MONITORING AND ASSESSMENT OF CONDITIONS AT A DIESEL OIL SPILL SITE IN BONNE BAY, NEWFOUNDLAND AND TESTS TO DETERMINE THE RELATIVE TOXICITY OF DIESEL FUEL TO THREE MARINE INVERTEBRATE SPECIES

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Monitoring and assessment of conditions at a diesel oil spill site in Bonne Bay, Newfoundland and tests to determine the relative toxicity of diesel fuel to three marine invertebrate species.

by

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A thesis submitted to the

School of Graduate Studies

in partial fulfillment of the

requirements for the degree of

Masters of Science

Interdisciplinary Program in Environmental Science

Memorial University of Newfoundland

May, 2004

St. John's Newfoundland and Labrador

Your file Votre référence
ISBN: 0-494-09931-3
Our file Notre référence
ISBN: 0-494-09931-3

Abstract

In 1999, 38000 litres of diesel oil was spilled in Gros Morne National Park, much of which leached onto on a small area of coastline. The goal of this project was to determine the effects of the diesel oil, and resulting clean-up procedures, on the coastal environment. Chapter 1 focuses on assessing conditions at the diesel oil spill site by examining hydrocarbon levels in sediments and organisms at the site, and by conducting a survey of the algal taxa present. Significant quantities of diesel were present for at least two years after the spill, both in sediments and biota. The oil spill site was also affected by uncharacteristically low-salinity conditions, as evidenced by the predominance of fresh-water tolerant algae. Chapter 2 focuses on determining the range of effects of the diesel oil using caged invertebrates, transplanted at a gradient from the point source of diesel and analyzed using binary logistic regression. Both distance from the source and length of time at the site (i.e. length of exposure to diesel oil and low-salinity conditions) affected the survival time of transplanted organisms; organisms transplanted closer to the source died faster than those farther away, though all organisms eventually perished. Chapter 3 examines the effects, combined and individual, of diesel and reduced salinity on the survival rates of three invertebrates commonly found along the Newfoundland coastline. Survival of these invertebrates, *Mysis stenolepis, Gammarus oceanicus,* and *Littorina obtusata*, was examined using one-way analysis of variance with a Tukey's test, two-factor analysis of variance, and regression analysis. Not all marine intertidal invertebrates react equally when exposed to diesel oil and reduced salinity, alone or combined, however, when considering that these organisms represent those potentially affected by a coastal oil spill, it can be concluded that even a short-term exposure could be devastating.

Acknowledgements

First I must thank my supervisor, Dr. Bob Hooper, for giving me the opportunity to work on a project that I loved, in a community I still love. I want to thank my supervisory committee members, Dr. Alan Whittick and Dr. Tom Knight, and thesis examiners, Dr. Grant Gardner and Dr. Paul Snelgrove, for their helpful comments while reviewing my thesis. I also want to thank several other people, who either aided in the identification of specimens or provided statistical advice, including Dr. D. Steele, Dr. L. Lye, Dr. D. Schneider, and Dr. R. Knoechel. I would also like to extend many thanks to my family for their support. Last, but not least, I want to thank my friends Mike Kelly, Ian King, Mary Ann Sheehan, Jon Edwards, William Coffey and Dave Kearsey – you all helped me enormously, whether it was with logistically-horrible fieldwork, tedious lab work, figure preparation or by just being a great friend!! Big Hug…..D

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Introduction and Overview

On August 27, 1999 a Quinnsway Transport (Mount Pearl, NL) tanker truck carrying 38000 litres of Imperial Oil diesel overturned while traveling through Gros Morne National Park, a UNESCO (United Nations Educational, Scientific, and Cultural Organization) World Heritage Site. The tanker truck overturned on the landward side of highway 430 at Rocky Barachois spilling the diesel oil into a roadside ditch. The diesel oil quickly penetrated the soil, flowing under the roadbed and into the adjacent waters of the East Arm of Bonne Bay. Containment booms were established in the roadside ditch and in the waters around the spill site to prevent extensive damage to the marine environment. Subsequent remediation measures, including the removal of approximately 1000 tonnes of contaminated soil and the deployment of oil-absorbing cloth, removed an estimated 12000 litres of fuel from the environment (Hooper *et al.*, 2001).

In October 1999 a semi-permanent rock berm was constructed across the affected cove to contain and recover the remaining diesel oil as it seeped out of the roadbed. The constructed rock berm was approximately 200 metres long and 9 metres wide, enclosed roughly 250 metres of shoreline, and rested a maximum of about 50 metres out from the base of Highway 430, into the East Arm of Bonne Bay. In total, the berm enclosed about 3600m^2 of coastline. In order to prevent sedimentation and slumping, scientists and consultants recommended that the berm be constructed with well-washed rock with the seaward side reinforced using large armour stone; The lagoon side of the berm was equipped with a polyvinylchloride (PVC) liner from the upper surface to approximately twelve inches below the low tide mark. This design would allow flushing of the lagoon,

while still containing the diesel (Hooper, pers. comm.). However, core material used by the contractor for the resulting berm structure was crushed and unsorted, with very high silt and clay content, and the seaward side of the berm was not properly armoured (Hooper *et al.*, 2001).

Construction of the rock berm was considered essential to keep environmental damage confined, particularly during the periods of severe weather and ice that are common to Bonne Bay, but the berm imposed its own environmental impact. Some mortality by habitat burial was anticipated due to the footprint associated with berm construction, however the resulting structure proved very susceptible to ice and storms. Significant slumping of the berm occurred and extensive areas of seabed were smothered as large quantities of clay escaped from the core material. Furthermore, porosity of the berm, which was initially adequate to facilitate flushing and minimize tidal differences in the lagoon, was reduced, resulting in as much as 15cm of tidal difference when compared to outside the berm. Inside the berm, salinity levels dropped to near freshwater conditions, temperatures fluctuated much faster than outside the berm, and wave action was reduced. The spill area was completely sheltered where it was previously subjected to occasional surf. The movement of beach sediments and gravel ceased, thus slowing the rate of oil residue removal. Overall, damage as a result of the spill was exacerbated by the presence of the berm (Hooper *et al.*, 2001).

Following the spill there was an immediate loss of several conspicuous invertebrate species, including *Mysis* spp., *Gammarus* spp., *Littorina saxatilis* and many smaller

organisms. Mortality was delayed, but none-the-less severe, for *Strongylocentrotus, Asterias, Ophiopholis* and *Metridium* spp.. The most tolerant of all organisms in the spill area were the common periwinkle, *Littorina littorea* and the rockweed, *Ascophyllum nodosum* (Hooper *et al.*, 2001).

Eel grass, *Zostera marina*, deteriorated on a continual basis apparently from effects related to the fine suspended sediments. There has been no successful eelgrass recolonization to date. Cancer and hermit crabs, sea anemones, sea urchins, and moon snails were eradicated from the site and had not re-colonized up to the time of this study. Sand dollars were eradicated from the site, but began immigrating from the margins of the sediment-covered area within a year of the spill. Scallops and mussels no longer occupy the heavily sediment-impacted zone (pers. obs).

Prior to the diesel oil spill and clean-up activities, the dominant fleshy algae at the site were the rockweeds *Ascophyllum nodosum* and *Fucus vesiculosus*. As with any oil spill in a sensitive area, intensive clean-up efforts followed this spill, and were focused initially on the manual removal of most of the diesel oil-contaminated intertidal rockweed algae (Hooper *et al.*, 2001). The low shore populations of these species initially showed good recovery from their post-spill clearance. Surviving bases produced new axes and surviving axes showed healthy growth for approximately a year. Eventually rockweeds began to deteriorate, showing signs of necrosis. *Chondrus* spp., which was abundant in the months after the spill, had completely disappeared by the following spring (pers.

obs.). Low salinity levels are believed to be the cause of devastation to these once-thriving populations (Hooper, pers. comm.).

Throughout this study, the mid-intertidal and high-intertidal zones were totally dominated by opportunistic blue-green algae (*Oscillatoria* spp., *Spirulina* spp., *Phormidium* spp., *Anabaena* spp., etc.), diatoms (*Melosira* spp., *Navicula* spp., etc.) and green algae (*Capsosiphon* spp., *Enteromorpha* spp., *Percursaria* spp., and numerous slimy coccoid species). A blue-green algal mat stretched across most of the lower east portion of the lagoon, while the west and upper east sections of the lagoon were covered by a diatom-based mat of brown slime (pers. obs.). Most of the species found within these mats were characteristic of low-salinity environments (Hooper, pers. comm.).

Crustose algae cover was almost 100% of available substrates on the lower east shore. Dominant crustose algal species were *Phymatolithon laevigatum, P. lenormandii, Hildenbrandia rubra, Pseudolithoderma* spp. and the lichen *Verrucaria* spp. Green algae, cyanobacteria and diatoms colonized the rock surfaces and mud within the lagoon itself, as a part of a microbial mat of bacteria, fungi and protozoans (Hooper *et al.*, 2001).

Initially, young herring and cunners were plentiful within the lagoon (Hooper *et al.*, 2001). During this study, however, herring and large cunners were totally absent, with dramatically decreasing numbers of small and juvenile cunners. No other fish were observed within the lagoon (pers. obs.).

Mussels were still abundant in late 1999 but began to die throughout 2000 and were not found within the lagoon by the end of this study (pers. obs.). Following the spill, adult periwinkles proved to be the most tolerant animals but all of the smaller, younger animals were killed. Adult common periwinkles (*Littorina littorea*) were present in moderate abundance in 1999. Population size did not change during 2000 and adults appeared healthy, but no reproduction or recruitment had occurred (Hooper *et al.*, 2001). Common periwinkles were dying by 2001. Other common shore species, including amphipods, barnacles and some snail species, were still absent from inside the lagoon prior to completion of the present study (pers. obs).

Colonization of the berm itself and of the area seaward of the berm has been monitored since construction. Very little life was present when the berm was completed in 1999. Colonial diatoms and filamentous brown algae like *Pilayella* spp. were the earliest berm colonizers, followed less than a year later by *Urospora, Ulothrix* and *Enteromorpha* spp. During the Summer 2000, however, early colonizing algae were joined by *Chordaria, Dictyosiphon, Polysiphonia, Ceramium* and young *Laminaria* spp. Animals such as cunners, crabs, hermit crabs, flounders and sand dollars moved in from surrounding areas as food abundance increased. Succession progressed on some large boulders but the accelerated erosion and slumping reversed much of the recovery process (Hooper *et al.*, 2001). Periwinkle and cunner populations did not recover to pre-spill sizes prior to completion of the present study (Hooper, pers. comm.).

Since construction, the seabed on the immediate seaward base of the berm has been covered with large amounts of clay and silt that washed from the berm, smothering the sessile fauna and causing much of the mobile epifauna to depart the site. Motile brown diatoms flourished. A few periwinkles climbed up the berm slope from the adjacent seabed and a few *Mysis* spp. hovered between the boulders. Cunners moved elsewhere, presumably due to lack of food and overall unfavourable conditions. All of the lobsters and most of the crabs (*Cancer irroratus*) and sea stars disappeared in the months after construction. Scallops that survived the initial impacts were all dead by the spring of 2001 (Hooper *et al.*, 2001).

A small quantity of suspended clay was dispersed farther offshore from the berm area after construction, coating nearby kelp beds. Before the spill, these beds were a combination of *Laminaria solidungula* and *L. longicruris,* as well as *Phycodrys, Ptilota, Phyllophora* and *Polysiphonia* spp. Most of these seaweeds were shaded and smothered by the silt. Only the *Laminaria solidungula* survived (Hooper *et al.*, 2001).

The present work is a part of a larger study on the evolution of a diesel oil spill site in an ecologically sensitive area (Hooper *et al.*, 2001). This study, however, describes the fate and effects of diesel oil in the coastal marine environment under very specific environmental conditions, and is divided into three main components. Chapter 1 examines the hydrocarbon-content of sediment and biota at the spill site over three years, as well as the algal community observed as a result of clean-up procedures and site succession. Chapter 2 examines the range of effects of diesel oil and reduced salinity on

three common invertebrates transplanted to the spill site and, finally, Chapter 3 documents toxicity tests focusing on the individual and combined effects of diesel oil and reduced salinity. This thesis focuses on conditions at the oil spill site up to and including 2001; the semi-permanent rock berm was removed in August 2002.

References

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Chapter 1. Preliminary assessment of a diesel oil spill site using (1) hydrocarbon content analysis of sediments and biota and (2) an algal survey.

1.1 Introduction

Petroleum-derived hydrocarbons represent one of the foremost pollutants in the marine environment (Khan, 1999), with an estimated 1.7 to 8.8 million tonnes per year entering the sea (Clark, 1992). Even in small amounts, oil causes a variety of negative effects in marine organisms (Castro and Huber, 2003). In this chapter I examine the extent of diesel oil contamination of a coastal ecosystem by monitoring the hydrocarbon content of biota and sediment from the area for two years after the oil was spilled; an algal survey of the site facilitates understanding current site conditions.

The chemical composition of petroleum products is complex and changes over time with release to the environment. They are highly complex mixtures of variable molecular weight hydrocarbons that contain both aromatics and aliphatics (Brzorad and Burger, 1994). In general, aromatic compounds are more toxic than aliphatics, and lower molecular weight compounds are more toxic than higher molecular weight compounds (Clark, 2001). Low molecular weight compounds are sometimes mistakenly considered less important because they are volatile and are readily lost to the atmosphere after a spill (Lytle and Peckarsky, 2001). Diesel fuel, in particular, consists mainly of saturated aliphatics as well as aromatic hydrocarbons (Song, 2000). The high concentrations of aromatic hydrocarbons in diesels (Connell and Miller, 1981; Nelson-Smith, 1972) make it particularly toxic (Clark, 2001; Miller, 1982). Also, biodegradation in the first several months after a spill reduces the straight-chain hydrocarbon fraction, leaving the aromatic

fraction intact. On a volume basis, the toxicity of weathered diesel can increase before the aromatics are degraded (Brzorad and Burger, 1994).

TPH (Total Petroleum Hydrocarbons) levels are used by approximately 75% of the American states in evaluating petroleum-contaminated sites and for developing clean up criteria (Weisman, 1998). Canada is currently developing TPH limits through the Total Petroleum Hydrocarbon Working Group. The TPH Working Group states that the use of TPH concentrations assumes that the resulting TPH levels are an accurate measurement of the petroleum-derived hydrocarbon concentration present (Weisman, 1998). Since methods for determining TPH levels in samples vary, caution is advised when using these criteria, and it is suggested that TPH be considered "estimate of the total concentration of petroleum hydrocarbons in a sample" (Hutcheson *et al.*, 1996). As such, comparisons of data from contaminated sites to data from pristine sample sites supplement TPH level data.

Chemical analyses are a fundamental primary step in the characterization of contaminated sites. Chemical analysis of sediments and tissues is believed to provide an integrated assessment of the presence and bioavailabilty of contaminants, as well as provide information on potential impacts (MacDonald *et al.*, 1997). Thus, sampling of various intertidal and subtidal sediments and invertebrates may be used to evaluate the range and persistence of oil spill damage.

Frequently, petroleum hydrocarbon levels in mussels are monitored after a petroleum contamination event (Amodio-Cocchieri and Cirillo, 2003; Law *et al.*, 2002; Baumard *et al.*, 1999; Short and Babcock, 1996), but other studies have focused on specific PAH concentrations in specimens such as limpets (Glegg *et al.*, 1999; Cripps and Shears, 1997), crustaceans (Law *et al.*, 2002; Lee and Page, 1997), and mussels (Amodio-Cocchiere and Cirillo, 2003; Law *et al.*, 2002; Baumard *et al.*, 1999; Short and Babcock, 1996). Sediment studies (La Rocca *et al.*, 1996) have described how hydrophobic and environmentally persistent chemicals such as petroleum hydrocarbons are primarily associated with suspended particles and consequently with bottom sediments. The degradation (Ke *et al.*, 2002), preservation (Delille and Pelletier, 2002), distribution (Pastor *et al.*, 2001) and origin (Nishigima *et al.*, 2001) of oil in sediments under various conditions have also been described.

Environmental assessment includes monitoring for the presence of pollutants in the field. The use of algae as ecological indicators of pollutants is diverse and well established, having been used at both the species and community level. Using algae as indicators has several intrinsic advantages. Algae are considered among the most important primary producers, they contribute significantly to near-shore ecosystems (Coelho *et al.*, 2000) by providing food and shelter for a variety of marine organisms (Knox, 2001; Stekoll and Deysher 2000). Also, algae provide important nursery areas for some fish species (Coelho *et al.*, 2000), and help buffer against large-scale changes in moisture (Knox, 2001), temperature and nutrient concentrations (Coelho *et al.*, 2000). Because of their importance to near-shore ecosystems, anthropogenic or environmental impacts that cause

large-scale disturbances in algal populations, such as oil spills (Coelho *et al.*, 2000; Crowe *et al.*, 2000) and low-salinity occurrences (Kamer and Fong, 2000; Kirst, 1989), can be devastating. Algae are not only ecologically important contributors to coastal systems, but they are sessile and therefore can be used to characterize one location over time, they are easily collected, and readily accumulate compounds from their surrounding water. Because of these advantages, the use of algae as both monitors of pollution and indicators of environmental quality has increased over the years (Levine, 1984).

Algae have been used as indicators of water quality (Maestrini *et al.*, 1984), soil fertility (Pipe and Shubert, 1984), and coastal conditions (Levine, 1984). The capacity of algae to take up heavy metals from the environment has resulted in the use of these organisms as indicators of heavy metal contamination in surrounding waters (Cai *et al.*, 1995; Brady *et al.*, 1994; Levine, 1984; Whitton, 1984). It has also resulted in many studies involving the use of algae to remove heavy metals from contaminated waters (Aderhold *et al.*, 1996; Leusch *et al.*, 1995). Algal populations, especially members of the Fucales, were extensively studied after the **Exxon Valdez** oil spill (Stekoll and Deysher, 2000; Stekoll and Deysher, 1996; De Vogelaere and Foster, 1994). Fucoid algae are particularly suitable monitors because they are dominant, perennial components of the North Atlantic intertidal zone (Wrabel and Peckol, 2000).

Oil spills may cause large-scale disturbances on seaweed covered rocky shores, but it is difficult to generalize about the degree of damage because of the variability of spills (De Vogelaere and Foster, 1994). Diesel oil is far more toxic than other types of oil (Carman

et al., 2000; Pulich et al., 1974; Gordon and Prouse, 1973), with intertidal algae being affected directly or indirectly by the oil spill. Sublethal effects could include reduced growth rates, inhibited reproduction (Stekoll and Deysher., 1996), or a decrease in photosynthesis (Pulich *et al.*, 1974; Gordon and Prouse, 1973). Initial reductions in populations can occur as a result of mortality caused by direct contamination, smothering, or clean-up activities.

Studies of algae, and subsequent cleanup activities, after major oil spills are quite common (Megharaj *et al.*, 2000; Stekoll and Deysher, 2000; Stekoll and Deysher, 1996; De Vogelaere and Foster, 1994; Cross *et al.*, 1987; Notini, 1978; Stirling, 1977), with a considerable portion of this knowledge stemming from research conducted after the **Exxon Valdez** oil spill. After the **Exxon** spill, considerable quantities of oil were mechanically removed from the shores, leaving algae populations devastated (Stekoll and Deysher, 2000; Stekoll and Deysher, 1996; De Vogelaere and Foster, 1994).

Although it is commonly assumed that clean-up procedures reduce damage and increase recovery rates, this is often not the case. Considerable injury to the intertidal community due to oiling or cleanup has been observed to lead to the need for a complete recolonization and restoration of these communities (Stekoll and Deysher, 1996). The absence of fucoids affects survival and recruitment of other intertidal algae, as well as intertidal invertebrates. A lack of *Fucus* canopy negatively affected recruitment of other Fucales after the **Exxon Valdez** oil spill (De Vogelaere and Foster, 1994). Oiled sites lacking a canopy of healthy, adult *Fucus* subjected germlings to increased heat and

desiccation stress (van Temelen *et al.*, 1997). De Vogelaere and Foster (1994) also reported that a lack of rockweed canopy negatively affected recolonization of barnacles and limpets due to the absence of suitable habitat. As well, the presence of fucoids on oiled beaches increases the surface area of the beach, allowing greater natural weathering of the oil. Fucoids, as well as other algae, also provide oxygen as a by-product of photosynthesis, which is often needed in the weathering process (Hooper *et al.*, 2001). However, clean-up efforts after an oil spill are often fueled more by political and social pressures than by concern for environmental damage (Foster *et al.*, 1990; Siva, 1979). Speedy responses, however, do not necessarily facilitate ecologically effective clean-up procedures.

Post-spill studies have shown a common trend: intertidal algae are mechanically or manually removed, and intertidal invertebrates, including grazers (herbivores), are killed due to oil-related toxicity. Consequently, it is also important to note the role of herbivory in the recovery process. Intertidal seaweed beds are maintained by the carnivory of whelks, which reduces filter feeder populations (Chapman and Johnson, 1990), and by herbivorous periwinkles, which reduce ephemeral algal populations (Williams *et al.*, 2000; Chapman and Johnson, 1990). Periwinkle snails preferentially consume early successional, ephemeral algae such as *Enteromorpha*. If not grazed upon, these early stages inhibit the appearance of later successional species like *Fucus vesiculosis* and *Ascophyllum nodosum* (Lubchenco, 1983). Fucoids, such as *Ascophyllum* and *Fucus*, form canopies that create habitat and provide food to a variety of intertidal organisms,

including gastropods, barnacles, and sponges fundamental to the structure of the intertidal community (Stekoll and Deysher, 2000; van Tamelen *et al.*, 1997; Lubchenco, 1983).

Salinity is a dominant environmental factor regulating aquatic community structure (Verschuren *et al.*, 2000; Kirst, 1989). As such, lowered salinity can have negative effects on many marine and estuarine organisms. Decreased salinity is associated with coral bleaching, mortality of reef organisms, the distribution of anemones, and reduced photosynthetic and growth rates of estuarine microalgae (Kamer and Fong, 2000).

Marine and freshwater habitats can be distinguished based on the variety of algae that occur in these environments. Exclusively freshwater divisions of algae do not occur, but some groups are more abundant and diverse in fresh water (Cyanobacteria, Chlorophyta and Charophyta) or marine water (Phaeophyta, Pyrrophyta and Rhodaphyta) (Wehr and Sheath, 2003).

The present study is a preliminary assessment of conditions at the diesel oil spill site in Gros Morne National Park and describes conditions at the site for over two years after the diesel oil spill. Specifically, extensive hydrocarbon-content analyses of sediment and biota are completed and examined to determine the extent of contamination and the degree of oil persistence in and around the lagoon. To delineate spill site conditions further, succession after the spill and the resulting algal community, as well as environmental conditions at the site are described. This assessment will serve to guide future studies at the site.

1.2 Materials and Methods

1.2.1 *Study Site*

Bonne Bay is a fjord located on the west coast of Newfoundland, surrounded by Gros Morne National Park of Canada, a UNESCO (United Nations Educational, Scientific, and Cultural Organization) World Heritage Site. Its outer region is split into the East Arm and South Arm, with relatively deep basins (up to 230 m). At the mouth of the East Arm is a shallow (15 m) sill that impedes deep circulation to the basin, while the South Arm is relatively open to the Gulf of St. Lawrence (Hooper, 1975).

The diesel oil spill site is located on the shore of a small, sheltered cove within the East Arm of Bonne Bay (Figure 1.1). The intertidal substratum along the shore to the east of the cove consists of waste shale and limestone rock, dumped during the reconstruction of Highway 430 in 1984-85. An outcrop of quartzite dominates the centre of this cove and is bounded on either side by unsorted sediments and angular rock fragments. Shale bedrock dominates the western shores. Between the center outcrop and the most eastern section of the lagoon are two large culverts that drain freshwater from the surrounding terrain (Hooper *et al.*, 2001).

The subtidal substrata are more varied. The eastern area of the study site consists of well-sorted, aerobic sand and gravel beds. Boulders and gravel from highway construction frequently overlie the natural substrate. The center of the upper subtidal spill area contains heterogeneous patches of angular gravel and boulders. The western upper subtidal contains bedrock and more angular boulders. The deeper subtidal zone shifts to finer gravels and sands at about 5 to 10 m depth. Dropstones are a prominent feature

throughout the subtidal. Bottom slope is slight along the east and increasingly steep towards the west (Hooper *et al.*, 2001).

1.2.2 *Sediment and Biota Sampling for Chemical Analysis.*

Sediment and biotic samples were collected from several areas in Bonne Bay, including Norris Point Beach, Gull Rock Lookout and the diesel oil spill site (Figure 1.1, Figure 1.2). Figures were created using MapInfo® and geo-referenced using a Garmin Model 12® GPS. Samples were collected into pre-cleaned sample bottles provided by Philip Analytical Services (Halifax, Nova Scotia). Intertidal sediment samples were collected directly into sample bottles, to a depth of 1- 2 cm below the silt component, if silt was present, using the sample bottle as a scoop. Subtidal sediment samples, that is, those collected outside the berm, were collected using 6.5x17.5cm transparent polycarbonate cores. For cores with a silt component, the silt of the sample and approximately 1cm below were removed to sample bottles for analysis. For cores without a clay component, the top 1-2cm of sediment was removed for analysis. Biotic samples were collected manually into sample bottles. Philip Analytical Services analyzed samples for a variety of aliphatic and polycyclic aromatic hydrocarbons.

Sediment samples were first collected after berm construction, approximately two months after the diesel oil spill, in October 1999. Samples collected at the spill site were from inside the berm, the base of the berm (duplicates taken) and 25 m (duplicates taken), 100 m, 200 m, 300 m, 400 m, and 500 m offshore from the base of the berm along a transect line (Figure 1.2). Samples were collected from Norris Point Beach for the purposes of

comparison. Samples were analyzed for BTEX (Benzene, Toluene, Ethylbenzene, Xylene), C_6-C_{10} (gas range hydrocarbons), $>C_{10}$-C_{21} (fuel range hydrocarbons), $>C_{21}$-C_{32} (lube range hydrocarbons), TEH (Total Extractable Hydrocarbons, $>C_{10}$-C_{32}) and TPH (Total Petroleum Hydrocarbons, C_6-C_{32}) (Table 1.1).

Samples of the blue mussel *Mytilus edulis* (Linnaeus, 1758) (Table 1.2.A), the periwinkle *Littorina littorea* (Linnaeus, 1758) (Table 1.2.C) and the fucoid algae *Ascophyllum nodosum* (Table 1.2.C), were also collected for hydrocarbon analysis at various locations inside the berm, at the base of the berm, out to 500 m from the berm base, and at the comparison site Gull Rock Lookout during the fall 1999. Samples were analyzed for $>C_{10}$-C_{21} (fuel range hydrocarbons), $>C_{21}$-C_{32} (lube range hydrocarbons), and TEH (Total Extractable Hydrocarbons, $>C_{10}$-C_{32}).

Sediment and biota samples were collected in July, September, October and November 2000 from several locations in and around the lagoon, as well as comparison beaches (Tables 1.3, 1.4 and 1.5). Sediment collected in July 2000 was from the east portion of the lagoon at the high water neap level (Table 1.3). Sediment collected in September 2000 was from the east portion of the lagoon at the low water level, east portion of the lagoon (east lagoon) at the high water neap level (same as July sample), and outside the berm on the pocket beach to the east (east beach) at the mean tide level (Figure 1.2). Samples were also collected from the west seep and the east seep, two areas of the lagoon identified by repeated visual observations to be the major sources of leaching diesel within the lagoon. These two areas were slightly west and east of a large rock outcrop

within the lagoon, hence becoming known as the west-of-outcrop and east-of-outcrop, or west and east, seeps (Figure 1.2). Sediment samples in July and September 2000 were collected from east lagoon, east seep, west lagoon, west seep and east beach (Figure 1.2), and analyzed for a range of hydrocarbons, including several PAHs (Table 1.3). In October 2000, a series of biotic samples were collected from east lagoon, east seep, east beach, west lagoon, west seep, and west beach (Figure 1.2). Only *Littorina* were sampled in 2000, and these were analyzed for fuel and lube range hydrocarbons, as well as total extractable hydrocarbons (TEH) (Table 1.4). Periwinkles were collected in November 2000 from two comparison sites, Norris Point Beach and Gull Rock Lookout, and at the west beach (Figure 1.1, Figure 1.2). Sediment samples collected in November 2000 were from the east lagoon and the east seep (Figure 1.2). A core sample of clay was also collected from outside of the berm. These samples were analyzed for TPH, TEH, BTEX, gas, fuel and lube range hydrocarbons (Table 1.5).

The final set of sediment and biota samples were collected in September 2001. Sediment samples were collected from east and west lagoon, as well as the east and west seeps, and east beach (Figure 1.2). Sediment was analyzed for TPH, THE, BTEX, gas, fuel and lube range hydrocarbons. Periwinkles were collected from the east seep, and the west and east beaches (Figure 1.2). Biota samples were analyzed for TEH, fuel and lube range hydrocarbons (Table 1.6).

1.2.3 *Algal Sampling*

Algal samples were collected and analyzed in late August and early September 2001 from three locations in Bonne Bay: Norris Cove, "Mike's" Cove and the diesel oil spill site (Figure 1.1). Seven samples were collected from within the lagoon (location 1), spanning the entire length of the berm. Comparison beaches, Norris Cove and "Mike's" Cove (locations 2 and 3, respectively), were selected based on similarity of substratum and algal species composition to those of the oil spill site prior to the spill, that is, both comparison beaches are predominantly platform, consisting of unsorted sediments and angular rock fragments covered by an extensive *Ascophyllum nodosum* (Le Jolis, 1863) and *Fucus vesiculosus* (Linnaeus, 1753) bed (Hooper, pers. comm.). Two random samples were collected from each reference beach. GPS units were recorded for each sample location using a Garmin Model 12® GPS (Table 1.8).

All samples were manually collected, with seawater from the immediate location, into Whirl Pak® sample bags, placed into coolers, and transported to Memorial University in St. John's, Newfoundland for analysis. Each sample was examined macroscopically and microscopically for the presence of algae and diatoms. Ten wet mount preparations were made from each sample and the presence of each alga and diatom identified was recorded. Wet mounts were viewed using a compound light microscope (Zeis® model 66525).

1.2.4 *Environmental Data*

Salinity and temperature profiles were completed in late August and early September 2001 using a Yellow Springs Instrument Model 85D® (Yellow Springs, Ohio) for temperature, salinity and conductivity. Measurements were taken within the lagoon and at reference beaches at the same locations algae were collected.

1.3 Results

1.3.1 Overview: Oil Spill Site

Hydrocarbon content analysis of sediments and biota collected from the oil spill site showed extremely high levels of petroleum contamination when compared to comparison locations; algal populations are indicative of a freshwater environment.

1.3.2 *Sediment and Biota Sampling for Hydrocarbon Content Analysis.*

Hydrocarbon content analysis of sediments collected approximately two months after the diesel oil spill showed a range of results. Sediment analysis at the comparison beach, Norris Point Beach, showed non-detectable limits of each parameter tested except for very low levels (24.6 mg/kg) of lube range hydrocarbons (Table 1.1). Similarly, samples collected from 100 m to 500 m away from the berm did not have detectable levels of hydrocarbon tested. The two samples collected at 25 m from the base of the berm did not show similar hydrocarbon contamination levels. The sample containing natural seabed sediment did not indicate the presence of hydrocarbons, whereas the samples composed of clay showed elevated levels of fuel (61.8 mg/kg) and lube (37.6 mg/kg) range hydrocarbons, for a TEH of 99.4 mg/kg (Table 1.1).

Two samples were also collected at the base of the berm. Again, the sample composed of natural seabed had no detectable limits of hydrocarbons, while the sample composed of clay showed levels of fuel (60.6 mg/kg) and lube (28.4 mg/kg) range hydrocarbons and TEH (89 mg/kg) similar to the sample at 25 m composed of clay (Table 1.1).

Sediment collected inside the berm, from high in the intertidal zone, was found to have levels of fuel (4790 mg/kg) and lube (305 mg/kg) range hydrocarbons orders of magnitude higher than all other samples collected at the same time. BTEX hydrocarbon contamination was not detectable in any of these samples (Table 1.1).

Mytilus, *Littorina* and *Ascophyllum* samples collected from the comparison location Gull Rock Lookout had no detectable levels of hydrocarbons (Table 1.2. A,B,C).

Mytilus collected at the base of the berm and out to 400 m from the berm all showed evidence of hydrocarbon contamination. At the base of the berm, 100 m, 200 m and 300 m from the base of the berm were very similar high levels of fuel (97, 104, 119, 87.8 mg/kg, respectively) and lube (non-detectable, 19.2 and 22.2 mg/kg and non-detectable, respectively) range hydrocarbons, especially when compared to Gull Rock Lookout. At 400 m and 500 m from the berm lube range hydrocarbons were undetectable and fuel range hydrocarbons decreased to 19.6 mg/kg, and non-detectable levels, respectively. Inside the berm, mussels had contamination levels (fuel range: 529 mg/kg; lube range: 44.8 mg/kg) that were extremely elevated compared to Gull Rock Lookout, or samples from outside the berm (Table 1.2. A.).

Littorina collected at 200 m, 400 m and 500 m from the base of the berm did not have detectable levels of hydrocarbons. *Littorina* collected from the base of the berm, and 100 m and 300 m from the base of the berm showed elevated levels of fuel range hydrocarbons (115, 44.2, 87.8 mg/kg respectively), especially when compared to Gull Rock Lookout, the comparison site. Periwinkles collected inside the berm also showed elevated levels of fuel (511 mg/kg) and lube (74.3 mg/kg) range hydrocarbons. These levels were similar to contamination levels observed in mussels at the same location (Table 1.2. B.).

Ascophyllum samples collected at 0 m, 100 m, 200 m, 300 m, 400 m and 500 m outside the berm did not have detectable levels of hydrocarbons. Three *Ascophyllum* samples collected inside the berm, however, showed high levels of fuel (179, 144 and 143 mg/kg) and lube (non-detectable, 33.6 and 31.6 mg/kg) range hydrocarbons (Table 1.2. C).

Hydrocarbon patterns in these biota samples did not correspond to patterns observed in sediments from the same areas, except for the rockweeds, which, like some sediment samples, did not have detectable levels of hydrocarbons present as close as the berm base. No obvious trend in biota contamination was observed, except that beyond 400 m from the berm base appears to be mostly hydrocarbon free.

Most sediment samples collected in July and September 2000 (Table 1.3) had extremely high levels of TPH, especially when compared to sediment collected at the east beach,

where the sediment (sample 6, Table 1.3) did not have any detectable levels of hydrocarbons.

Sediment collected in July 2000 was composed primarily of mixed drift algae. Benzene, ethylbenzene, and xylene were not detectable upon analysis; however toluene was present in detectable quantities (0.970 mg/kg). Gas range hydrocarbons were also present in elevated proportions (4.3 mg/kg). TPH (98000 mg/kg) levels were exceedingly high, mostly due to elevated fuel (89000 mg/kg) and lube (8800 mg/kg) range hydrocarbons, even when compared to samples taken inside the berm the previous year (Table 1.3). This sediment sample, collected nearly one year after the original spill, had the highest TPH value of all sediment collected throughout the entire sampling regime.

Naphthalene was not present in detectable quantities; however its derivatives 1- and 2-methylnaphthalene were present in measurable quantities (0.15 and 0.17 mg/kg, respectively), as were the PAHs acenaphthylene (2.3 mg/kg), acenaphthene (15 mg/kg), phenanthrene (5.9 mg/kg), anthracene (0.50 mg/kg), pyrene (2.3 mg/kg), benz[a]anthracene (0.11 mg/kg), chrysene (0.41 mg/kg), fluoranthene (0.92 mg/kg) and fluorene (13 mg/kg) (Table 1.3). As well, 2-methylnaphthalene, acenaphthene, phenanthrene, anthracene, pyrene, benz[a]anthracene, chrysene, fluoranthene and fluorene were present in quantities exceeding Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME, 2001).

The two samples collected in September 2000 at the east lagoon showed similar types of contamination, but these levels were lower than those collected in July 2000 in the same area. Like the July sample, the first sample collected in September from the east lagoon was composed of drift algae and soil (sample 2, Table 1.3), whereas a second sample (sample 3, Table 1.3) was primarily marine sediment. Sample 2, the drift algae, showed extremely high TPH (21000mg/kg) levels, primarily from elevated levels of fuel (17000 mg/kg) and lube (4100 mg/kg) range hydrocarbons. PAHs phenanthrene (0.3 mg/kg), pyrene (0.6 mg/kg), acenaphthene (0.4 mg/kg) and fluorene (0.7 mg/kg) were also detected in elevated quantities, all at levels that exceeded Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME, 2001). Sample 3, the marine sediment, also had high levels of TPH (17000 mg/kg) from fuel (11000 mg/kg) and lube (5700 mg/kg) range hydrocarbons, similar to levels found in the above drift algae. As well, levels of the PAHs acenaphthene (0.3 mg/kg), pyrene (0.4 mg/kg), and chrysene (0.3 mg/kg) were elevated (Table 1.3), exceeding Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME, 2001).

Sediment collected at the west seep (sample 5, Table 1.) showed elevated levels of gas (3.5 mg/kg), fuel (520 mg/kg) and lube (64 mg/kg) range hydrocarbons, for a TPH level of 590 mg/kg. The only detectable PAHs were 1-, and 2-methylnaphthalene, which were both present at levels of 0.09 mg/kg, and acenaphthene and phenanthrene, which were also present in measurable quantities (0.27 and 0.11 mg/kg, respectively) (Table 1.3). 2-Methylnaphthalene, acenaphthene and phenanthrene levels exceeded Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME, 2001).

The east seep appeared to be the most contaminated of the remaining September 2000 sediment samples. Elevated levels of ethylbenzene (0.086 mg/kg), xylene (0.196 mg/kg), gas (8.9 mg/kg), fuel (11000 mg/kg) and lube (830 mg/kg) range hydrocarbons were observed, for a TPH level of 12000 mg/kg. Other detectable PAHs were naphthalene (0.25 mg/kg), 1-methylnaphthalene (1.9 mg/kg), 2-methylnaphthalene (2.0 mg/kg), acenaphthylene (0.46 mg/kg), acenaphthene (2.5 mg/kg), fluorene (1.7 mg/kg), phenanthrene (0.71 mg/kg), and pyrene (0.27 mg/kg) (Table 1.3). Naphthalene, 2-methylnaphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, and pyrene levels exceeded Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME, 2001).

Littorina periwinkles collected in October 2000 from the east beach (sample 2, Table 1.4) did not have detectable limits of fuel or lube range hydrocarbons. These results complemented sediment analysis from one month earlier. Specimens from the west beach (sample 1, Table 1.4) showed elevated levels of fuel (32 mg/kg) and lube (17 mg/kg) range hydrocarbons, obviously higher than those at the comparison beach, but still lower in magnitude than samples from inside the berm (Table 1.4).

Periwinkles from west lagoon in October 2000 (sample 5, Table 1.4) showed fuel (200 mg/kg) and lube (91 mg/kg) range hydrocarbon levels much higher than those found in other parts of the lagoon at that time. Periwinkles from east lagoon also showed elevated levels of fuel (100 mg/kg) and lube (38 mg/kg) range hydrocarbons (sample 3, Table 1.4).

Periwinkles collected in October 2000 at the west seep (sample 6, Table 4) and east seep (sample 4, Table 1.4) also had elevated levels of fuel (82 and 130 mg/kg, respectively) and lube (31 and 40 mg/kg, respectively) range hydrocarbons. As with sediment samples, periwinkles collected from the east seep had the highest contamination levels of both seeps (Table 1.4).

Periwinkles collected in November 2000 from comparison beaches (Norris Point Beach and Gull Rock Lookout) had no detectable limits of hydrocarbons, with the exception of low (21 mg/kg) levels of lube range hydrocarbons at Norris Point Beach. Analysis of periwinkles from west beach showed elevated levels of fuel (27 mg/kg) and lube (19 mg/kg) range hydrocarbons levels (Table 1.5) which were nearly identical to samples collected one month earlier in the same location.

Sediment collected from east lagoon in November 2000 showed elevated levels of fuel (2600 mg/kg) and lube (360 mg/kg) range hydrocarbons, for a TPH of 2900 mg/kg (sample 3, Table 1.5). Hydrocarbon levels in sediment collected from the east seep, however, were still greater than an order of magnitude higher than sediment collected from east lagoon. This sediment, collected in duplicate, from the east seep had levels of fuel (86000 and 74000mg/kg) and lube (7200 and 6200 mg/kg) range hydrocarbons, for TPH levels of 93000 and 80000 mg/kg, higher than nearly all earlier samples. Xylene (1.41 and 0.91 mg/kg) and gas range hydrocarbons (73 and 58 mg/kg) levels were also elevated in these samples (sample 4, Table 1.5). Analysis of clay from outside the berm

showed elevated limits of lube (29 mg/kg) range hydrocarbons only, for total levels of TPH of 45 mg/kg (sample 2, Table 1.5).

Periwinkles collected during the final sampling period, September 2001, from west and east beaches did not have detectable levels of hydrocarbons. However, those collected at the east seep still had elevated levels of petroleum hydrocarbons present, with fuel and lube range hydrocarbon loading of 110 and 57 mg/kg, respectively (Table 1.6). *Littorina littorea* were continually collected from three areas throughout the study: the east beach, the west beach and the area known as the east seep. These areas repeatedly showed non-detectable, decreasing and steady levels of hydrocarbon contamination, respectively.

Sediment samples collected from east portion in September 2001 had elevated levels of petroleum hydrocarbons, with primarily fuel (9200, 2600 and 2800 mg/kg) and lube (2200, 570, and 640 mg/kg) range hydrocarbons elevated, for TPH values of 11000, 3200 and 3500 mg/kg (Table 1.6).

Finally, sediment collected at the west and east seeps in September 2001 still showed extremely high levels of fuel (550 and 6900 mg/kg) and lube (510 and 690 mg/kg) range hydrocarbons, though the east seep remained much greater. The east seep also had detectable levels of xylene (0.172 mg/kg) (Table 1.6). These final sediment tests confirm that the east seep is the main source of leaching hydrocarbons.

1.3.3 *Environmental Data*

During summer 2001, at the time of algal sample collection, the surface layer inside the berm had very low salinity (Table 1.8). Salinity profiles taken during the summer showed a range of 3.1 to 4.9 salinity units (su) at the surface for the entire lagoon area (Table 1.8). Salinity profiles taken at comparison beaches show that coastlines in the area have the typical range of salinities, at about 29 salinity units. Comparison site 8, which was adjacent to a fresh water stream running across Norris Cove beach, had a dramatically lower salinity (Table 1.8).

Throughout the summer, temperatures within the lagoon showed faster fluctuations and a wider range of temperatures than outside the lagoon. At the time of sample collection, temperatures inside the lagoon were about 4°C warmer than comparison beaches (Table 1.8).

1.3.4 *Algal Sampling*

Thirteen genera of diatoms and other algae were identified from eleven samples collected: seven samples from within the lagoon and four samples from comparison beaches (Table 1.7). Within the lagoon the species that was found at most of the sites was the fucoid *Ascophyllum nodosum* (Le Jolis, 1863). This alga was found at four of seven sample locations within the lagoon. At comparison beaches *Ascophyllum nodosum* (Le Jolis, 1863) and *Fucus vesiculosus* (Linnaeus, 1753) were the most prevalent species, having been found at all four sampling locations (Table 1.7).

Ascophyllum nodosum was found at four sampling locations within the lagoon and four sampling locations at comparison beaches. At two locations (locations 2 and 6, Table 1.7) within the lagoon, only the holdfasts of *A. nodosum* remained, the rest of the algae having been cut off during clean-up procedures after the oil spill (Hooper, pers. comm.). The two remaining locations (locations 3 and 5, Table 1.7) were *A. nodosum* beds that were transplanted during the summer of 2000 as a part of a phytoremediation experiment. So in fact, while *A. nodosum* was the alga found most often at sampling locations, healthy, naturally occuring rockweeds were not observed inside the lagoon.

At both comparison beaches *Ascophyllum nodosum* was the predominant alga, with much smaller amounts of the fucoid *Fucus vesiculosus* present. *Fucus* was not found within the lagoon (Table 1.7) at the time of sampling, though it had been present prior to clean-up (Hooper, pers. comm.).

Only one other algal species was observed at a comparison location. *Enteromorpha intestinalis* was found at site 8, which bordered a freshwater stream running along Norris Cove beach. *Enteromorpha intestinalis* was found at three locations within the lagoon (Table 1.7). An unidentified species of *Enteromorpha* was also found within the algal mat inside the lagoon. This species was "abnormal and not easy to assign to species" (Hooper, pers. comm.).

Chaetomorpha capillaries, despite being found at only two locations (Table 1.7), covered an extensive area within the lagoon. The two locations where it was found were large,

bright green algal mats covering several square metres inside the lagoon. This was also true for the diatoms *Navicula* spp. and *Nitzschia* spp. While only found at three of the seven sampling locations within the lagoon, these two freshwater diatoms were found to be part on an extensive diatom-based blanket of brown slime covering the west and upper east sections of the lagoon (Table 1.7). *Chaetomorpha capillaris, Navicula* spp. and *Nitzschia* spp. were not found at any reference location (Table 1.7).

The rhodophyte *Hildenbrandia* and the diatom *Melosira,* common freshwater genera, were both found at three locations within the lagoon (Table 1.7). The chlorophyte *Ulothrix* was found at two locations within the lagoon (Table 1.7). These three genera were not found at comparison sites (Table 1.7).

1.4 Discussion

1.4.1. *Hydrocarbon Content Analysis*

Diesel oil has extensively contaminated subtidal and intertidal organisms and sediments within the immediate area of the spill. Contamination is indicated by consistently high petroleum hydrocarbon levels in biota and sediment sampled from within area, as compared to their absence from comparison locations. Hydrocarbon content analysis of biota samples indicates very little change in contamination levels over the two years of sampling.

Littorina littorea were sampled throughout the study, as it was the only intertidal animal that survived past the initial sampling period. Nelson-Smith (1972) describes several

studies where the survival of *L. littorea* in response to an oil spill was observed to be much greater than that of other intertidal invertebrates, including other littorinids, limpets and dogwelks.

Mussels in the area of the spill survived the initial oiling with very little mortality, and continued to survive throughout 1999. As 2000 progressed, however, the number of mussels dying increased until they were completely wiped out of the immediate area, making it impossible to sample them continuously throughout this study (Hooper *et al.*, 2001).

Hydrocarbon levels found in mussels after the Gros Morne diesel oil spill appear to be related to proximity to the oiled beach and the rock berm, a trend also observed after the Exxon Valdez spill (Short and Babcock, 1996). This trend was also noted in contamination levels in the periwinkle *Littorina littorea* collected at increasing distances from the spill. These organisms showed overall decreasing, though somewhat variable, contamination levels farther from the spill.

A. nodosum were not found to be contaminated outside the berm. Because these samples were collected from the subtidal zone outside the berm, and thus exposed to the action of waves, little or no hydrocarbon contamination was expected. Furthermore, fucoids are protected by a slimy covering that likely prevents the adhesion of oil (Notini, 1978; Nelson-Smith, 1974), making it difficult to incorporate or bioaccumulate hydrocarbons into tissues. Elevated hydrocarbon levels associated with rockweeds inside the berm were

likely due to constant exposure to fresh diesel fuel leaching from the roadbed. This diesel oil covered the rockweeds with receding tides and was not washed off due to the reduced wave action associated with the berm.

Littorina littorea that were extensively sampled more than one year after the initial spill still showed levels of contamination an order of magnitude greater than those from comparison beaches. Since diesel oil appeared to be leaching directly into only two of the sample areas, the high levels of hydrocarbons suggest transport by water or wind from the two main seeps within the lagoon, pooling at the ends of the lagoon, contaminating the periwinkles throughout the area. The presence of large quantities of drift algae, especially at the ends of the lagoon, supports the theory that wind or water transport is a factor in the movement of materials in the lagoon.

Previous studies (Amodio-Cocchiere and Cirillo, 2003; Law *et al.*, 2002; Baumard *et al.*, 1999; Glegg *et al.*, 1999; Cripps and Shears, 1997; Lee and Page, 1997 Short and Babcock, 1996) have primarily focused on specific hydrocarbons, making it impossible to make direct comparisons to the Total Petroleum Hydrocarbon (TPH) data obtained in this study. Instead, TPH levels at affected sites were compared to remote, or comparison, sites. At all affected sites during the sampling period of two years, hydrocarbon contamination in periwinkles was generally an order of magnitude greater when compared to contamination at the comparison beaches, with the exception Norris Point beach. Contamination at Norris Point beach was likely due to the amount of boat traffic in the area, as the public wharf, which receives moderate amounts of marine traffic, is

perpendicular to Norris Point beach. This agrees with Wang *et al.* (2001), who found that PAHs in coastal sediments increased in concentration adjacent to higher traffic areas.

Diesel oil contaminated sediment samples show hydrocarbon patterns clearly different from pristine samples. In general, sediment sampled from affected areas up to two years after the spill contained elevated petroleum hydrocarbon levels, indicating that considerable quantities of diesel oil were still present in the lagoon two years after the spill had occurred. Pools of diesel oil were observed to surface from disturbed sediments and an unmistakable oily odor was present throughout the study.

Hydrocarbon contamination was usually highest at the east end of the lagoon and at the east seep throughout this study. Since there was not any indication that diesel was leaching directly into the east end of the lagoon, it is believed that hydrocarbons present at this sampling location originated from the two obvious seeps within the lagoon, and were washed or blown to the eastern end of the lagoon. As such, the east seep was considered the main source of leaching diesel oil within the lagoon for future experiments.

Sediment sampled outside the berm and lagoon showed a pattern of contamination due to differences associated with the heterogeneous distribution of contaminants in various sediment types. Samples showing higher levels of hydrocarbons were primarily composed of clay associated with berm construction, as opposed to the natural seabed sediment. This is similar to the pattern reported by Pastor *et al.* (2001), who found elevated contamination levels in the muddier sediments one year after a spill. Since no

diesel was observed outside the berm after construction, this clay likely adsorbed hydrocarbons at the surface during berm construction, as suggested by La Rocca *et al.* (1996), and subsequently settled to the seabed outside the berm

Analysis indicates less weathering of petroleum had occurred inside the berm than outside the berm. This suggests that hydrocarbons in sediments inside the berm were more recent, originating from unweathered oil leaching from under the roadbed. In contrast, hydrocarbons in sediments outside the berm were in place since berm construction, and were therefore subjected to longer exposure.

The sediment samples collected a year after the spill, which were tested both for specific polycyclic aromatic hydrocarbons (PAHs) and TPH levels, showed elevated TPH levels from all areas of the lagoon when compared to comparison beaches. Sediment collected from the east end of the lagoon, as well as both seeps, also had levels of individual PAHs that exceeded acceptable limits set by the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME, 2001). The presence of light aliphatics and aromatics, such as alkyl-naphthalenes, a year after the spill suggests that the oil had only entered the marine environment recently, as naphthalene in sediments is easily depleted by volatilization from oil, dissolution from sediments and bacterial degradation in a brief amount of time (Ke *et al.*, 2002). A similar study (Delille and Pelletier 2002) showed that when diesel oil was spilled, but remained trapped below the surface, resurfacing hundreds of meters from the source, the original distribution of hydrocarbons was well preserved.

1.4.2. *Environmental Data*

An above average snowfall during the winter 2000-2001 led to greater than normal freshwater input into the lagoon, giving a freshwater surface layer more than a metre deep in some locations (Hooper *et al.*, 2001). Salinity profiles of comparison beaches, however, show the typical range of salinities for Bonne Bay (Hooper, pers. comm.), except for site 8 at Norris Cove beach, which was adjacent to a fresh water stream.

1.4.3. *Algae*

In the present study, intertidal algae populations were primarily affected by post-spill procedures. Intensive clean-up efforts followed the diesel spill in Gros Morne National Park in August 1999, and focused initially on the manual removal of most of the diesel-contaminated intertidal fucoid algae (Hooper *et al.*, 2001).

Ascophyllum nodosum, and to a much lesser extent *Fucus vesiculosis,* were common seaweeds at the oil spill site before clean-up procedures began (Hooper *et al.*, 2001), but were present inside the lagoon at the time of sampling as either remnants of the clean-up procedures (variable sized holdfasts attached to rocks (*A. nodosum*)), in large transplanted patches that were necrotic and discolored (*A. nodosum*), or were completely absent (*F. vesiculosis*). As a part of the clean-up, these algae had been cut off near the bases or plucked from the rocks (pers. obs.), presumably because they were covered in oil and assumed by clean-up crews to be dead or damaged beyond recovery (Hooper *et al.*, 2001). Fucoids, however, are surprisingly hardy and resistant to oil-related toxicity, likely due a mucilage layer that prevents adhesion of oil (Notini, 1978; Nelson-Smith, 1974).

Necrosis and discoloration of transplanted patches is believed to be a result of low salinity levels found inside the lagoon, as supported by Kirst (1989) who found striking changes in morphology occurred in marine algae growing in low salinity waters, primarily as a result of osmotic and ionic stress.

During the present study, *Littorina littorea* populations appeared to be relatively resistant to the diesel oil. Populations were reduced, but not eliminated. The periwinkles *L. saxatalis* and *L. littorea*, however, were completely eliminated and had not recolonized the lagoon three years after the spill. The absence of these two grazers has allowed massive algal mats composed primarily of *Chaetomorpha, Oscillatoria, Capsosiphon, Phormidium, Spirulina, Navicula, Nitzschia*, and other genera, to flourish (Hooper *et al.*, 2001), halting succession by preventing the appearance of later successional species like the fucoids *A. nodosum* and *F. vesiculosis* (Lubchenco, 1983).

The absence of fucoids, due to removal or halted succession, affects the survival and recruitment of other intertidal algae (van Temelen, 1997) and invertebrates (Vogelaere and Foster, 1994), which appeared to be a factor at the present site. Barnacles, limpets and amphipods had not recolonized the lagoon up to three years after the spill (pers. obs.) likely due, at least in part, to lack of suitable habitat.

"Growth and distribution of marine algae are primarily controlled by light, temperature, nutrients, water movement and salinity" (Kirst, 1989). Construction of the berm, though necessary to prevent the spreading of oil, interfered with most of these factors.

Temperatures inside the lagoon fluctuated more rapidly and to a larger degree than comparison beaches; flushing of the lagoon was reduced due to slumping of the berm; surface salinity was a degree of magnitude lower than comparison beaches and nutrient levels were low for all of the afore-mentioned reasons (Hooper *et al.*, 2001). This finding is in agreement with Kamer *et al.* (2000), who reported that physical modifications resulting in reduced tidal flow and circulation, and therefore mixing, could have prolonged adverse effects on estuarine organisms. The berm was initially very effective and flushing rates were adequate inside the lagoon to allow some regeneration of algae from holdfasts (pers. obs.); however this effect was short-lived. This was reflected in the algae taxa that were found within the lagoon. *Enteromorpha, Capsosiphon, Hildenbrandia, Melosira, Navicula, Nitzschia* and others found within the lagoon are predominantly freshwater or low salinity taxa (Wehr and Sheath, 2003).

1.5 Summary

Upon completion of the preliminary assessment of the diesel oil spill site in Gros Morne National Park using hydrocarbon content analysis of sediments and biota, as well as an algal survey, the following can be concluded: (1) Hydrocarbon content analysis of sediment collected from the diesel oil spill site and surrounding areas indicates that contamination was localized, (2) Hydrocarbon content analysis of sediment collected inside the berm almost a year after the spill revealed levels exceeding Canadian Sediment Quality Guidelines for the Protection of Aquatic Life, (3) Hydrocarbon content analysis of sediment collected from the diesel oil spill site indicates that fresh diesel oil was present inside the berm at the site up to two years after the spill, whereas diesel quantities

outside the berm had decreased drastically, (4) Visual observations indicate diesel was not escaping through the berm, (5) Visual observations and hydrocarbon content analysis of sediment collected from inside the berm indicates that the primary source of leaching diesel oil is the area known as the east seep, (6) Hydrocarbon content analysis of biota from the diesel oil spill site indicates that organisms were impacted locally by the presence of diesel oil, as evidenced by the accumulation of hydrocarbons in tissues and dramatic population reductions, (7) Environmental data in the form of salinity and temperature monitoring show salinity of surface water is lowered and temperature fluctuations are common, (8) Algal survey data from inside the berm are indicative of an area stressed by an uncharacteristically low-salinity environment, (9) Algal survey data indicate algal succession inside the berm has been halted due to the absence of herbivores and, finally, (10) Further testing must be done to determine the viability of the site while it is contained by the rock berm, and determine the effects of combined stresses (diesel oil and low salinity) on nearshore communities.

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Table 1.1. Hydrocarbon levels in sediment samples collected in October 1999 and analyzed by Philip Analytical Services.

Location	TPH (C_6-C_{32}) mg/kg	TEH (C_{10}-C_{32}) (mg/kg)	Benzene mg/kg	Toluene mg/kg	Ethyl-benzene mg/kg	Xylene mg/kg	C_6-C_{10} mg/kg	>C_{10}-C_{21} mg/kg	>C_{21}-C_{32} mg/kg
EQL (mg/kg)	32.5	30	0.025	0.025	0.025	0.050	2.5	15.0	15.0
Inside berm	**5090**	**5090**	nd	nd	nd	nd	nd	**4790**	**305**
Base berm/clay	89	89	nd	nd	nd	nd	nd	**60.6**	**28.4**
Base berm/sed	nd	nd	nd	nd	nd	nd	nd	nd	nd
25m/clay	**99.4**	**99.4**	nd	nd	nd	nd	nd	**61.9**	**37.6**
25m/sed	nd	nd	nd	nd	nd	nd	nd	nd	nd
100m	nd	nd	nd	nd	nd	nd	nd	nd	nd
200m	nd	nd	nd	nd	nd	nd	nd	nd	nd
300m	nd	nd	nd	nd	nd	nd	nd	nd	nd
400m	nd	nd	nd	nd	nd	nd	nd	nd	nd
500m	nd	nd	nd	nd	nd	nd	nd	nd	nd
Norris Pt. Beach	nd	nd	nd	nd	nd	nd	nd	nd	24.6

Notes: EQL = Estimated Quantitation Limit for routine analysis

nd = not detected above standard EQL

C_6-C_{10} = Gas Range Hydrocarbons; >C_{10}-C_{21} = Fuel Range Hydrocarbons; >C_{21}-C_{32} = Lube Range Hydrocarbons; TPH = Total Petroleum Hydrocarbons (C_6-C_{32}, less BTEX).

Sediment results are expressed on a dry weight basis.

Table 1.2. A. Hydrocarbon levels in *Mytilus edulis* samples collected in November 1999 and analyzed by Philip Analytical Services.

	TEH (C_{10}-C_{32}) mg/kg	>C_{10}-C_{21} mg/kg	>C_{21}-C_{32} mg/kg
EQL	30	15	15
Gull Rock Lookout	nd	nd	nd
Inside Berm	**574**	**529**	**44.8**
Berm Base (0 m)	**97**	**97**	nd
100 m	**97**	**97**	nd
200 m	**141**	**119**	**22.1**
300 m	**87.8**	**87.8**	nd
400 m	nd	**19.6**	nd
500 m	nd	nd	nd

Notes: EQL = Estimated Quantitation Limit for routine analysis

nd = not detected above standard EQL

nd () = not detected at the elevated EQL shown in parentheses

C_6-C_{10} = Gas Range Hydrocarbons; >C_{10}-C_{21} = Fuel Range Hydrocarbons; >C_{21}-C_{32} = Lube Range Hydrocarbons; TEH = Total Extractable Hydrocarbons

Biota results are expressed on a wet weight basis

Table 1.2. B. *Littorina littorea*.

	TEH (C_{10}-C_{32}) mg/kg	>C_{10}-C_{21} mg/kg	>C_{21}-C_{32} mg/kg
EQL	30	15	15
Gull Rock Lookout	nd	nd (30)	nd (30)
Inside Berm	**586**	**511**	**74.3**
Berm Base (0 m)	**115**	**115**	nd (30)
100 m	**44.2**	**44.2**	nd
200 m	nd	nd (40)	nd (40)
300 m	**144**	**97.7**	**46.6**
400 m	nd	nd (80)	nd (80)
500 m	nd	nd (60)	nd (60)

Table 1.2. C. *Ascophyllum nodosum.*

	TEH (C_{10}-C_{32}) mg/kg	**>C_{10}-C_{21} mg/kg**	**>C_{21}-C_{32} mg/kg**
EQL	30	15	15
Gull Rock Lookout	nd	nd	nd
Inside Berm	**179**	**179**	nd (40)
	178	**144**	**33.6**
	175	**143**	**31.6**
Berm Base (0 m)	nd	nd	nd
100 m	nd	nd	nd
200 m	nd	nd	nd
300 m	nd	nd (30)	nd (30)
400 m	nd	nd	nd
500 m	nd	nd (20)	nd (20)

Table 1.3. Hydrocarbon levels in sediment samples collected in July and September 2000 and analyzed by Philip Analytical Services.

Hydrocarbon Tested	EQL	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
TPH (mg/kg)	32	98000	21000	17000	12000	590	nd
Benzene (mg/kg)	0.025	nd	nd	nd	nd	nd	nd
Toluene (mg/kg)	0.025	0.970	nd	nd	nd	nd	nd
Ethylbenzene (mg/kg)	0.025	nd	nd	nd	0.086	nd	nd
Xylene (mg/kg)	0.050	nd	nd	nd	0.196	nd	nd
C_6-C_{10} (less BTEX) (mg/kg)	2.5	4.3	nd	nd	8.9	3.5	nd
>C_{10} – C_{21} (mg/kg)	15	89000	17000	11000	11000	520	nd
>C_{21} – C_{32} (mg/kg)	15	8800	4100	5700	830	64	nd
Naphthalene (mg/kg)	0.05	nd	nd	nd	**0.25**	nd	nd
2-Methylnaphthalene (mg/kg)	0.05	**0.17**	nd	nd	**2.0**	**0.09**	nd
1- Methylnaphthalene (mg/kg)	0.05	0.15	nd	nd	1.9	0.09	nd
Acenaphthylene (mg/kg)	0.05	**2.3**	nd	nd	**0.46**	nd	nd
Acenaphthene (mg/kg)	0.05	**15.0**	**0.4**	**0.3**	**2.5**	**0.27**	nd
Fluorene (mg/kg)	0.05	**13**	**0.7**	nd	**1.7**	nd	nd
Phenanthrene (mg/kg)	0.05	**5.9**	**0.3**	nd	**0.71**	**0.11**	nd
Anthracene (mg/kg)	0.05	**0.50**	nd	nd	nd	nd	nd
Fluoranthene (mg/kg)	0.05	**0.92**	nd	nd	nd	nd	nd
Pyrene (mg/kg)	0.05	**2.3**	**0.6**	**0.4**	**0.27**	nd	nd
Benz[a]anthracene (mg/kg)	0.05	**0.11**	nd	nd	nd	nd	nd
Chrysene (mg/kg)	0.05	**0.41**	nd	**0.3**	nd	nd	nd
Benzo[b]fluoranthene (mg/kg)	0.05	nd	nd	nd	nd	nd	nd
Benzo[k]fluoranthene (mg/kg)	0.05	nd	nd	nd	nd	nd	nd
Benzo[a]pyrene (mg/kg)	0.05	nd	nd	nd	nd	nd	nd
Perylene (mg/kg)	0.06	nd	nd	nd	nd	nd	nd
Indeno[1,2,3-cd]pyrene (mg/kg)	0.05	nd	nd	nd	nd	nd	nd
Dibenz[a,h]anthracene (mg/kg)	0.05	nd	nd	nd	nd	nd	nd
Benzo[ghi]perylene (mg/kg)	0.05	nd	nd	nd	nd	nd	nd

Sample descriptions:

- Sample 1: collected July 10, 2000; soil, drift algal mixture, HW neap level, east lagoon.
- Sample 2: collected September 19, 2000; same as sample 1.
- Sample 3: collected September 19, 2000; low water level, east lagoon.
- Sample 4: collected September 19, 2000; mean tide level, east seep.
- Sample 5: collected September 19, 2000; mean tide level, west seep.
- Sample 6: collected September 19, 2000; mean tide level, beach east of the berm.

Notes: EQL = Estimated Quantitation Limit for routine analysis.
nd = not detected above standard EQL.
C_6-C_{10} = Gas Range Hydrocarbons; >C_{10}-C_{21} = Fuel Range Hydrocarbons; >C_{21}-C_{32} = Lube Range Hydrocarbons; TPH = Total Petroleum Hydrocarbons (C_6-C_{32}, less BTEX).
Sediment results are based on a wet weight basis.

Table 1.4. Hydrocarbon levels in *Littorina littorea* samples collected in October 2000 and analyzed by Philip Analytical Services.

Hydrocarbon Tested	EQL	Sample 1	Sample 2	Sample 2 (duplicate)	Sample 3	Sample 4	Sample 5	Sample 6
TEH (mg/kg)	30	**49**	nd	nd	**138**	**170**	**291**	**113**
$>C_{10} - C_{21}$ (mg/kg)	15	**32**	nd	nd	**100**	**130**	**200**	**82**
$>C_{21} - C_{32}$ (mg/kg)	15	**17**	nd	nd	**38**	**40**	**91**	**31**

Sample descriptions:

- Sample 1: beach west of the berm, outside.
- Sample 2: beach east of berm, outside.
- Sample 3: east lagoon.
- Sample 4: east seep.
- Sample 5: west lagoon.
- Sample 6: west seep.

Notes: EQL = Estimated Quantitation Limit for routine analysis.
nd = not detected above standard EQL.
$>C_{10}$-C_{21} = Fuel Range Hydrocarbons; $>C_{21}$-C_{32} = Lube Range Hydrocarbons;
TEH = Total Extractable Hydrocarbons.
Biota results are expressed on a wet weight basis.

Table 1.5. Hydrocarbon levels in sediment and biota samples collected in November 2000 and analyzed by Philip Analytical Services.

Hydrocarbon Tested	EQL	Sample 1	Sample 2	Sample 3	Sample 4	Sample 4 (duplicate)	Sample 5	Sample 6
TPH (mg/kg)	32	-	**45**	**2900**	**93000**	**80000**	-	-
TEH (mg/kg)	30	nd	**44.6**	-	-	-	**46**	nd
Benzene (mg/kg)	0.025	-	nd	nd	nd	nd	-	-
Toluene (mg/kg)	0.025	-	nd	nd	nd	nd	-	-
Ethylbenzene (mg/kg)	0.025	-	nd	nd	nd	nd	-	-
Xylene (mg/kg)	0.050	-	nd	nd	**1.41**	**0.91**	-	-
C_6-C_{10} (less BTEX) (mg/kg)	2.5	-	nd	nd	**73**	**58**	-	-
>C_{10} – C_{21} (mg/kg)	15	nd	nd	**2600**	**86000**	**74000**	**27**	nd
>C_{21} – C_{32} (mg/kg)	15	nd	**29**	**360**	**7200**	**6200**	**19**	**21**

Sample descriptions:

- Sample 1: *L. littorea* periwinkles; Gull Rock Lookout.
- Sample 2: sediment; clay material off berm.
- Sample 3: sediment; east lagoon.
- Sample 4: sediment; east seep.
- Sample 5: *L. littorea* periwinkles; beach outside berm to the west.
- Sample 6: *L. littorea* periwinkles; Norris Point Beach.

Notes: EQL = Estimated Quantitation Limit for routine analysis.
nd = not detected above standard EQL.
- = parameter not requested in this sample.
C_6-C_{10} = Gas Range Hydrocarbons; >C_{10}-C_{21} = Fuel Range Hydrocarbons; >C_{21}-C_{32} = Lube Range Hydrocarbons; TPH = Total Petroleum Hydrocarbons (C_6-C_{32}, less BTEX); TEH = Total Extractable Hydrocarbons.

Table 1.6. Hydrocarbon levels in sediment and biota samples collected in September 2001 and analyzed by Philip Analytical Services.

HC Tested	EQL	Sample 1	Sample 2	Sample 2 (duplicate)	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sampl 9
TPH (mg/kg)	32	**11000**	**3200**	**3500**	nd	**1100**	**7600**	-	-	-	nd
TEH (mg/kg)	30	-	-	-	-	-	-	**167**	nd	nd	-
Benzene (mg/kg)	0.025	nd	nd	nd	nd	nd	nd	-	-	-	nd
Toluene (mg/kg)	0.025	nd	nd	nd	nd	nd	nd	-	-	-	nd
Ethyl-benzene (mg/kg)	0.025	nd	nd	nd	nd	nd	nd	-	-	-	nd
Xylene (mg/kg)	0.050	nd	nd	nd	nd	nd	**0.172**	-	-	-	nd
C_6-C_{10} (less BTEX) (mg/kg)	2.5	nd	nd	nd	nd	nd	nd	-	-	-	nd
>C_{10} – C_{21} (mg/kg)	15	**9200**	**2600**	**2800**	nd	**550**	**6900**	**110**	nd	nd	nd
>C_{21} – C_{32} (mg/kg)	15	**2200**	**570**	**640**	nd	**510**	**690**	**57**	nd	nd	nd

Sample descriptions:

- Sample 1: sediment; neap high tide level at the east end of the lagoon.
- Sample 2: sediment; low neap level at the east end of the lagoon.
- Sample 3: sediment; low neap level at the west end of the lagoon.
- Sample 4: sediment; west seep.
- Sample 5: sediment; east seep.
- Sample 6: *L.littorea* periwinkles; east seep.
- Sample 7: *L.littorea* periwinkles; beach outside west end of the berm.
- Sample 8: *L.littorea* periwinkles; beach outside east end of the berm.
- Sample 9: sediment; beach outside east end of the berm.

Notes: EQL = Estimated Quantitation Limit for routine analysis.
nd = not detected above standard EQL.
- = parameter not requested in this sample.
C_6-C_{10} = Gas Range Hydrocarbons; >C_{10}-C_{21} = Fuel Range Hydrocarbons; >C_{21}-C_{32} = Lube Range Hydrocarbons; TPH = Total Petroleum Hydrocarbons (C_6-C_{32}, less BTEX); TEH = Total Extractable Hydrocarbons.

Table 1.7. Algal taxa found at each sample location within the lagoon at the oil spill site and at comparison beaches in Bonne Bay, Newfoundland and Labrador.

Algae	**Lagoon sampling locations**							**Comparison locations**			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Chaetomorpha	✔	-	-	-	✔	-	-	-	-	-	-
Oscillatoria	✔	✔	-	-	✔	-	-	-	-	-	-
Phormidium	-	✔	-	-	✔	-	-	-	-	-	-
Spirulina	-	✔	-	-	-	-	-	-	-	-	-
Anabaena	-	✔	-	-	-	-	-	-	-	-	-
Melosira	✔	-	-	✔	-	✔	-	-	-	-	-
Enteromorpha	-	✔	-	✔	-	✔	-	✔	-	-	-
Ulothrix	-	-	-	-	-	✔	✔	-	-	-	-
Navicula	✔	✔	-	✔	-	-	-	-	-	-	-
Nitzschia	✔	✔	-	✔	-	-	-	-	-	-	-
Hildenbrandia	✔	✔	-	-	-	-	✔	-	-	-	-
Ascophyllum nodosum	-	✔	✔	-	✔	✔	-	✔	✔	✔	✔
Fucus vesiculosus	-	-	-	-	-		-	✔	✔	✔	✔

✔ - algae is present; - - algae is not present

Table 1.8. Site locations and environmental data within the lagoon at the oil spill site and at comparison beaches summer 2001 in Bonne Bay, Newfoundland and Labrador.

Site	GPS Co-ordinates	Salinity (su)	Temperature (°C)
Lagoon			
1	N 49° 28.972, W 57° 44.299	4.9	20.3
2	N 49° 28.970, W 57° 44.276	3.6	20.2
3	N 49° 28.966, W 57° 44.265	4.5	20.1
4	N 49° 28.964, W 57° 44.241	3.9	20.1
5	N 49° 28.962, W 57° 44.235	4.2	20.2
6	N 49° 28.955, W 57° 44.202	3.1	20.5
7	N 49° 28.925, W 57° 44.178	4.6	20.4
Norris Cove			
8	N 49° 29.850, W 57° 50.367	4.9	15.1
9	N 49° 29.848, W 57° 50.360	29	17.0
Mike's Cove			
10	N 49° 29.063, W 57° 45.009	29.7	16.3
11	N 49° 29.059, W 57° 45.002	29.7	16.4

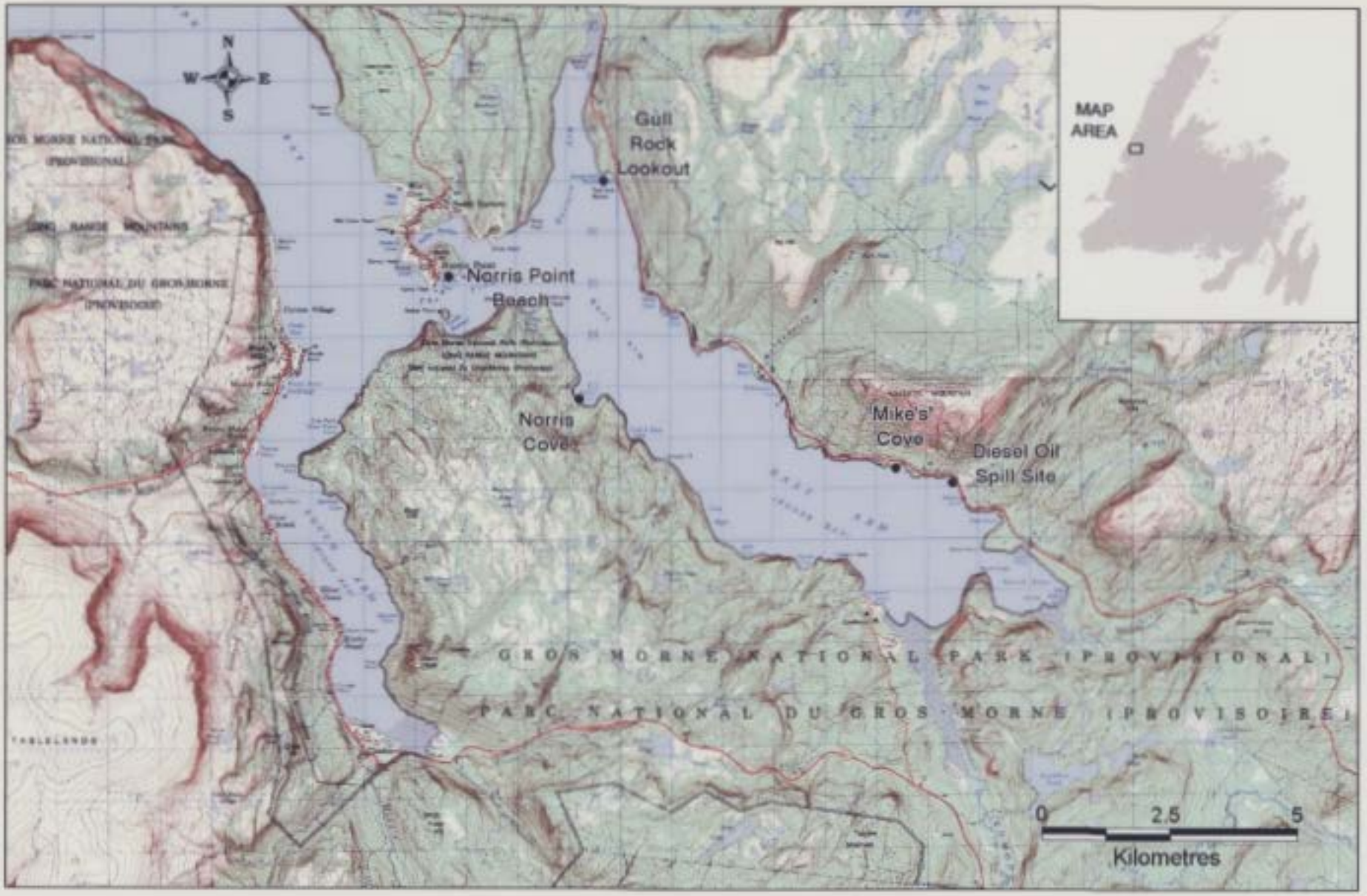

Figure 1.1. Bonne Bay location map showing the diesel oil spill site and comparison locations.

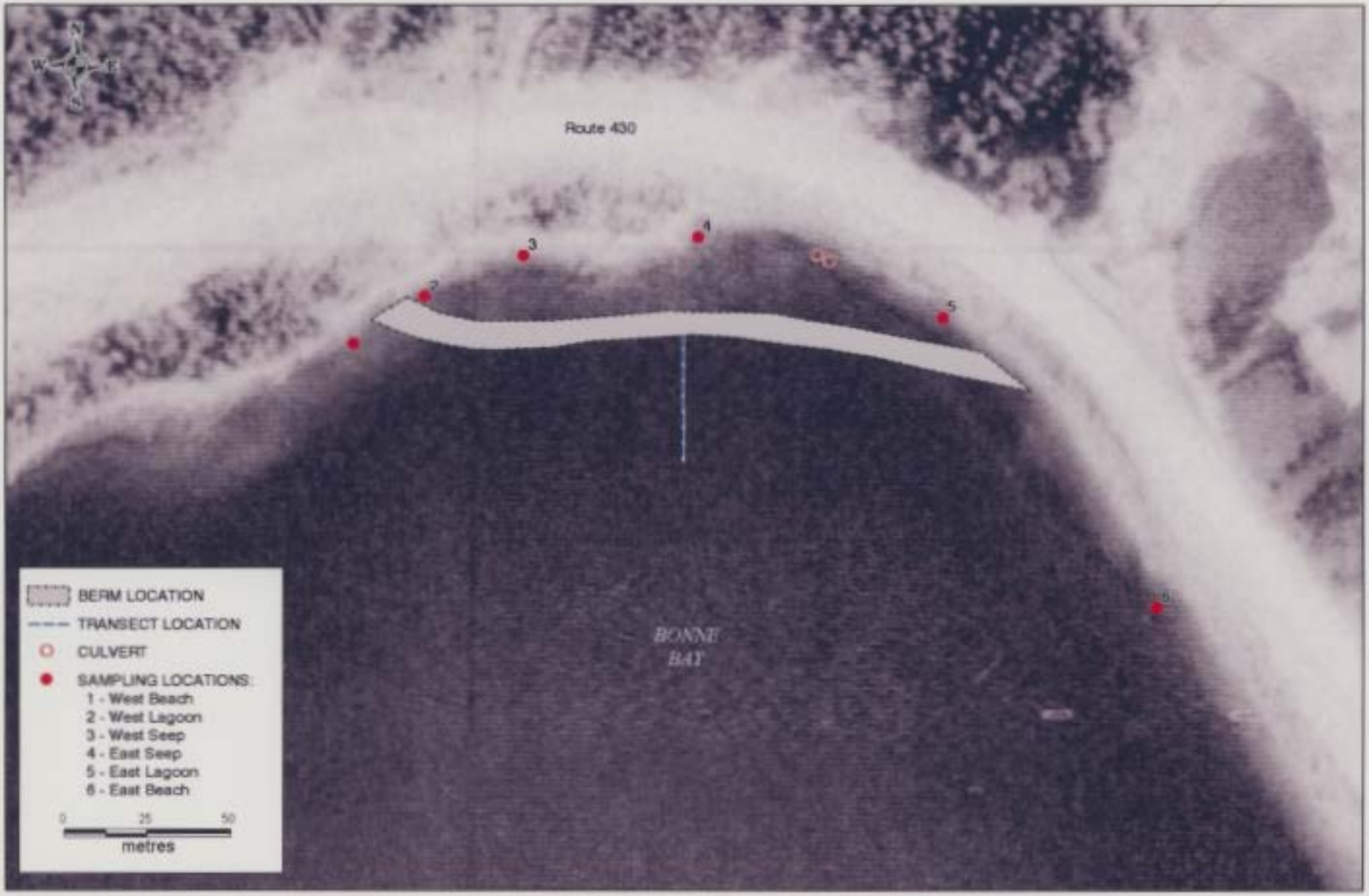

Figure 1.2. Diesel oil spill site in Bonne Bay, NL showing the location of the semi-permanent rock berm and sampling locations.

Chapter 2. Determination of the range of effects of hydrocarbon contamination and low-salinity conditions, using gradient analysis, at a diesel oil spill site.

2.1 Introduction

This chapter uses gradient analysis to examine the spatial and temporal extent of impacts due to a diesel oil spill and low-salinity conditions inside the berm at the diesel oil spill site in Gros Morne National Park. Previous studies (Chapter 1) focused on determining overall site conditions, both inside the berm and at nearby sites, through hydrocarbon content analysis and an algal survey. The previous study determined that the most significant source of leaching diesel was the east seep and that definite effects were observed due to diesel toxicity and lowered salinity. These results form the basis of the present experiment.

Diesel oil is chemically complex and changes over time with release to the environment. All petroleum products are highly complex mixtures that contain aromatics, aliphatics and variable molecular weight hydrocarbons (Brzorad and Burger, 1994). Diesel oil in particular consists mainly of saturated and aromatic hydrocarbons (Song, 2000). The high concentrations of aromatic hydrocarbons in diesel oils (Connell and Miller, 1981; Nelson-Smith, 1972) make it particularly toxic (Clark, 2001; Miller, 1982). Also, biodegradation in the first several months after a spill reduces the straight-chain hydrocarbon fraction, leaving the aromatic fraction intact. So, on a volume basis, the toxicity of weathered diesel oil can increase before the aromatics are degraded (Brzorad and Burger, 1994).

"Temperature and salinity are key environmental variables that rule estuarine organisms' life history" (Neuparth *et al.*, 2002), however few studies have addressed these affects in combination with other stress factors. Independently, changes in temperature or salinity can result in such widespread effects as reduced life span and altered metabolic activity in estuarine organisms (Neuparth *et al.*, 2002; Tedengren *et al.*, 1988). Two schools of thought exist as to the impact on organisms of altered environmental factors when in combination with other stress factors, for instance an oil spill. The first is that organisms with a wider tolerance to salinity changes, that is, estuarine organisms, will pre-adapt to tolerate other stresses, including pollution (Jernelov and Rosenberg, 1976). The contrary view is that organisms living near the limits of their tolerance range with respect to temperature and salinity, as estuarine organisms often are, are more susceptible to any added stress (McLusky *et al.*, 1986). The latter theory was supported by Tedengren and Kautsky (1987) in studies on diesel oil in combination with low salinity, as well as Tedengren *et al.* (1988) in studies on diesel oil and cadmium combined with salinity stress, and finally, McLusky *et al.* (1986), who studied the effects of temperature and salinity on the toxicity of heavy metals.

The Coastal Resource Coordinator's Bioassessment Manual suggests that chemical analyses are a crucial first step in site assessments, however on their own they offer little information on impacts to organisms. It is also suggested that, as a part of an impact evaluation, the bioavailability of contaminants must be tested; that it is not sufficient just to be aware of their presence (MacDonald *et al.*, 1997). In fact, Costa *et al.* (1998) stated that bioassays are the only way to determine the toxicity of contaminated sediments. A

bioassay based on *in situ* sediment toxicity using caged organisms, as will be used in this study, can determine the ecological effects due to the presence of a toxic substance (MacDonald *et al.*, 1997).

Contamination monitoring programs using sentinel organisms were developed to estimate the state of various polluted areas. Where sentinels are not available, caging technology is applicable. Monitoring contamination using caged organisms is a developing technology, but basically involves placing caged animals at various locations in the area to be monitored. The use of caged mussels as bioindicators of contaminants such as PAHs originated with a program called "Mussel Watch" (Piccardo *et al.*, 2001). Primarily used to monitor spatial and temporal contamination trends, this procedure has evolved as a widely-practiced monitoring technique, involving mussels (Piccardo *et al.*, 2001; Baumard *et al.*, 1999; Baumard *et al.*, 1998; Mersch *et al.*, 1996), as well as clams (Fukuyama *et al.*, 2000). MacDonald *et al.* (1997) states that the most commonly used marine and estuarine organisms for toxicity tests are amphipods, mysids and bivalves; however, it is ecologically relevant to use other locally available organisms.

Bioassays are widely used in the monitoring of effects of marine pollution, either through the use of bioaccumulation studies, community studies, toxicity tests, or other appropriate studies (MacDonald *et al.*, 1997). Regardless of the chosen test, it is important to design the most sensitive sampling methodology. Until recently, identifying pollution-induced changes has been based on a Before-After Control-Impact (BACI) sampling design, which was considered the most effective for detecting changes due to anthropogenic

disturbances. This design involved the random collection or placement of samples at control and impact locations (Ellis *et al.*, 2000; Ellis and Schneider, 1997; Underwood, 1994; Underwood, 1992). The evolution of sampling to detect environmental impacts has led away from BACI designs, towards what is now considered the most reliable method in the detection of anthropogenic disturbance - gradient designs. Gradient designs require collecting or placing samples according to distance, rather than random placement. These designs are considered more powerful than randomized sampling, especially in situations where the contaminant disperses with distance from a point source (Ellis *et al.*, 2000; Ellis and Schneider, 1997), as is the case with the diesel oil spill in Gros Morne National Park. Gradient designs have been used in a variety of situations, from detecting benthic effects related to oilfields in the North Sea (Gray *et al.*, 1990; Ellis and Schneider, 1997), to PAH, PCB and heavy metal contamination in Sydney Harbour, Nova Scotia (Zajdlik *et al.*, 2000) and the effects of a relatively small diesel oil spill on stream invertebrates (Lytle and Peckarsky, 2001).

Invertebrate communities form the foundation of marine ecosystems and are frequently subjected to stress from both oil pollution and environmental variables, especially in the intertidal region, which is exceptionally vulnerable to oil spills (Suchanek, 1993). Evaluating the effects of a complex mixture like diesel oil on the coastal environment requires information on the acute and chronic toxicities on several species representing different modes of life and habitat (Gulec *et al.*, 1997). Several species are recommended for the purposes of toxicity testing; however, locally available species often provide greater ecological relevance (MacDonald *et al.*, 1997).

Amphipods are ecologically important organisms, comprising a significant portion of aquatic biomass and diversity worldwide (Costa *et al.*, 1998). An enormous amount of work has been completed using amphipods for testing and monitoring of environmental stresses. Bulnheim (1984) studied the physiological responses of five amphipod species to a variety of environmental stresses, while others studied the effects of salinity stress (Steele and Steele, 1991), oil and dispersants (Gulec *et al.*, 1997) and toxic sediment (Costa *et al.*, 1998) on various amphipods. Amphipods are considered good bioindicators of the impacts due to oil spills mainly due to their sensitivity to the aromatic portion of oil (Gesteira and Dauvin, 2000). *Gammarus oceanicus*, often the most abundant marine littoral amphipod (Halcrow, 1981), is found on sheltered to slightly exposed rocky shores from the Gulf of Maine to Newfoundland (Steele, 1976; Steele and Steele, 1972). Nearly three decades ago, Linden (1976) studied the effects of oil on *G. oceanicus,* while more recently Aunaas *et al.* (1991) studied the effects of both oil and oil dispersants on *G. oceanicus.*

Mysids are an important part of estuaries, as producers and consumers, contributing significantly to the standing stock of omnivores in many estuaries (Roast *et al.*, 1998). The use of mysid shrimp has become widely accepted in toxicity testing and environmental monitoring, in fact, Nimmo and Hamaker (1982) stated "their utility as a model organism can be applied to evaluate the ecological impact of pollutants on larval crustaceans, particularly the commercially important species of shrimps, lobsters and crabs". Mysids are frequently used in laboratory studies, and in the past have been used

to determine the effects of trace metals (Roast *et al.*, 2000), petroleum hydrocarbons (Riebel and Percy, 1990), and salinity and cadmium toxicity (De Lisle and Roberts, 1988) on various species. As well, laboratory studies on the interactions of salinity, temperature and age on growth have provided much-needed baseline data on these important organisms (McKenney and Celestial, 1995). *Mysis stenolepis* is one of only four species of littoral mysids found in Atlantic estuaries (Dadswell, 1975), and very little information exists on this organism with respect to bioassays; however, Roast *et al.* (1998) promotes the use of local, indigenous species.

Littorinid gastropods are common throughout the world. They comprise a significant portion of many intertidal and shallow subtidal environments and, through grazing effects, often play a vital role in shaping these ecosystems (Mill and McQuaid, 1995, Lubchenco, 1983). Previous research has focused on responses of various other gastropods to environmental salinity changes (Sokolova *et al.*, 2000 a; Sokolova *et al.*, 2000 b; Marigomez, 1991), a variety of anthropogenic stresses (Crowe *et. al.*, 2000) and oil (Chapman *et al.*, 1988). Over the last few decades, however, the use of littorinids in studying the effects of pollution and the development of their use as sentinel species in pollution monitoring has led to the notion that these organisms are an "ideal group on which to work" (Mill and McQuaid, 1995).

Gammarus oceanicus, Mysis stenolepis and *Littorina obtusata* are abundant intertidal organisms along the coastline affected by the spill (Hooper, pers. comm.), but were

eradicated in the weeks after the spill, and had not recolonized the area up to two years after the spill (Hooper *et al.*, 2001).

Monitoring activities in the two years after the spill have shown little recolonization or recovery of the diesel oil spill site in Gros Morne National Park. This has lead to an experiment to determine how widespread and severe conditions are inside the berm, more specifically, to investigate the effects of a diesel oil spill using a sampling scheme that enabled the determination of both the spatial and temporal extent of impacts on invertebrates.

2.2 Materials and Methods

2.2.1 *Study Site*

Bonne Bay is a fjord located on the west coast of Newfoundland, within the boundaries of Gros Morne National Park of Canada. The diesel oil spill site is located on the shores of a small, sheltered cove (49° 28' N, 57° 44' W) (Figure 2.1) of a deep fjord basin within the park. The intertidal substratum along the east shore of the cove consists of waste shale and limestone rock. An outcrop of quartzite dominates the center of the cove and is bounded on either side by unsorted sediments and angular rock fragments. Shale bedrock dominates the western shores. At the time of this experiment, the spill site was enclosed by a man-made rock berm to prevent the spread of diesel throughout the East Arm of Bonne Bay. This rock berm was approximately 200 m long and 9 m wide, enclosed close to 250 m of shoreline and rested about 50 m out from the base of Highway 430, into the East Arm of Bonne Bay (Figure 2.2). Figures were created using MapInfo® Version 6.0

and Corel Draw® Version 10 and all features were geo-referenced using a Garmin Model 12® GPS. A more complete description of the oil spill site is given in Chapter 1.

This experiment was conducted at the diesel spill site and two comparison beaches during July and August 2001 (Figure 2.1). Comparison beaches were Norris Cove (49° 29' N, 51° 50' W) and Mike's Cove (49° 29' N, 51° 45' W), Bonne Bay, Newfoundland and Labrador. Comparison beaches were selected based on the similarity of exposure and beach structure to pre-spill, oil spill site conditions: moderately exposed, predominantly *Ascophyllum nodosum* covered, rocky platform beaches.

2.2.2 *Test Organism Collection*

All test species (*Gammarus oceanicus*, *Mysis stenolepis* and *Littorina obtusata)* were collected from Norris Cove beach in Bonne Bay, Newfoundland, Canada. Water temperature and salinity at collection times were approximately 15 °C and 30±1 salinity units (su), respectively. All organisms were collected within two days of the beginning of the experiment.

Gammarus oceanicus and *Littorina obtusata* were collected by hand from the *Ascophyllum nodosum* and *Fucus vesiculosis* belt, within the rocky intertidal zone. *G. oceanicus* and *L. obtusata* were collected into plastic bags containing seawater and *A. nodosum,* respectively. *Mysis stenolepis* were collected from the subtidal zone to depths of 1 m, using a dip net, and were transferred from the dip net to a plastic holding unit containing seawater. All test organisms were immediately transferred to the Bonne Bay

Marine Station, where they were placed into aerated holding aquaria. All specimens were kept for 24 hours at 15±1 °C and 30±0.5 salinity units before being used in this experiment. Holding seawater was changed after 24 hours using an 80% water replacement regime. Each holding aquaria was provided with *A. nodosum* attached to a rock as a source of food and/or cover.

Several arbitrarily selected test organisms for each species were measured using a dissecting microscope and a rule to ensure test organisms were of similar sizes. *Gammarus oceanicus* were measured to be 15 mm - 17 mm in length; *Mysis stenolepis* were 2.5 - 2.7 cm in length; *Littorina obtusata* were 5 – 6 mm in height and 3-4 mm across the opercular opening.

2.2.3 *Environmental Data*

Salinity and temperature profiles were completed late August and early September 2001 using a Yellow Springs Instrument Model 85D® (Yellow Springs, Ohio) for temperature, salinity and conductivity. Measurements were taken within the lagoon and at reference beaches at transplant locations.

2.2.4 *Sampling Design*

Three common shoreline species were manually transplanted onto the oil spill site beach, as well as the two reference beaches. Specimens were transported to each beach in coolers, where they were placed in enclosures. Enclosures consisted of standard insect mesh sewn with plastic line and were approximately 40 cm x 20 cm in size.

The most significant source of leaching diesel oil within the lagoon had been previously determined by hydrocarbon content sediment analysis (Chapter 1). Beginning at the source, two enclosures per species were placed at geometric distances radiating west 0m (source), 1m, 2m, 4m, 8m, 16m, 32m, and 64 m, and east 1m, 2m, and 4m, for a total of six enclosures (2 enclosures x 3 species) per distance (Figure 2.2).

At the oil spill site, twenty *Gammarus oceanicus* were placed in each amphipod enclosure, along with a small amount of sediment from the immediate area. Ten *Mysis stenolepis* were placed in each mysid shrimp enclosure. Twenty *Littorina obtusata* were placed in each periwinkle enclosure, along with a few small rocks and algae from the immediate area if any was present. If algae were not present in the immediate area, it was omitted from the enclosure in order to replicate localized conditions. This gave a total of 22 enclosures per species at the oil spill site, with six enclosures at each distance; *Gammarus oceanicus* and *Littorina obtusata* were placed within what was the *Ascophyllum nodosum* and *Fucus vesiculosis* zone prior to the spill, but was mainly remnants during the experiment while *Mysis stenolepis* were placed in the shallow subtidal zone. Specimens were not transplanted as far to the east as to the west due to the lack of appropriate substrate for the test organisms, i.e. the east portion of the lagoon was not a platform beach, but was actually a steep embankment leading directly into the lagoon.

Five locations were selected at each of two comparison beach sites, for a total of ten comparison locations. At the comparison beaches, twenty *Gammarus oceanicus* were

placed in each amphipod enclosure, along with a small amount of sediment from the immediate area. Ten *Mysis stenolepis* were placed in each mysid shrimp enclosure. Twenty *Littorina obtusata* were placed in each periwinkle enclosure, along with algae from the immediate area. This gave a total of 20 enclosures per species (ten per each comparison beach site), with six enclosures at each location. Enclosures were placed, in duplicate, at random distances from each other along the two comparison beaches.

Placement of the transplants was along one of two transect lines per comparison beach and represented positions where these organisms are naturally found: *Gammarus oceanicus* and *Littorina obtusata* were placed within the *Ascophyllum nodosum* and *Fucus vesiculosis* zone, while *Mysis stenolepis* transplants were placed in the shallow subtidal zone.

The response criterion was survival. Dead organisms were considered to be those showing any decomposition or significant discoloration, those failing to show movement, and missing organisms, which were assumed to have died and decomposed. Surviving specimens were counted approximately every seven days at low tide, for twenty-eight days or until none remained. This response criterion is based on Costa *et al.* (1998).

2.2.5 *Data Analysis*

The data were analyzed using one-way analysis of variance (ANOVA) with a Tukey's test and graphs of confidence limits of the mean, non-parametric Kruskal-Wallis tests, and Binary Logistic Regression on Minitab© Release 12. Each species was analyzed

separately. Tukey's tests can be interpreted by comparing the signs of the numbers in the resulting figure, that is, like signs show that there is significant difference, while unlike signs indicate there is no significant difference. Graphs of confidence limits of the means show that factors are statistically similar if confidence limits overlap.

For the purposes of statistical analyses, the oil spill was referred to as location 1, Norris Cove Beach was referred to as location 2, and Mike's Cove was referred to a location 3. Transplants were placed at various sites within these locations.

First, survivorship data was analyzed using ANOVA with a Tukey's test. For this test, transplant sites within the berm were grouped as an impact location (location 1) and sites at both reference beaches were grouped to give two non-impacted locations (location 2 = Norris Cove beach; location 3 = Mike's Cove), without reference to distance or time. ANOVA was used to determine if these locations showed the same levels of survivorship for all transplanted organisms, while Tukey's tests were used to determine which sites differed in survivorship. Ryan-Joiner normality tests were performed to examine if survivorship data followed a normal distribution (α=0.05). Normality test results (p-value <0.01) and normal probability plots indicated the survivorship data were not normal and must be transformed. Rank-transformation was used due to the frequency of zero values, after which data were normal (p-value >0.1). For the ANOVA, the null hypothesis was Ho: survivorship at location 1 = location 2 = location 3 and the alternate hypothesis was Ha: survivorship at location 1 $\neq$ location 2 $\neq$ location 3. The tolerance for making a type I error (α) was set at 5%.

Non-parametric (Kruskal-Wallis) tests were also performed on survivorship data prior to transformation (α= 5%) for the scenario described above.

Binary logistic regression was used on impact site data to investigate the factors that might have caused differences in survivorship. Parameters tested were time, and distance from the source. Survivorship data were rank-transformed and distance data were log-transformed for normality. The data were then used to formulate a specific model of survivorship within the berm, using distance and time as predictors. This model can be used to predict survivorship at a series of distances, over a period of four weeks for each organism.

2.3 Results

2.3.1 *Overview: Oil Spill Site and Comparison Beach Conditions*

Survival decreased for *Littorina obtusata* and *Gammarus oceanicus* transplanted onto the oil spill location and two comparison locations over the four weeks of the experiment. Transplanted *Mysis stenolepis* all died after one week, therefore statistical analyses could not be performed.

2.3.2 *Environmental Data*

The general trend for salinity data was that at both comparison beaches salinity was approximately a third higher than at the oil spill site, with the exception of the site that bordered the freshwater stream (site 1), which showed salinity similar to surface values at

the oil spill location (Table 2.1). Within the oil spill location, salinity was drastically lower on the surface, and fresh water was entering the lagoon through the site next to the culverts.

Temperatures at the oil spill site were approximately 3-4 degrees higher at the surface than those observed at comparison beaches (Table 2.1). Once again, the exceptions were the freshwater stream (Norris Cove Beach, site 1) and culvert (Oil spill, Culvert) sites, where temperatures were nearly 5 degrees below those observed on the surface with the lagoon.

2.3.3 *One-way Analysis of Variance and Kruskal-Wallis Tests*

Using a tolerance of 5% for making a Type I error for ANOVA and Kruskal-Wallis tests, a clear and like pattern of survival was observed for both *Littorina obtusata* and *Gammarus oceanicus*.

One-way analysis of variance tests (Table 2.2) of all locations showed that survivorship is not statistically identical at the three locations for *Littorina obtusata* or *Gammarus oceanicus*. Tukey's tests and graphs of the confidence limits of the means for both *Littorina obtusata* and *Gammarus oceanicus* (Figure 2.3, Figure 2.7, respectively) showed that the oil spill location was statistically different with respect to survivorship from both comparison locations, while the two comparison locations were not statistically different from each other. Kruskal-Wallis tests (Table 2.2), which were consistent with ANOVAs, confirmed that median survivorship of *Littorina obtusata* at the oil spill

location was about one third that observed at comparison locations, while the median survivorship of *Gammarus oceanicus* at the oil spill location was an order of magnitude less than that observed at comparison locations. In summary, survival of *Littorina obtusata* and *Gammarus oceanicus* was statistically similar and greater at both comparison locations, as compared to the diesel oil spill site.

2.3.4 *Binary Logistic Regression*

Survivorship data for both *Gammarus oceanicus* and *Littorina obtusata* transplanted into the oil spill location were analyzed using binary logistic regression. The models obtained from these analyses (Table 2.3) allow survivorship to be predicted from the parameters time and distance; p-values obtained from these analyses (Table 2.3) indicate there is sufficient evidence that the parameters are not zero using a significance level of α= 5%, that is, time and distance have an effect on survivorship. Figures 2.3 A and B show that organisms closer to the source of leaching diesel died more quickly than those farther away.

2.3.5 *Reproduction*

Gammarus oceanicus transplanted onto comparison beaches produced 804 young after the first week, 221 after the second week and 20 after the third week. *Gammarus oceanicus* transplanted onto the oil spill beach produced 10 young after the first week, 68 after the second week and 2 after the third week (Appendix).

2.4 Discussion

2.4.1 *Environmental Data and Survivorship Analysis*

Animals that inhabit estuaries are often exposed to variable temperature and salinity environments (Castro and Huber, 2003; Knox, 2001). Variations in temperature may affect survival, growth and metabolic activity, while salinity variations may impose additional osmotic stress (McLusky *et al.*, 1986). In fact, physiological adaptation to less than ideal environmental conditions imposes energetic costs that may affect other physiological needs, such as reproduction or growth, possibly leading to life history impacts (Neuparth *et al.*, 2002). Neuparth *et al.* (2002) discovered that a simple 5°C reduction in temperature led to a shorter life span, generation time and life expectancy, and faster growth, higher age at maturity and population growth rate in *Gammarus locusta*. Furthermore, it is a widely accepted school of thought that organisms existing under these extreme conditions are more vulnerable to anthropogenic stresses, such as oil spills (Tedengren *et al.* 1988; Tedengren and Kautsky, 1987; McLusky *et al.*, 1986).

Environmental conditions such as reduced salinity and varying temperatures, as well as the stress of diesel oil toxicity, characterize the physical environment within the lagoon at the oil spill location. These conditions led to poor survivorship of transplanted, caged organisms at this location. Generally speaking, organisms transplanted into the oil spill location were adversely affected as shown by total mortality after four weeks. In addition, impaired reproduction was seen in lagoon-transplanted organisms, specifically, amphipods. Impaired reproductive ability in amphipods has been noted in other uncharacteristically hypo-saline conditions (Neuparth *et al.*, 2002), and in organisms

exposed to oil (Linden, 1976). Lee *et al.* (1977) also found that fewer young were produced by amphipods exposed to oil, noting that it may be a factor of fewer adult survivors, in addition to impaired reproductive ability associated with the oil.

Mortality inside the lagoon at the diesel oil spill site reached 100% by week four of the experiment for both *L. obtusata* and *G. oceanicus*. Distance was shown to be a significant factor in survival, though all organisms had died by the end of four weeks regardless of the distance from the source of seeping diesel. The fact that mortality reached 100% regardless of the distance from the point source is believed to be a result of uniformly low salinity conditions throughout the lagoon. Additionally, diesel pooling at various locations within the lagoon (Chapter 1) may have prevented a more pronounced gradient of effects from being observed. However, organisms closer to the source of diesel had faster mortality than those farther away from the pollution source.

The cause of massive mortality of *Mysis stenolepis* in the first week of the experiment, especially at comparison locations, is unknown. Cages used for transplanting were largely untouched at the oil spill location, therefore it was assumed that the animals died and decomposed within the first week. At the comparison beaches, cages were found to have large holes, suggesting that test organisms were preyed upon by other intertidal organisms.

Time was a factor in survivorship at the oil spill location. A significant difference in survivorship among several of the weeks for both tests species was observed.

Physiological adaptations, such as osmoregulation in amphipods (Aunaas *et al.,* 1991) or behavioral mechanisms, such as the ability to shut off from the environment in gastropods, and avoidance responses in amphipods (Crowe *et al.,* 2000) may allow organisms to tolerate and compensate for environmental irregularities in the short term (Bulnheim, 1984), however exposure to adverse conditions with time was unavoidable, and the results severe inside the lagoon.

Costa *et al.* (1998), in a proposed acute sediment toxicity test for marine amphipods based on ASTM (American Society for Testing and Materials) guidelines, recommends the use of 90% control survival to accept the test as "regular". If survival is less than this, "insufficient health condition" of test animals may be the cause. Survival at comparison beaches for *Littorina obtusata* was in the range of 85% - >95%, while for *Gammarus oceanicus* survival was much lower, in the range of 70% - 74%. Declining numbers of adult survivors was likely a result of the high productive output at comparison locations, as Steele (1976) describes the reproductive life cycle of *G. oceanicus* to include successive broods of young, a resting stage, then die-off, beginning in August.

When discussing anthropogenic-related discharges and their effects, often there is no distinction made between contamination (raised levels of a contaminant as compared to background levels) and the effects of this contamination. Olsgard and Gray (1995) suggest that the effects of contaminants on biota be called pollution. Using this definition, it can be said that even though a point source of contamination was identified, and distance was shown to be a significant parameter for survival time, a gradient of pollution

relating to the diesel oil was not observed in that all organisms eventually perished. This is perhaps due to the existence of environmental covariables. Ellis *et al.* (2000) explains that while gradient designs are the most appropriate for environmental assessment of point source data, the presence of variation not related to the impact can defeat the use of gradient designs. Here, the presence and subsequent slumping of the berm, causing hypo-saline conditions and fluctuating temperatures, can be considered environmental covariables that masked the gradient associated with the diesel oil, but also imposed effects of their own.

2.5 Summary

An *in situ* bioassay involving transplanted, caged, marine intertidal invertebrates was used to determine the extent of damage to the coastline in Gros Morne National Park as a result of the spill and post-spill containment procedures. This experiment demonstrated that the coastline was negatively affected by toxicity relating to the diesel fuel and hypo-saline conditions created by the presence of a semi-permanent rock berm, as evidenced by the massive mortality of transplanted animals. Distance from the known point source of diesel was shown to be statistically significant for survivorship, despite the fact that all organisms died within four weeks. The farther the organism was from the source, the longer it survived. Time was shown to be a significant factor in survival. Oil contaminants that were contained within the lagoon exercised their effects in conjunction with the environmental stresses of uniformly low salinity conditions and fluctuating temperatures, thereby reducing the gradient of effects, but still leading to the assumption that the closer the organism was to the pollution source, the faster it died.

Conditions inside the berm cannot support typical marine life. However, when leaching diesel reaches a minimum and the rock berm can be removed, problems associated with salinity and temperature will be remedied, reducing the impact to one associated with minute amounts of leaching diesel.

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Table 2.1. Salinity and temperature data from the oil spill location, Norris Cove Beach and Mike's Cove.

Site	Temperature (°C)	Salinity (su)
Diesel Oil Spill Location		
Surface		
64m	18.1	3.1
Culvert	15.4	0.1
32m	19.0	2.5
16m	19.7	3.4
8m	19.5	4.2
4m	19.5	4.0
2m	19.1	3.5
1m	19.0	3.5
0m	19.0	3.6
1m	19.0	3.6
2m	19.2	3.6
4m	19.6	3.3
8m	20.1	3.1
16m	20.0	3.1
32m	20.2	3.6
64m	20.3	4.6
Deep		
64m	20.0	21.0
Culvert	-	-
32m	20.2	20
16m	20.8	20.1
8m	20.6	20.0
4m	20.5	20.0
2m	20.0	21.0
1m	20.0	21.0
0m	20.1	21.0
1m	20.1	21.0
2m	20.1	21.0
4m	20.7	21.0
8m	20.8	21.1
16m	21.0	22.0
32m	21.6	20.7
64m	20.6	21.7
Norris Point Beach		
Surface		
1	15.1	4.9
Deep		
1	17.0	29.0

Surface/Deep		
2-5	17.0	29.0
"Mike's" Cove		
Surface/Deep		
6-10	16.3	29.7

Table 2.2. Results of one-way ANOVAs (analysis of variance) and Kruskal-Wallis tests on survivorship at the three locations (location 1 = oil spill location; location 2 = Norris Point Beach; location 3 = Mike's Cove), $\alpha = 5\%$. Significant values are in bold.

Variable	**Hypothesis**	**Anova p-value**	**K-W p-value**	**Conclusion**
Littorina obtusata				
Survivorship at each location	Ho: location 1 = location 2 = location 3; Ha: location 1 ≠ location 2 ≠ location 3	**<0.001**	**<0.001**	Reject Ho
Gammarus oceanicus				
Survivorship at each location	Ho: location 1 = location 2 = location 3; Ha: location 1 ≠ location 2 ≠ location 3	**<0.001**	**<0.001**	Reject Ho

Table 2.3. Analysis of survivorship at the oil spill location, using binary logistic regression. P-values for parameters that significantly contribute to survival are in bold.

VARIABLE	REGRESSION COEFFICIENT	P-VALUE	MODEL
Littorina obtusata			
Distance (logDistance)	0.34301	**<0.001**	ln(p/1-p)= 4.8433 + 0.34301 logDistance – 2.9712 week
Time (week)	-2.9712	**<0.001**	
Gammarus oceanicus			
Distance (logDistance)	0.38247	**<0.001**	ln(p/1-p)= 1.3925 + 0.38247 logDistance – 1.74346 week
Time (week)	-1.74346	**<0.001**	

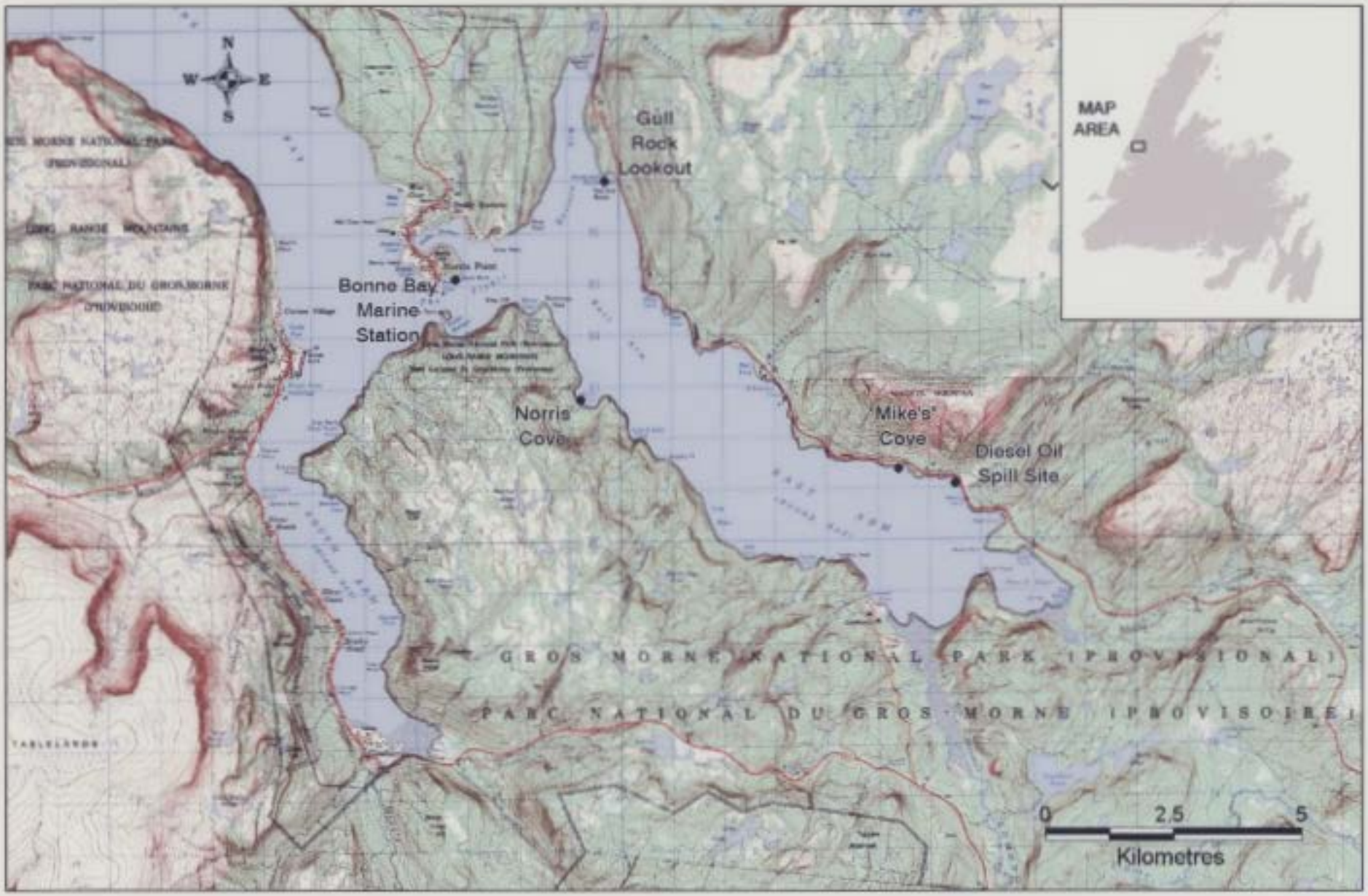


Figure 2.1. Bonne Bay location map showing the diesel oil spill site and sampling locations.

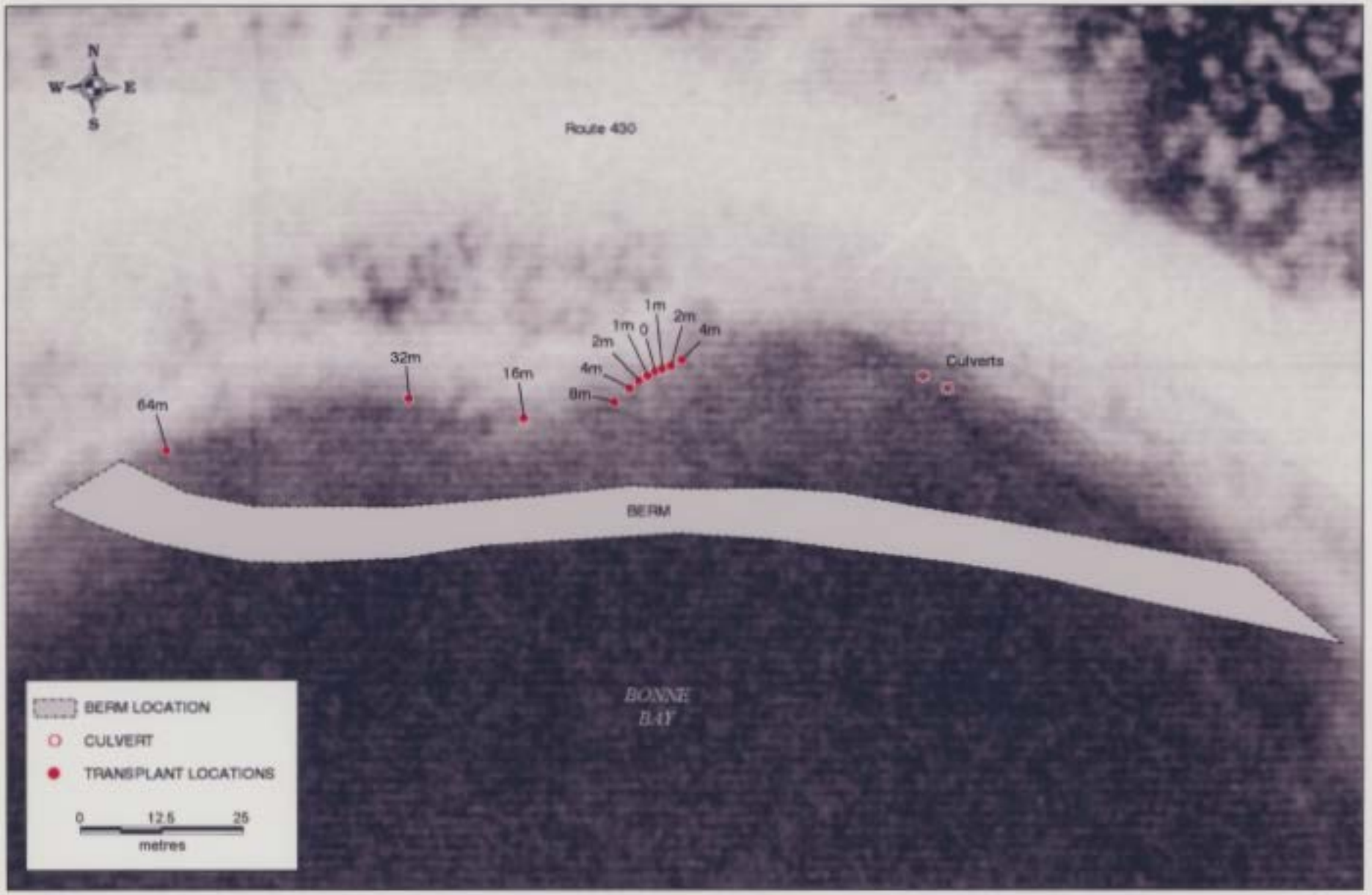


Figure 2.2. Diesel oil spill site in Bonne Bay, NL showing the semi-permanent rock berm and transplant locations.

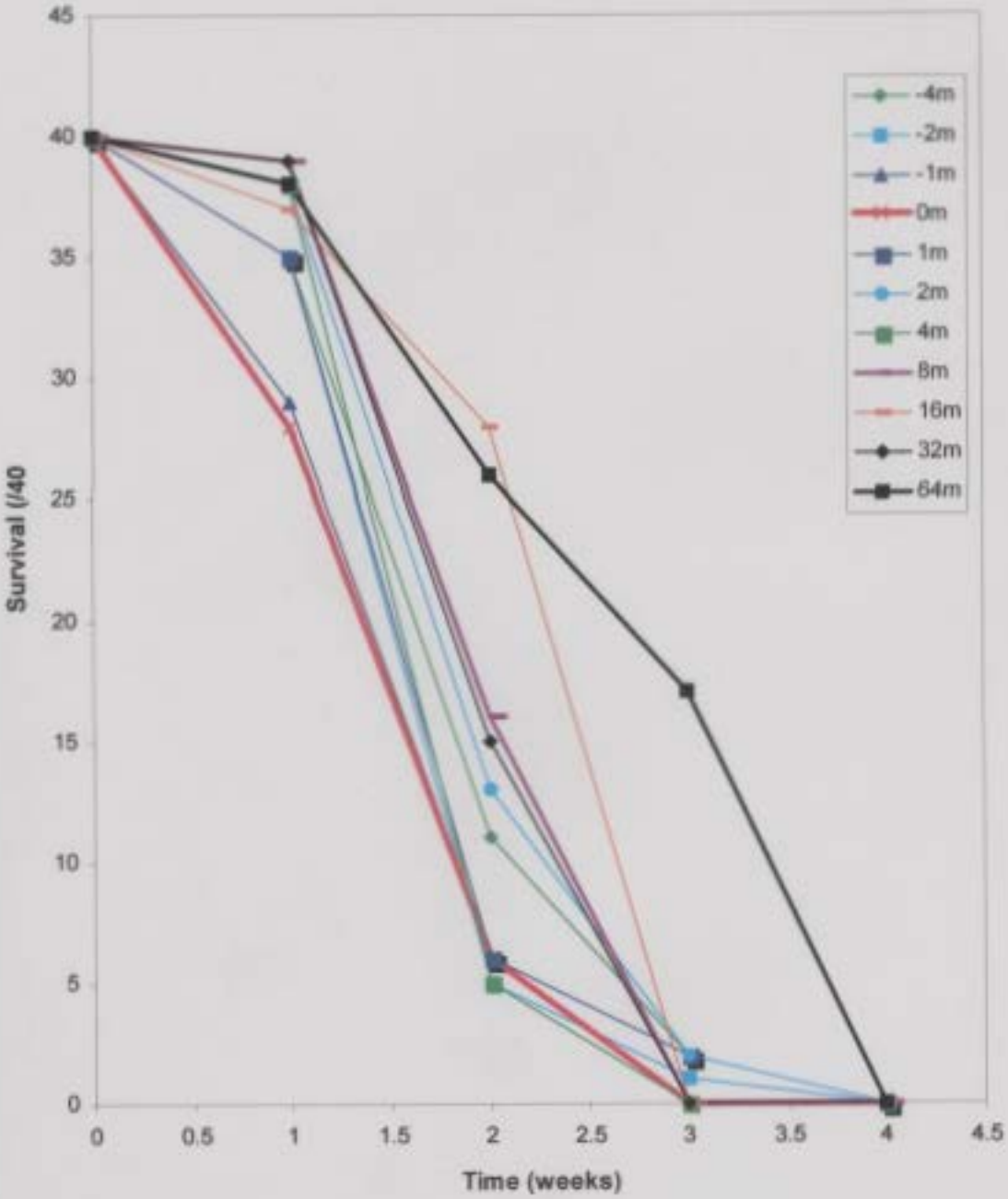
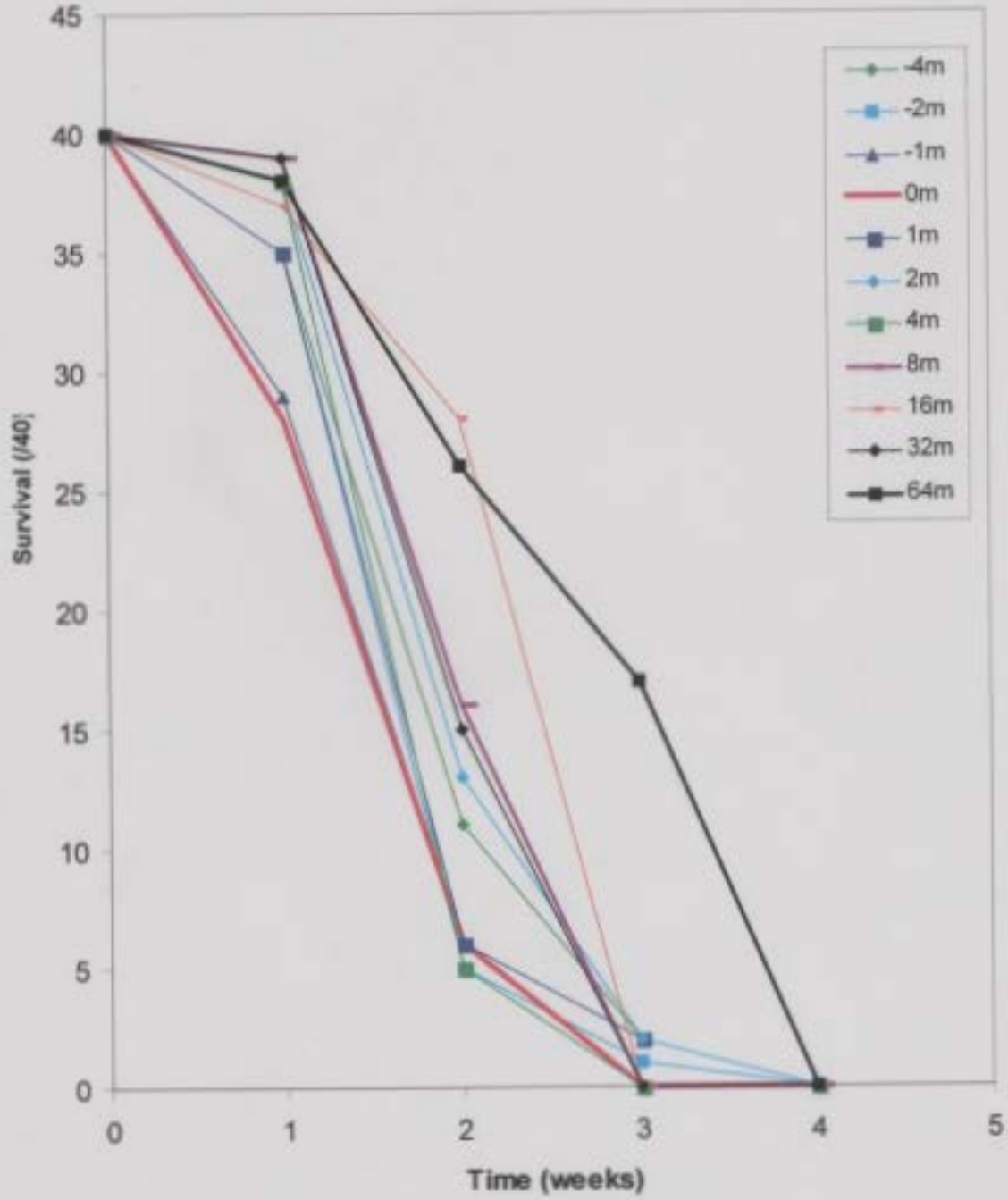


Figure 2.3.A. Survivorship of transplanted, caged *Littorina obtusata* at the diesel oil spill site over 4 weeks and at 11 distances.



B. Survivorship of transplanted, caged *Gammarus oceanicus* at the diesel oil spill site over 4 weeks and at 11 distances.

A. Individual 95% confidence limits for the mean

Location	N	Mean	StDev	--------+---------+---------+--------
1	55	41.19	33.06	(---*----)
2	25	67.78	18.60	(------*-------)
3	25	64.20	21.32	(-------*------)
				--------+---------+---------+--------
				45 60 75

B. Tukey's pairwise comparisons

Intervals for (column level mean) - (row level mean)

	1	2
2	-42.46 -10.72	
3	-38.88 -7.14	-15.03 22.19

Figure 2.4. A. Plot of the confidence limits of the means and B. Plot of Tukey's pairwise comparisons from one way analysis of variance for rank transformed survivorship of *Littorina obtusata* at the three locations (location 1 = oil spill, location 2 = Mike's Cove, location 3 = Norris Cove Beach) (Ho: location 1 = location 2 = location 3; Ha: location 1 ≠ location 2 ≠ location 3; α=5%), from Minitab© Release 12.

A. Individual 95% confidence limits for the mean

B. Tukey's pairwise comparisons

	1	2
2	-44.79 -15.56	
3	-49.35 -20.12	-21.70 12.58

Figure 2.5. A. Plot of the confidence limits of the means and B. Plot of Tukey's pairwise comparisons from one way analysis of variance for rank transformed survivorship of *Gammarus oceanicus* at the three locations (location 1 = oil spill, location 2 = Mike's Cove, location 3 = Norris Cove Beach) (Ho: location 1 = location 2 = location 3; Ha: location 1 ≠ location 2 ≠ location 3; α=5%), from Minitab© Release 12.

Appendix 2.1

Raw data of survivorship of transplanted invertebrates at the diesel oil spill location.

Week	Distance	Survivorship	Offspring
Mysis stenolepis			
0	64	20	-
0	32	20	-
0	16	20	-
0	8	20	-
0	4	20	-
0	2	20	-
0	1	20	-
0	0	20	-
0	1	20	-
0	2	20	-
0	4	20	-
1	64	0	-
1	32	0	-
1	16	0	-
1	8	0	-
1	4	0	-
1	2	0	-
1	1	0	-
1	0	0	-
1	1	0	-
1	2	0	-
1	4	0	-
Littorina obtusata			
0	64	40	-
0	32	40	-
0	16	40	-
0	8	40	-
0	4	40	-
0	2	40	-
0	1	40	-
0	0	40	-
0	1	40	-
0	2	40	-
0	4	40	-
1	64	38	-
1	32	39	-
1	16	37	-
1	8	39	-

1	4	38	-
1	2	38	-
1	1	35	-
1	0	28	-
1	1	29	-
1	2	35	-
1	4	35	-
2	64	26	-
2	32	15	-
2	16	28	-
2	8	16	-
2	4	5	-
2	2	13	-
2	1	6	-
2	0	6	-
2	1	6	-
2	2	5	-
2	4	11	-
3	64	17	-
3	32	0	-
3	16	0	-
3	8	0	-
3	4	0	-
3	2	2	-
3	1	2	-
3	0	0	-
3	1	0	-
3	2	1	-
3	4	2	-
4	64	0	-
4	32	0	-
4	16	0	-
4	8	0	-
4	4	0	-
4	2	0	-
4	1	0	-
4	0	0	-
4	1	0	-
4	2	0	-
4	4	0	-
Gammarus oceanicus			
0	64	40	-
0	32	40	-
0	16	40	-
0	8	40	-

0	4	40	-
0	2	40	-
0	1	40	-
0	0	40	-
0	1	40	-
0	2	40	-
0	4	40	-
1	64	30	-
1	32	20	-
1	16	20	-
1	8	20	-
1	4	12	-
1	2	8	-
1	1	0	10
1	0	2	-
1	1	0	-
1	2	4	-
1	4	0	-
2	64	22	-
2	32	18	-
2	16	12	-
2	8	18	68
2	4	0	-
2	2	3	-
2	1	0	-
2	0	0	-
2	1	6	-
2	2	6	-
2	4	0	-
3	64	18	-
3	32	8	-
3	16	10	-
3	8	14	2
3	4	0	-
3	2	4	-
3	1	0	-
3	0	0	-
3	1	4	-
3	2	0	-
3	4	0	-
4	64	0	-
4	32	0	-
4	16	0	-
4	8	0	-
4	4	0	-

4	2	0	-
4	1	0	-
4	0	0	-
4	1	0	-
4	2	0	-
4	4	0	-

Appendix 2.2

Raw data for survivorship of transplanted invertebrates at comparison locations (sites 1-5 = Norris Cove Beach; sites 6-10 = Mike's Cove).

Week	Site	Survivorship	Offspring
Mysis gaspensis			
0	1	20	-
0	2	20	-
0	3	20	-
0	4	20	-
0	5	20	-
0	6	20	-
0	7	20	-
0	8	20	-
0	9	20	-
0	10	20	-
1	1	0	-
1	2	0	-
1	3	0	-
1	4	0	-
1	5	0	-
1	6	0	-
1	7	0	-
1	8	0	-
1	9	0	-
1	10	0	-
Littorina obtusata			
0	1	40	-
0	2	40	-
0	3	40	-
0	4	40	-
0	5	40	-
0	6	40	-
0	7	40	-
0	8	40	-
0	9	40	-
0	10	40	-
1	1	39	-
1	2	38	-
1	3	40	-
1	4	37	-
1	5	40	-
1	6	38	-

1	7	37	-
1	8	36	-
1	9	40	-
1	10	40	-
2	1	37	-
2	2	36	-
2	3	35	-
2	4	35	-
2	5	36	-
2	6	37	-
2	7	36	-
2	8	34	-
2	9	37	-
2	10	36	-
3	1	37	-
3	2	35	-
3	3	34	-
3	4	35	-
3	5	35	-
3	6	35	-
3	7	34	-
3	8	33	-
3	9	35	-
3	10	34	-
4	1	36	-
4	2	35	-
4	3	34	-
4	4	34	-
4	5	34	-
4	6	34	-
4	7	33	-
4	8	33	-
4	9	35	-
4	10	33	-
Gammarus oceanicus			
0	1	40	-
0	2	40	-
0	3	40	-
0	4	40	-
0	5	40	-
0	6	40	-
0	7	40	-
0	8	40	-
0	9	40	-
0	10	40	-

1	1	26	72
1	2	30	150
1	3	34	34
1	4	26	138
1	5	28	342
1	6	32	8
1	7	28	12
1	8	32	12
1	9	28	4
1	10	32	32
2	1	26	26
2	2	28	50
2	3	30	-
2	4	26	120
2	5	28	10
2	6	32	2
2	7	28	4
2	8	32	-
2	9	28	-
2	10	28	-
3	1	26	-
3	2	28	10
3	3	30	-
3	4	26	10
3	5	28	-
3	6	30	-
3	7	28	-
3	8	30	-
3	9	28	-
3	10	26	-
4	1	26	-
4	2	28	-
4	3	30	-
4	4	26	-
4	5	30	30*
4	6	28	-
4	7	26	-
4	8	30	-
4	9	28	-
4	10	26	-

* went from 28 – 30 over a week

Chapter 3. What are the individual and combined effects of diesel oil and reduced salinity on three common shoreline invertebrates?

3.1 Introduction

Salinity is a dominant environmental factor regulating aquatic community structure (Verschuren *et al.*, 2000). In near-shore habitats, salinity may change rapidly within a very short time, posing a challenge to marine organisms, which are mostly adapted to a narrow salinity range (Levinton, 2001). The ranges of salinity encountered in marine habitats differ greatly from place to place. In the open ocean, salinity varies between 33 and 37 salinity units (full oceanic salinity adjacent to Newfoundland is usually between 30 and 32 salinity units (Hooper, pers. comm.)), while in near-shore waters and estuaries the seawater is further diluted by rivers. These effects are further complicated by tidal actions. As a result, salinity may range from full strength seawater to nearly fresh water (Kirst, 1989). In order to operate efficiently under these conditions, marine organisms must maintain fairly constant chemical conditions within the cell using specific biochemical reactions. Anything that causes significant changes in cellular chemistry could therefore harm a marine organism (Levinton, 2001).

Petroleum products are highly complex mixtures of aromatics. Diesel fuel consists mainly of saturated and aromatic hydrocarbons. Saturated hydrocarbons are generally long-chain alkanes with carbon numbers ranging from C_{10} - C_{20}. There can be lighter and heavier components present in diesel oil, but usually in very small quantities. Aromatic components in diesel oil include alkylated benzenes, naphthalenes, phenanthrenes, chrysenes and others (Song, 2000). The high concentrations of aromatic hydrocarbons in

diesel oils (Connell and Miller, 1981; Nelson-Smith, 1972) make this fuel particularly toxic (Clark, 2001; Miller, 1982) because of the carcinogenic qualities associated with aromatics (Brzorad and Burger, 1994). Also, biodegradation in the first several months after a spill reduces the straight-chain hydrocarbon fraction, leaving the aromatic fraction intact. So, on a volume basis, the toxicity of weathered diesel oil can increase before the aromatics are degraded (Brzorad and Burger, 1994).

Organisms exposed to petroleum hydrocarbons from an oil spill are initially affected mechanically. Heavy oils smother surfaces and hinder movement, inhibiting respiration and feeding (Moore and Dwyer, 1974). Hydrocarbons affect organisms at the cellular level also. Intercellular membranes that regulate essential metabolic processes, like osmoregulation, are disrupted, disturbing the control over passage of materials in and out of the cell (Nelson-Smith, 1972).

A number of experiments on the toxicity of diesel oil have been performed in the last several years. In some cases, an actual diesel oil spill allowed for crucial field studies to be conducted. One such case was the spillage of 2000-3000 tons of diesel oil into the East Lamma Channel in Hong Kong, which allowed researchers to determine the relative sensitivities of several rocky shore species to diesel oil, as well as describe the ecological changes that took place as a result (Stirling, 1977). The accidental release of 600,000 litres of diesel oil into Arthur Harbor, Antarctica, when the *Bahia Paraiso* ran aground in 1989 allowed for intensive studies on the water, organisms and sediments in an area considered to be one of the last pristine areas on earth (Kennicutt *et al.*, 1991). A more recent Antarctic event allowed for studies on a minor, localized spill when 1000 litres of

diesel oil was spilled from the Faraday Research Station. Toxic effects were seen immediately, but were short-term (Cripps and Shears, 1997). In each of the above-mentioned field studies, the effects of diesel oil spillage were seen for at least one year (Cripps and Shears, 1997; Kennicutt *et al.*, 1991; Stirling, 1977).

There is a large amount of literature on the toxicity of diesel oil and other oils from laboratory studies as well, many of which have focused on how oil affects individual or groups of species. Gesteira and Dauvin (2000) and Roast *et al.* (1998) recommended the use of amphipods and mysids for toxicity testing. Neff *et al.* (2000) studied the weathering properties, chemical composition and toxicity of Australian diesel oil on six different species of marine animals. Other studies considered the effects of remediation techniques, including dispersants (Gulec *et al.*, 1997; Fisher and Foss, 1993; Butler *et al.*, 1982), burning (Cohen and Nugegoda, 2000) and biological degradation of oil (Delille and Pelletier, 2002; Eriksson *et al.*, 1998). Field and lab studies aid in the development of effective spill response strategies and remediation techniques for dealing with spills (Neff *et al.*, 2000).

Contamination of coastal waters by oil spills is an issue that draws considerable scientific attention. Much research has been conducted in the last 30 years, ranging from oil-in-water toxicity tests (Tatem *et al.*, 1978; Linden, 1976), and sediment toxicity tests (Ho *et al.*, 2000), to impacts of oil on invertebrate communities and populations (Suchanek, 1993). Included in the repertoire of essential oil toxicity studies are acute toxicity tests. Miller (1982) suggested short-term toxicity studies, as part of a multi-faceted approach, be used to evaluate the effects of petroleum hydrocarbons on marine organisms.

The Coastal Resource Coordinator's Bioassessment Manual (MacDonald *et al.*, 1997) describes a toxicity test as a process that exposes organisms to complex samples under controlled conditions to determine if adverse effects occur. Short-term toxicity studies can be used to establish the tolerance ranges and lethal exposure levels (Connell and Miller, 1981) of whole samples, as opposed to chemical components (MacDonald *et al.*, 1997). Specifically, acute toxicity tests are used to determine the concentration of a sample that produces a specific adverse effect on a specified percentage of test organisms (ASTM, 1996). LC_{50} (the concentration which is lethal to 50% of the test population) (Nelson-Smith, 1972) are the most common tests because death is usually simply determined for most organisms, and 50% mortality is the most reproducible and easily determined measure of toxicity. Test duration is usually 24, 48 or 96 hours and can be conducted using one of four techniques: static, recirculation, renewal and flow-through techniques (ASTM, 1996). Each technique offers advantages and disadvantages, however static systems are believed to provide a better simulation of a field situation where both the sediments and water column have been contaminated (Ho *et al.*, 2000), as was the case in Bonne Bay.

Estuarine and intertidal zones are frequently exposed to oil spills, as well as to lowered salinity (Butler *et al.*, 1982). McLusky *et al.* (1986) suggest that salinity is one of the principal environmental factors affecting the inhabitants of estuaries and coastal waters and studying its affects in combination with other pollutants may help determine the effects seen in these ecosystems. Despite the considerable amount of information available on oil toxicity tests, there is relatively little data examining how lowered

salinity affects responses to oil toxicity, and in particular, diesel oil (Tedengren *et al.*, 1988; Tedengren and Kautsky, 1987).

Two opposing theories exist as to how and why estuarine organisms respond to lowered salinity in combination with a known toxicant. First is the theory that organisms living near the limits of their salinity tolerance, or any stress, will be more susceptible to an additional stress (McLusky *et al.*, 1986). The contrary view is that organisms with a wider tolerance to salinity changes, i.e. estuarine organisms, will pre-adapt to tolerate other stresses, including pollution (Jernelov and Rosenberg, 1976).

McLusky's (1986) theory was supported by Tedengren *et al.* (1988) in an experiment on the combined effects of altered salinity, cadmium and diesel oil, where it was found that exposure to diesel oil in combination with lowered salinity showed a synergistic effect. Tedengren *et al.* (1988) also suggested the reason for this is that organisms from low-salinity conditions, for example estuaries, are more exposed to toxic substances in the water as they generally process more water during osmoregulation. Since the organism must pass a relatively larger amount of a specific substance through their bodies, toxic or accumulative effects may be more pronounced.

Jernelov and Rosenberg's (1976) opposing theory was supported in an experiment by Butler *et al.* (1982) where reducing the salinity did not affect the lethal toxicity of oil for all organisms tested; however, sub-lethal effects were observed.

The present study tests these two theories by examining the effects of diesel oil combined with lowered salinity for three common intertidal organisms: amphipods, mysids, and littorinid gastropods. Amphipods are ecologically important organisms, comprising a significant portion of aquatic biomass and diversity worldwide (Costa *et al.*, 1998). A large amount of literature exists concerning the use of amphipods in testing and monitoring of environmental stresses. Bulnheim (1984) studied the physiological responses of five amphipod species to a variety of environmental stresses, while others studied the effects of salinity stress (Steele and Steele, 1991), oil and dispersants (Gulec *et al.*, 1997) and toxic sediment (Costa *et al.*, 1998) on various amphipods. Amphipods are considered good bioindicators of the impacts due to oil spills mainly due to their sensitivity to the aromatic portion of oil (Gesteira and Dauvin, 2000). *Gammarus oceanicus* (Phylum Arthropoda, Subphylum Crustacea, Class Malacostraca, Subclass Eumalacostraca, Order Amphioda) (Pearse *et al.*, 1994), often the most abundant marine littoral amphipod (Halcrow, 1981), is found on sheltered to slightly exposed rocky shores from the Gulf of Maine to Newfoundland (Steele, 1976; Steele and Steele, 1972). Nearly three decades ago, Linden (1976) studied the effects of oil on *G. oceanicus,* while more recently Aunaas *et al.* (1991) studied the effects of both oil and oil dispersants on *G. oceanicus.*

Mysids are an important part of estuaries, as producers and consumers, contributing significantly to the standing stock of omnivores in many estuaries (Roast *et al.*, 1998). The use of mysid shrimp has become widely accepted in toxicity testing and environmental monitoring, in fact, Nimmo and Hamaker (1982) stated "their utility as a model organism can be applied to evaluate the ecological impact of pollutants on larval

crustaceans, particularly the commercially important species of shrimps, lobsters and crabs". Mysids are frequently used in laboratory studies, and in the past have been used to determine the effects of trace metals (Roast *et al.*, 2000), petroleum hydrocarbons (Riebel and Percy, 1990), and salinity and cadmium toxicity (De Lisle and Roberts, 1988) on various species. As well, laboratory studies on the interactions of salinity, temperature and age on growth have provided much-needed baseline data on these important organisms (McKenney and Celestial, 1995). *Mysis stenolepis* (Phylum Arthropoda, Subphylum Crustacea, Class Malacostraca, Subclass Eumalacostraca, Order Mysidacea) (Pearse *et al.*, 1994) is one of only four species of littoral mysids found in Atlantic estuaries (Dadswell, 1975). Despite the fact that relatively little is known about this species as compared to other mysid species, Roast *et al.* (1998) promotes the use of local, indigenous species for testing.

Littorinid gastropods, like *Littorina obtusata* ((Phylum Mollusca, Class Gastropoda, Subclass Prosobranchia, Order Megogastropoda) (Pearse *et al.*, 1994), are common throughout the world. They comprise a significant portion of many intertidal and shallow subtidal environments and, through grazing effects, often play a vital role in shaping these ecosystems (Mill and McQuaid, 1995, Lubchenco, 1983). Previous research has focused on responses of various other gastropods to environmental salinity changes (Sokolova *et al.*, 2000 a; Sokolova *et al.*, 2000 b; Marigomez, 1991), a variety of anthropogenic stresses (Crowe *et al.*, 2000) and oil (Chapman *et al.*, 1988). Over the last few decades, however, the use of littorinids in studying the effects of pollution and the

development of their use as sentinel species in pollution monitoring has led to the notion that these organisms are an "ideal group on which to work" (Mill and McQuaid, 1995).

G. oceanicus, M. stenolepis and *L. obtusata* are abundant intertidal organisms along the coastline affected by the spill (Hooper, pers. comm.), but were eradicated after the spill, and had not recolonized the area up to two years after (Hooper *et al.*, 2001). This has lead to experiments to determine the individual and combined effects of diesel oil and reduced salinity. The aim of these tests was to facilitate an understanding of conditions at the site.

3.2 Materials and Methods

3.2.1 *In vitro Bioassays*

Experiments were performed at the Bonne Bay Marine Station, Norris Point, Newfoundland and Labrador (Figure 2.1), July - October 2001 and 2002. Lethal bioassay range finding tests were performed in 2001, while lethal bioassays combining diesel oil and reduced salinity were completed in 2002. Figures were created using MapInfo Professional® Version 6.0 and Minitab© Release 12.

3.2.2 *Test Organism Collection*

All test species (*Gammarus oceanicus*, *Mysis stenolepis* and *Littorina obtusata)* were collected from Norris Cove beach (49° 29' N, 51° 50' W) in Bonne Bay, Newfoundland and Labrador (Figure 2.1). Water temperature and salinity at collection times were 6 –15 °C (depending on the time of year) and 30 salinity units. All organisms collected were used within 10 days or released, and additional organisms were collected for different

experiments. This meant that several collections were done during months of experimentation.

Gammarus oceanicus and *Littorina obtusata* were collected by hand from the *Ascophyllum nodosum* and *Fucus vesiculosis* belt, within the rocky intertidal zone. *G. oceanicus* and *L. obtusata* were collected into plastic bags containing seawater and *A. nodosum,* respectively. *Mysis stenolepis* were collected from the subtidal zone to depths of 1 m, using a dip net, and were transferred from the dip net to a plastic holding unit containing seawater. All test organisms were transferred to the Bonne Bay Marine Station by boat immediately and placed into aerated holding aquaria. All specimens were kept for 24 hours at 15±1 °C and 30±0.5 salinity units before being used in this experiment. Holding seawater was changed after 24 hours using an 80% water replacement regime. Each holding aquaria was provided with *A. nodosum* attached to a rock as a source of food and/or cover.

Several test organisms were randomly measured using a dissecting microscope and a ruler. *Gammarus oceanicus* were measured to be 15 mm - 17 mm in length; *Mysis stenolepis* were 2.5 - 2.7 cm in length; *Littorina obtusata* were 5 – 6 mm in height and 3-4 mm across the opercular opening.

3.2.3 *Experimental Design: Acute Lethal Bioassay (LC_{50}) Range Finding Experiments*

Acute lethal bioassay range finding experiments were performed to determine the approximate 24 or 48 hour LC_{50} values of fresh diesel-in-seawater mixtures for three

common intertidal species: *Gammarus oceanicus*, *Mysis stenolepis* and *Littorina obtusata.* These were used to determine suitable test concentrations of diesel oil exposure for subsequent testing. Testing spanned over several months to include various sizes and life stages of organisms.

Diesel oil was purchased from Walsh's Esso service station in Norris Point, stored in an airtight glass container and placed in the dark. Diesel oil was exposed to light and air only while being measured and transferred to test aquaria. Seawater was obtained from the flow-through seawater system at the Bonne Bay Marine Station, which was equipped with a 20μm filter. Salinity and temperature of seawater were 30±0.5 salinity units and 15±1 °C, respectively, as measured with a Yellow Springs Instruments 85® (Yellow Springs, Ohio) salinity, temperature, oxygen and conductivity meter. All seawater and diesel volumes were measured using graduated cylinders and Gilson 1000, 200 and 20 micropipettes. Starting diesel-in-seawater test concentrations were arbitrarily set at 1 ml/L and adjusted higher or lower with each test, depending on the response of test specimens. Diesel-in-seawater mixtures were prepared in 4 L glass test aquaria by transferring a measured volume of seawater into the aquaria, then adding the required volume of fresh diesel to attain the appropriate test concentration. Diesel-in-seawater test mixtures were prepared in 3 L volumes for *G. oceanicus* and *M. stenolepis* and 1 L volumes for *L. obtusata.* Diesel-in-seawater mixtures were shaken vigorously by hand for one minute before specimens were introduced into the mixture. The given diesel exposure concentrations are calculated nominal concentrations since measurements of the actual hydrocarbon concentrations in seawater were not made. These concentrations refer

to the hypothetical situation where oil and water are completely miscible. In reality, most oil re-establishes as a surface film after the initial shaking, causing test organisms to be exposed to a greater concentration of oil at the surface than in the liquid phase. To avoid exposure to the surface film, test organisms were added immediately after shaking, before a surface film was established.

At each concentration tested, four 4 L aquaria were used: two control aquaria and two experimental aquaria. Control aquaria contained 3 liters of clean seawater (15±1 °C and 30±0.5 salinity units, ambient measurements), as taken from the seawater system, and 20 test specimens. Experimental aquaria contained the test mixture and 20 test specimens. The duration of the experiments were 24 or 48 hours, depending on the species tested (*G. oceanicus* and *M. stenolepis:* 24 hours; *L. obtusata:* 48 hours). Mixtures were not adjusted during the exposure period, i.e. conditions were based on a static system. After the initial 24 or 48-hour exposure period, specimens were transferred to aquaria containing clean seawater for an additional 24 or 48 hours.

3.2.4 *Experimental Design: Lethal Bioassay of Effects of Diesel Oil and Reduced Salinity*

Lethal bioassays of effects of diesel oil and reduced salinity were performed to determine the effects on the survivorship ability of three common intertidal species: *Gammarus oceanicus*, *Mysis stenolepis* and *Littorina obtusata.*

Stress factors were applied in isolation and combination to reveal any cumulative effects. The experimental regime included eight 4 L aquaria for each species and concentration

tested: two aquaria contained seawater at ambient salinity (30±0.5 salinity units); two contained seawater at reduced salinity (20.5±0.5 salinity units); two contained diesel mixed with ambient salinity (30±0.5 salinity units) seawater; and two contained diesel mixed with reduced salinity seawater (20.5±0.5 salinity units). The duration of the experiment was 24 or 48 hours, depending on the length of the corresponding LC_{50} range finding tests. Concentrations of diesel used were also based on range finding tests for each of the three species. Reduced salinity water was prepared by diluting ambient, filtered seawater from the flow-through seawater system with distilled water and mixing until uniform. Temperature was maintained at 15±1 °C. Temperature and salinity measurements were taken with a Yellow Springs Instruments Model 85® salinity, oxygen and conductivity meter.

Diesel oil-in-seawater mixtures were prepared using the same method as the previous experiment. Given volumes of ambient or reduced salinity seawater were placed in 4 L glass aquaria and the complementary volume of diesel was added to make 1 or 3 L of test solution, depending on the species (*G. oceanicus* and *M. stenolepis* were subjected to 3 L of test solution, while *L. obtusata* were subjected to 1 L test solution). All volumes of water and diesel oil were measured using graduated cylinders and Gilson 1000, 200 or 20 micropipettes. Diesel oil-in-seawater mixtures were shaken by hand vigorously for one minute before test specimens were added. The given exposure concentration is again a calculated nominal concentration since measurements of the actual hydrocarbon concentrations in ambient seawater or reduced salinity seawater were not made. After the

appropriate exposure period specimens were transferred to 4 L glass aquaria containing ambient seawater.

3.2.5 *Response Criteria*

Survivorship at the end of the test period was based on lethal responses. For *Gammarus oceanicus*, response criteria followed those outlined by Costa *et al.* (1998). Dead animals were identified by physical necrosis or discoloration, absence of pleopod movement, or lack of response to gentle external stimulation. Missing organisms were assumed to have died and decomposed, or been eaten.

Response criteria for *Mysis stenolepis* were similar to that for *Gammarus oceanicus,* and followed those criteria outlined by Riebel and Percy (1990). Dead animals were identified by physical necrosis or discoloration, absence of limb movement, or lack of response to gentle external stimulation. Missing organisms were assumed to have died and decomposed, or been eaten.

Response criteria for *Littorina obtusata* followed those outlined by Chapman *et al.* (1988). The criterion for death when snails had extended feet was failure to respond and withdraw into their shells with the touch of forceps. When the foot was withdrawn, death was based on the inability to keep operculum closed against gentle outward force with forceps.

3.2.6 *Data Analysis*

Data was analyzed by one-way analysis of variance (ANOVA) with a Tukey's test, two-factor analysis of variance (ANOVA), and regression analysis using Minitab© Release 12. Tukey's tests can be interpreted by comparing the signs of the numbers in the resulting table, that is, like signs show that there is a significant difference, while unlike signs indicate there is no significant difference. Graphs of confidence limits of the means show that factors are statistically similar if confidence limits overlap. Regression analysis was used to predict LC_{50} values for test conditions involving diesel. Each species was analyzed separately.

Ryan-Joiner normality tests were performed for each test to examine if survivorship data followed a normal distribution (α=0.05). Normality test results (p-value <0.01), normal probability plots and boxplots indicated the survivorship data were normal and did not require transformation.

One-way ANOVA and Tukey's tests were performed on all three species separately to determine if there was a difference in survivorship between the four test conditions: ambient salinity, reduced salinity, ambient salinity with diesel, and reduced salinity with diesel. The null hypotheses for these tests were Ho: Survivorship when exposed to test condition 1 = Survivorship when exposed to test condition 2 = Survivorship when exposed to test condition 3 = Survivorship when exposed to test condition 4; Ha: Survivorship when exposed to test condition 1 ≠ Survivorship when exposed to test

condition 2 ≠ Survivorship when exposed to test condition 3 ≠ Survivorship when exposed to test condition 4. The tolerance for making a type I error (α) was set at 5%.

Two-way ANOVA tests were also performed on all three species separately to determine which factors of concentration or salinity, or their interaction, affected survivorship. The null hypotheses for these tests were Ho: Survivorship is equal at all test concentrations; survivorship is equal at reduced and ambient salinity; there is no interaction between concentration and salinity; Ha: Survivorship is not equal at all test concentrations; survivorship is not equal at low and ambient salinity; there is an interaction between concentration and salinity. The tolerance for making a type I error (α) was set at 5%.

Regression analysis was used to predict the effects of salinity and diesel on survivorship and for the prediction of LC_{50} and 95% CI and slope of the line 95% CI.

3.3 Results

3.3.1 *Overview: Bioassay Survivorship*

Test results showed that *Mysis stenolepis* was the most sensitive to diesel oil, and *Littorina obtusata* was the least sensitive. The sensitivity of *Gammarus oceanicus* to diesel oil was less than *Mysis stenolepis* and greater than *Littorina obtusata*, and proved to be the only animal of the three tested to show effects compounded by reduced salinity.

3.3.2 *Range Finding Tests*

The results of range finding tests provided the basis for reduced salinity tests and were documented in combination with these results (Appendix 3.7). *Mysis stenolepis* showed the lowest tolerance to diesel oil, with *Gammarus oceanicus* tolerating diesel oil at a concentration an order of magnitude higher. *Littorina obtusata* showed the widest range and greatest tolerance to the diesel oil.

3.3.3 *Survivorship of* ***Mysis stenolepis***

A one-way analysis of variance test (Table 3.1) of *Mysis stenolepis* survivorship showed that survivorship was not statistically equal when exposed to the four test conditions (p-value: <0.001) (Table 3.1). Tukey's tests and graphs of confidence limits of the mean (Figure 3.4) demonstrate where the differences exist. These tests show that survivorship was not statistically different for *Mysis stenolepis* exposed to diesel oil mixed with reduced salinity or ambient salinity water; survivorship of *M. stenolepis* was not significantly different when exposed to reduced salinity or ambient salinity water without the diesel oil; survivorship when exposed to diesel oil, despite the salinity of the water, was significantly different from when there was no diesel oil exposure. Graphs of confidence limits of the means demonstrate that survivorship was in fact least for *Mysis stenolepis* exposed to diesel oil mixed with reduced salinity water, followed by diesel oil mixed with ambient salinity water, low salinity water alone, and finally, ambient salinity water.

Results from two-way analysis of variance test results showed that the concentration of the diesel oil (p-value: <0.001), but not the salinity of the water (p-value: 0.100) or the interaction of these two factors (p-value: 0.311), had a significant effect on survivorship (Table 3.2).

Regression analysis showed that the LC_{50} values, with 95% confidence intervals, were not different for ambient (3.426 μL/L) or reduced salinity (2.924 μL/L) water mixed with diesel oil due to overlapping confidence limits (Table 3.3, Figure 3.2). Furthermore, the slopes of the regression lines for ambient (-4.786) or reduced salinity (-7.719) water mixed with diesel oil were not different (Table 3.3, Figure 3.1), leading to the conclusion that while diesel oil had a significant effect on the survivorship of *Mysis stenolepis,* these effects were not compounded by the added stress of reduced salinity.

3.3.4 *Survivorship of* ***Gammarus oceanicus***

A one-way analysis of variance test (Table 3.1) on *Gammarus oceanicus* showed that survivorship was not statistically equal (p-value: <0.001) when exposed to the four test conditions. Tukey's tests and graphs of confidence limits of the mean (Figure 3.5) display these differences. These tests show that survivorship was significantly different for *Gammarus oceanicus* exposed to diesel oil mixed with reduced salinity and ambient salinity water. Survivorship of *Gammarus oceanicus* was not significantly different when exposed to reduced salinity or ambient salinity water without the diesel oil; survivorship when exposed to diesel oil, despite the salinity of the water, was significantly different from when there was no diesel oil exposure. As with *M. stenolepis*, graphs of confidence

limits of the means demonstrate that survivorship was least for *Gammarus oceanicus* exposed to diesel oil mixed with reduced salinity water, followed by diesel oil mixed with ambient salinity water, ambient salinity water alone, and finally, reduced salinity water, however the latter showed very little difference.

Results from two-way analysis of variance test results (Table 3.2) showed that the interaction between concentration of the diesel oil and the salinity of the water had a significant effect on survivorship (p-value: <0.001, in all cases).

Regression analysis showed that the LC_{50} values, with 95% confidence intervals, were different for ambient (42.70 µL/L) or reduced salinity (-5.03 µL/L) water mixed with diesel oil (Table 3.3, Figure 3.2). The slopes of the regression lines for ambient (-4.786) or reduced salinity (-7.719) water mixed with diesel oil were not different (Table 3.3, Figure 3.1) for *Gammarus oceanicus* due to overlapping confidence limits.

3.3.5 *Survivorship of* ***Littorina obtusata***

A one-way analysis of variance test (Table 3.1) on *Littorina obtusata* showed that survivorship was not statistically equal when exposed to the four test conditions (p-value: <0.001). Tukey's tests and graphs of confidence limits of the mean (Figure 3.6) show where the differences exist. These tests show that survivorship was not statistically different for *Littorina obtusata* exposed to diesel oil mixed with reduced salinity or ambient salinity water; survivorship of *L. obtusata* was not significantly different when exposed to reduced salinity or ambient salinity water alone; survivorship when exposed

to diesel oil, despite the salinity of the water, was significantly different from when there was no diesel oil exposure.

Results from two-way analysis of variance test results (Table 3.2) on *Littorina obtusata* showed that the concentration of the diesel oil (p-value: <0.001), but not the salinity of the water (p-value: 0.833) or the interaction of these two factors (p-value: 0.833), had a significant effect on survivorship.

Regression analysis showed that the LC_{50} values, with 95% confidence intervals, were not different for ambient (384.3 μL/L) or reduced salinity (395.7 μL/L) water mixed with diesel oil due to overlapping confidence limits (Table 3.3, Figure 3.2). Furthermore, the slopes of the regression lines for ambient (-0.030) and reduced salinity (-.033) water mixed with diesel oil were nearly identical (Table 3.3, Figure 3.1), leading to the conclusion that while diesel oil had a significant effect on the survivorship of *Littorina obtusata,* these effects were not compounded by the added stress of reduced salinity.

3.4 Discussion

3.4.1 *Survivorship of* ***Mysis stenolepis***

Mysis stenolepis were the most sensitive to diesel oil of the three animals tested, however survivorship was not further affected by reduced salinity. LC_{50} values obtained were similar to those obtained for other shrimp species, which were around 3.5 ppm for adults, however, sensitivity to oil increased with lowered salinity in that study (Fisher and Foss, 1993).

Mysids are used frequently in acute toxicity studies (Roast *et al.*, 2000). Most of the available information, however, is related to the toxicity of trace metals (Roast *et al.*, 2000; De Lisle and Roberts, 1988), as opposed to hydrocarbon toxicity. Data that is available on the toxicity of hydrocarbons is sparse and not directly comparable to this study due to different test procedures and test organisms (Riebel and Percy, 1990). Mysids are known to be a highly adaptive group of crustaceans, frequently exposed to reduced salinity conditions (McKenney and Celestial, 1995). Since mysids in the Bonne Bay area are usually found in areas of extremely low salinity like Deer Arm estuary (pers. obs.), they are assumed to have adapted to reduced salinity. From the present study it can be concluded that diesel oil toxicity is not significantly affected by salinity in the short term.

3.4.2 *Survivorship of* ***Gammarus oceanicus***

Gammarus oceanicus, an osmoconformer at increased salinities and an osmoregulator at reduced salinities (Aunaas *et al.*, 1991), was found to be the only species of the three tested that showed a significant decrease in survivorship with reduced salinity. These results are in agreement with a similar study by Tedengren *et al.* (1988) who found that the effects due to diesel oil exposure were aggravated by any changes in salinity, but that the effect was more pronounced if the salinity was reduced. Tedengren *et al.* (1988) also stated that it was their belief that diesel oil directly affects the osmoregulatory activity of *Gammarus* spp., which is crucial at reduced salinities, leading to the added negative effects.

LC_{50} values of *Gammarus oceanicus* exposed to oil are below those found by Linden (1976) and above those found by Lee *et al.* (1977) for similar oil types. This is likely due to differences in test procedures, and nevertheless shows that *Gammarus oceanicus* is sensitive to diesel oil exposure. Also, a negative value was obtained for the LC_{50} of diesel oil mixed with reduced salinity water. This it a predicted value based on the trend in the regression, and should be considered only an indicator that reduced salinity caused a decrease in survivorship of *Gammarus oceanicus* in comparison to ambient salinity water. Finally, the fact that results from the regression analysis demonstrated survivorship of *Gammarus oceanicus* is not different in diesel oil and ambient or reduced salinity water must be considered. These results are in disagreement with results of other tests obtained for the survivorship of *Gammarus oceanicus* due to the fact that two outlying concentration and survivorship values were removed to facilitate a better fit to the regression. Therefore, it was concluded that diesel oil had a significant effect on the survivorship of *Gammarus oceanicus* and these effects were compounded by the added stress of reduced salinity.

3.4.3 *Survivorship of* ***Littorina obtusata***

Littorina obtusata, a common intertidal gastropod, were affected by diesel oil only at very high concentrations, which were orders of magnitude greater than the other two organisms tested. *Littorina obtusata* were also not additionally affected by a decrease in salinity. Though similar studies have not been conducted on this particular species, *Polinices spp.*, also intertidal gastropods, have been studied (Gulec and Holdway, 1999; Gulec *et al.*, 1997; Chapman *et al.*, 1988). These studies found that *Polinices spp.* were

not suitable for short-term toxicity studies due to the ability to resist toxicity by retracting into its shell and remaining isolated from the toxic compound (Gulec *et al.*, 1997). It is therefore assumed that the diesel oil exposure prior to *Littorina obtusata* retracting into its shell was sufficient to cause the negative effects observed in the present study. Furthermore, if the snail sufficiently sealed itself off from the diesel oil, than it was no longer exposed to the reduced salinity. It is therefore assumed that the short exposure time to the reduced salinity did not affect survivorship, that only the diesel oil did, as intertidal snails are frequently exposed to brief periods of reduced salinity (Knox, 2001).

3.5 Summary

Survivorship of the three common marine invertebrates *Mysis stenolepis*, *Gammarus oceanicus* and *Littorina obtusata* was negatively affected by short-term exposure to diesel oil-in-water mixtures under acute toxicity test conditions. LC_{50} values for diesel oil mixed with ambient salinity water were lowest for *M. stenolepis,* an order of magnitude higher for *G. oceanicus* and several orders of magnitude higher for *L. obtusata,* i.e. *M. stenolepis* were found to be the most sensitive to diesel, followed by *G. oceanicus* and *L. obtusata.* Predicted LC_{50} values, calculated from regression analysis, for diesel oil mixed with reduced salinity water showed the same trend, though survivorship was not found to be significantly different from that in ambient salinity for *M. stenolepis* and *L. obtusata; G. oceanicus* was found to be significantly affected when salinity was reduced to approximately two thirds of the ambient salinity, that is, the combination of stresses only caused an increased impact in the amphipods.

Patterns of survivorship indicate that diesel oil has a significant effect on the three organisms tested, however, the added stress of reduced salinity does not further impact all three marine invertebrate species.

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Table 3.1. Results of one-way ANOVAs (analysis of variance) tests on survivorship of three marine invertebrates exposed to four tests conditions. Test condition 1: reduced salinity water + diesel oil; test condition 2 : ambient salinity water + diesel oil; test condition 3 : reduced salinity water; test condition 4 : ambient salinity water; α = 5%, from Minitab© Release 12.

HYPOTHESES		
Ho: Survivorship when exposed to test condition 1 = Survivorship when exposed to test condition 2 = Survivorship when exposed to test condition 3 = Survivorship when exposed to test condition 4; Ha: Survivorship when exposed to test condition 1 ≠ Survivorship when exposed to test condition 2 ≠ Survivorship when exposed to test condition 3 ≠ Survivorship when exposed to test condition 4.		
ORGANISM	**P-VALUE**	**CONCLUSION**
Mysis stenolepis	<0.001	Reject Ho; all four test conditions are not equal with respect to survivorship
Gammarus oceanicus	<0.001	Reject Ho; all four test conditions are not equal with respect to survivorship
Littorina obtusata	<0.001	Reject Ho; all four test conditions are not equal with respect to survivorship

Table 3.2. Results of two-way ANOVAs (analysis of variance) tests on survivorship of three marine invertebrates exposed to various diesel oil-in-seawater mixtures, α = 5%, from Minitab© Release 12. Significant values are in bold.

HYPOTHESES		
Ho: Survivorship is equal at all test concentrations; survivorship is equal at reduced and ambient salinity; there is no interaction between concentration and salinity; Ha: Survivorship is not equal at all test concentrations; survivorship is not equal at low and ambient salinity; there is an interaction between concentration and salinity.		
ORGANISM	**P-VALUE**	**CONCLUSION**
Mysis stenolepis	a) Concentration: **<0.001**	a) Reject Ho; survivorship is not equal at each concentration
	b) Salinity: 0.100	b) Do not reject Ho; survivorship is not statistically different at low and ambient salinity
	c) Interaction: 0.311	c) Do not reject Ho; no significant effect due to interaction
Gammarus oceanicus	a) Concentration: **<0.001**	a) Reject Ho; survivorship is not equal at each concentration
	b) Salinity: **<0.001**	b) Reject Ho; survivorship is not equal at low and ambient salinity
	c) Interaction: **<0.001**	c) Reject Ho; significant effect due to interaction
Littorina obtusata	a) Concentration: **<0.001**	a) Reject Ho; survivorship is not equal at each concentration
	b) Salinity: 0.833	b) Do not reject Ho; survivorship is not statistically different at low and ambient salinity
	c) Interaction: 0.833	c) Do not reject Ho; no significant effect due to interaction

Table 3.3. Regression analysis for the prediction of LC_{50} and 95% confidence interval (CI) and slope of the regression line 95% CI for three marine invertebrates exposed to diesel oil mixtures at ambient and reduced salinity, from Minitab© Release 12.

ORGANISM	SALINITY	LC_{50} and 95% CI	REGRESSION LINE SLOPE and 95% CI
Mysis stenolepis	Ambient	3.426 µL/L (3.184 µL/L, 3.669 µL/L)	-4.786 (-5.765, -3.811)
	Reduced	2.924 µL/L (2.613 µL/L, 3.234 µL/L)	-7.719 (-12.843, -2.595)
Gammarus oceanicus	Ambient	42.70 µL/L (34.80 µL/L, 50.59 µL/L)	-0.161 (-0.214, -0.107)
	Reduced	-5.03 µL/L (-28.61 µL/L, 18.55 µL/L)	-0.123 (-0.196, -0.050)
Littorina obtusata	Ambient	384.2 mL/L (336.9 mL/L, 431.4 mL/L)	-0.030 (-0.040, -0.021)
	Reduced	395.7 mL/L (217.6 mL/L, 573.7 mL/L)	-0.033 (-0.044, -0.023)

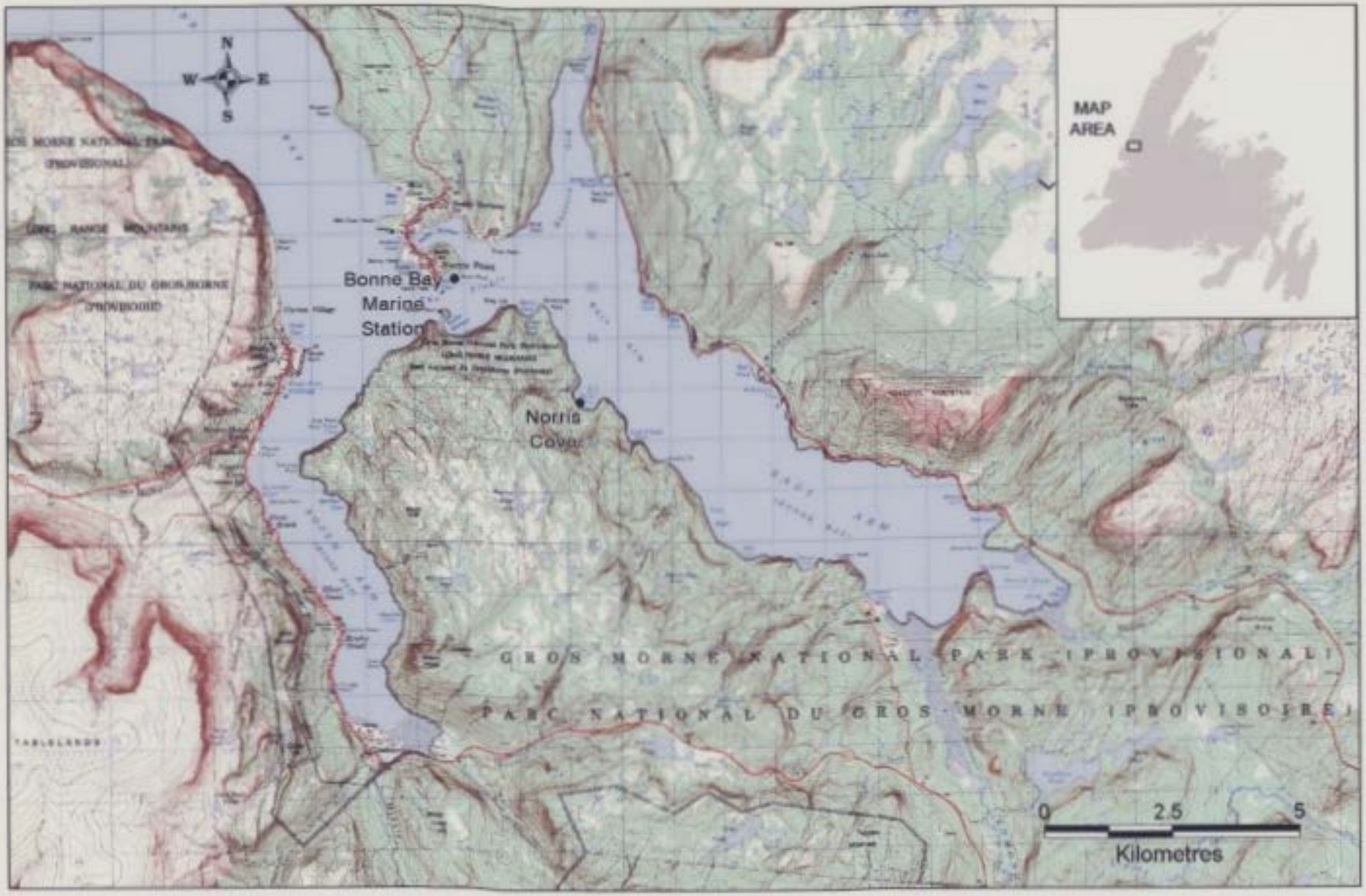
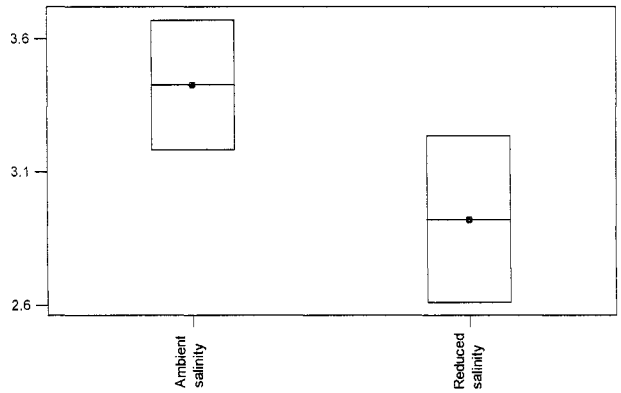
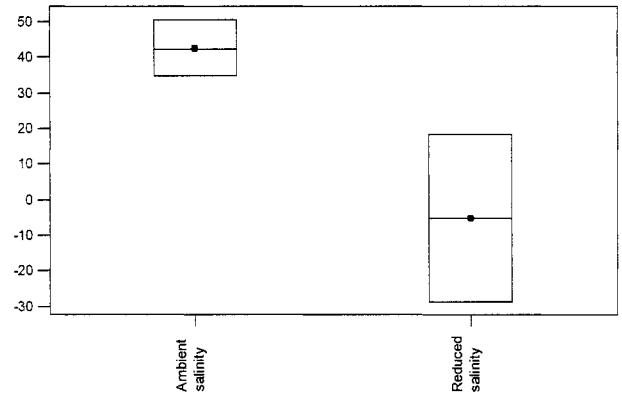


Figure 3.1. Bonne Bay location map showing Norris Cove and the Bonne Bay Marine Station, the sites of organism collection and toxicity testing, respectively.

A. Mysis gaspensis



B. Gammarus oceanicus



C. Littorina obtusata

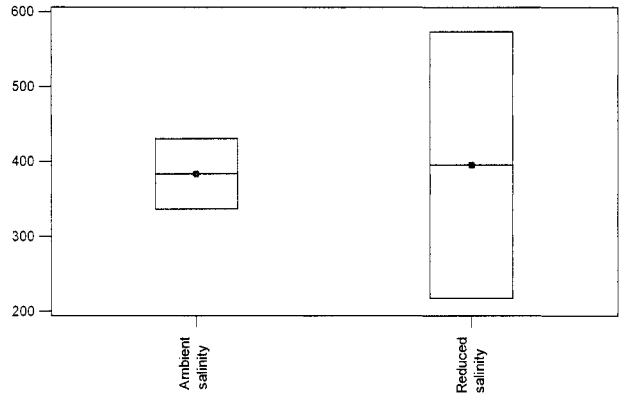
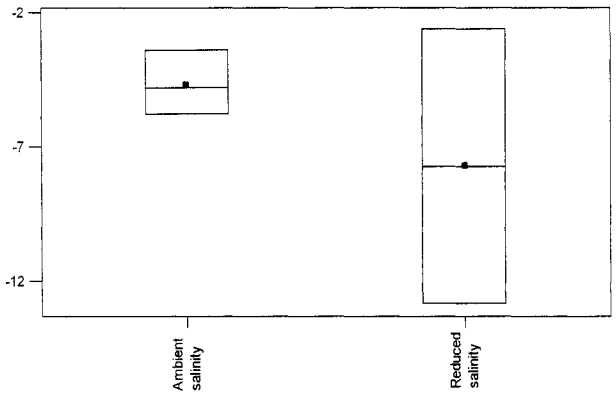
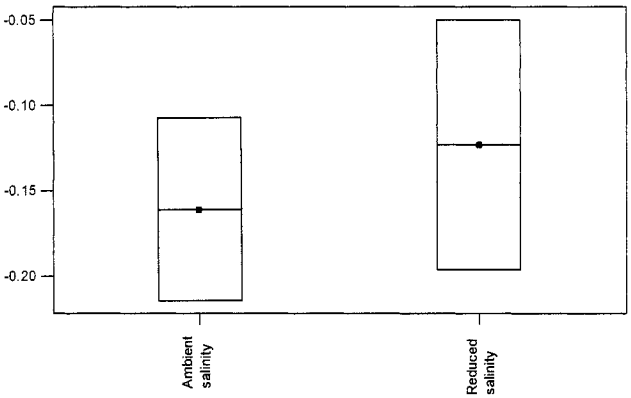


Figure 3.2. Boxplots of the LC_{50} values, with 95% confidence intervals, for three test species. The top and bottom limits of the box represent the confidence intervals and the red circles represent the slopes.

A. Mysis gaspensis



B. Gammarus oceanicus



C. Littorina obtusata

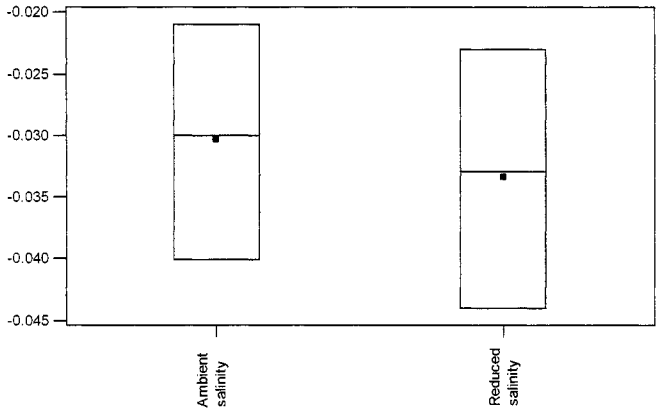


Figure 3.3. Boxplots of the slopes of the regression lines, with 95% confidence intervals, for three test species. The limits of the box represent the confidence intervals and the red circles represent the slopes.

A. Individual 95% confidence limits for the mean

Condition	N	Mean	StDev	----------+---------+---------+------
1	10	8.200	6.033	(----*-----)
2	10	11.900	5.971	(-----*----)
3	10	18.400	0.966	(-----*----)
4	10	19.300	1.059	(-----*----)
				----------+---------+---------+------
				10.0 15.0 20.0

B. Tukey's pairwise comparisons

	1	2	3
2	-8.886		
	1.486		
3	-15.386	-11.686	
	-5.014	-1.314	
4	-16.286	-12.586	-6.086
	-5.914	-2.214	4.286

Figure 3.4. A. Plot of the confidence limits of the means and B. Plot of Tukey's pairwise comparisons from one-way analysis of variance tests on survivorship of *Mysis stenolepis* exposed to four tests conditions. Test condition 1: reduced salinity water + diesel oil; test condition 2 : ambient salinity water + diesel oil; test condition 3 : reduced salinity water; test condition 4 : ambient salinity water; $\alpha = 5\%$, from Minitab© Release 12.

A. Individual 95% confidence limits for the mean

Condition	N	Mean	StDev	----------+---------+---------+------
1	12	1.917	2.151	(-*--)
2	12	10.833	4.324	(-*-)
3	12	19.750	0.452	(--*-)
4	12	18.583	1.311	(-*-)

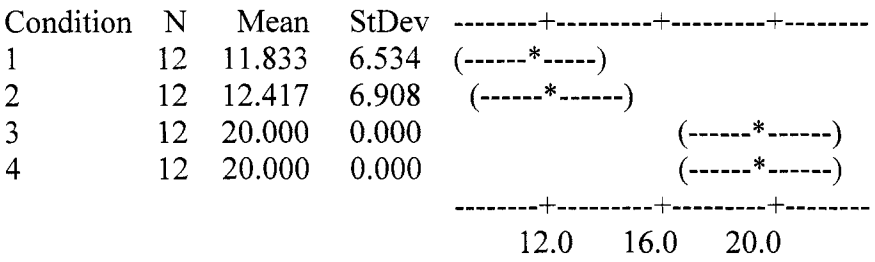
----------+---------+---------+------
6.0 12.0 18.0

B. Tukey's pairwise comparisons

	1	2	3
2	-11.658 -6.175		
3	-20.575 -15.092	-11.658 -6.175	
4	-19.408 -13.925	-10.492 -5.008	-1.575 3.908

Figure 3.5. A. Plot of the confidence limits of the means and B. Plot of Tukey's pairwise comparisons from one-way analysis of variance tests on survivorship of *Gammarus oceanicus* exposed to four tests conditions. Test condition 1: reduced salinity water + diesel oil; test condition 2 : ambient salinity water + diesel oil; test condition 3 : reduced salinity water; test condition 4 : ambient salinity water; α = 5%, from Minitab© Release 12.

A. Individual 95% confidence limits for the mean



B. Tukey's pairwise comparisons

	1	2	3
2	-5.771 4.605		
3	-13.355 -2.979	-12.771 -2.395	
4	-13.355 -2.979	-12.771 -2.395	-5.188 5.188

Figure 3.6. A. Plot of the confidence limits of the means and B. Plot of Tukey's pairwise comparisons from one-way analysis of variance tests on survivorship of *Littorina obtusata* exposed to four tests conditions. Test condition 1: reduced salinity water + diesel oil; test condition 2 : ambient salinity water + diesel oil; test condition 3 : reduced salinity water; test condition 4 : ambient salinity water; $\alpha = 5\%$, from Minitab© Release 12.

Appendix 3.1

A. Toxicity test results for survivorship of *Mysis stenolepis*. *M. stenolepis* were 15 – 17 mm long adults; ambient salinity water = 30±0.5 su, 15±1°C, > 60% oxygen saturation; low salinity water: 20.5±0.5 su, 15±1°C, > 60% oxygen saturation.

	LC_{50} test results of fresh diesel oil in ambient salinity water (Range Finding Tests)				**LC_{50} test results of fresh diesel oil in reduced salinity water**			
Concentration (µl/L)	**Survivorship (/20)**	**trials**	**Average %**	**30 su water**	**Survivorship (/20)**	**trials**	**Average %**	**21 su water**
1.5	15	40	85%	20	-	-	-	-
	19			20	-		-	-
1.67	17	80	91.25 %	20	-	-	-	-
	16			20	-		-	-
	20			-	-		-	-
	20			-	-		-	-
2.00	15	40	77.5%	18	-	-	-	-
	16			19	-		-	-
2.33	19	40	92.5%	20	15	40	72.5%	17
	18			19	14			19
2.66	18	80	87.5%	20	12	40	57.5%	18
	17			20	11			17
	17			20	-			-
	18			20	-			-
3.00	12	80	68.75%	17	3	40	47.5%	18
	13			18	16			19
	16			-	-			-
	14			-	-			-
3.33	7	80	48.75%	19	0	40	15%	19
	4			20	6			20
	11			-	-			-
	17			-	-			-
4.00	5	40	27.5%	20	2	40	12.5%	19
	6			20	3			18
5.00	0	40	0%	20	-	-	-	-
	0			20	-		-	-
6.00	0	40	0%	20	-	-	-	-
	0			20	-		-	-
5.00*	18		95%	20	15	40	73%	17
	20			20	14			18
8.00*	16		65%	20	16	40	55%	20
	10			20	6			17

*** larger size class**

B. Toxicity test results for survivorship of *Gammarus oceanicus. G. oceanicus* were 2.5 – 2.7 cm long adults; ambient salinity water = 30±0.5 su, 15±1°C, > 60% oxygen saturation; low salinity water: 20.5±0.5 su, 15±1°C, > 60% oxygen saturation.

	LC_{50} test results of fresh diesel oil in ambient salinity water (Range Fining Tests)				**LC_{50} test results of fresh diesel oil in reduced salinity water**			
Concentration (µl/L)	**Survivorship (/20)**	**trials**	**Average %**	**30 su water**	**Survivorship (/20)**	**trials**	**Average %**	**21 su water**
5	20	40	100%	20	-	-	-	-
	20			20	-	-	-	-
7	20	40	98%	20	-	-	-	-
	19			20	-	-	-	-
8	20	40	100%	20	-	-	-	-
	20			20	-	-	-	-
12	20	40	100%	20	-	-	-	-
	20			20	-	-		-
15	15	80	70%	19	7	40	28%	19
	15			20	4			20
	14			-	-			-
	13			-	-			-
18	19	40	95%	20	-		-	-
	19			20	-			-
20	7	40	38%	19	4	40	13%	16
	8			20	1			18
22	18	40	95%	20	-		-	-
	20			20	-			-
25	19	40	80%	20	-		-	-
	13			20	-			-
30	11	80	74%	20	2	40	10%	20
	13			20	2			19
	17			-	-			-
	18			-	-			-
40	14	80	49%	20	0	40	0%	20
	12			20	0			19
	0			20	-			-
	13			19	-			-
45	16	80	53%	19	0	40	5%	19
	15			20	2			17
	4			20	-		-	-
	7			20	-		-	-
50	3	40	18%	20	0	40	2.5%	19
	4			20	1			17
135	1	40	2.5%	20	-		-	-
	0			19	-		-	-
405	1	40	2.5%	20	-		-	-
	0			20	-		-	-
1215	0	40	0%	20	-		-	-
	0			19	-		-	-

C. Toxicity test results for survivorship of *Littorina obtusata. L obtusata* were 5 – 6 mm in height, with 3 –4 mm opercular openings; ambient salinity water = 30±0.5 su, 15±1°C, > 60% oxygen saturation; low salinity water: 20.5±0.5 su, 15±1°C, > 60% oxygen saturation.

	LC_{50} test results of fresh diesel oil in ambient salinity water (Range Finding Tests)				**LC_{50} test results of fresh diesel oil in reduced salinity water**			
Concentration (ml/L)	**Survivorship in 30± 0.5 salinity units**	**trials**	**Average %**	**Control (no diesel oil)**	**Survivorship in 20.5±0.5 salinity units**	**trials**	**Average %**	**Control (no diesel oil)**
100	20	40	100%	20	20	40	100%	20
	20			20	20			20
200	18	40	87.5%	20	14	40	75%	20
	17			20	16			20
300	19	120	60%	20	15	80	70%	20
	19			20	17			20
	10			20	16			20
	6			20	8			20
	9			20	-			-
	9			20	-			-
400	6	40	60%	20	4	40	20%	20
	6			20	4			20
500	4	80	27.5%	20	4	40	20%	20
	4			20	4			20
	8			20	-			-
	6			20	-			-
600	6	40	22.5%	20	-	-	-	-
	3			20	-	-	-	-
700	0	40	12.5%	20	-	-	-	-
	5			20	-	-	-	-

Conclusions

Significant quantities of diesel were present at the diesel oil spill site up to two years after the spill. The accumulation of hydrocarbons in biota and dramatic population reductions indicated evident that organisms were impacted by the presence of diesel. Finally, Chapter 1 indicated the oil spill site was an area stressed by an uncharacteristically low-salinity environment.

Analysis of survival of transplanted, caged, marine intertidal invertebrates demonstrated that the coastline was negatively affected by toxicity relating to the diesel fuel and hypo-saline conditions created by the presence of a semi-permanent rock berm. From Chapter 2 it was concluded that current conditions inside the berm could not support normal marine life.

Toxicity tests indicate that all marine intertidal organisms do not react equally to stresses such as diesel oil and reduced salinity, or the combination of these factors, in the short term. However, by considering these organisms as a part of a community and examining what the overall effects of diesel and reduced salinity, alone or in combination, are on this community, it can be concluded that even short-term exposure to these conditions is devastating.