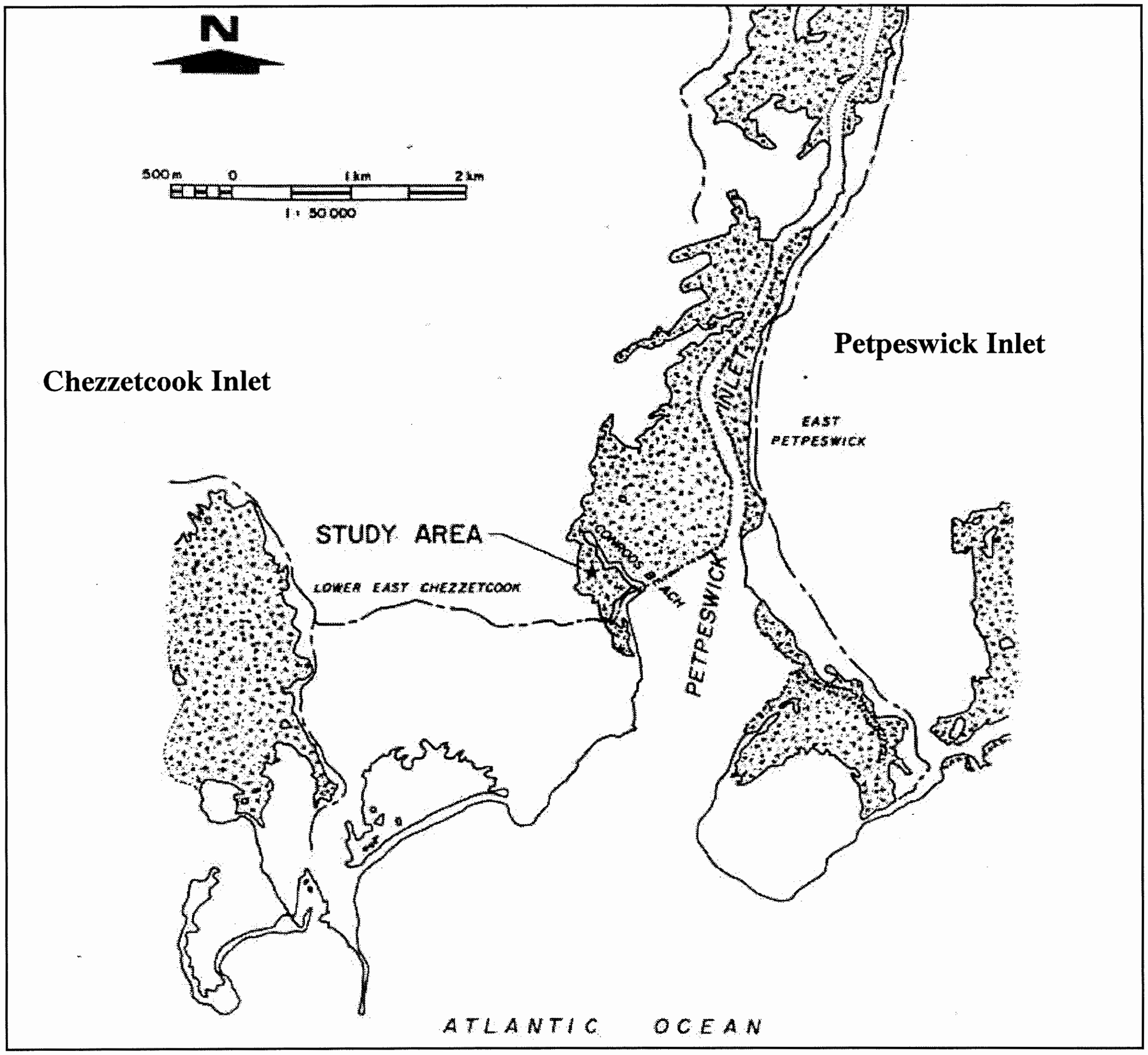


Figure 1.2 Conrod's Beach, Petpeswick, Nova Scotia (Lane et al., 1987)

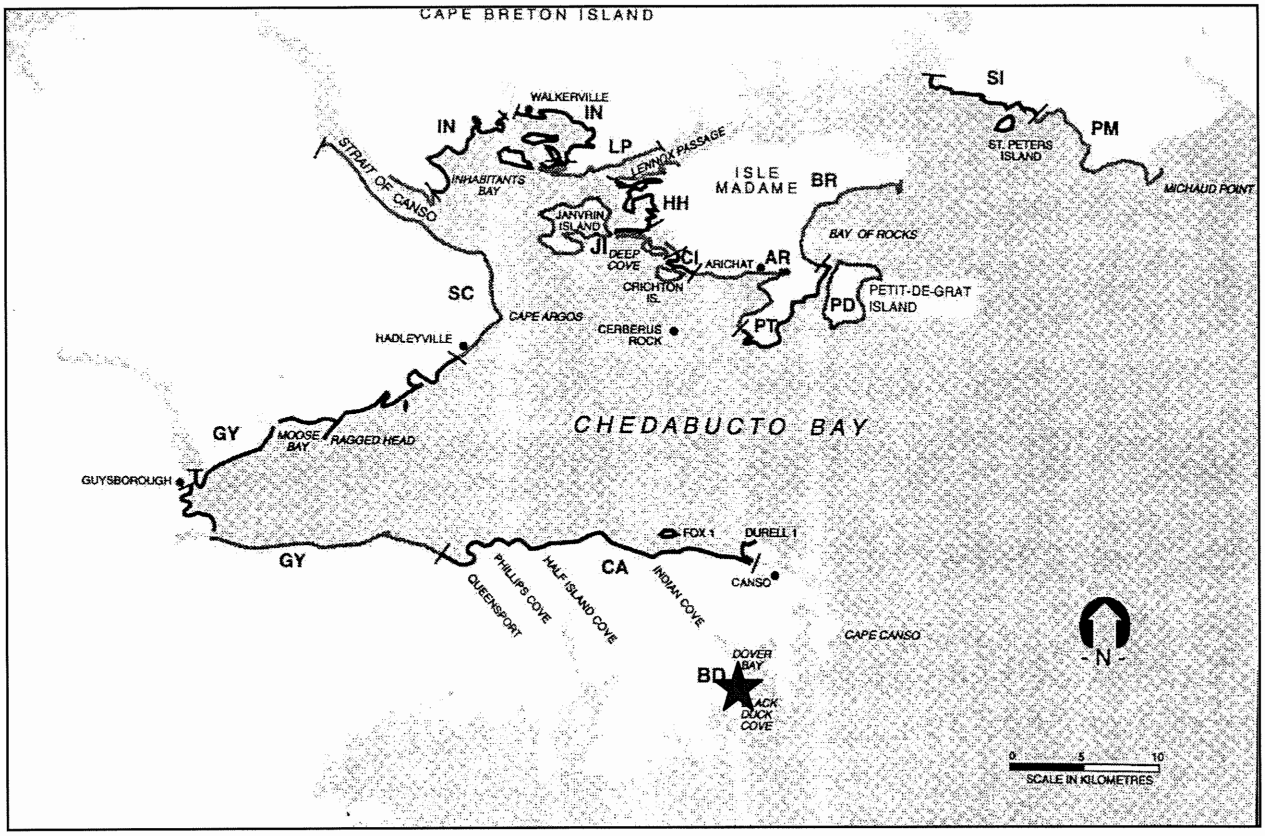


Figure 1.3 Black Duck Cove, Nova Scotia (Owens et al., 1994). The dark line along the coast represents the 15 areas studied by Owens et al. (1994). Each area has an associated prefix. The star indicates the location of the current study.

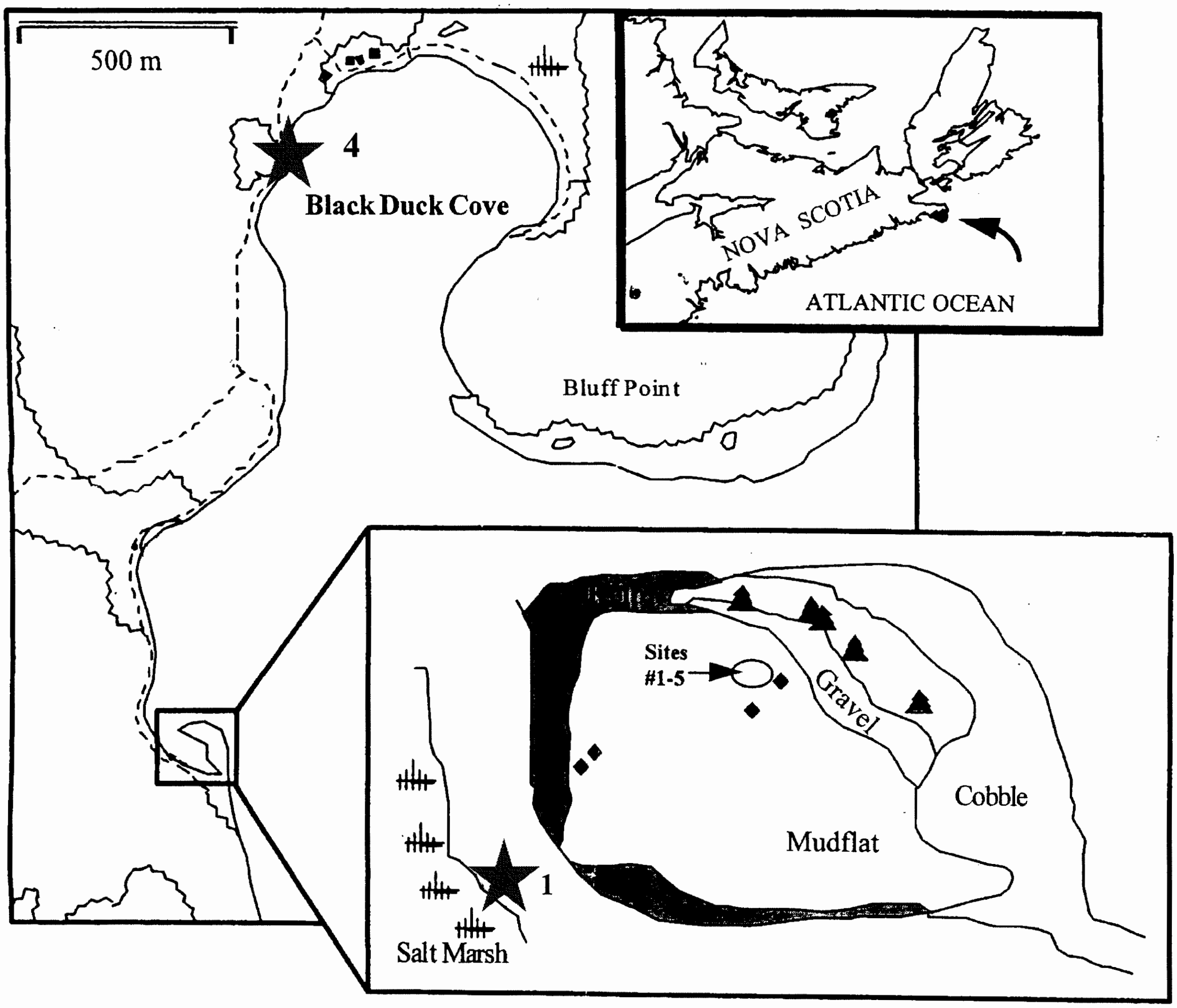


Figure 1.4 Sampling sites for Core 1 and Core 4 in Black Duck Cove. The star indicates the location of sampling for the corresponding core (Owens et al., 1994).

flats. There is an abundance of vegetation here, although Owens et al. (1994) suggest that this is uncommon due to the coarse sediment.

1.4 Background

1.4.1 Foraminifera Species as Indicators

Foraminifera belong to the Phylum Protozoa and Class Sarcodina. These unicellular organisms are relatively simple in organization with a protoplasmic encapsulated cell and a secreted (calcium carbonate) or agglutinated shell (cemented detrital material) called a test that may be divided into chambers (Loeblich and Tappan 1964). When the Foraminifera die their test becomes fossilized in the geological record and an environmental history is created.

Agglutinated tests are resistant to low oxygen and pH levels thus preserve in marsh sediments. Few foraminiferal species thrive in a marsh environment due to the temperature, salinity, and pH fluctuations; therefore, marshes are dominated by a few marine species. The same species of foraminifera occur at the same latitudes worldwide, provided the salinity of the water is the same. (Scott et al., 2001).

Foraminifera are also used as indicators because of the relatively easy and inexpensive sampling, processing, and examining techniques that they entail. A 10cc sample allows for an accurate statistical analysis of the foraminiferal species present.

1.4.1.1 Sensitivity

Although sensitive, foraminifera are one of the last organisms to completely die out following contamination and can be found in transition zones that contain no other marine organisms (Schafer, 1971). After the stress occurs, some species of foraminifera die out while others sustain deformities in their hard tests, which are then preserved in the geological record. Both of these effects indicate the presence of unnatural environmental factors.

1.5 Thesis Organization

This thesis is divided into six chapters. The first chapter, Introduction, provides a background on the study areas as well as foraminifera, and the use of foraminifera as environmental indicators. The purpose of this study is also stated in this chapter. The second chapter, Background, summarizes the previous work done in the areas of study. The background compares local studies with other estuarine studies in oil and other types of pollution studies such as heavy metals and chemical contaminants. The third chapter, Methods, explains the methodology of this study. A detailed analysis of sampling, processing, and counting is explained. The fourth chapter, Results, describes the results obtained from the experiment in Petpeswick Inlet and the results from the oil spill analysis in Black Duck Cove. The population and species abundance as well as the living versus total populations and deformities are discussed in the fifth chapter, Discussion. These results will be compared to previous studies. The responses to various treatments in Petpeswick Inlet are indicated in this chapter as well as the ability to detect and monitor the oil spill in Black Duck Cove. The last chapter presents the conclusions of the thesis based on the results and discussion. The goals of this project will be reexamined in final chapter: the ability to monitor the effects of oil on foraminifera as well as determining if foraminifera may be used to indicate the occurrence of an over 30-year-old oil spill and the amount of remediation that transpired since that oil spill.

CHAPTER 2: PREVIOUS WORK

2.1 Introduction

Numerous studies concerning foraminifera and their role as environmental indicators have been conducted. Foraminiferal assemblages have been used to assess anthropogenic disturbances such as oil spills, sewage discharge, heavy metal contamination and a wide variety of other perturbations at an increasing rate over the last 30-40 years (Scott et al., 2001).

2.2 Local Estuarine Studies

2.2.1 Petpeswick Inlet

Sabean (2001) examined the foraminferal assemblages, living and total, of Petpeswick Inlet after the artificial oil spill was created. The results showed fluctuations within the assemblages due to both natural and anthropogenic changes. The results obtained from Sabean's research are expected to be similar to those found in this study. Chezzetcook Inlet, N.S., is located directly west of Petpeswick Inlet, N.S. While both have a similar environment, Chezzetcook Inlet has been intensely studied, and serves as a valid comparison for marsh foraminiferal assemblages.

Scott and Medioli (1980a) used surface samples to determine the distribution of marsh foraminifera within the Chezzetcook Inlet, as well as other marsh areas in Nova Scotia. The living and total foraminiferal assemblages show the seasonal variance in the living populations of foraminiferal species (Scott and Medioli, 1980b). The purpose of this study was to view the reliability of the total population as an environmental indicator. Two of the marsh zones used in Scott and Mediolis study, IIA and IIB, are very similar in content to the zones in the Petpeswick Inlet study. Again, variations in dominant species due to seasonal changes were noted.

2.2.2 Black Duck Cove

Although there have been no studies completed concerning foraminifera found in the Black Duck Cove area, other investigations have been conducted. After the oil spill it was decided that some areas would be cleaned manually and others, including Black Duck Cove, would be left for a natural self-cleaning process. Over the next two years McLean and Betancourt (1973 In: Buckley et al. 1974) studied the physical and chemical changes seen in Black Duck Cove. They concluded that most of the physical changes took place in the first year after the oil was exposed to the natural environment.

Buckley et al. (1974) studied the Chedabucto Bay area and found that because Black Duck Cove was a low energy environment, some areas within the cove contained oil that was not altered and remained in the fluid state during the high summer temperatures. Other areas that were covered by tidal activity showed oil residue that was not as mobile. The chemical and physical characteristics showed the percentage of hydrocarbons decreased by 20% within one and a half years.

Owens et al. (1994) conducted a field study on the shores of Chedabucto Bay to determine where visible oil residue was located. They suggested that the oil remaining was due to the physical, biophysical, and biological processes within the Bay. Black Duck Lagoon, within Black Duck Cove, has a heavy concentration of oil remaining as an asphalt pavement and pooled between the coarse sediment.

Thick asphalt layers were also found nine years after the Baffin Island Oil Spill Project conducted in Cape Hatt, Baffin Island, North West Territories. In this experiment a variety of experimental spills were released onto the nearshore water, which became stranded within the low energy environment. The purpose was to compare the fate of oil and monitor the persistence. It was found that natural process removed 90%-95% of the surface oil (Humphrey et al., 1992).

2.3 Pollution studies

Environmental conditions such as temperature, salinity, solubility of carbonate, depth, nutrition, substrate, dissolved oxygen, illumination, pollution, water motion, trace elements, and ecological variance can cause morphological deformities within foraminiferal tests (Boltovskoy et al., 1991). An example of this is found in Petpeswick Inlet where numerous *Miliammina fusca* were deformed in oiled plots (Sabean, 2001). It was found that foraminiferal test deformation depends on the degree and type of pollutants (Samir and El-Din, 2001). That is, highly contaminated areas show foraminifera tests to be highly deformed as a response to certain forms of pollution. Also, morphological changes are often species specific even if similarly stressed (Boltovskoy et al., 1991). It is important to compare the pre-pollution assemblages to the post-pollution present-day assemblage found in the estuary to do a fair analysis on the effects of the pollutant (Alve, 1995).

CHAPTER 3: METHODS

3.1 Petpeswick Inlet

3.1.1 Sampling Grid

On June 6th and 8th, 2000, the Department of Fisheries and Oceans laid three sets of six plots (I, II, III), which were 3m x 3m, and labeled IA-IIIF, with each letter corresponding to a different oil treatment (see Figure 3.1). The treatments included: A, Control with nutrient enrichment (no oil added); B, Control without nutrients (no oil added); C, Natural attenuation control (no treatments added); D, Bioremediation by nutrient enrichment (NH_4NO_3 + $Ca(H_2PO_4)_2\,H_2O$); E, Bioremediation by nutrient enrichment (NH_4NO_3 + $Ca(H_2PO_4)_2\,H_2O$) with surface vegetation continuously cut back to ground level; F, Bioremediation by nutrient enrichment (NH_4NO_3 + $Ca(H_2PO_4)_2\,H_2O$) and oxygen enrichment by agricultural disking (Sabean, 2001). Each of the oiled plots (C, D, E, and F) had 12L of medium sulphurous light weathered crude oil applied over a two-day period (Sabean, 2001).

3.1.2 Sample Collection

Sampling was conducted for the present study on July 9th and August 15th, 2001 at low tide. One sample was taken from each of the eighteen plots in a random spot where vegetation, usually *Spartina alterniflora,* was apparent. To collect the samples a 10cc stainless steel core, developed by Scott (1977) was used. One end of the corer had a serrated edge to cut through roots. A garden trowel was used to aid in the extraction of the material, and a knife was used to cut the top 1cm of the surface sediments. The samples were then stored in individual plastic containers, brought back to the lab at Dalhousie University, and left in the refrigerator until processed.

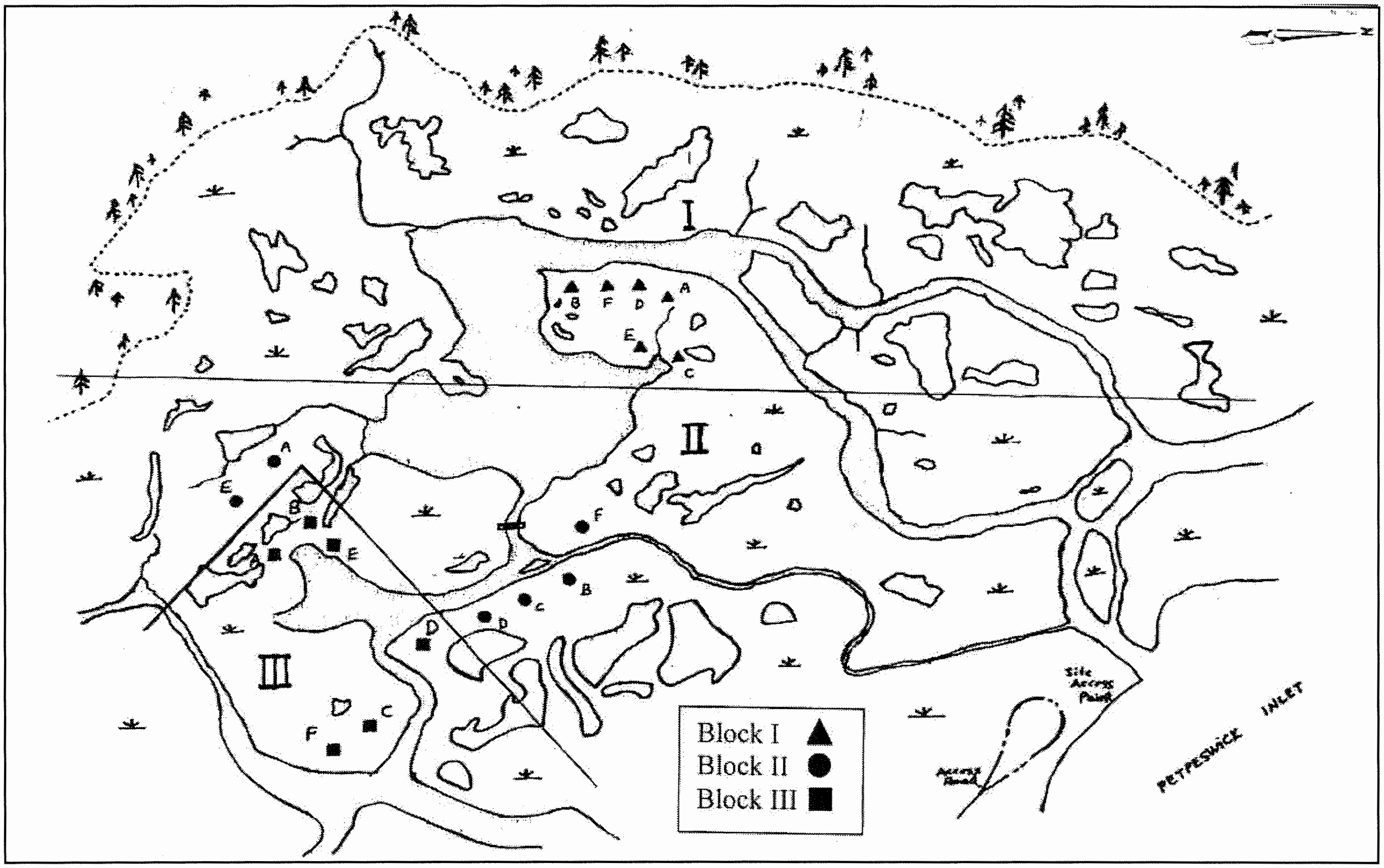


Figure 3.1 Map of study plots within Conrod's Beach (Department of Fisheries and Oceans, 2000)

3.1.3 Processing

The samples were refrigerated in the lab until processed. During processing each sample was washed through a >500μ sieve and a >63μ sieve to eliminate large debris as well as silt and clay. A mild detergent was used on the oiled samples to break up mud clumps and clean the sieve screen. The clean samples were placed in a sealed plastic container and had formaldehyde and Rose Bengal stain added. The formaldehyde was used to kill the specimen as well as fix the tissues. The Rose Bengal is used to stain the protoplasm of the specimen, which enables detection of species living at the time of sample collection.

3.2 Black Duck Cove

3.2.1 Sample Collection

The surface samples were collected at Black Duck Cove in the same manor as the Petpeswick Inlet samples, collecting at low tide using the Scott surface marsh sampler, a knife and a trowel. Sampling was conducted in Black Duck Cove on September 18th, 2001. In addition, four core samples from different floral zones. The purpose of the cores was to determine if an oil spill could be detected thirty years later by looking for deformities within the foraminiferal assemblages.

3.2.2 Processing

The surface samples were processed the same way as the Petpeswick Inlet samples. The surface and core samples were brought back to the lab at Dalhousie University. The surface samples were washed in a mild detergent and then fixed with formaldehyde. The core samples were described and then cut into 1cm sections and placed in a clean plastic container. The sections were cleaned and fixed the same way as the surface samples. The weight percentage of organic matter in each core sample was determined using the loss on ignition method: heating at 400°C for one hour and weighing before and after ignition.

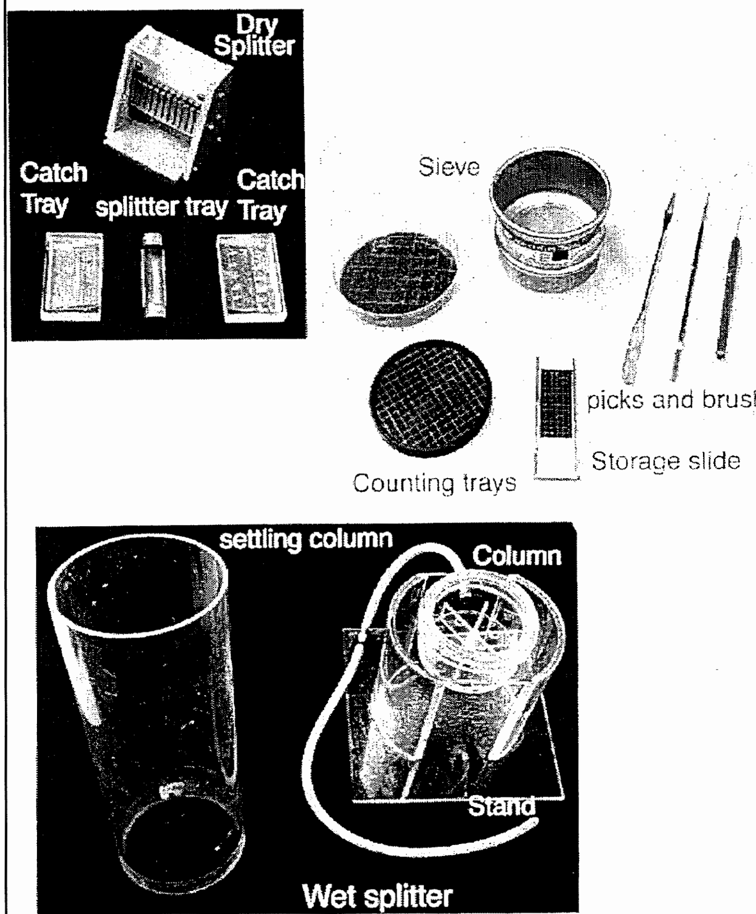
3.3 Examination

3.3.1 Splitting

The sample may contain thousands of foraminifera that may be split into equally sized sub-samples. Although the samples can not be dried, they can be split using a settling column splitter (Figure 3.2) that divides the sample into eight equal divisions due to water turbulence and gravity (Scott and Hermelin 1993). One eighth of a sample is observed under the microscope and counted. The values are multiplied by eight to give a representative value to the entire sample.

3.3.2 Identifying and Counting

It is necessary to count 200-300 specimens to get a representative sample. After splitting the sample the contents are placed on a petri dish and placed under a stereomicroscope with magnifications of 20-60x. Each sample is counted and identified by aid of photographic plates, textbooks and sample trays. These identifications were verified by D. B. Scott. Not only are the different species noted and counted but also the number of dead and living are also counted.



Sampling Handling Devices

Figure 3.2 Foraminiferal processing and splitting equipment (after Scott et al., 2001)

3.4 Photography

Foraminifera with major deformities were mounted on the image stage of a Dynaphot® Scanning Light microscope and photographed using Fuji 64T color35mm slide film, while pictures in the field were taken with a Polaroid digital 320 camera. All images were scanned into Microsoft photo editor for editing.

3.5 Data Presentation

The data compiled during this project are presented in Microsoft Excel spreadsheets and graphs. Tables, shown in Appendix A, show the total foraminifera numbers including number of species (T), number of living (L) and total species (T) as well as the abundance and deformities within each species (Td and Ld). The number of individuals within the 10 cc sample was used to establish the percentage abundance for each species of foraminifera.

CHAPTER 4: RESULTS

4.1 Petpeswick Inlet

4.1.1 Description of Results Format

Samples were taken from the 18 plots in Petpeswick Inlet on July 9, 2001 and August 15, 2001 (weeks 57 and 62). The samples were counted and the data were inserted into tables made by Sabean (2001) with counts from weeks -1-20. The –1 represents samples taken one week before the actual experiment began. The results were then graphed using Microsoft Excel. The total living (L) and total (T) (living plus dead) number of species per 10 cc, as well as the number of individuals living (L) and total individual values (T) are recorded as whole numbers (see Appendix A-1 and A-2). The living (L) and total (T) numbers for individual species are shown as relative percentages. To get this value, the number of living individuals (L) for each species was divided by the total number of living individuals (T) for the entire sample and then multiplied by 100. Similarly, the total number of individuals (T) (living plus dead) in each species was divided by the total number of individuals within that particular sample and then multiplied by 100.

Species with deformation were also counted and are shown as a percentage calculated by dividing the number of living deformed individuals (Ld) for each species by the number of living (L) individuals within that species and multiplying by 100. The total deformed (Td) (living plus dead) for each species was divided by the total number of individuals (T) of that species and then multiplied by 100 to get a percentage. The number of living (or total) deformed individuals was divided by the total living (or total) of the species and not the sample because the total values were too high to show any significant deformation within the individual species, and some species showed very little deformation while others show a significant amount.

The samples showed a maximum of nine different species present: *Miliammina fusca, Pseudothurammina limnetis, Haplophragmoides maniliaensis*, *Tiphotrocha comprimata, Trochammina inflata, Trochammina macrescens f. macrescens* and *f. polystoma*, and *Trochammina ochracea* as well as inner linings. The most common species found in each sample was *Miliammina fusca* and *Trochammina macrescens f. polystoma*; *Miliammina fusca* contained most of the deformation ((Ld) and (Td)) present. *Trochammina macrescens f. polystoma* deformation was below 5%, well under the standard of 10% that indicates an environmental disturbance (Scott et al., 2001). The deformation rate of *Miliammina fusca* was considerably higher, especially within the oiled plots (see Appendix A-1 and A-2). For the purpose of this study only *Miliammina fusca* will be discussed in detail.

The number of living individuals per 10 cc, the percentage of living and total *Miliammina fusca,* as well as the percentage of living and total deformed in *Miliammina fusca* in weeks 57 and 62 were plotted in graphs as a continuation from Sabean (2001). Weeks -1-20 show the variability due to different treatments (see Figure 4.1-4.6). The total number of individuals per 10 cc in weeks -1-20 was not available and will therefore not be included in this report. Although there are three plots for each treatment, they have been averaged to one value and then discussed and graphed.

4.1.2 Treatment A- Control plot with no oil

The total number of living individuals increase slightly from ~1500 individuals/10cc (week 57) to ~1800 individuals/10cc (week 62) (see Figure 4.1a). The total living *Miliammina fusca* decreased from ~16% (week 57) to ~11% (week 62) (see Figure 4.1b). The living deformed *Miliammina fusca* increased from 0% (week 57) to ~1% (week 62) (see Figure 4.1c). Total *Miliammina fusca* decreased from ~23% (week 57) to 12.5% (week 62) (see Figure 4.1d). The percentages of total deformed *Miliammina fusca* increased from 0% in week 57 to ~3% in week 62 (see Figure 4.1e).

4.1.3 Treatment B- Control plot with nutrient enrichment, no oil

The total number of living individuals increased from ~900 in week 57 to ~1200 in week 63 (see Figure 4.2a). Percentage of living *Miliammina fusca* decreased from ~15% (week 57) to ~8 (week 62) (see Figure 4.2b). The living deformed *Miliammina fusca* stayed constant at 0% over week 57 and week 62 (see Figure 4.2c). Total *Miliammina fusca* decreased from ~19% (week 57) - 16% (week 62) (see Figure 4.2d). The percentages of total deformed *Miliammina fusca* stayed constant at 0% through week 57 to week 62 (see Figure 4.2e).

4.1.4 Treatment C- Oiled plot with no treatments (natural attenuation)

The total number of living individuals decreased from ~900 in week 57 to ~450 in week 63 (see Figure 4.3a). Percentage of living *Miliammina fusca* decreased from ~11% (week 57) to ~8% (week 62) (see Figure 4.3b). The living deformed *Miliammina fusca* increased from 8% in week 57 to 11% in week 62 (see Figure 4.3c). Total *Miliammina fusca* decreased from ~30% (week 57) - 19% (week 62) (see Figure 4.3d). The percentages of total deformed *Miliammina fusca* increased from 14% in week 57 to 21% in week 62 (see Figure 4.3e).

Figure 4.1 Foraminifera results from Treatment A-Control plot (no oil). Note the bars represent standard deviation.

a) Number of living individuals averaged from Treatment IA, IIA, IIIA- Control plot (no oil). Data from weeks –1 to 20 from Sabean (2001).

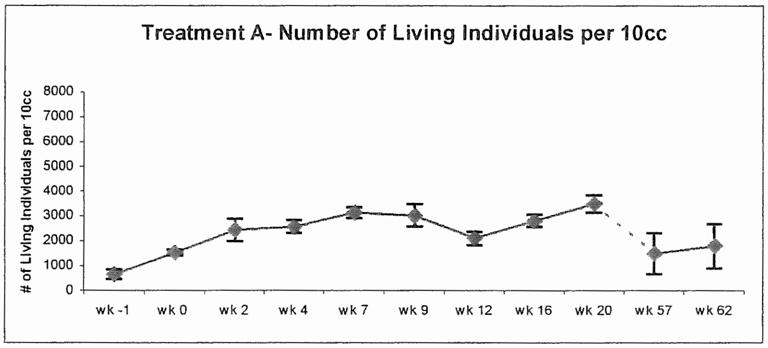
b) Percentage of living *Miliammina fusca* averaged from Treatment IA, IIA, and IIIA- Control plot (no oil). Data from weeks –1 to 20 from Sabean (2001).

c) Percentage of living deformed *Miliammina fusca* averaged from Treatment IA, IIA, and IIIA- Control plot (no oil). Data from weeks –1 to 20 from Sabean (2001).

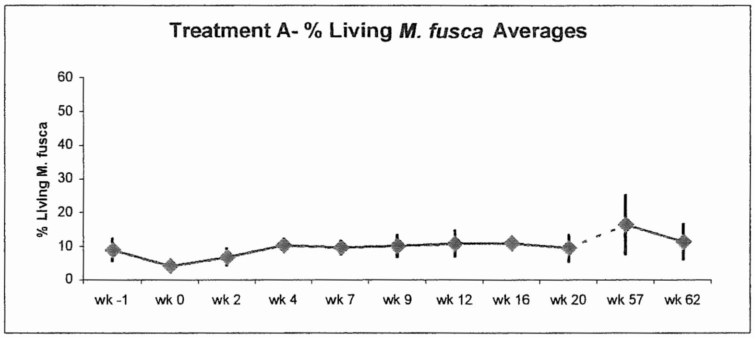
d) Percentage of total *Miliammina fusca* averaged from Treatment IA, IIA, and IIIA- Control plot (no oil). Data from weeks –1 to 20 from Sabean (2001).

e) Percentage of total deformed *Miliammina fusca* averaged from Treatment IA, IIA, and IIIA- Control plot (no oil). Data from weeks –1 to 20 from Sabean (2001).

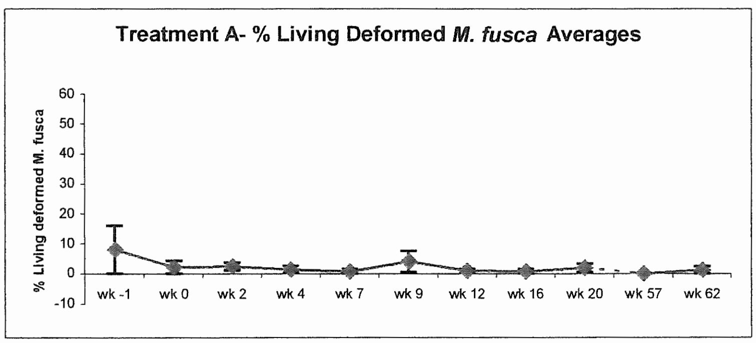
4.1a



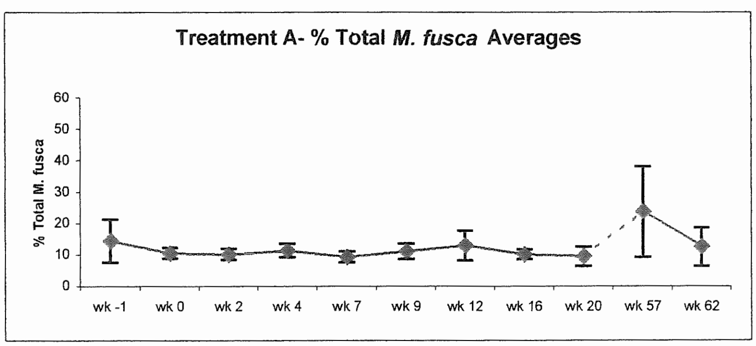
4. 1b



4.1c



4.1d



4.1e

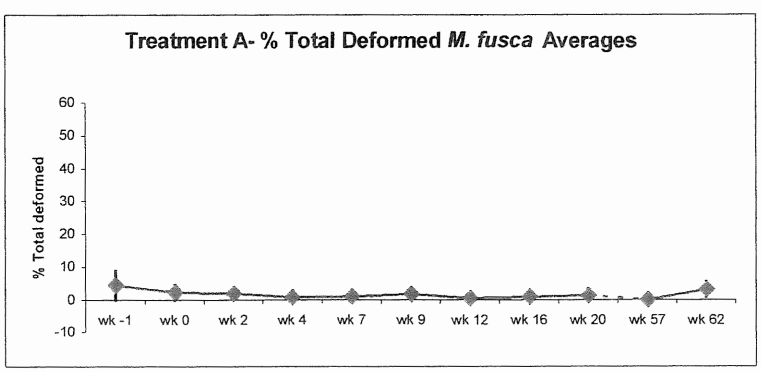


Figure 4.2 Foraminifera results from Treatment B- Control plot with nutrient enrichment (no oil). Note the bars represent standard deviation.

a) Number of living individuals averaged from Treatment IB, IIB, IIIB Control plot with nutrient enrichment (no oil). Data from weeks –1 to 20 from Sabean (2001).

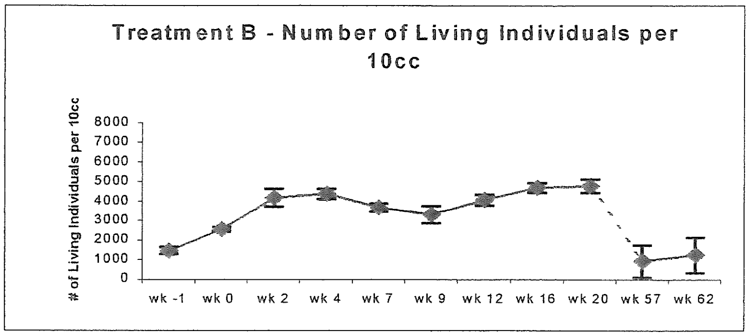
b) Percentage of living *Miliammina fusca* averaged from Treatment IB, IIB, and IIIB- Control plot with nutrient enrichment (no oil). Data from weeks –1 to 20 from Sabean (2001).

c) Percentage of living deformed *Miliammina fusca* averaged from Treatment IB, IIB, and IIIB- Control plot with nutrient enrichment (no oil). Data from weeks –1 to 20 from Sabean (2001).

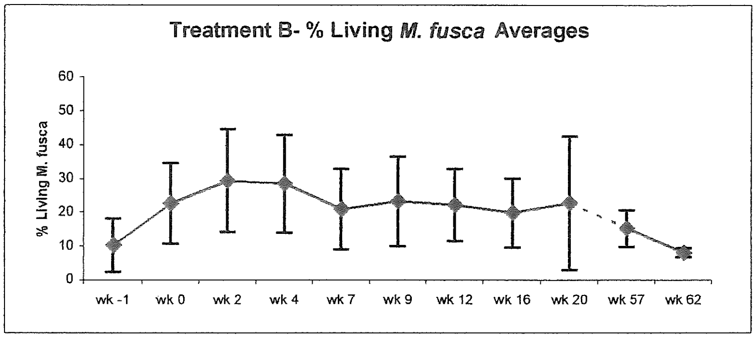
d) Percentage of total *Miliammina fusca* averaged from Treatment IB, IIB, and IIIB- Control plot with nutrient enrichment (no oil). Data from weeks –1 to 20 from Sabean (2001).

e) Percentage of total deformed *Miliammina fusca* averaged from Treatment IB, IIB, and IIIB- Control plot with nutrient enrichment (no oil). Data from weeks –1 to 20 from Sabean (2001).

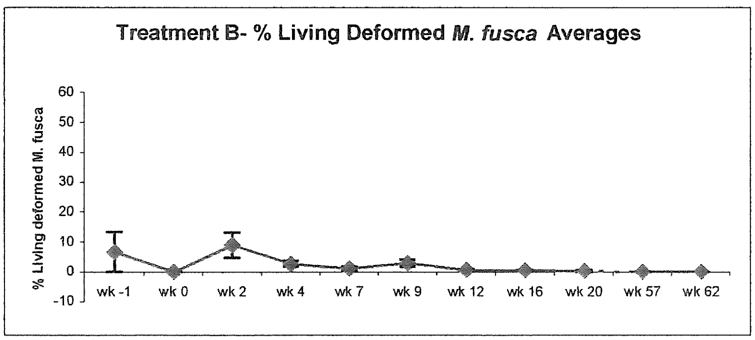
4.2a



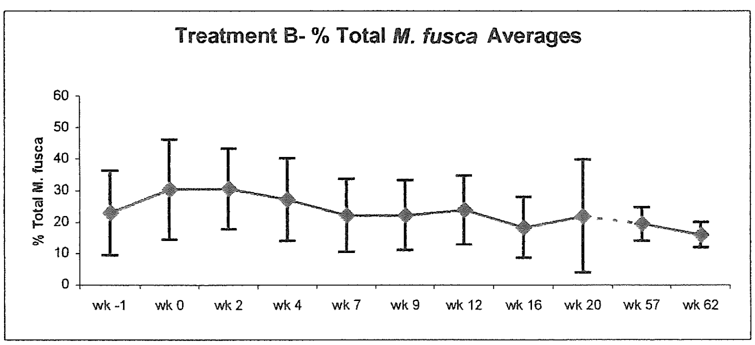
4.2b



4.2c



4.2d



4.2e

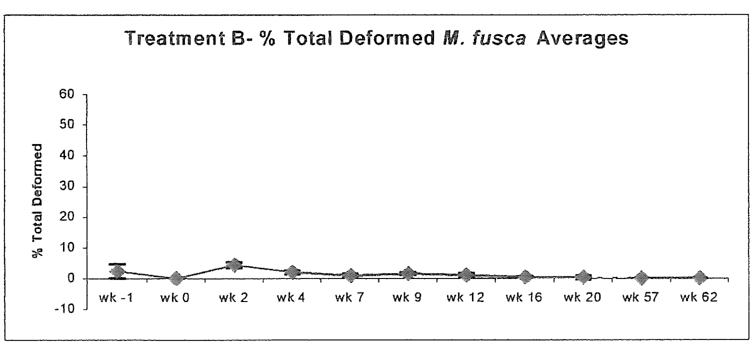


Figure 4.3 Foraminifera results from Treatment C- natural attenuation (oiled plot). Note the bars represent standard deviation.

a) Number of living individuals averaged from Treatment IC, IIC, IIIC- natural attenuation (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

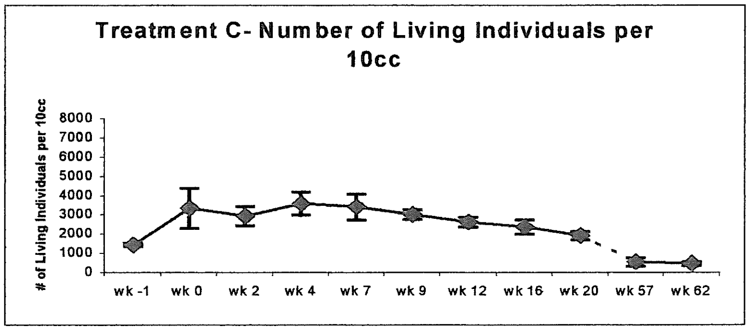
b) Percentage of living *Miliammina fusca* averaged from Treatment IC, IIC, and IIIC- natural attenuation (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

c) Percentage of living deformed *Miliammina fusca* averaged from Treatment IC, IIC, and IIIC- natural attenuation (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

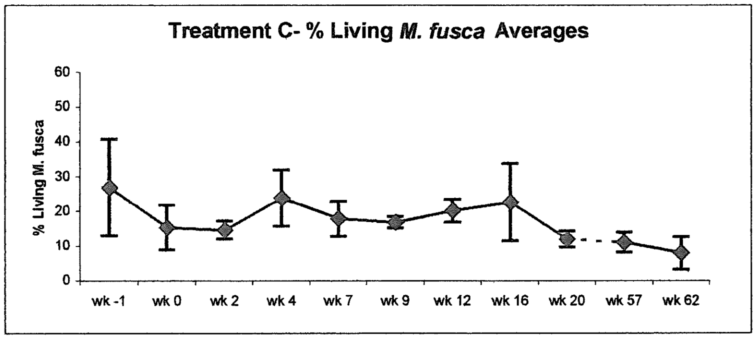
d) Percentage of total *Miliammina fusca* averaged from Treatment IC, IIC, and IIIC- natural attenuation (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

e) Percentage of total deformed *Miliammina fusca* averaged from Treatment IC, IIC, and IIIC- Control plot- natural attenuation (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

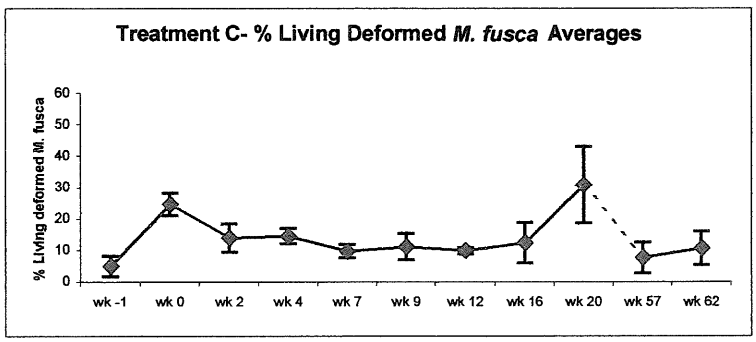
4.3a



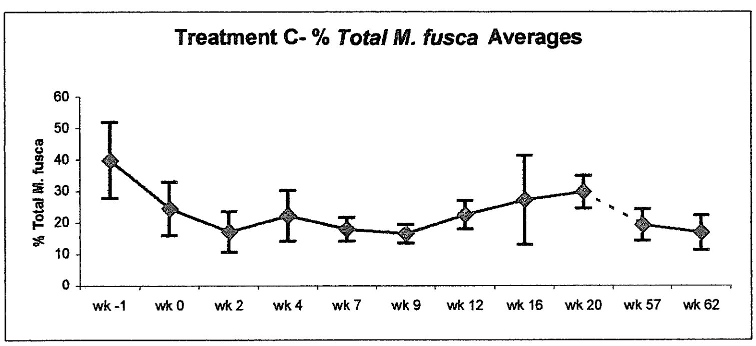
4.3b



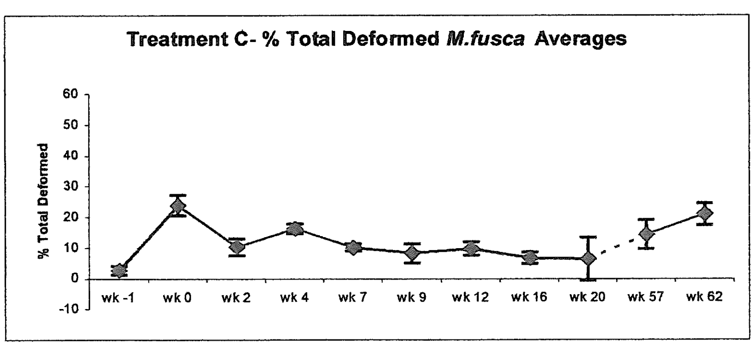
4.3c



4.3d



4.3e



4.1.5 Treatment D- Oiled plot with nutrient enrichment

The total number of living individuals increased from ~350 in week 57 to ~500 in week 63 (see Figure 4.4a). Percentage of living *Miliammina fusca* increased from ~13% (week 57) to ~17% (week 62) (see Figure 4.4b). The living deformed *Miliammina fusca* increased from 12% in week 57 to 18% in week 62 (see Figure 4.4c). Total *Miliammina fusca* decreased from ~22% (week 57) to 13% (week 62) (see Figure 4.4d). The percentages of total deformed *Miliammina fusca* decreased from 26% in week 57 to 14% in week 62 (see Figure 4.4e).

4.1.6 Treatment E- Oiled plot with nutrient enrichment and cut plants

The total number of living individuals increased from ~400 in week 57 to ~800 in week 63 (see Figure 4.5a). Percentage of living *Miliammina fusca* increased from ~31% (week 57) to ~22% (week 62) (see Figure 4.5b). The living deformed *Miliammina fusca* decreased from 7% in week 57 to 4% in week 62 (see Figure 4.5c). Total *Miliammina fusca* decreased from ~50% (week 57) to 23% (week 62) (see Figure 4.5d). The percentages of total deformed *Miliammina fusca* decreased from 9% in week 57 to 8% in week 62 (see Figure 4.5e).

4.1.7 Treatment F- Oiled plot with nutrient enrichment and agricultural disking

The total number of living individuals increased from ~250 in week 57 to ~500 in week 63 (see Figure 4.6a). Percentage of living *Miliammina fusca* increased from ~8% (week 57) to ~11% (week 62) (see Figure 4.6b). The living deformed *Miliammina fusca* decreased from 3% in week 57 to 0% in week 62 (see Figure 4.6c). Total *Miliammina fusca* stayed constant at 13.5% for weeks 57 and 62 (see Figure 4.6d). The percentages of total deformed *Miliammina fusca* decreased from 7% in week 57 to 0% in week 62 (see Figure 4.6e).

Figure 4.4 Foraminifera results for Treatment D- Nutrient enrichment (oiled plot). Note the bars represent standard deviation.

a) Number of living individuals averaged from Treatment ID, IID, IIID- Nutrient enrichment (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

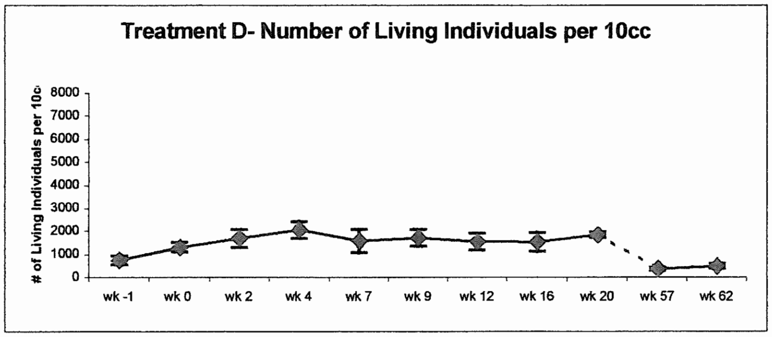
b) Percentage of living *Miliammina fusca* averaged from Treatment ID, IID, and IIID- Nutrient enrichment (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

c) Percentage of living deformed *Miliammina fusca* averaged from Treatment ID, IID, and IIID- Nutrient enrichment (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

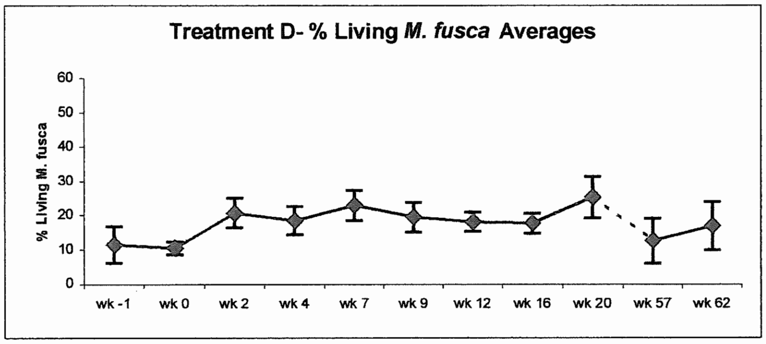
d) Percentage of total *Miliammina fusca* averaged from Treatment ID, IID, and IIID- Nutrient enrichment (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

e) Percentage of total deformed *Miliammina fusca* averaged from Treatment ID, IID, and IIID- Nutrient enrichment (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

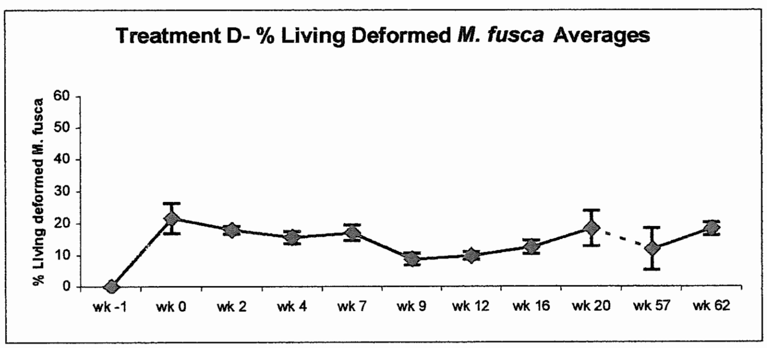
4.4a



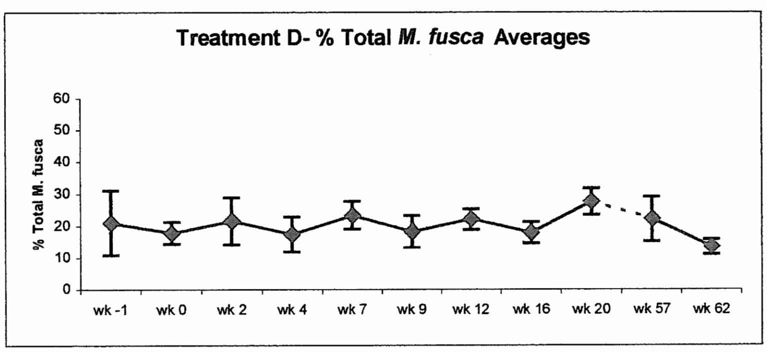
4.4b



4.4c



4.4d



4.4e

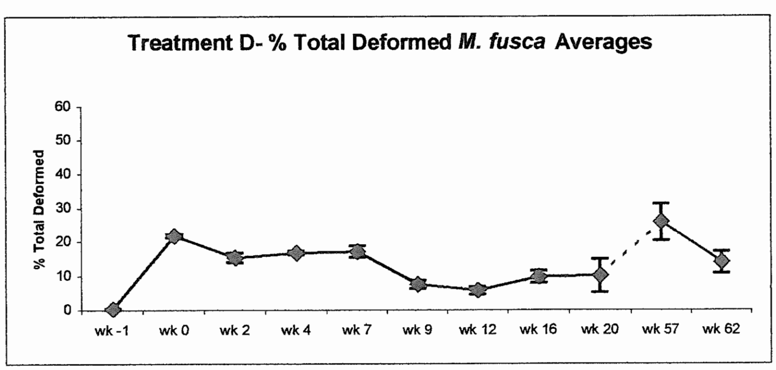


Figure 4.5 Foraminifera results for Treatment E- Nutrient enrichment and cut plants (oiled plot). Note the bars represent standard deviation.

a) Number of living individuals averaged from Treatment IE, IIE, IIIE- Nutrient enrichment and cut plants (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

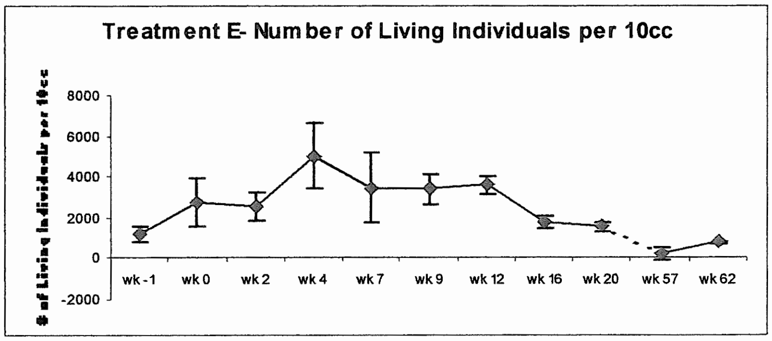
b) Percentage of living *Miliammina fusca* averaged from Treatment IE, IIE, and IIIE- - Nutrient enrichment and cut plants (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

c) Percentage of living deformed *Miliammina fusca* averaged from Treatment IE, IIE, and IIIE-- Nutrient enrichment and cut plants (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

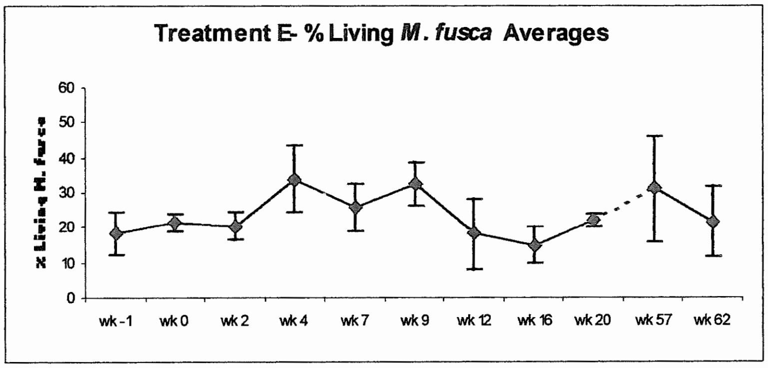
d) Percentage of total *Miliammina fusca* averaged from Treatment IE, IIE, and IIIE- - Nutrient enrichment and cut plants (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

e) Percentage of total deformed *Miliammina fusca* averaged from Treatment IE, IIE, and IIIE- - Nutrient enrichment and cut plants (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

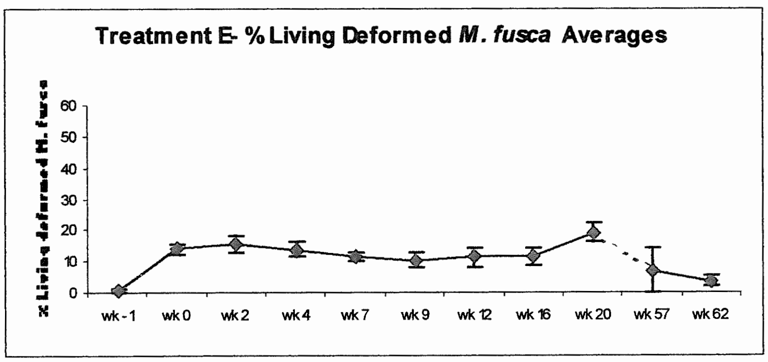
4.5a



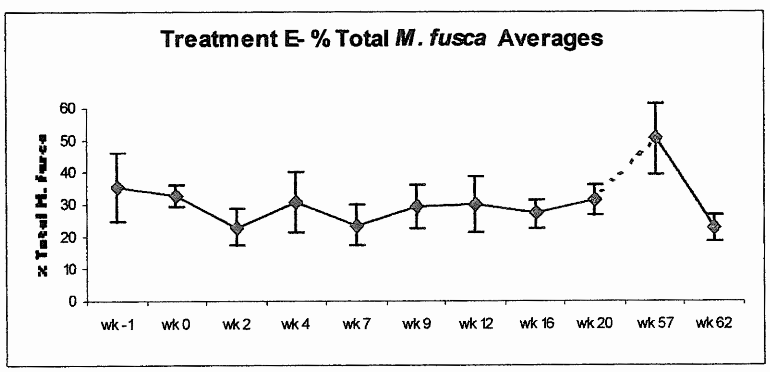
4.5b



4.5c



4.5d



4.5e

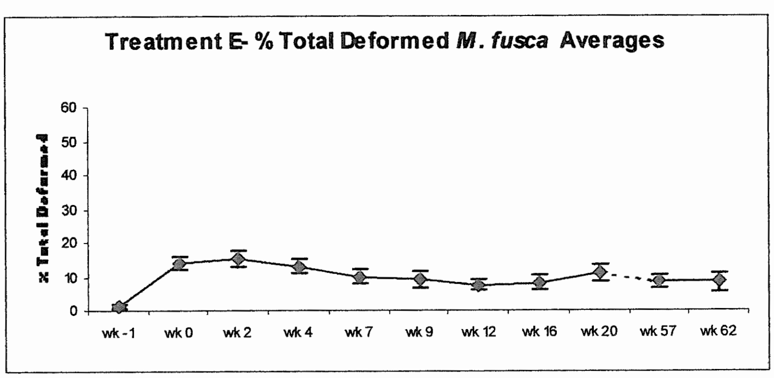


Figure 4.6 Foraminifera results for Treatment F- nutrient enrichment and agricultural disking (oiled plot). Note the bars represent standard deviation.

a) Number of living individuals averaged from Treatment IF, IIF, IIIF nutrient enrichment and agricultural disking (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

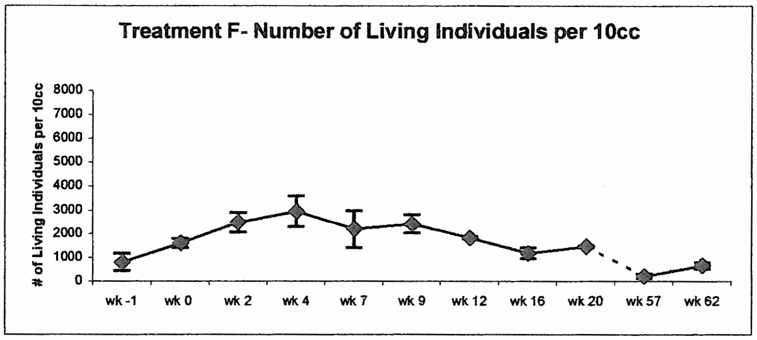
b) Percentage of living *Miliammina fusca* averaged from Treatment IF, IIF, and IIIF- nutrient enrichment and agricultural disking (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

c) Percentage of living deformed *Miliammina fusca* averaged from Treatment IF, IIF, and IIIF- nutrient enrichment and agricultural disking (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

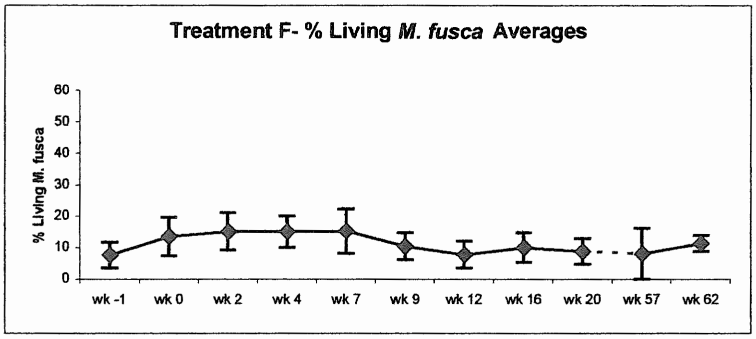
d) Percentage of total *Miliammina fusca* averaged from Treatment IF, IIF, and IIIF- nutrient enrichment and agricultural disking (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

e)Percentage of total deformed *Miliammina fusca* averaged from Treatment IF, IIF, and IIIF- nutrient enrichment and agricultural disking (oiled plot). Data from weeks –1 to 20 from Sabean (2001).

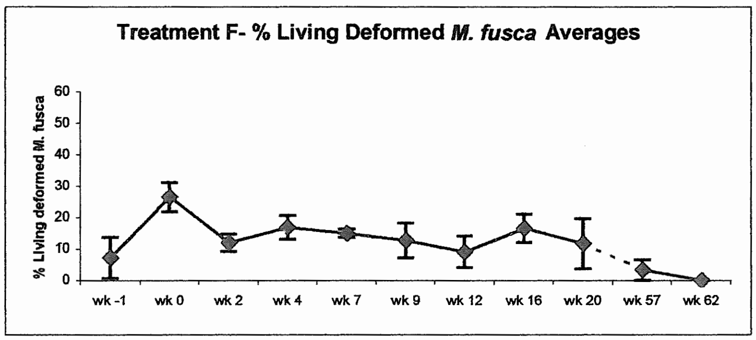
4.6a



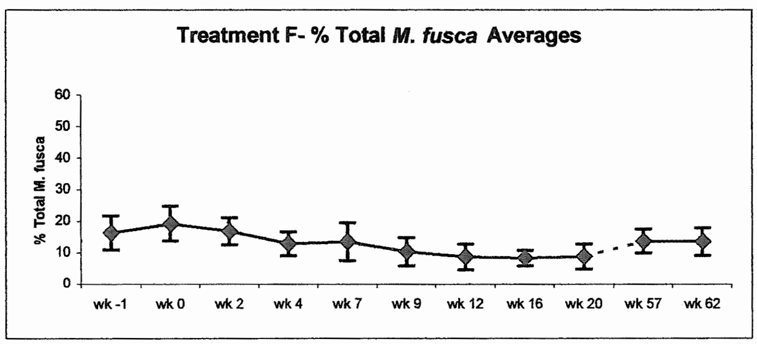
4.6b



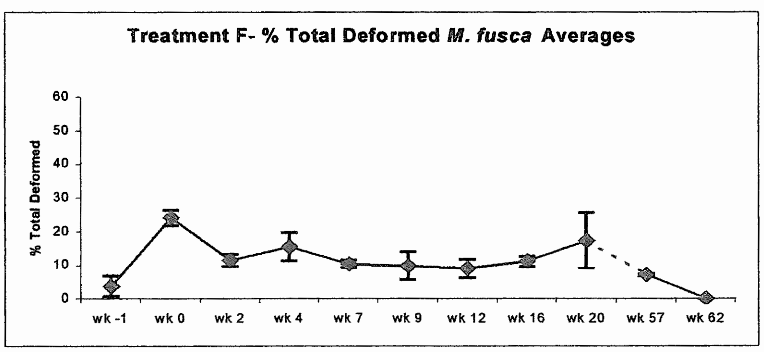
4.6c



4.6d



4.6e



4.2 Black Duck Cove

4.2.1 Description of Results Format

Four cores and eight surface samples were collected from Black Duck Cove on September 18, 2001. All eight surface samples along with cores number 1 and 4 were examined. The data were placed in a Microsoft Excel worksheet and graphed using Kaleidagraph. All calculations including total (T) and total living (L) are done the same as Petpeswick Inlet (see Section 4.1.1).

The cores showed a maximum of nine species: *Miliammina fusca, Elphidum williamsoni, Haplophragmoides maniliaensis, Tiphotrocha comprimata, Trochammina inflata, Trochammina macrescens f. macrescens* and *f. polystoma*, and *Trochammina ochracea* as well as inner linings. Similar to Petpeswick Inlet, overall the two main species found were *Miliammina fusca* and *Trochammina macrescens f. polystoma*. These two species were also the species where deformities were most common. Again the species *Trochammina macrescens f. polystoma* did not have sufficient deformities to be significant for the purpose of this study, therefore although it is graphed, only *Miliammina fusca* is discussed as well as graphed.

4.2.2 Surface Samples

Eight surface samples were extracted from different marsh zones in order to provide a general surface analysis. Four surface samples were taken near Core 2, where the core was not examined, and four near Core 4. The samples extracted from near Core 4 will be discussed (see Appendix B-3).

The low marsh had a total individual number of ~3800 individuals/10cc while the total living was ~1400 individual/10cc. The dominant species was *Miliammina fusca*, whose living and total constituted over 73% of the total population. There were no significant deformities in any species. This sample corresponds to Core 4 for depth at 0 cm.

In the lower-mid marsh the total number of individuals was ~6500 /10cc and the total living was ~2700 individuals/10cc. The most common living species was *Trochammina macrescens f. polystoma* while *Miliammina fusca* was the most common total species. There were no significant deformities in any species.

The upper-mid marsh had a total of ~4000 individuals/10cc and a total living number of ~2000 individuals/10cc. The most common species, both living and total percentage wise, was *Trochammina macrescens f. polystoma,* with no significant deformities within any species.

The number of total individuals in the upper marsh was ~2100 individuals/10cc and the total number of living individuals was ~1400 individuals/10cc. The most common species, both total and total living was *Trochammina macrescens f. polystoma.* There was no substantial deformation present in any species.

4.2.3 Core Samples

Core 1 showed a distinct oil layer between 10 and 15cm. The results show that the total number of individuals (T) was ~2500 on the surface and increased slightly in the next two centimeters to ~3300 individuals/10cc. In the 3-8 centimeter interval the total (T) was constant at ~1000 individuals/10cc. Below the 8-9 centimeter interval the total (T) was constantly low with a maximum of 200 individuals/10cc (10-11cm) and a minimum of 11 individuals/10cc (18-19cm) (see Figure 4.7). The number of living individuals/10cc (L) showed a similar trend with a high surface value (~700) increasing over the next two centimeters and then decreasing rapidly to the 8-9 cm. After this, the number of total living individuals decreased to ~0 (see Figure 4.7).

Total *Miliammina fusca* constitutes over 60% of the total population in the top eight centimeters; from a maximum of 98% (2-3 cm), to a minimum of 63% (4-5 cm) (see Figure 4.7). The percentage of living *Miliammina fusca* is only 34% in the top 1cm but increases to 98% in the 2-3cm interval. The percentage stays above 60% until the 9-10cm interval, drops to 20% in the 10-11 cm interval, and then remains at 0% after this. The percentage of total deformed *Miliammina fusca* is 2% at the surface (0-1cm) and remains below 3.5% until the 7-8cm interval. It increases to 6% at the 8-9cm interval and then decreases to 4% at the 9-10 interval. The 10-11cm interval shows a 15% deformation value, which drops to zero at the 11-12 cm mark. The total deformed then rises to 33% at 12-13cm, drops to zero at 13-14cm and again increases to 33% at 14-15cm. After this the percent deformed is constant at 0% for the remainder of the core. The living deformed *Miliammina fusca* is 0% at the surface, and then remains below 3.4% until the 7-8cm interval. At this interval the living deformation rate increases to 5-6% until 8-9cm. The living deformed then increase rapidly to 20% at 9-10cm then 50% at 10-11cm. The remainder of the core had a 0% value. However, these percentages are deceptive because the total numbers are very low.

Only the total (T) was counted for core 4, which was extracted from the lower-mid marsh. The surface centimeter had a total of ~4600 individuals/10cc, which increased to ~8800 individuals/10cc at 7-8cm. The 9.5-10.5cm interval showed a total of ~2800 individuals/10cc decreasing in the12.5-13.5cm interval to ~2300 individuals/10cc. The total number of individuals at 14.5-15.5cm dropped to ~900 individuals/10cc and then dropped drastically in the16-17cm interval to only ~93 individuals/10cc. It stayed constant until 20-21cm where it increased to ~240 individuals/10cc.

Figure 4.7 Foraminifera results for Core 1, Black Duck Cove

Total number of individuals /10cc (T) compared with depth (cm).

Total number of living individuals/10cc (L) compared with depth (cm).

Total percentage (T) of *Trochammina macresens f. polystoma* in relation to depth (cm).

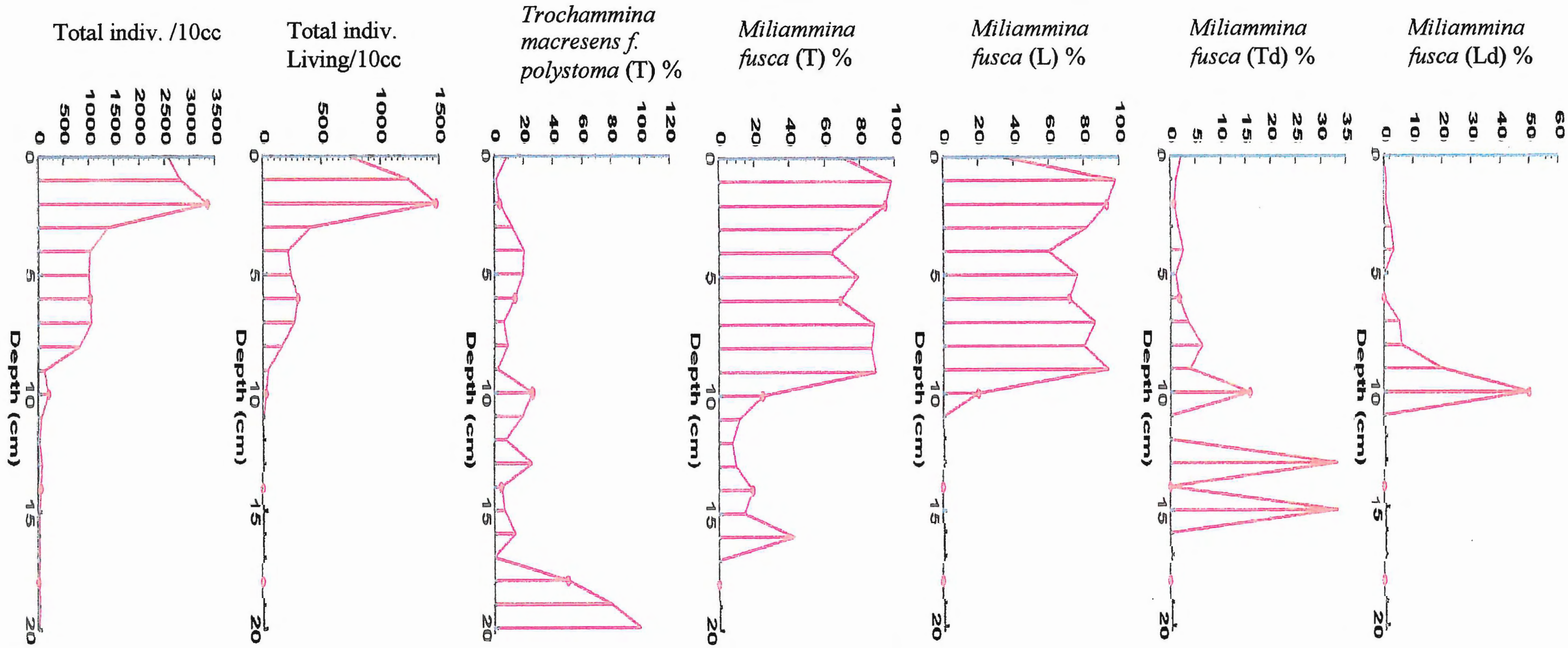
Total percentage (T) of *Miliammina fusca* compared with depth (cm).

Total percentage of living (L) *Miliammina fusca* compared with depth (cm).

Percentage of total deformed (Td) *Miliammina fusca* which represent the percentage out of total *Miliammina fusca* only compared with depth (cm).

Percentage of total living deformed (Ld) *Miliammina fusca* which represent the

Percentage out of living *Miliammina fusca* only compared with depth (cm).


Total indiv. /10cc
Total indiv. Living/10cc
Trochammina macresens f. polystoma (T) %
Miliammina fusca (T) %
Miliammina fusca (L) %
Miliammina fusca (Td) %
Miliammina fusca (Ld) %
Depth (cm)

At 21-22cm the total number of individuals increased to ~460/10cc, decreased to ~230/10cc at the next centimeter, and increased to ~420 individuals/10cc at the 23-24cm. In the 24-25cm interval, the number of individuals increased to ~1500/10cc then decreased to ~280/10cc at the 25-26cm interval (see Figure 4.8).

The total percentage of *Miliammina fusca* stayed between 50% and 65% from 1-9.5cm, then increased to over 80% within the 12.5-14.5cm interval, then decreased to under 80% until 19cm where it remained above 80% for the remainder of the core. The percentage deformed (Td) was consistently below 4% from 0-12.5cm. It then increased to 7.5% at 14.5-15.5 cm, dropped to 0% at 16-17cm, and increased to 8.3% at 17-18cm. After this, the deformity percentage dropped to 0 at 18-19cm, and increased to 8.3% at the 19-20cm interval. 2.3% of the total *Miliammina fusca* was deformed at 20-21cm, then decreased to below 1% for the remainder of the core. Again some of these percentages are deceptive because of the low absolute abundances.

4.3 Deformation

Deformed *Miliammina fusca* in both Petpeswick Inlet and Black Duck Cove show a variety of morphological changes. Deformities found include twisted tests and apertures as well as twinning. Other species showed deformation by chamber enlargement, and twisted chambers. Figure 4.9a shows a normal *Miliammina fusca* specimen, and figure 4.9b and 4.9c shows different forms of deformation extracted from two different samples.

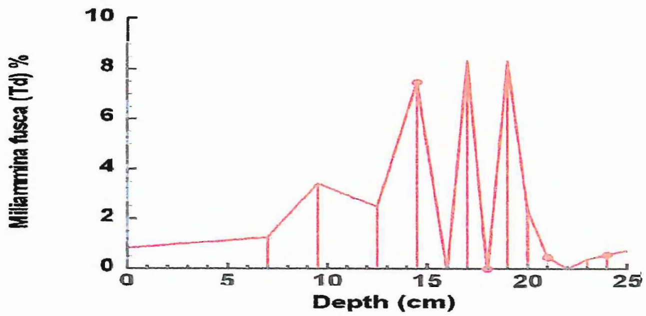
Figure 4.8 Foraminifera results for Core 4, Black Duck Cove

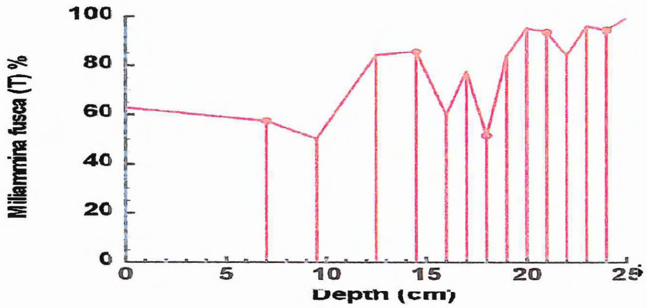
Total number of individuals /10cc (T) compared with depth (cm).

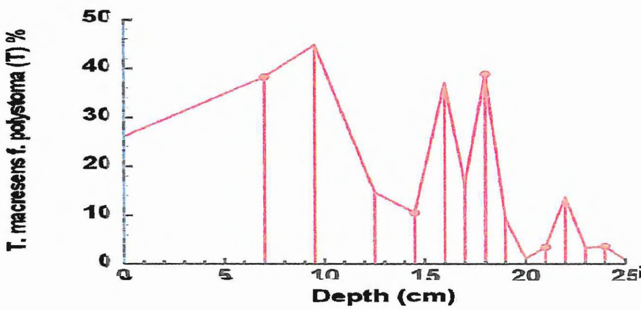
Total percentage (T) of *Trochammina macresens f. polystoma* in relation to depth (cm).

Total percentage (T) of *Miliammina fusca* compared with depth (cm).

Percentage of total deformed (Td) *Miliammina fusca* which represent the percentage out of total *Miliammina fusca* only compared with depth (cm).


Miliammina fusca (Td) %
10
8
6
4
2
0
0
5
10
15
20
25
Depth (cm)


Miliammina fusca (T) %
100
80
60
40
20
0
0
5
10
15
20
25
Depth (cm)


T. macresens f. polystoma (T) %
50
40
30
20
10
0
0
5
10
15
20
25
Depth (cm)

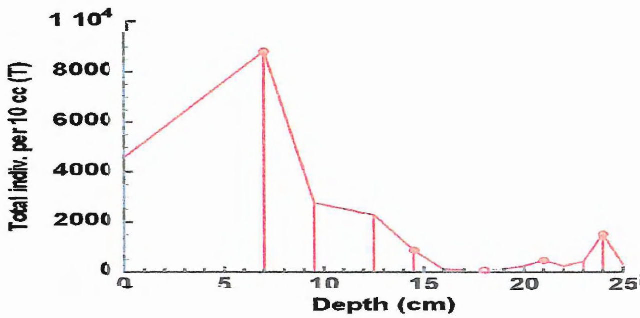

Total indiv. per 10 cc (T)
1 10⁴
8000
6000
4000
2000
0
0
5
10
15
20
25
Depth (cm)

Figure 4.9a Normal *Miliammina fusca* specimen (~200μ)

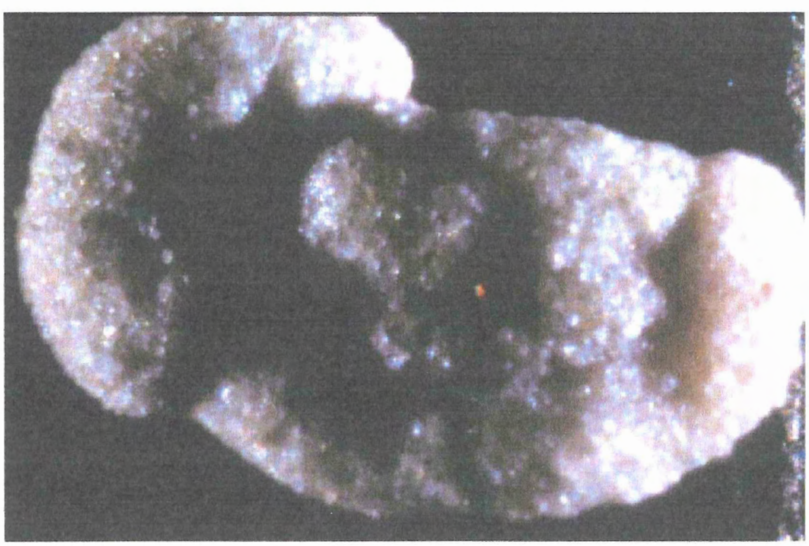
Figure 4.9b Deformed *Miliammina fusca* specimen (~200μ)

Figure 4.9c Deformed *Miliammina fusca* specimen (~200μ)

4.9a

4.9b

4.9c



CHAPTER 5: DISCUSSION

5.1 Petpeswick Inlet

Two species were found to dominate the foraminiferal assemblage in Petpeswick Inlet, *Trochammina macrescens f. polystoma* and *Miliammina fusca*. Of these two species only *Miliammina fusca* is discussed in detail because of the deformation that occurred within the oiled plots. There appears to be no assemblage response in any of the plots (i.e. no species were reduced or excluded). The only effect from the oil appears to be the deformation of *Miliammina fusca.* It appears that *Trochammina macrescens f. polystoma* is more tolerant to stress therefore deformation did not occur. This may be a result of *Trochammina macrescens f. polystoma* is in the lowest, or least stressful, part of its range, while *Miliammina fusca* is in the highest, or most stressed part of its range.

Treatment A, control plot with no oil, showed a very low deformity percentage within *Miliammina fusca* over weeks 57 and 62. Total percent of deformities remained below 3.2% over both weeks, much lower then the 10% deformed, which would indicate a polluted or stressed environment. The deformation rate with no oil or nutrients is naturally low, and the number of individuals is high.

Treatment B, control plot with no oil and nutrient enrichment for the first 22 weeks, shows that at week 57 and week 62 there are no deformities present. It appears that the nutrient enrichment has little or no effect on deformities present in *Miliammina fusca*.

Treatment C, oiled plot with natural attenuation, showed over 10% deformation in living and total *Miliammina fusca*. This indicates that the oil is still affecting the foraminifera, mainly *Miliammina fusca*, even after 14 months of recovery.

Treatment D, oiled plot with nutrient enrichment, follows a similar trend as Treatment C. The percent deformed (living and total) is over 10%. This indicates that nutrient enrichment does not affect *Miliammina fusca* and after 62 weeks the oil still has an effect on the species.

Treatment E, oiled plot with nutrient enrichment and cut plants, showed a constant percentage of deformed *Miliammina fusca* at 10%. This again shows that cutting the plants and adding nutrients does not positively affect the foraminifera. It is interesting to note that even when the percentage of total and living *Miliammina fusca* increases the deformation rate remains constant.

Treatment F, oiled plot with nutrient enrichment and agricultural disking showed the most favorable results. Deformities were at 7% in week 57 and decreased to 0% in week 62. Treatment D shows that nutrient enrichment is not decreasing deformation thus the agricultural disking must be responsible. Perhaps mixing the topsoil, thus adding oxygen, increases bacteria growth. The bacteria may have begun to break down the oil while providing the foraminifera with food. However, the total number of individuals is the lowest of the treatments in week 62 and the second lowest (next to Treatment D) in week 57.

It is clear that all of the oiled plots are still being affected by the oil, except for Treatment F, which included agricultural disking. The treatments with no oil (Treatments A and B) contain very few deformations indicating that the stress is at natural levels in these plots.

5.1.1 Comparison with previous work

Although several experiments concerning foraminiferal responses were discussed in Chapter 2, it is important to compare the results of this study with Sabean (2001). As previously mentioned, this study is a continuation of Sabean's who participated in the DFO experiment through weeks -1-20. During this time similar results were obtained including the species present, as well as the most common species. Significant deformation also occurred in only *Miliammina fusca*. The oiled treatments, which responded a few days after the oil was applied, showed total and live deformed greater than 5%, and in most cases greater than 10%. This occurred throughout the 20 weeks, but showed the highest deformity rate directly after the oil was applied. One difference found in this study is that Treatment F showed the best response with deformities very low. This was not the result in Sabean's (2001). Another difference between this study and Sabean's (2001) is the large reduction of total living individuals within the oiled plots in weeks 57 and 62 (see Appendix A-1 and A-2). This may indicate the oil caused a delayed reaction affecting foraminiferal reproduction. In both studies the control plots (no oil) showed little or no deformities.

Previous experiments studied the effects of oil pollution on benthic foraminifera, such as a study conducted by Whitcomb (1978). He demonstrated that the tests of various foraminiferal species showed deformities that ranged from 10%-15%, after chronic hydrocarbon pollution in the tidal flat complexes of the lower York River, Virginia. It was suggested that the deformed tests may have been a result of starvation due to a decrease in diatom numbers because of the toxicity of the oil (Whitcomb, 1978).

5.2 Black Duck Cove

Before the oil spill, Core 1 shows a very low count of foraminifera. This is because the sediment consists mainly of sand, which does not support the large populations observed in marshes. At this depth the foraminiferal assemblage was almost entirely *Trochammina macrescens f. polystoma*. After the oil spill occurs (10-15cm) the foraminifera population increases and *Miliammina fusca* is established. The 5cm interval of oil may represent a 10-year span if deposition was constant. At the 9-10cm interval the total number of individuals present is still low but the value increased, as did the total percentage of deformities within *Miliammina fusca*. There are three main peaks of deformities within the total percentage deformed *Miliammina fusca*: 15-16cm, 13-14cm, and 10-11cm. These intervals are within the observed oil layer (10-15cm). After the 10-11cm interval, the total deformation present decreased to below 10% indicating an apparently less stressed environment. Although there are few foraminifera living at 10cm below the surface, there are still high percentages of *Miliammina fusca* deformed (~60%). The low abundance numbers and high numbers of deformities indicate that the foraminiferal population is still being adversely affected even over 30 years after the oil spill.

Although a distinct oil layer was not observed within Core 4, it can be inferred. Between the depths of 14.5-15.5cm and 16-17cm the total number of individuals decreased by half. The population does increase at depths greater than 21cm. Assuming the deposition rate is constant the oil had effected the foraminiferal population for ~9 years. There are three peaks with high deformation rates in *Miliammina fusca*, 14.5-15.5, 17-18cm and 19-20cm all within *Miliammina fusca.* The total percentage deformed peaks are all under 10%, however the total population was extremely low. It is suggested that the oil was deposited around the 20-21cm interval, as this is when the total number of individuals/10cc decreased rapidly. The oil continued to effect the foraminiferal population until 12.5-13.5cm where the deformation is low. The oil is suggested to have killed most of the foraminiferal population, as well as deforming the tests of some *Miliammina fusca*.

Core 1 and Core 4 show a decrease in the total number of individuals/10cc when the Bunker C oil was deposited. There were three peaks of deformities in *Miliammina fusca* in both cores, peaking directly after the Bunker C oil was deposited until the population increased and the oil either was not present or detoxified naturally. A thick oil layer was observed in Core 1 but not in Core 4. Also, Core 4 deformation peaks are below 10%, but they did occur simultaneously with lowering of population thus indicating a disturbance. The decrease in population appears to occur for ~9-10 years in both cores, assuming constant deposition. The ecosystem at Black Duck Cove now appears healthy with the foraminiferal population established, and percentage of *Miliammina fusca* deformed below 5%, thus presently unstressed.

5.3 Comparison of Experimental to Real Oil Spill

Based on the results found in Petpeswick Inlet and Black Duck Cove, it can be observed how foraminifera respond after a recent controlled spill and an over 30-year-old spill. Both areas showed a very rapid response to the oil, however they were different. In Petpeswick Inlet the weathered crude oil that was applied hardly lowered the number of individuals but it did increase the deformities. Black Duck Cove, where Bunker C oil was distributed, the oil virtually wiped out the foraminiferal population and also increased deformities. A large amount of oil as well as a heavier oil, such as Bunker C oil, appears to be more detrimental to the foraminiferal populations; the relatively small amounts of weathered light crude oil does not effect foraminiferal assemblages and population numbers nearly as much. At this point it is impossible to determine whether it was the increased volume of oil or the type of oil that caused the increase in disturbances.

Another difference within these two studies is the physical setting. Black Duck Cove is rockier with boulders trapping the oil. Petpeswick Inlet, on the other hand, had plots chosen for the experiment that was grass covered and appeared healthy. This may have enhanced the recovery process at Petpeswick Inlet. Both areas show natural fluctuations over time but this is to be expected in coastal salt marshes (Scott and Medioli, 1980b).

The important question that must be addressed is what caused the increased stress at Black Duck Cove compared to Petpeswick Inlet: the type or the volume of oil spilled? This can be determined experimentally but remains to be done.

CHAPTER 6: CONCLUSIONS

6.1 Conclusions

1. In Petpeswick Inlet, after 62 weeks, deformities were still present in the dominant species *Miliammina fusca* within Treatments C, D and E. These deformities accounted for >5% of the total *Miliammina fusca* population. Treatment F had a low deformity rate as did both unoiled plots (<5%)
2. Of the *in situ* bioremediation treatments applied to the plots, only agricultural disking had an effect on the recovery of foraminifera after 14 months. This was shown in Treatment F. Nutrient enrichment had no positive effect on the foraminifera nor did cutting the plants. Leaving the oil untreated also did not reduce the deformation percentage amongst the *Miliammina fusca*.
3. Black Duck Cove shows that the total number of individuals/10 cc decreases rapidly when a large volume of Bunker C oil was deposited. The oil effected the population growth for 5 cm in Core 1, which may equal ~10years. Deformations increased rapidly within *Miliammina fusca* after the oil was deposited and decreased again as the total number of individuals increased.
4. Current conditions at Black Duck Cove show that although there is an asphalt like layer of oil on the beach, the foraminifera are established and not unnaturally deformed (<5%).
5. Comparing samples from Petpeswick Inlet to Back Duck Cove shows that in a large oil spill with a harsh oil, like Bunker C from *the Arrow*, foraminifera populations are destroyed, where a small spill of light weathered crude oil is not as detrimental.

6. Foraminifera are cost-effective, reliable indicators of oil spills for both recent and past coastal salt marsh environments. Foraminifera provide a good fossil record, which shows the recovery that occurred. If one knows the rate of deposition the exact time needed for recovery can be found by examining a core in that area

6.2 Future Work

In regard to Petpeswick Inlet it is advised that samples be taken each year for several years to view the long-term effects of the oil spill. This would allow for a continuation of the comparison between the treated and non-treated plots. It may be beneficial to conduct a similar experiment that shows the responses of foraminifera with different volumes or weights of oil applied.

More cores from Black Duck Cove would be advisable within different marsh zones to view the distance that the oil reached. It would also show if different zones had a quicker/slower remediation time. Cores from other salt marshes that were also affected by Bunker C oil from a past spill should be examined and compared with Black Duck Cove.

Taxonomy

The following list is all species of benthic foraminifera found in this study, and are listed in alphabetical order by genus. The classification of foraminifera genera is in accordance with Scott et al. (2001).

Eggerella advena (Cushman)

Verneuilina advena Cushman, 1922, p.141.
Eggellera advena (Cushman). Cushman, 1937, p.51,pl.5, figs 12-15; Phleger and Walton, 1950,p.277,p1.1, figs. 16-18; Scott et al., 1977,pl.2, fig. 7; Scott and Medioli, 1980a, p.40, pl.2, fig.7: Scott et al., 1991, p.385, pl.2, figs. 1, 2.

Miliammina fusca (Brady)

Quinqueloculina fusca Brady, 1870, p.286, pl.11, figs. 4.4a, b.
Miliammina fusca (Brady) Phleger and Walton, 1950, p.280, pl.1, figs.19a, b; Phleger, 1954, p.642,pl.2 figs.22,23; Scott et al., 1977, p.1579, pl.2, figs. 8, 9; Schafer and Cole, 1978, p.28, pl.12, fig.2; Scott and Medioli, 1980a, p.40, pl.2, figs.1-3; Scott et al., 1991, p.386, p1.1, fig. 14.

Pseudothurammina limnetis (Scott and Medioli)

Astrammina sphaerica (Heron-Allen and Earland), Zaninetti et al., 1977, pl.1, fig. 9.
Thurammina (?) limnetis Scott and Medioli, 1980a, p.43, pl.1, figs. 1-3.
Pseudothurammina limnetis Scott and others, *In* Scott et al., 1981, p.126; Scott et al., 1991, p.386, pl.2.

Tiphotrocha comprimata (Cushman and Brönnimann)

Trochammina comprimata Cushman and Brönnimann, 1948, p.41, pl.8, figs. 1-3; Phleger, 1954, p.646, pl.3, figs. 20,21.
Tiphotrocha comprimata (Cushman and Brönnimann). Saunders, 1957, p.11,pl.4, figs. 1-4; Scott et al., 1977, p. 1579, pl.4, figs.3, 4; Scott and Medioli, 1980a, p.44, pl.5, figs.1-3;Scott et al., 1990, pl.1, figs.10a, b; Scott et al., 1991, p.388, pl.2 figs. 5, 6.

Trochammina inflata (Montagu)

Nautilas inflatus Montagu, 1808, p.81, fig.3.
Rotalina inflata Williamson, 1958, p.50, pl.4, figs.93, 94.
Trochammina inflata (Montagu). Parker and Jones, 1859, p.347; Phleger, 1954, p.646, pl.3, fig. 24; Scott and Medioli, 1980a, p.44, pl.3, figs. 1-12;Scott et al., 1990, p.733, pl.1, figs.1a, b, 2a-c; Scott et al., 1991, p.388,pl.2, figs. 10, 11; Scott et al., 1995,p.294, p.294, figs.6.6-8.

Trochammina macrescens (Brady)

Trochammina inflata (Montague) var. macrescens Brady, 1870, p.290, p1.11, fig.5; Scott,1976, p.320, pl.1, figs.4-7; Scott et al., 1977, pl.4, figs.6-7.

Jadammina polystoma Barenstein and Brand, 1938, p.381, figs.1,2.

Trochammina macrescens Brady. Parker, 1952, p.460, pl.3, fig.3; Phleger, 1954, p.646, pl.3, fig.24; Scott and Medioli, 1980a, p.44, pl.3, figs.1-12; Scott et al., 1990, p.733, pl.1, figs. 1a, b, 2a-c; Scott et al., 1991, p.388, pl.2, figs.10-11; Scott et al., 1995, p.294, p.294, figs.6.6-8.

Trochammina ochracea (Williamson)

Rotalina ochracea Williamson, 1858, p.55, pl.4, fig.112, pl.5 fig.113.

Trochammina squamata Parker and Jones, 1865, p.407, pl.15, figs.30, 31; Scott and Medioli, 1980a, p45, pl.4, figs.6, 7.

Trochammina squamata Parker and Jones and related species. Parker, 1952, p.460, pl.3, fig.5.

Trochammina ochracea (Willamson). Crushman, 1920, p. 75, pl.15, fig.3; Scott and Medioli, 1980a, p.45, pl.4, figs.4, 5.

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APPENDIX A
Petpeswick Inlet
Data Tables

Table A-1 Table of results for foraminiferal occurrences for Treatments A-F for July 9, 2001. The living and total (living plus dead) number of the total number of species per 10cc, and the living and total (live plus dead) number of the total number of individuals per 10cc are included. The relative percentage of living and total (live plus dead) foraminiferal species are included, as is the percentage of living and total per individual species. The percentage of living deformed and total deformed within an individual species represent the percentage out of the living and total for that species only.
L = Living
T= Total (live plus dead)
Ld = Living deformed
Td= Total (live plus dead) deformed

	IA	IIA	IIIA	IB	IIB	IIIB	IC	IIC	IIIC	ID	IID	IIID	IE	IIE	IIIE	IF	IIF	IIIF
Total # of species (L)	3	4	4	3	3	4	3	4	3	3	3	4	3	2	2	2	2	4
/10cc (T)	5	5	5	6	5	6	6	5	6	4	5	4	4	4	5	5	6	5
Total # of individuals (L)	2432	960	1024	492	816	1488	768	536	328	448	292	320	592	261	256	268	144	332
/10cc (T)	4504	2472	3080	1204	1576	3296	2088	1616	1584	1792	768	844	2312	637	2960	896	1624	1308
Trochammina inflata (L)	5.26	1.67	1.56	5.69	0.98	7.53	6.25	7.46	7.32	1.79	0.00	5.00	12.16	0.00	0.00	8.96	5.56	16.87
(Ld)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	4.44	4.21	2.34	3.65	2.03	6.31	3.83	4.95	5.05	0.89	0.00	5.21	5.88	0.00	0.00	8.04	2.96	11.62
T(d)	0.00	0.00	0.00	0.00	0.00	7.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Trochammina macrescens f. polystoma (L)	88.16	87.50	62.50	66.67	86.27	81.72	72.92	79.10	82.93	98.21	75.34	75.00	60.81	90.82	37.50	89.55	88.89	55.42
(Ld)	0.37	1.90	1.27	0.00	0.00	0.66	1.43	3.77	0.00	0.00	0.77	0.00	2.22	0.00	0.00	0.00	0.00	0.00
(T)	83.30	77.99	41.30	48.84	77.16	72.82	62.84	58.91	80.81	88.84	68.23	57.82	27.34	64.44	32.16	73.66	79.31	65.14
(Td)	0.21	0.83	0.82	0.00	1.56	0.33	5.49	1.68	1.25	0.00	3.31	1.61	1.27	0.00	0.00	0.00	1.86	0.47
Miliammina fusca (L)	6.58	10.00	33.59	26.02	9.80	9.68	16.67	8.96	7.32	0.00	21.92	16.25	25.68	8.16	59.38	0.00	0.00	24.10
(Ld)	0.00	0.00	0.00	0.00	0.00	0.00	6.25	16.67	0.00	0.00	12.50	23.08	21.05	0.00	10.53	0.00	0.00	10.00
(T)	10.59	9.06	52.73	29.24	11.17	17.96	21.84	26.24	9.60	8.48	26.56	31.75	65.40	28.45	57.30	13.84	6.90	19.88
(Td)	0.00	0.00	0.00	0.00	0.00	0.00	5.26	16.98	21.05	36.84	23.53	19.40	12.17	10.00	6.60	6.45	7.14	7.69
Trochammina ochracea (L)	0.00	0.83	0.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.37	0.00	0.00	0.00	0.00	0.00	0.00	1.20
(T)	1.77	6.47	2.60	11.30	4.57	1.70	3.83	5.94	1.52	0.89	2.08	1.42	1.04	2.93	0.27	2.23	3.94	1.22
Tiphotocha comprimata (L)	0.00	0.00	0.00	0.00	0.00	1.08	0.00	1.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	0.00	0.00	0.00	0.33	0.00	0.49	0.38	1.49	1.01	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.49	0.00
Pseudothurammina limnetis (L)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	0.36	0.32	0.52	3.32	1.52	0.73	4.21	0.00	0.51	0.45	1.04	0.00	0.00	1.67	0.27	0.45	2.96	0.31
Inner Linnings (L)	0.00	0.00	1.56	1.63	2.94	0.00	4.17	2.99	2.44	0.00	1.37	3.75	1.35	1.02	3.13	1.49	5.56	2.41
(T)	0.00	1.94	0.52	3.32	3.55	0.00	3.07	2.48	1.52	0.45	1.56	3.79	0.35	2.51	0.81	1.79	3.45	1.83
Haplophragmoides manilianensis (L)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.52	0.00	0.00	0.00	0.00	0.00	0.00

Table A-2 Table of results of foraminiferal occurrences for Treatments A-F for August 15, 2001. Same format as Table A-1.

	IA	IIA	IIIA	IB	IIB	IIIB	IC	IIC	IIIC	ID	IID	IIID	IE	IIE	IIIE	IF	IIF	IIIF
Total # of species (L)	5	4	4	3	4	5	2	2	3	3	4	4	4	3	4	3	4	4
/10cc (T)	7	6	6	5	6	5	5	4	4	4	6	6	6	5	4	4	5	4
Total # of individuals (L)	1248	2344	1792	1208	1376	1152	424	640	296	400	372	672	768	744	824	123	896	456
/10cc (T)	2312	3008	2288	1768	2208	2080	1744	1776	1728	820	1156	1752	5216	1832	1808	554	1912	1024
Trochammina inflata (L)	4.49	11.95	1.79	5.30	6.40	9.03	0.00	2.50	5.41	1.00	1.08	3.57	2.08	5.38	20.39	15.22	5.36	6.14
(Ld)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.76	0.00	0.00	0.00
(T)	3.11	12.23	1.40	5.88	10.14	5.38	2.29	3.60	14.35	0.49	1.38	4.11	2.76	5.24	18.58	11.07	2.93	5.47
T(d)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.38	0.00	0.00	0.00
Trochammina macrescens f. polystoma (L)	75.64	85.67	79.02	83.44	84.88	82.64	88.68	96.25	72.97	84.00	80.65	63.10	73.96	91.40	38.83	76.09	77.68	78.95
(Ld)	0.00	0.00	0.56	0.00	0.68	0.00	0.00	0.00	0.00	2.63	0.00	1.89	0.00	0.00	2.50	0.00	0.00	0.00
(T)	71.97	80.85	73.43	74.66	77.54	83.46	76.61	80.63	58.33	87.32	82.35	70.78	52.30	79.48	32.74	82.81	81.17	79.69
(Td)	0.00	0.33	0.48	0.00	0.94	0.00	0.60	0.56	0.79	1.12	0.84	0.65	0.88	0.00	1.35	0.00	0.52	0.00
Miliammina fusca (L)	17.31	1.02	15.63	10.60	6.98	6.25	7.55	0.00	16.22	10.00	9.68	30.95	22.92	3.23	38.83	6.52	15.18	12.28
(Ld)	3.70	0.00	0.00	0.00	0.00	0.00	50.00	0.00	16.67	20.00	11.11	20.69	9.09	0.00	15.00	0.00	11.76	7.14
(T)	20.76	3.46	19.58	13.57	8.33	8.08	16.97	11.26	25.46	6.34	7.27	21.46	44.02	13.10	47.35	4.81	11.72	12.89
(Td)	1.67	7.69	0.00	0.00	0.00	0.00	18.92	28.00	16.36	15.38	0.09	14.00	6.27	0.00	7.69	0.00	7.14	6.06
Trochammina ochracea (L)	0.00	0.00	0.00	0.00	0.58	0.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.97	0.00	0.89	0.88
(T)	2.08	1.60	1.75	3.62	1.45	2.31	1.38	4.05	0.93	3.41	4.84	0.91	0.46	1.31	0.88	0.83	2.93	1.17
Tiphotocha comprimata (L)	1.28	0.68	1.34	0.00	0.00	0.69	0.00	0.00	0.00	0.00	1.08	0.00	1.04	0.00	0.00	0.00	0.00	0.00
(T)	0.69	0.80	1.40	0.00	0.36	0.38	0.00	0.00	0.00	0.00	1.04	0.00	0.15	0.00	0.00	0.00	0.00	0.00
Pseudothurammina limnetis (L)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	0.35	0.27	0.35	0.90	0.72	0.00	0.92	0.00	0.00	0.00	0.35	0.91	0.15	0.44	0.00	0.00	0.42	0.00
Inner Linnings (L)	0.64	0.68	2.23	0.66	1.16	0.69	3.77	1.25	5.41	5.00	7.53	1.19	0.00	0.00	0.97	2.17	0.89	1.75
(T)	0.69	0.80	2.10	1.36	1.45	0.38	1.83	0.45	0.93	2.44	2.77	1.37	0.15	0.44	0.44	0.48	0.84	0.78
Haplophragmoides manilianensis (L)	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.19	0.00	0.00	0.00	0.00	0.00	0.00
(T)	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00

APPENDIX B
Black Duck Cove
Data Tables

Table B-1 Table of results for foraminiferal occurrences for Core 1, Black Duck Cove. The living and total (living plus dead) number of the total number of species per 10cc, and the living and total (live plus dead) number of the total number of individuals per 10cc are included. The relative percentage of living and total (live plus dead) foraminiferal species are included, as is the percentage of living and total per individual species. The percentage of living deformed and total deformed within an individual species represent the percentage out of the living and total for that species only.

L = Living
T= Total (live plus dead)
Ld = Living deformed
Td= Total (live plus dead) deformed

Depths	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Total # species (L)	4	2	2	4	4	2	4	4	2	2	3	2	0	2	2	0	0	0	1	0	0
/10cc (T)	6	4	4	4	6	4	4	4	4	4	5	4	4	4	4	4	3	1	2	3	1
Total # individuals (L)	720.00	1240.00	1472.00	408.00	208.00	236.00	296.00	264.00	160.00	42.67	26.66	8.00	0.00	5.34	5.34	0.00	0.00	0.00	5.33	0.00	0.00
/10cc (T)	2576.00	2816.00	3352.00	1380.00	1024.00	1000.00	1024.00	1048.00	820.00	122.67	205.32	69.33	34.68	82.68	56.01	37.34	18.67	32.00	10.66	40.00	10.67
Trochammina macrescens f. polystoma (L)	14.44	1.94	7.07	12.75	32.69	23.73	18.92	10.61	20.00	0.00	60.02	0.00	0.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	9.01	1.42	3.82	12.17	19.92	19.20	14.06	6.49	9.27	2.18	25.97	19.23	7.70	25.81	4.77	7.15	14.30	0.00	50.00	80.00	100.00
Miliammina fusca (L)	34.44	98.06	92.93	81.37	59.62	76.27	71.62	86.36	80.00	93.74	19.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(Ld)	0.00	0.80	0.58	2.41	3.23	0.00	0.00	5.26	6.06	20.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	70.81	98.01	94.51	77.97	63.28	78.80	68.75	88.17	86.34	89.13	24.67	11.54	7.70	9.68	19.05	14.27	42.85	0.00	0.00	0.00	0.00
(Td)	2.19	1.16	0.76	1.49	2.47	1.02	1.70	3.46	6.21	3.85	15.79	0.00	0.00	33.33	0.00	33.33	0.00	0.00	0.00	0.00	0.00
Trochammina ochracea (L)	0.00	0.00	0.00	3.92	1.92	0.00	8.11	1.52	0.00	0.00	0.00	33.38	0.00	0.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	4.66	0.28	1.19	8.70	11.72	0.80	16.02	4.58	3.90	4.34	41.56	57.70	76.90	61.28	71.42	71.42	42.85	100.00	0.00	13.33	0.00
Elphidium williamsoni (L)	43.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	12.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Inner linning (L)	7.78	0.00	0.00	1.96	0.00	0.00	1.35	1.52	0.00	6.26	19.99	66.63	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
(T)	2.48	0.28	0.00	1.16	1.17	1.20	1.17	0.76	0.49	4.35	6.49	11.54	7.70	0.00	0.00	7.15	0.00	0.00	50.00	0.00	0.00
Haplophragmoides manilianensis (L)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Trochammina macrescens (L)	0.00	0.00	0.00	0.00	5.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	50.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	0.00	0.00	0.48	0.00	3.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.23	4.77	0.00	0.00	0.00	0.00	6.68	0.00
Trochammina inflata (L)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(T)	0.00	0.00	0.00	0.00	0.39	0.00	0.00	0.00	0.00	0.00	1.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table B-2 Table of results for foraminiferal occurrences for Core 4, Black Duck Cove. Same format as B-1, except living foraminiferal was not counted.

Depth (cm)	**0**	**7**	**9.5**	**12.5**	**14.5**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
Total # species (T)	4	4	5	4	4	3	3	3	3	4	4	4	3	3	2
Total# of individuals (T)	4592	8832	2784	2288	872	93.33	82.67	82.67	114.67	237.33	460	234.67	416	1512	276
Trochammina macrescens f. polystoma (L)	26.13	38.32	44.83	14.69	10.55	37.04	16.18	38.83	9.3	1.12	3.48	13.65	3.37	3.7	0.72
Miliammina fusca (T)	62.89	57.52	50.29	84.27	85.78	59.83	77.67	51.63	83.92	95.4	93.91	84.19	96.15	94.71	99.28
(Td)	0.83	1.26	3.43	2.49	7.48	0	8.33	0	8.33	2.35	0.46	0	0.38	0.56	0.73
Trocha ochracea (T)	0.35	0	0.57	0.7	3.21	2.85	6.45	9.68	0	1.12	2.17	1.14	0.48	0.53	0
Inner linning (T)	0	0.18	0	0	0	0	0	0	0	0	0	0	0	0	0
Trochammina inflata (T)	10.63	3.99	4.02	0.35	0	0	0	0	6.98	2.24	0.43	1.14	0	0	0
Eggerella advena (T)	0	0	0.29	0	0.46	0	0	0	0	0	0	0	0	0	0

Table B-3 Table of results for foraminiferal occurrences in surface samples at Black Duck Cove, near core 4. Same format as B-1.

	lowmarsh (mudflat)	lower mid marsh (*S. alterna*)	upper mid marsh (mixed flora)	high marsh
Total # species (L)	5	3	4	2
/10cc (T)	6	6	5	3
Total # off individuals (L)	1360	2664	2064	1384
/10cc (T)	3784	6536	4072	2144
Trochammina inflata (L)	10	20.42	34.11	0
(T)	4.23	15.67	33.79	0.37
Trochammina macrescens f. polystoma (L)	14.71	59.76	63.18	99.42
(Ld)	0	1.01	0	0
(T)	13.11	31.21	57.17	97.76
(Td)	2.7	0.78	0.34	0.76
Miliammina fusca (L)	73.53	19.82	1.94	0.58
(Ld)	2.4	0	0	0
(T)	79.92	52.39	7.86	1.87
(Td)	1.59	0.93	2.5	0
Trochammina ochracea (L)	0.59	0	0	0
(T)	1.69	0.37	0.59	0
Trochammina macrescens (L)	1.18	0	0	0
(T)	0.63	0.12	0	0
inner linning (L)	0	0	0.78	0
(T)	0.42	0.6	0.59	0