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Hibernia Production Phase Environmental Effects Monitoring Program – Year Seven (2009) Volume I - Interpretation

Prepared for

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Final Report

File No. 1051516

Date: March 15, 2012

EXECUTIVE SUMMARY

As a Condition of Approval for the Hibernia project (Canada-Newfoundland and Labrador Offshore Petroleum Board (C-NLOPB) Condition 12), an Environmental Effects Monitoring (EEM) program was designed and implemented with input from international and national specialists reflecting the current scientific knowledge of the day. The design of the EEM Program drew on a number of information sources, including the Hibernia Baseline Characterization Program (HMDC 1995) and dispersion model results for drill cuttings and produced water (Seaconsult 1993). The public and regulatory agencies were consulted during the development of the Hibernia EEM program, and the resulting EEM design document was formally approved by the C-NLOBP in 1997. The EEM program was designed to detect changes on the quality of the receiving environment through examination of sediment quality and subsequent biological uptake and took into consideration platform operating conditions at that time. The main goal of the EEM program was to determine the area demonstrably affected by Hibernia Project activities.

Operational EEM programs were conducted in 1998, 1999, 2000, 2002, 2004, 2007 and 2009. Seabed sediments and commercial fish species from the Hibernia Field (Study Area) are sampled in each program year to assess environmental effects. The sediment samples are collected for physical, chemical and toxicity testing. The selected commercial fish species, American plaice (a common flatfish species), are sampled to test for contaminants (body burden), taint and various health indices. This report interprets and discusses the results from the seventh year of sampling for the program conducted in 2009.

During the 2009 EEM program, seafloor sediments were sampled at 32 locations in a sampling net radiating out 6,000 m from the Hibernia platform along four of the radii (north, east, west and south) and out to 1,000 m from the other four radii (northeast, southeast, southwest and northwest). In addition, there are two control stations located at a distance of 16,000 m from the GBS on the north and west radii for use as field reference locations for the toxicity testing.

Statistical tests were conducted on the data to detect project-induced contamination in the marine environment over time and space. The tests are designed to detect statistically significant differences between samples taken at various distances from the Hibernia platform, between samples taken at similar locations in different years, between samples at the Reference Area and the Hibernia Area, and between samples within a single station.

Hydrocarbons and barium, both constituents of drill muds, were used as indicator chemicals to estimate the zone of influence for project activities. Concentrations of hydrocarbons and barium were elevated near the platform discharge points and concentrations decreased with distance from the platform as expected. The results of the 2009 Hibernia EEM program found continued improvements in the sediment contaminant concentrations as compared to previous years' data. The 2009 hydrocarbon concentrations have decreased to concentrations comparable to the 1998 data or 1994 baseline data. The 2009 barium concentrations have increased to concentration similar to the 2002 barium concentrations. Partial re-injection of synthetic-based mud (SBM) drilling wastes commenced in March 2000 with greater than 95 percent re-injection

being achieved in September 2002. The continued improvement observed in sediment contaminant concentrations is directly attributed to these operational changes that were instituted on the Hibernia platform commencing in 2000.

Sediment toxicity tests were subjected to rigorous statistical analyses. There were 7 Microtox responses in 2009 at stations 1-6000, 3-500, 3-2000, 4-1000, 7-2000, 7-3000 and 7-6000. The Microtox responses for the 1999, 2000, 2002 and 2004 data correlated with sediment particle profiles that were comprised of 15 percent silt and clay fractions. However, this relationship was not observed for 1994, 1998, 2007 and 2009 data. An in-depth analyses of the Microtox responses indicated that the Microtox responses correlated with elevated strontium, TIC/TOC and ammonia levels as well as particle size profiles and no causal link between the observed Microtox responses and Hibernia platform discharges can be demonstrated.

In 2009, positive responses for juvenile polychaete survival and growth and amphipod survival occurred at stations 7-2000 and 7-3000. A toxic response for juvenile polychaete survival was also observed at stations 2-250. Statistical analyses found that these positive bioassay responses were not correlated with Hibernia operational discharges.

A water chemistry program has been undertaken as part of the Hibernia EEM Program since 2004. Sample locations for the 2009 water chemistry program were chosen based on CTD profiles that indicated the potential for produced water to be located at a particular location. The presence of elevated metals, hydrocarbons and PAH concentrations was detected near the Hibernia platform. The 2004, 2007 and 2009 water column program validated dilution ratios that were predicted to occur within 50 m of the discharge point as per produced water modelling (Lorax Environmental 2004). The observation of elevated concentration of contaminants were of such a limited spatial extent and magnitude, that it was concluded that the observed elevated parameters are localized adjacent to the produced water discharge area (essentially within 50 m from the discharge point). This finding is in agreement with results of international programs to date.

In 2004, only lube range hydrocarbons (C21-C32) were present in most American plaice livers from both the Hibernia and Reference Areas. In 2007 and 2009, both the lube and fuel range hydrocarbons were detected in fish livers from Hibernia and Reference areas. There were no statistically significant differences between the Hibernia and Reference areas in the levels of lube and fuel range hydrocarbons detected in the livers. There were differences in metal concentrations detected between the Hibernia and Reference areas. It is important to note that there is no pattern to the differences in metal concentrations detected and these differences are attributed to inter-annual variations and not attributed to Hibernia platform discharges. The results of the fish health study indicated the health status of American plaice collected at the Hibernia Area is similar to those collected at the Reference Area. The taints assessments (by triangle test and hedonic scaling) found that American plaice were not tainted at either the Hibernia or Reference areas.

The Hibernia EEM program was designed to be scientifically comprehensive and has, by the detection of the changes in sediment quality, proven to be effective in detecting project-induced chemical and biological changes over space and time. Further improvements in sediment contaminant concentrations occurred in 2009. The improvements in sediment contaminant

concentrations (since 2002) are directly attributed to the re-injection of Hibernia's synthetic based drill cuttings, solids and muds.

In conclusion, changes attributable to the project during the 2009 Hibernia EEM program were limited. The spatial extent and magnitude of chemical contaminants continue to improve and are comparable to 1998 or baseline data (1994), dependent upon the parameter. Within the water column, a potential zone of effects is of limited spatial extent and magnitude. Metal and hydrocarbon body burdens (as measured in American plaice) are not affected by project activities. Biological resources (as measured in American plaice) were not tainted by project activities. Fish health indicators (as observed in American plaice) were similar between Hibernia and Reference areas.

ACKNOWLEDGEMENTS

The Hibernia EEM program (2009) was led by Stantec Consulting Ltd. (Stantec) (St. John's, Newfoundland and Labrador) under contract to Hibernia Management and Development Company Ltd. (HMDC) and under the guidance of Robert Dunphy (HMDC).

Stantec led data collection, with participants including Matthew Hynes, Barry Wicks, Doug Rimmer, John Pennell, Kristian Greenham and James Loughlin. Fugro Jacques Geosurvey's Inc. provided geopositional services for sediment and water collections. Chemical analyses of sediment, water and tissues were conducted by Maxxam Analytics (Halifax, Nova Scotia and St. John's, Newfoundland and Labrador). Particle size analysis was conducted by Stantec. Sediment toxicity tests were supervised by Trudy Toms of Stantec - Laboratory Division. Fish taste tests were performed at the Marine Institute of Memorial University. Fish health indicator analyses were supervised by Dr. Anne Mathieu of Oceans Ltd. (St. John's, Newfoundland and Labrador). Sediment quality, water quality and body burden data were analyzed by Dr. Malcolm Stephenson, Sam Salley, Virginia Soehl and Angus Campbell. Project management was executed by Sandra Whiteway. The Stantec analysis and reporting team included Barry Wicks, Beverley Best, Theresa Tobin, Karen Williams and Stephen Rowe. Sandra Whiteway (Stantec) reviewed the document before final printing.

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1.0 INTRODUCTION

1.1 Project Setting

Hibernia is located near the northeast corner of the Grand Banks, approximately 315 km east-southeast of St. John's, Newfoundland and Labrador, and approximately 35 km northwest of the Terra Nova Oil Field. The White Rose site is located approximately 50 km east-northeast of the Hibernia platform (Figure 1.1).

The Hibernia field was discovered in late 1979. Following construction of the Hibernia platform at Bull Arm, Newfoundland, from 1990 to 1997, tow out to the Grand Banks occurred in May 1997. The platform was installed on the seafloor and drilling commenced in June 1997. Throughout the summer of 1997, additional subsea infrastructure (the offshore tanker loading system) was installed. First oil occurred on November 17, 1997. The development is operated by the Hibernia Management and Development Company Limited (HMDC).

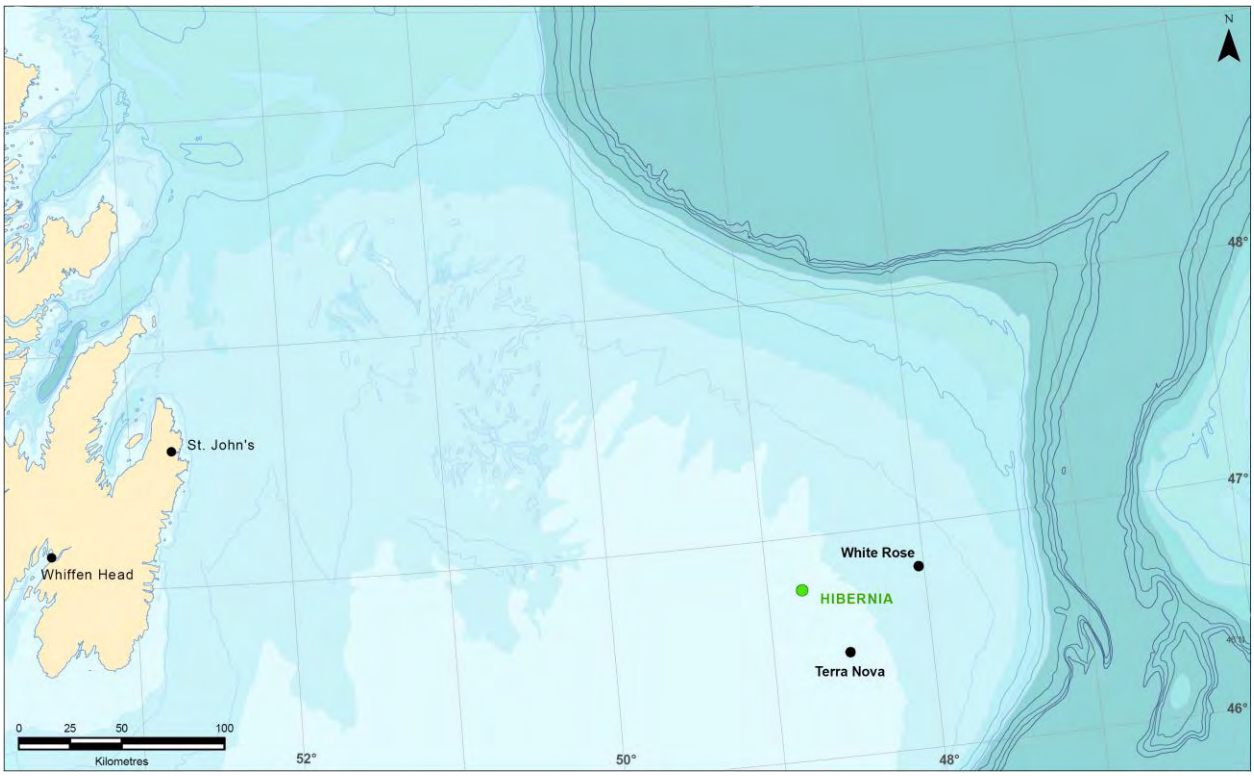
Photo 1 Hibernia Gravity Base Structure

Hibernia uses a single fixed platform, or gravity-based structure (GBS) (Photo 1), to complete drilling of at least 64 and possibly up to 83 or more development wells that will be required during the life of the project. Hibernia crude is shipped from the platform to the IMTT Transshipment Terminal at Whiffen Head, Placentia Bay, NL, by the purpose-built shuttle-tankers *Kometik*, *Mattea* and the *Vinland*. The tankers and the transshipment terminal are completely independent of the Hibernia drilling and production operation.

Regulated discharges to the marine environment during the production phase include liquid discharges (produced water, storage displacement water, platform drainage water, sea water return, sanitary and domestic wastes), drill cuttings, solids and muds.

On average, water-based muds (WBMs) are used for the first 300 to 400 m of the well depths, and the remainder of wells are drilled using a synthetic-based mud (SBM) system due to challenges posed in extended reach and directional drilling.

Figure 1.1 Location of the Hibernia Field



1.2 Project Commitments

In the Environmental Impact Statement (EIS) prepared for the Hibernia project (Mobil 1985), a commitment was made by the Hibernia partners to undertake environmental effects monitoring (EEM). In submitting its development plan for the Hibernia Project in 1985, Mobil Oil, on behalf of Hibernia, stated:

> *"Effects monitoring will be under taken to detect changes in the environment surrounding the project that can be attributed to the project."*

The Canada-Newfoundland and Labrador Offshore Petroleum Board (C-NLOPB) approval of the Hibernia Development Plan was announced in June 1986. This project approval was subject to a number of conditions, including:

Condition 12

> *"It is a condition of the approval of the Hibernia Development Plan that prior to production, the Proponent submit, for the Board's approval, its plans for environmental compliance and effects monitoring programs."*

The EEM Plan for the Hibernia Development Project's production phase was submitted to the C-NLOPB by HMDC in April 1996 (HMDC 1996). Final C-NLOPB approval of the EEM Plan was obtained in June 1996.

The EEM program is one of a series of environmental protection initiatives outlined in the HMDC Operational Plan, which forms an integral part of production operations, including environmental compliance monitoring and emergency response management. Environmental compliance monitoring characterizes effluent to verify conformance to the discharge limits that are described in the Environmental Protection Plan associated with the authorized work or activity.

The EEM program functions to detect project-induced changes in the marine environment. The outcomes should be reviewed by the operator to determine if outcomes match the expected outcomes predicted in the environmental assessment.

1.3 Environmental Effects Monitoring Objectives

HMDC established a set of specific objectives to be met in the development and application of its 1996 EEM Design Program. These objectives are:

- fulfill regulatory information requirements and address legitimate public concerns;
- provide early warning of potential project-induced environmental effects;
- meet the project needs;
- be scientifically defensible;
- be cost-effective, making optimal use of personnel, technology and equipment;
- use the data which will be collected for assessment and, where necessary, to modify operational practices and procedures; and
- analyze and interpret data so that the results are understandable both to the public and non-scientists.

The EEM program is designed to provide the primary means to determine and quantify project-induced detectable changes in the surrounding environment. Where such meaningful changes occur, the EEM program will provide the context for evaluating these changes in terms of scientific meaning and regulatory frameworks, and will assist in identifying any required modification to operations.

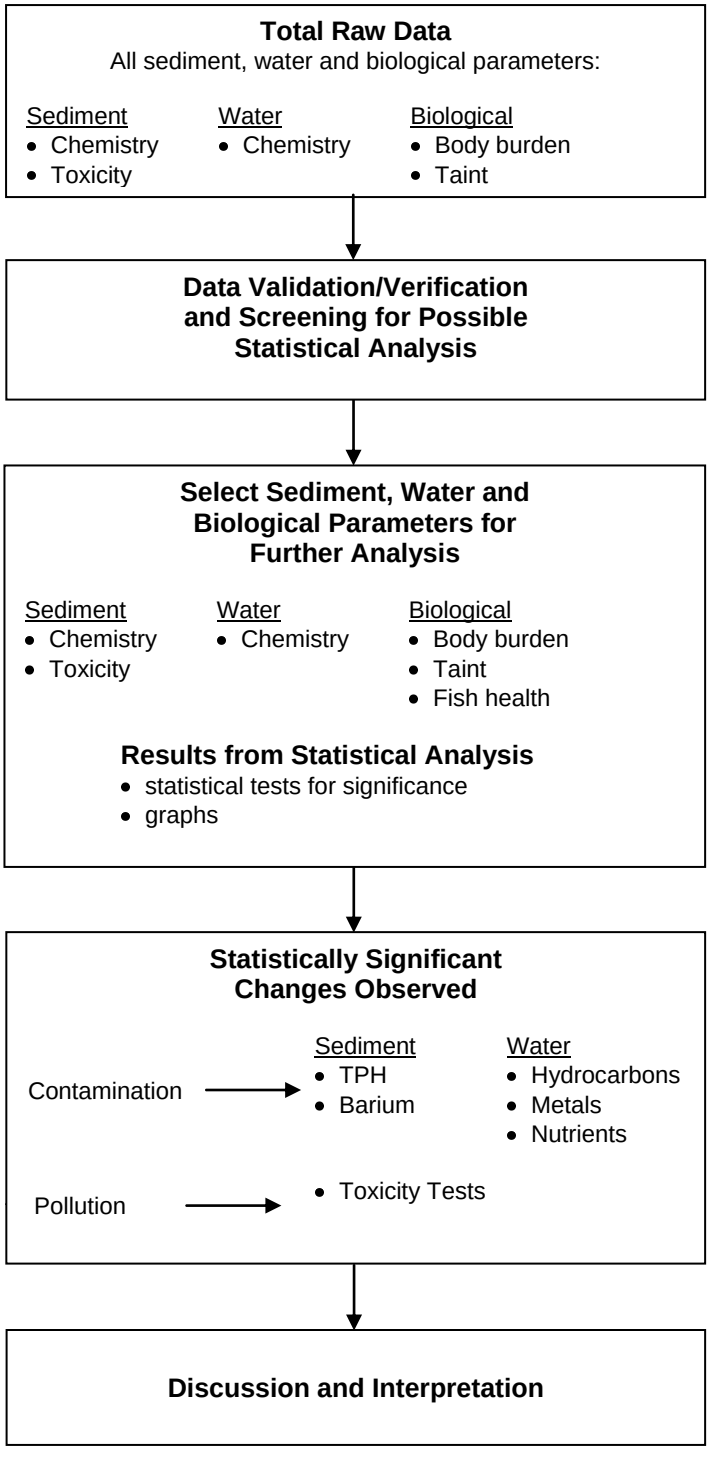
1.4 Evaluation and Interpretation of the 2009 Hibernia Environmental Effects Monitoring Data

The step-wise process employed by the study team to evaluate and interpret data collected for the EEM program is illustrated in Figure 1.2. The data collected for the 2009 Hibernia EEM program consisted of sediment chemistry, sediment toxicity, water chemistry, taint, fish health and analysis of potential contaminant substances (body burden) in tissues of American plaice (*Hippoglossoides platessoides*). The data collected was subjected to a verification and validation process, prior to statistical analysis, to ensure they are of acceptable quality. The data were screened to determine their usefulness to:

- describe potential contamination from Hibernia's process discharges (produced water, storage displacement water, platform drain water, sea water return) and drilling discharges (drilling muds, drill cuttings and solids); and

- to evaluate their suitability for statistical analysis, specifically whether the data set for a particular parameter contained a sufficient number of data points above the reportable detection limit (RDL) to conduct statistical analyses.

Figure 1.2 Process for Evaluating and Interpreting Environmental Effects Monitoring Data



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The data collected during the 2009 EEM program were evaluated using a number of data interpretation and statistical tests, including:

- data screening to evaluate which parameters were routinely detectable, or where most values were below detection limits, whether there was a pattern of detection that would be consistent with releases from the Hibernia platform;
- 2-dimensional (2-D) colour contour plotting to visually represent the data and aid in the evaluation of temporal and spatial trends; and
- statistical tests of significance based on Analysis of Variance (ANOVA) methods.

The statistical analysis pays special attention to parameters that can plausibly be linked to operations of the Hibernia platform. These include some parameters for which statistically significant changes have been observed in past EEM programs, specifically, hydrocarbons, barium and Microtox for the previous Hibernia EEM Programs (HMDC 1995; 1999; 2000; 2001a; 2003a, 2005 and 2007). In addition to continuing to closely monitor these three variables, the analysis of the 2009 Hibernia EEM Program also reviews the temporal and spatial trend analysis of other chemical constituents from all EEM programs to date (HMDC 1994, 1998, 1999, 2000, 2002, 2004, 2007 and 2009).

A glossary of acronyms used in this report is provided in Appendix A.

2.0 REGULATED/APPROVED DISCHARGES

The OWTG (National Energy Board (NEB) *et al.* 2010) outline the recommended practices and standards for the treatment and disposal of wastes associated with offshore oil operations. A formal review of the OWTG (NEB *et al.* 2010) is undertaken every five years to ensure the guidelines reflect current scientific and technical knowledge. The limits noted in the following sections are based on the current OWTG (NEB *et al.* 2010). The location of discharge points for regulated discharges are shown in Figures 2.1 and 2.2, respectively.

2.1 Contamination Versus Pollution

The United Nations Group of Experts on Scientific Aspects of Marine Environmental Protection (GESAMP) recognizes the term *contamination* to refer to *elevated levels of a chemical as compared to background levels* (GESAMP 1993). They also recognize the term *pollution* to refer to the *effects of the contamination on the biota.* The term contamination and the derivation contaminant will be used as per the GESAMP definition in this report. The definitions for contamination and pollution as recognized by GESAMP (1993) clearly demonstrate that contamination (i.e., levels elevated as compared to background levels) does not necessarily indicate that an ecological effect has occurred.

Biological effects to marine life would be the principle concern associated with platform effluent discharge. Such effects may be either biological or physical in nature and can be direct or indirect. For example, biological effects may occur when marine life is exposed to contaminated water or sediment. The magnitude of the resulting effect is dependent upon such factors as the nature of the contaminant, its concentration in the marine environment and the degree of exposure (i.e., related to dose/response).

2.2 Liquid Discharges

2.2.1 Produced Water

Produced water is defined as “all water separated from crude oil or gas during the primary processing of oil and gas on offshore production platforms” (Environment Canada 1990) and consists of both injected sea water and formation water. This definition encompasses formation water, injection water (sea water injected to enhance oil recovery) and production and injection water treatment chemicals (HMDC 1994). Hibernia produced water (HMDC 2001b) contains traces of zinc and manganese, with larger amounts of barium, boron, calcium, magnesium, and strontium. It also includes high levels of total dissolved solids, and elevated ammonia and hardness levels. It contains a variety of hydrocarbon fractions, including selected polycyclic aromatic hydrocarbons (PAHs), as well as naturally occurring radionuclides. All chemicals are screened for offshore use in accordance with the C-NLOPB Offshore Chemical Management System (OCMS) guidelines (NEB *et al.* 2009).

Figure 2.1 Hibernia Platform Sea Water Inlet and Discharge Locations

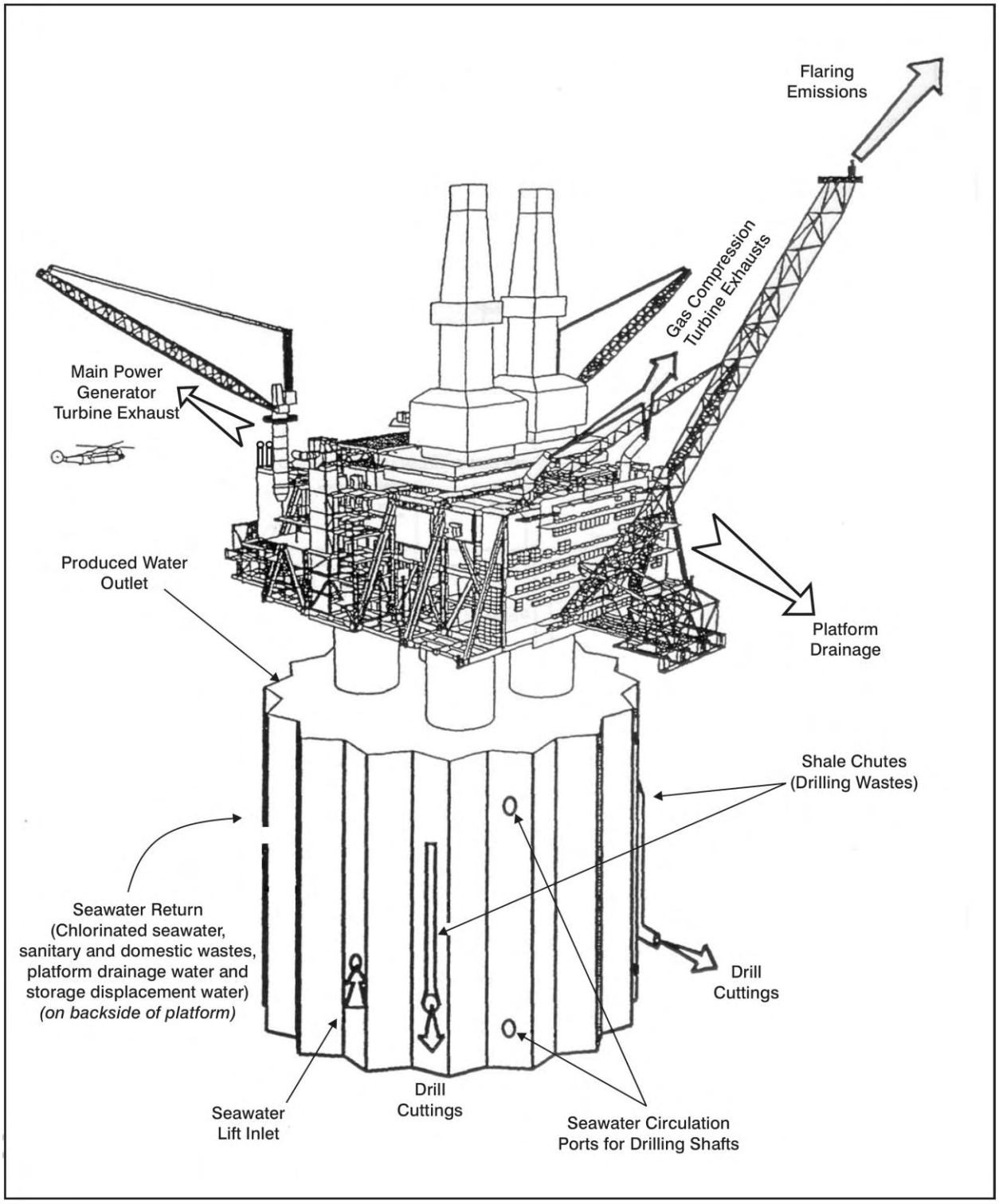
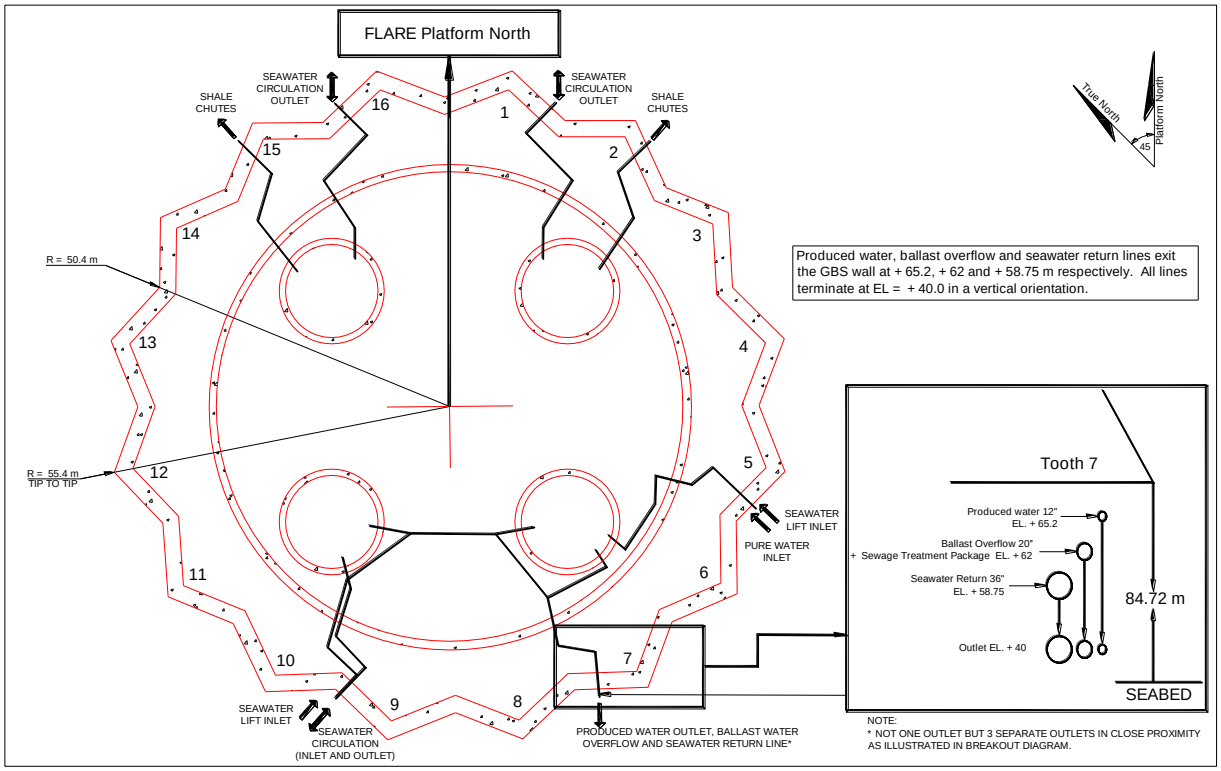


Figure 2.2 Hibernia Platform: Cross Sectional Sea Water Inlet and Discharge Locations



Formation water is comprised of reservoir interstitial water present under natural conditions, having a chemical composition that is in equilibrium with the resident oil under conditions of high temperature and pressure (HMDC 1994). The chemical composition of the formation water can vary greatly between fields and even between wells (Thomas 1984). The most common chemical constituents associated with formation waters are calcium, magnesium, sodium, bicarbonate, sulphates and chloride. The hydrocarbon content of formation water generally consists of some aromatics and light fraction hydrocarbons. The chemical constituents of concern associated with formation water are entrained oil or petroleum hydrocarbons, metals, and low dissolved oxygen concentrations (Abernathy 1989).

Injection water (sea water injected into the reservoir for pressure maintenance) forms a component of produced water after break-through has occurred, that is, after water from injection wells has swept through the reservoir to producing wells (HMDC 1994). The injection water used at the Hibernia field will consist of deaerated sea water that may be treated with processing chemicals to remove trace oxygen, control biological growth and minimize corrosion (HMDC 1994). The treatment chemicals (which vary in doses) may become part of the produced water through two processes.

Data on Hibernia's produced water (Table 2.1) collected in November 2000, 2002, 2004, 2007 and 2009 are compared with produced waters from the North Sea and the United States (Canadian Association of Petroleum Producers (CAPP) 2000). Produced water production

began in the year 2000 and a detailed chemical and toxicological characterization was undertaken by HMDC.

Table 2.1 Hibernia's Produced Water Chemical Constituents

Component (mg/L)	North Sea Data*	US Data	Hibernia 2000 Data Note ***	Hibernia 2002 Data Note ***	Hibernia 2004 Data Note ***	Hibernia 2007 Data Note ***	Hibernia 2009 Data Note ***
Total Oil	2 - 220	2.3 – 359	11	28.20	27.3	22.6	16
Dissolved Oil	≤ 760	≤ 200	11 - 12.5	-	-	-	-
Benzene	0.4 – 5 (oil) 0.3 – 440 (gas)	0.18 - 14.0	8	8.42	18.2	13.5	14.4
Toluene	0.01 – 2 (oil) 4 – 145 (gas)	0.16 – 7.95	4.29	4.62	9.64	7.9	8.9
Xylene	0.1 – 7 (oil) 0.8084 (gas)	-	1.05	1.9	3.63	2.5	2.8
Ethylbenzene	-	0.025 - 0.56	0.192	0.394	0.71	0.5	0.6
Naphthelenes	0.07 – 0.1	0.018 – 0.30	0.076 (0.128**)	0.210 (0.427**)	0.300	0.335	0.118
2,4 Dimethylphenol	-	0.016 – 0.50	-	-	0.29	-	-
Phenol	2 - 23	0.20 – 3.40	-	-	12	-	10.4
COD	130 - 15800	182 – 3000	1,110	-	-	-	-
BOD	28 - 6700	126 - 1920	480	-	-	-	-
pH	-	-	-	7.9	7.5	-	-
Turbidity (NTU)	-	-	-	629	71.6	-	-

Notes: Methodology is not indicated for North Sea and US data.
* Compiled from sources cited in Stephenson *et al.* 1994.
** HMDC (2001b): Napthalenes including 1-Methylnaphthalene, 2-Methylnaphthalene.
\- No Data.
*** Data provided from one sample.

Produced water, which includes formation water, injection water and process water, has a regulatory daily upset limit of 60 mg/L 24-hour average oil concentrations. The regulatory limit for the volume weighted 30-day rolling average oil discharge was reduced to 30 mg/L on January 1, 2008, from a previous regulatory limit of 40 mg/L. Hibernia produced water for the periods covered by the 1998, 1999, 2000, 2002, 2004, 2007 and 2009 EEM programs are summarized in Table 2.2. Produced water throughput was sporadic until July 1999, when flow became constant. Produced water had a daily mean oil concentration of 20.9 mg/L (September 2007 through September 2009), ranging from 0 to 70.0 mg/L (the higher value occurring for a brief period during system start-up). The mean volume weighted 30-day rolling average oil discharge (September 2007 through September 2009) was 21.0 mg/L and associated ranges were between 11.4 to 34.3 mg/L.

The daily average effluent flow for the period September 1, 2007 to August 31, 2009 is illustrated in Figure 2.3. All exceedances were reported directly to C-NLOPB (HMDC, pers. comm.). The data for the oil in water frequency diagram for September 2007 to August 2009 is illustrated in Figure 2.4. Frequency represents the number of times a range of oil in water concentrations occurred, as determined by compliance monitoring.

Table 2.2 Produced Water – Oil in Water (OIW)

Discharge Period	Daily Mean Oil Concentration (mg/L) (Reg. Limit 60 mg/L daily)	30-Day Rolling Average (mg/L) (Reg. Limit 30 mg/L)
Nov. 1997 - Sept. 1998	NA	NA
Sept. 1998 - Aug. 1999	20.4	21.1
July 1999 - July 2000	24.5	31.0
Aug 2000 - July 2002	28.99	29.4
Aug 2002 - Aug 2004	28.8	28.6
Sept 2004 - Aug 2007	27.4	27.3
Sept 2007 – Aug 2009	20.9	21.0

Figure 2.3 Daily Produced Water Discharge Volume in $m^3 \times 10^3$ (September 01, 2007 to August 31, 2009)

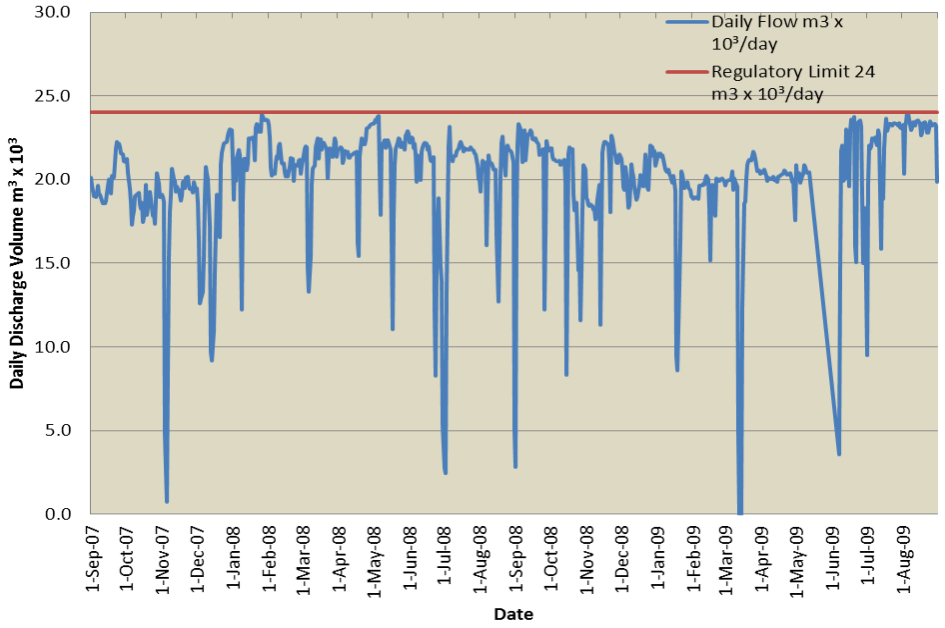
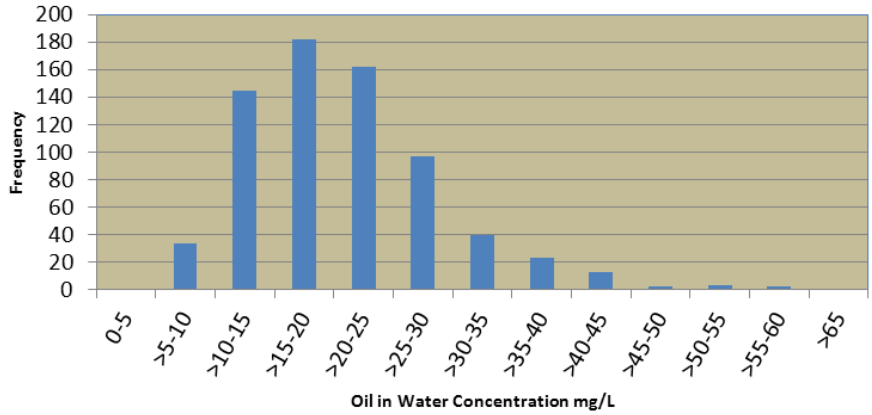


Figure 2.4 Oil In Water (mg/L) Frequency Diagram (September 01, 2007 to August 31, 2009)



2.2.2 Storage Displacement Water

Storage displacement water, or deballast water, is the chlorinated sea water that is pumped into and subsequently discharged from the GBS oil storage cells for the purposes of displacing crude oil during transfer to a pipeline or tanker (Environment Canada 1990). As more oil is produced and pumped into the top of the cell, the underlying ballast water (storage displacement water) is displaced by deballast pumps (HMDC 1994). During tanker loading, crude oil is pumped from the top of the storage cells to the tanker and seawater is pumped into the base of the cells. Storage displacement water discharge rates occur on an 1:1 basis with barrels of oil produced.

The main components of the storage displacement water are sea water, and dispersed and dissolved hydrocarbons. The maximum allowable oil in water (OIW) limit is an instantaneous 15 mg/L. The data for the Hibernia displacement water for the periods covered by the 1998, 1999, 2000, 2002, 2004, 2007 and 2009 EEM programs are summarized in Table 2.3.

The Hibernia OIW (September 2007 through August 2009) discharge concentrations ranged from 0.0 to 3.1 mg/L. The daily average effluent flow ranged from 0 to 25,755 m^3/day. Storage displacement water values were within expected parameters, with no observed exceedances (HMDC pers. comm.).

Table 2.3 Storage Displacement Water Discharges (1997-2009)

Discharge Period	Daily Mean Oil Concentration (mg/L) (Reg. Limit 15 mg/L daily)	Daily Average Effluent Flow (m^3/day)
Nov. 1997 - Sept. 1998	2.17	0 - 28,000
Sept. 1998 - Aug. 1999	1.45	0 - 27,965
July 1999 - July 2000	2.1	0 - 39,967
Aug 2000 - July 2002	1.73	0 - 39,954
Aug 2002 - Aug 2004	1.1	0 - 36,269
Sept 2004 - Aug 2007	0.7	0 - 35,108
Sept 2007 – Aug 2009	0.5	0 - 25,755

Typically, storage displacement water is rapidly diluted to levels below toxic and sublethal thresholds within short distances from the outfall (Thomas 1984). Effluent modelling studies indicated that oil and residual chlorine concentration would reach maximum concentrations during the summer, when the receiving water is thermally stratified, resulting in limited dispersion (Seaconsult 1993). The storage displacement water may be trapped at 35 to 38 m below the surface, with the worst case scenario yielding a zone of potential contaminants no larger than 300 to 400 m from the platform (Seaconsult 1993).

2.2.3 Deck Drainage Water

Deck drainage is defined as all water (salt and fresh) resulting from precipitation, deck washings, tank and equipment operations, and runoff from curbs, gutters and drains (Environment Canada 1990). This waste stream exhibits intermittent and variable content and flow rate characteristics. Deck drainage may contain precipitation, sea water (ocean spray, firewater and service water), small amounts of hydrocarbons, drilling muds, lubricants, sealing fluids, chemicals, detergents and solids (Thomas 1984). At Hibernia, deck drain effluent is

collected and treated by two separate systems on the platform referred to as process area and drilling area drains, with each having dedicated treatment systems.

In areas where spillage from materials or emulsified oils resulting from machinery cleaning is possible, the streams are segregated and treated for removal of dispersed hydrocarbons. Some dissolved chemicals and oils reach the receiving environment regardless of the precautions taken (HMDC 1994).

2.2.3.1 Process Area Drainage Water

The open hazardous area drains route all drainage wastes servicing the process area to the open hazardous area drains tank; whereas, the open non-hazardous area drains, which service mainly the utility area, empty into the open non-hazardous area drain tank (HMDC 1994).

The distinction between hazardous area and non-hazardous area drains is based on the area in which the drains occur and the potential safety risks posed to personnel. The terms do not necessarily reflect characteristics of substances that could potentially enter the system.

Hazardous Area Drains:	*Drains occurring in those areas of the Hibernia platform that contain highly pressurized systems. Typically, hazardous area drains are associated with crude processing areas.*
Non-Hazardous Area Drains:	*Drains occurring in those areas of the Hibernia platform that are of lower or atmospheric pressurized systems.*

Oil, water and solids are separated by gravity separation. The oily layer of the water compartment is designed to flow over a weir into the oil compartment of the same tank.

The accumulated oil is transferred to the low-pressure separator enabling oil recovery. The water is transferred to the oily water treatment package that uses disk-stacked bowl-type centrifuges to separate oil, water and solids.

The process area hazardous drains collect effluents mainly from the process area, but also includes small areas within the wellhead and mud modules not serviced by drilling drains. These effluents may contain oily water from the processing equipment, pig launchers and receivers, as well as contaminated water from the chemical laydown area on the weather deck of the processing module (HMDC 1994).

The process area non-hazardous drains include water from potable and service water facilities, chemical injection package water, coarse water strainers, diesel storage tanks, pipe rack area and the weather deck of the living quarters module. The oil side of the non-hazardous drain tank is pumped to the oil side of the hazardous drain tank. The helideck drain and fuel tote tank storage area drains are routed directly overboard in order to prevent the possibility of highly volatile jet fuel entering the drains system (HMDC 1994).

The data for the Hibernia process area drainage water for the periods covered by the 1998, 1999, 2000, 2002, 2004, 2007 and 2009 EEM programs are summarized in Table 2.4. The data (September 2007 through August 2009) for the process area drainage water can be characterized

as having an oil concentration ranging from 0 to 98.9 mg/L, with an average oil concentration of 4.8 mg/L and daily effluent volume discharges ranging from 0 to 56.2 m^3. The maximum allowable daily mean oil concentration for one day was 40 mg/L. In June 2007, this limit was reduced to an instantaneous limit of 15 mg/L. All exceedences were reported to the C-NLOPB.

Table 2.4 Process Area Drainage Water Discharges (1997-2009)

Discharge Period	Daily Mean Oil Concentration (mg/L) (Reg. Limit 15 mg/L)	Daily Average Effluent Flow (m^3/day)
Nov. 1997 - Sept. 1998	8.97	79 - 214
Sept. 1998 - Aug. 1999	7. 2	79 - 1,510
July 1999 - July 2000	6.4	92 - 443
August 2000 - July 2002	5.9	0 - 566
Aug 2002 - Aug 2004	5.0	3.1 - 235
Sept 2005 - Aug 2007	2.7	0 - 107
Sept 2007 – Aug 2009	4.8	0 - 56.2

2.2.3.2 Drilling Area Drainage Water

The drainage from both the hazardous and non-hazardous drilling areas is collected and transferred to the hazardous area drain collection tank and safe area (non-hazardous) drain collection tank, respectively (HMDC 1994). Both tanks are equipped with agitators to keep solids in suspension. Pumps transfer the contents of the tanks to decanter centrifuges that remove solids which are then pumped to the cuttings reinjection system. The fluid phases from the two decanters are directed to disk-stacked centrifuges for polishing prior to discharge.

The drilling area hazardous drains collect effluents associated with the drilling floor operations, including rotary table, drag chains, blow-out preventer units, mixing hoppers, bulk tanks, centrifuges, cuttings cleaning and container storage. These effluents contain sea water, rainwater, chemicals and mud components, cuttings, weighing agents, lubricants and crude hydrocarbons (HMDC 1994). Prior to entering the hazardous area drains system, drainage from the drill floor operations is collected in a closed ended catch tank located in both M71 and M72 drill rigs. The catch tanks are emptied as needed via a manually-operated submersible pump. Relatively clear liquids in the upper level are directed to the decanter and disk-stacked centrifuges via the hazardous area drains for treatment prior to discharge. The lower layer, which contains high levels of suspended solids, is directed to the cuttings reinjection holding tank and is subsequently reinjected 1.6 km into the subsurface.

The drilling area non-hazardous drains collects effluents originating from the pipe rack, casing storage areas, logging units, cement units, mud pumps, mud lab and drilling storage areas. These effluents consist of sea water, minimal hydrocarbons and mud components (HMDC 1994).

The data for the Hibernia drilling area drainage water for the periods covered by the 1998, 1999, 2000, 2002, 2004, 2007 and 2009 EEM programs are summarized in Table 2.5. The drilling area drainage water (September 2007 through August 2009) can be characterized as having an oil concentration ranging from 0 to 14.8 mg/L, with average daily oil concentration of 5.8 mg/L.

This resulted in oil discharges (calculated) ranging (September 2007 through August 2009) from 0 to 0.69 (kg/day), with daily effluent volume discharges ranging from 0 to 60 m^3.

Table 2.5 Drilling Area Drainage Water Discharges (1997-2009)

Discharge Period	Daily Mean Oil Concentration (mg/L) *(Reg. Limit 15 mg/L)	Daily Average Effluent Flow (m^3/day)
Nov. 1997- Sept. 1998	152.3	5 - 224
Sept. 1998 – Aug. 1999	56.1	10 – 198
July 1999 – July 2000	19.7	12.9 - 190
Aug 2000 – July 2002	15.5	0 – 267.7
Aug 2002 – Aug 2004	9.3	1.1 – 214
Sept 2004 - Aug 2007	6.2	0 – 88.4
Sept 2007 – Aug 2009	5.8	0 - 60

Note:
* Regulatory Limit = 40 mg/L until June 2007, when the limit became 15 mg/L.

2.2.4 Sea Water Return Discharge

The sea water return (cooling water) stream is dominated by chlorinated sea water which is discharged with the storage displacement water and domestic sewage, and is intermittently mixed with process and drains effluent. The sea water return will contain residual chlorine used for corrosion control purposes. It may also be characterized by high biochemical oxygen demand (BOD). Chlorinated water supplied to the platform and not used through other systems is typically discharged to the receiving environment via the sea water return line (HMDC 1994).

The data for the Hibernia sea water return discharge water for the periods covered by the 1998, 1999, 2000, 2002, 2004, 2007 and 2009 EEM programs are summarized in Table 2.6. The regulatory limit for total residual chlorine from the Hibernia platform is 2.0 mg/L. The sea water return discharge total residual chlorine concentrations collected by grab samples (September 2007 through August 2009) ranged from 0 to 1.65 mg/L, with average daily chlorine concentrations of 0.46 mg/L.

Table 2.6 Sea Water Return Water Discharges (1997-2009)

Discharge Period	Mean Residual Chlorine Concentration (mg/L)
Nov. 1997 – Sept. 1998	1.03
Sept. 1998 – Aug. 1999	0.61
July 1999 – July 2000	0.54
Aug 2000 – July 2002	0.49
Aug 2002 – Aug 2004	0.8
Sept 2004 – Aug 2007	0.71
Sept 2007 – Aug 2009	0.46

2.2.5 Sanitary and Domestic Wastes

Sanitary wastes refer specifically to human wastes, while domestic wastes refer to all liquids originating from domestic facilities, such as kitchens, laundry rooms, showers and wash basins

(Environment Canada 1990). Raw sewage is macerated to a particle size of 6 mm or less in a sewage treatment package on the Hibernia platform consistent with the OWTG (NEB *et al.* 2010) requirement. Domestic effluents are treated to remove grease, screened plastics and metals (HMDC 1994).

2.3 Drilling Discharges

Drilling mud is a solution of suspended solids and dissolved materials in a carrier liquid such as synthetic oil (SBMs) or water (WBMs). Drilling muds are re-conditioned and recycled to minimize waste discharges and cost and to maximize operational efficiencies. Drill cuttings and solids are formation particles that are discharged to the marine environment, in the case of water based systems, or reinjected (SBMs) after separation from the drill fluid through processing through a vibrating shaker screen system.

2.3.1 Water-based Muds

WBMs employ fresh or saltwater as the continuous liquid phase. WBMs are generally used on the upper hole sections of a well, above the 340-mm drill string casing to a planned depth of approximately 1,645 and 1,220 m on the Hibernia and Avalon formations, respectively (HMDC 1994). Muds generally are composed of barite, bentonite or other clays, silicates, lignite, caustic soda, sodium carbonate/bicarbonate, inorganic salts, surfactants, corrosion inhibitors, lubricants and other additives for unique drilling problems (Thomas 1984; GESAMP 1993).

Whole WBMs remaining from a drilling mud change out may be discharged without treatment (NEB *et al.* 2010). The water-based drilling fluids carrying drill cuttings and solids will be routed through the solids control treatment system to remove the cuttings and recondition the drilling mud. The drilling mud re-conditioning system treats the fluids to remove formation solids and to replenish chemicals and weighting agents consumed during drilling operations (HMDC 1994). Processed drill cuttings and solids are discharged via two shale chutes on the northeast and northwest quadrants on the Hibernia platform, approximately 20 m from the base of the platform (HMDC 1994). A portion of the reconditioned mud is reused in drilling applications.

2.3.2 Synthetic-based Mud

SBMs refer to a drilling fluid whose continuous phase is composed of one or more fluids produced by the reaction of a specific purified chemical feedstock, rather than the physical separation processes such as fractionation, distillation and minor chemical reactions. Synthetic-based fluids (SBFs) typically have a total PAH concentration of less than 10 mg/kg (< 0.001 percent) and are non-acutely toxic in most or all marine toxicity tests. Examples of synthetics include C16-C18 internal olefins, poly-alpha olefins (PAOs), linear alpha olefins, esters, low viscosity esters, as well as other SBFs such as paraffinic fluids (i.e., saturated hydrocarbons or alkanes).

The drilling mud (Paradril-IA35) used at Hibernia is a SBM with PureDrill IA-35 as the base fluid, together with weighting agents, wetting agents, emulsifiers and other additives. PureDrill IA-35 synthetic drilling fluid is classified as a high purity synthetic alkane consisting of isoalkanes and cycloalkanes (Petro-Canada Technical Bulletin). PureDrill IA-35 is a clean, colourless, odourless

fluid that is safe to handle. It has an aromatic content of < 0.01 percent and a PAH content of < 0.001 ppm. It is non-toxic to human, plant and marine life.

PureDrill IA-35 has undergone an evaluation using the OCMS. The fluid was screened from a facility, human health and environmental perspective. PureDrill IA-35 base fluid is a component of a whole mud system called ParaDrill that received a Group E classification by the Offshore Chemical Notification System (OCNS) classification system employed in the United Kingdom. The Group E classification is the best rating achievable under the OCNS system and is assigned to chemicals that have relatively low toxicity and/or does not bioaccumulate or readily biodegrades.

The toxicity data for PureDrill IA-35 (Petro-Canada Technical Bulletin; Harris 1998) are:

- mysid shrimp 96-hour LC50 of >500,000 ppm;
- rainbow trout 96-hour LC50 of >400,000 ppm;
- amphipod (*Corophium volutator*) 10-day LC50 of 2,633 mg/L;
- *Macoma* 20-day LC50 of >50,000 mg/L;
- echinoid fertilization (*Lytechinius pictus*) IC50 (20 minutes) of >100 percent; and
- bacterial bioluminescence (Microtox test using *Vibrio fischeri*) EC50 of >100 percent.

Toxicity studies conducted by the Department of Fisheries and Oceans (DFO) using American plaice, winter flounder (*Pleuronectes americanus*) and the amphipod (*Rhepoxynius abronius)* on Hibernia drill cuttings and solids (J. Payne, pers. comm.; Payne *et al.* 2001a; 2001b) found:

- no acute toxicity in juvenile American plaice exposed for 30 days to Hibernia cuttings approximating hydrocarbon concentrations found 200 to 500 m from platforms in the North Sea;
- no acute toxicity in adult winter flounder exposed to Hibernia cuttings for 90 days; and
- in a dose response study using amphipods, a toxic response at 5,000 ppm hydrocarbon concentration only. The cuttings demonstrated a low acutely toxicity potential and extrapolations have been carried out to determine possible size of toxic zones that would occur in the field. The extrapolations indicate little or no risk of toxicity as close as 1,000 m or less from the platform.

Cuttings re-injection equipment was installed and commissioned in 2001, with greater than 95 percent re-injection achieved by fourth quarter 2002. Since the fourth quarter 2002, essentially all SBM drill cuttings and solids were re-injected into dedicated sub-surface disposal zones located 1.6 km beneath the seabed. Overboard discharges would only occur in the event of a malfunction with the cuttings re-injection equipment or when drilling through formations or material not conducive to grinding and slurrification by the cuttings reinjection system.

The data for the Hibernia drilling cuttings discharge for the periods covered by the 1998, 1999, 2000, 2002, 2004, 2007 and 2009 EEM programs are summarized in Table 2.7. SBMs for the period of September 2007 through August 2009 were reinjected and as such were not discharged.

Table 2.7 Drill Cutting Discharges (1997-2009)

Discharge Period	Average Oil Concentration (g/100 g)	48 Hour Rolling Average Oil Concentration (g/100 g)	Daily Cuttings Discharged (tonnes)
Nov. 1997- Sept. 1998	16.1	0 - 33.9	10.6
Sept. 1998 – Aug 1999	11.4	0 - 20.43	4.5
July 1999 – July 2000	17.7	0 – 27.3	10 4
Aug 2000 – July 2002	19.3*	0 - 22.1	4.86
July 2002 – Aug. 2004*	21.1	0 – 9.92	1.02
Sept. 2004 – Aug. 2007	0	0	0
Sept. 2007 – Aug. 2009	0	0	0

Note:
* Due to re-injection of drill cuttings, the average synthetic oil concentration is applicable to retained synthetic oil on the cuttings that were discharged, only.

In March 2001, Hibernia commenced partial re-injection of drill wastes. Up to August 31, 2007, 194,058 m^3 of fluids (combined drill wastes and slurrification liquids) had been re-injected. Re-injected fluids consist of drill cuttings, solids and liquid waste from a variety of sources.

3.0 ENVIRONMENTAL EFFECTS MONITORING PROGRAMS

3.1 Baseline Characterization Program

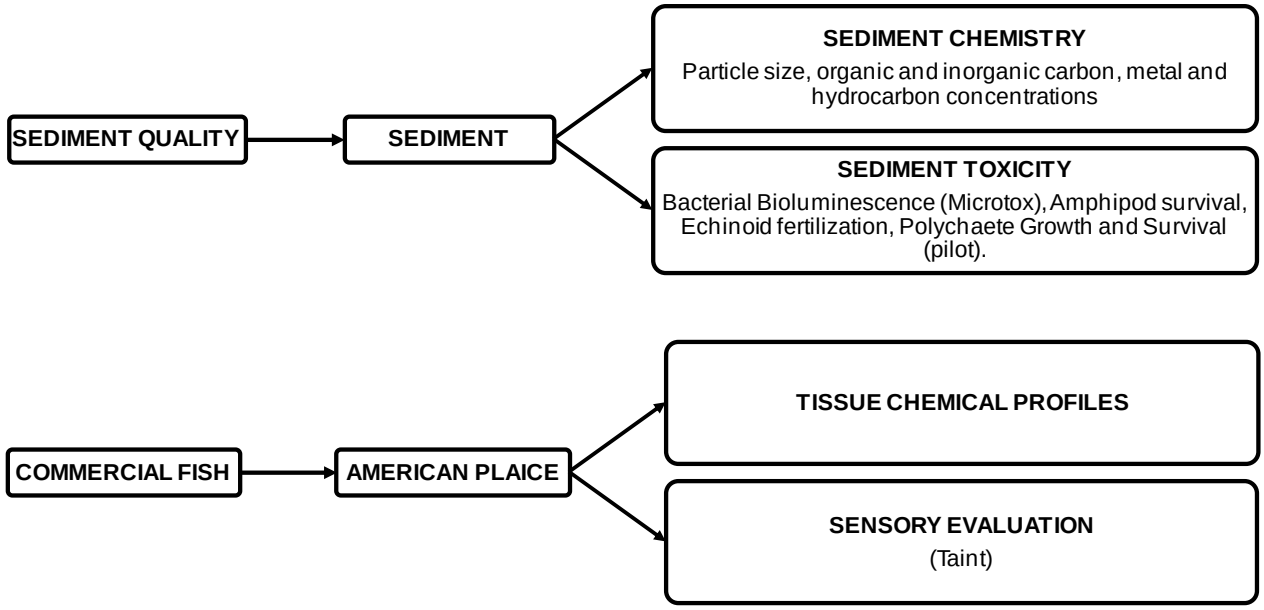
The baseline component of the Hibernia EEM Program commenced in August, 1994, and was conducted as per the C-NLOPB-approved program design specified in *Hibernia Project Offshore Environmental Effects Monitoring Program Design - Baseline Survey* (JWEL 1994).

The baseline component of the EEM program had three primary objectives:

- resolve outstanding questions with respect to the EEM program design;
- establish an understanding of background (pre-operational) conditions in the study area; and
- determine natural/existing spatial variation in monitoring parameters.

The baseline program was divided into two sampling programs: a) sediment survey to determine the baseline levels of candidate parameters and sediment toxicity; and b) biological survey to identify and obtain candidate species for subsequent organoleptic sensory evaluation (taste testing) for taint and body burden analysis. Components of the baseline program are provided in Figure 3.1. Further details on the baseline program are provided in *Hibernia Development Project - Baseline Environmental Effects Monitoring Data Report* (HMDC 1995).

Figure 3.1 Baseline Environmental Effects Monitoring Components



3.2 Hibernia Environmental Effects Monitoring Data Collection

The Hibernia Project began production in November 1997 and the first Production Phase EEM Program was initiated in the summer of 1998. The scheduling of the Hibernia EEM sediment and biological cruises are listed in Table 3.1. Further details on the results of each EEM program are contained within the related EEM program reports (HMDC 1995; 1999; 2000; 2001a; 2003a; 2005, 2009).

Table 3.1 Hibernia Environmental Effects Monitoring Programs Sediment and Biological Cruise Schedules

EEM Program (Year)	Sediment Cruise Dates	Biological Cruise Dates
1994	Aug. 31 – Sept. 10	December 4 – 6
1998	Aug. 26 – Sept. 01	December 16 – 23
1999	July 25 – 30 & Sept. 01 – Oct. 04	June 8 –10
2000	July 8 –18	July 5 - 6
2002	July 06 – July 14	June 29 – July 01
2004	Aug 22 – Aug 28	July 13 – July 14
2007	Aug 17 - Aug 29	July 17 - July 20
2009	July 30 – Aug 04	June 30 – July 03
* The 1999 sediment cruise was split into two cruises due to equipment losses and manufacturing of new equipment.		

3.3 Monitoring Hypotheses

The purpose of the EEM program is to detect project-induced changes to the surrounding environment. The monitoring hypotheses developed for the Hibernia production phase EEM program were based on biological endpoints designed to measure effects and to assist in the assessment of effects predictions. The chemical characterisation of sediment and biota body burdens was used to determine the cause for the rejection of a null hypothesis in favour of the alternative hypothesis.

The following monitoring hypotheses were developed for the Hibernia production phase EEM program (HMDC 1996):

H_0 No.1: Operational discharges from the Hibernia platform will not result in major biological effects (as measured by the amphipod survival assay) beyond the predicted impact zone of a 1,000 m radius around the production platform.

H_A No. 1: Operational discharges from the Hibernia platform will result in major biological effects (as measured by the amphipod survival assay) beyond the predicted impact zone of a 1,000 m radius around the production platform.

H_0 No.2: Operational discharges from the Hibernia platform will not result in minor biological effects (as measured by Microtox and/or juvenile polychaete growth assays) beyond 4,000 m.

H_A No. 2: Operational discharges from the Hibernia platform will result in minor biological effects (as measured by Microtox and/or juvenile polychaete growth assays) beyond 4,000 m.

H_0 No.3: Operational discharges from the Hibernia platform will not result in tainting (as measured by organoleptic evaluations) of fishery resources outside of the fishing exclusion zone.

H_A No. 3: Operational discharges from the Hibernia platform will result in tainting (as measured by organoleptic evaluations) of fishery resources outside of the fishing exclusion zone.

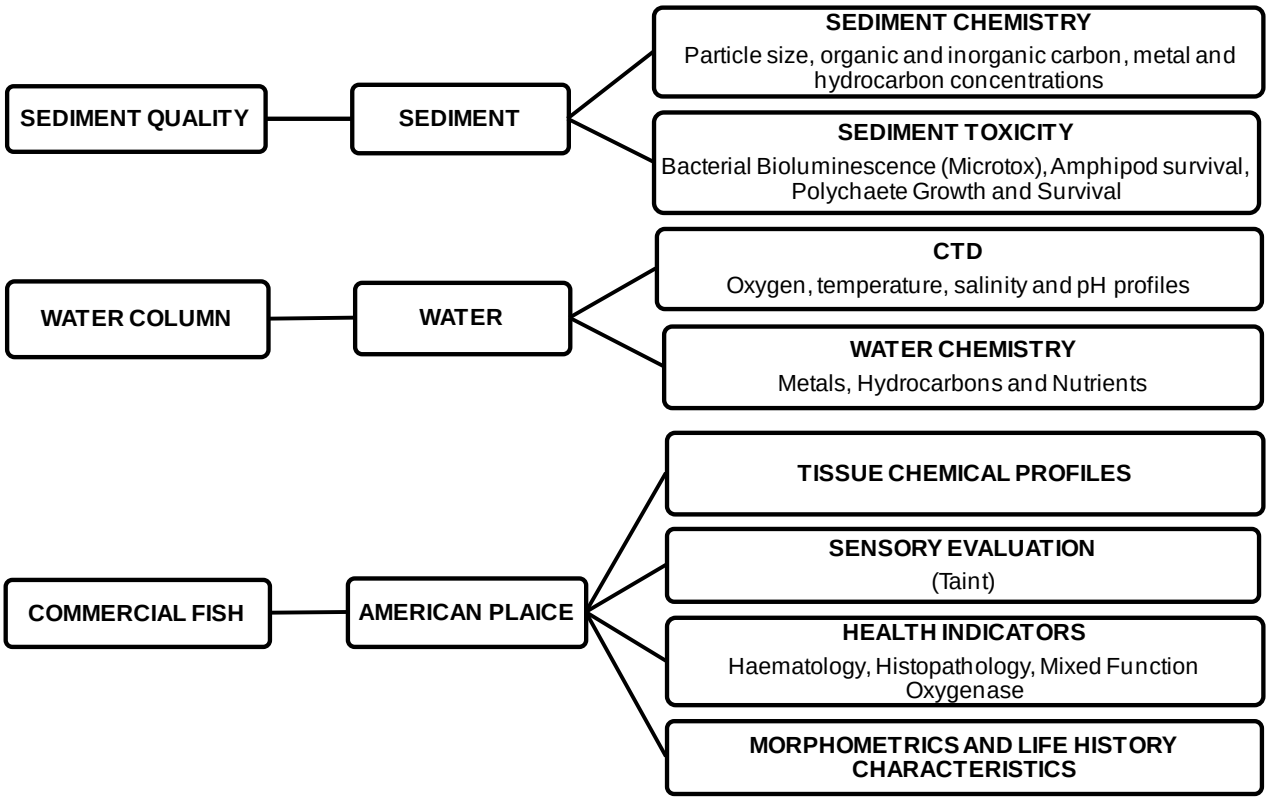
3.4 Program Design Evolution

The *Hibernia Development Project Production Phase Environmental Effects Monitoring Plan* (HMDC 1996) built upon data and information collected during the Hibernia baseline EEM program (HMDC 1995). The development and design of the Hibernia Baseline EEM Program was finalized after extensive consultation with regulatory agencies, and regional, national and international experts in the offshore oil EEM Programs.

The design of an EEM program depends upon the specific objectives of the program. Program objectives can change over time in response to evolving knowledge about the project, and its potential or observed interactions with the environment. Therefore, it is reasonable that the EEM program design should evolve or change over time, in keeping with the priorities and objectives of the program.

Various changes or modifications to the core Hibernia EEM program have resulted due to the program recommendations based on data analysis and information obtained from the Production Phase EEM programs (HMDC 1999; 2000; 2001a; 2003a; 2005). Components of the current 2009 program are illustrated in Figure 3.2. Further details on the current Hibernia EEM program are provided in the revised Hibernia Development Project Production Phase Environmental Effects Monitoring Plan (HMDC 2003b).

Figure 3.2 2009 Environmental Effects Monitoring Components



3.5 Sampling Frequency and Survey Timing

Sediment and biological surveys for the EEM program (HMDC 1996) were conducted on an annual basis for the first three years of production (1998, 1999, and 2000) and have been planned for every second year thereafter (2002, 2004, 2006, etc.). For the 2006 EEM, the C-NLOPB approved deferring the program until 2007. Sediment surveys typically take place during August or early September of each sampling year. The biological surveys were conducted for the baseline study (1994) and the 1998 study during late fall or early winter of the same years, with limited success, and in the summer of 1999 to optimize the opportunity for the collection of American plaice. The success of the 1999 summer survey was repeated for the 2000 biological survey with even greater success. The biological survey will continue to be conducted in the summer for subsequent EEM programs.

3.5.1 Boundaries

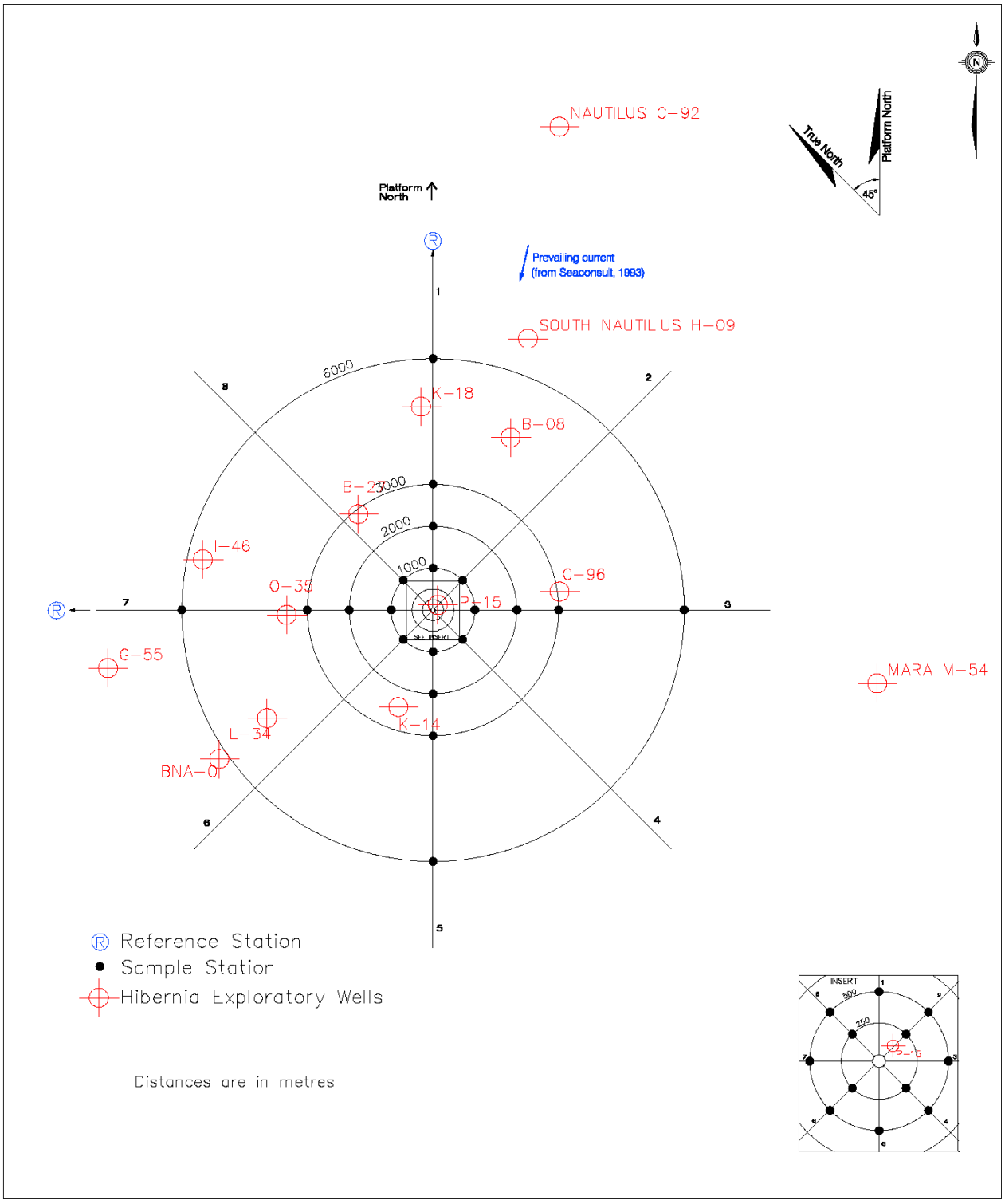
The temporal and spatial boundaries of the EEM program determine its scope and scale, as well as the location and frequency of sampling. The boundaries of the EEM program were determined with careful consideration of the temporal and spatial aspects of the project and its potential environmental effects, as well as the distribution of the environmental components of interest over time and space.

Based on the recommendations from previous EEM programs and in response to evolving knowledge about the project, and its potential or observed interactions with the environment, the

sediment sampling net has evolved over the years. In an effort to refine the sediment sampling program with respect to statistical considerations, scale for environmental contaminants, and sampling effort, changes to the sediment sampling program were implemented for the 2004 and 2007 programs. All changes to the Hibernia EEM program will be regularly reviewed for continued relevance.

Hibernia can be considered to have a single point source for discharges. The sampling design was based on a gradient to background approach with eight radii extending out from the platform. The revised EEM Sediment Sample Net for the 2004, 2007 and 2009 programs (Figure 3.3) extends out to 6,000 m from the platform along four of the radii (north, east, west and south) and out to 1,000 m from the other four radii (northeast, southeast, southwest and northwest). In addition, there are two control stations located at a distance of 16,000 m from the GBS on the north and west radii for use as field reference locations for the toxicity testing. Samples were collected at 34 pre-determined stations along this sampling grid, which was originally developed as part of the Hibernia Environmental Effects Monitoring Program (HMDC 1996) and modified in 2003 (HMDC 2003b).

Figure 3.3 Sample Net Design for the Hibernia EEM Program 2009



In the 2002 EEM program, Hibernia sediment data were described as Near-field, Mid-field and Far-field for sections of the sediment chemistry statistical analyses. This allowed a more focused assessment of potential environmental contaminants from the Hibernia platform. The "Field" distance descriptors used for the 2002, 2004, 2007 and 2009 statistical analyses to describe distances from the Hibernia platform are provided in Table 3.2.

Table 3.2 Hibernia EEM Field Distance Definitions

Field Distance Descriptors	Distance from Hibernia Platform		
	2002	2004	2007 and 2009
Near-Field	250 m ≤ distance ≤ 1,000 m	250 m ≤ distance ≤ 1,000 m	250 m ≤ distance ≤ 1,000 m
Mid-Field	1,500 m ≤ distance ≤ 3,000 m	2,000 m ≤ distance ≤ 3,000 m	2,000 m ≤ distance ≤ 3,000 m
Far-Field	4,000 m ≤ distance ≤ 8,000 m	6,000 m	6,000 m

4.0 HIBERNIA 2009 SEDIMENT CHEMISTRY PROGRAM

A description of detailed sampling methods and the presentation of raw data are provided in Volume II of this report. The following sections present the results of the data analysis, with supporting figures and key results from the statistical analyses that were carried out for the 2009 Hibernia EEM program. The rationale and approach to the detailed statistical analysis for the Hibernia 2009 EEM sediment chemistry program is provided in Appendix B.

4.1.1 Data Collection

Sediment chemistry data were collected from 46 locations in both 1994 and 1998, 58 stations in 1999, 60 stations in 2000 and 58 stations in 2002. In 2000, two stations at 32 km from the GBS along radials 1 and 7 were sampled, in addition to the two reference stations at 16 km that were sampled for all EEM sediment programs to date (1994, 1998, 1999, 2000, 2002, 2004, 2007 and 2009). The 32-km stations were sampled only in 2000. A total of 34 stations were sampled for sediment chemistry in 2004, 2007 and 2009 (Figure 3.3). No replicates were taken at these stations because they were found not to add significantly to the statistical power of the analyses for the 2002, 2004, 2007 and 2009 EEM programs (HMDC 2003b, HMDC 2005, HMDC 2009).

At each location for the 2009 EEM sediment stations, as in previous EEM sediment studies, a sample was collected within a 50-m radius of the station coordinates. These samples were then sent to the laboratory to determine the concentrations of the following substances:

- hydrocarbons (TPHs, PAHs and alkyl PAHs);
- total metals;
- total inorganic carbon and total organic carbon (TIC and TOC);
- barium weak acid leachable metal;
- sulphide and ammonia; and
- particle sizes.

The detection level for chemical analysis that is cited in this report, and as for the 2007 data, is the reportable detection limit (RDL). The RDL is the lowest concentration of a specific chemical that the laboratory can accurately measure for a specific method, within specified limits of precision and accuracy. In 2002 and 2004, the detection limit was defined as the Estimated Quantification Limit (EQL). The EQL is the concentration of a substance (analyte) that produces a signal sufficiently greater than the blank that it can be detected within specified levels during routine operating laboratory conditions (American Public Health Association, APHA 1998). Data collected in 1994, 1998 and 1999 were reported with detection limits that were defined as the limit of quantification (LOQ). There is no difference in how the LOQ, EQL and RDL are calculated; therefore, they are interchangeable values. The change in terminology was implemented in order to make it more consistent with terminology that is widely used in North America.

A summary of the chemical data for which analytes were detected in the 2009 Hibernia EEM Sediment Sampling Program is presented in Table 4.1 (data associated with the complete suite of chemicals that were analyzed are contained in Volume II, Appendix H). The analysis of two field replicate samples (taken at the reference stations) is included in Table 4.1 for a total of 36 sediment samples that were collected and analyzed. The RDL, the total number of samples analyzed, the number of samples that had values above the RDL and the associated mean, standard deviation, median and minimum and maximum numbers are identified in Table 4.1. Calculated descriptive statistics were based on samples with values above the RDL or equal to the RDL if not detected analytically. The reason was to obtain a meaningful descriptive statistic (e.g., mean) for the measured analyte and which would not be less than the RDL for that analyte. The use of these descriptive statistics are intended only for visual screening purposes to assist in retaining analytes of interest for further data analyses. Other methods to address non-detected values (reported as below the RDL) and used in data to test for statistical significance are described further below in respective sections of this report. The rationale and justification for the use of these methods is presented in Appendix B.

4.1.2 Data Screening

The first step in the data analysis used for this study was to conduct a visual screening of the chemical data. This screening was conducted to select chemical constituents for further analysis (Table 4.2) by using the following criteria:

- retain if the majority of data values were above the RDL; or
- where a large fraction (typically 50 percent or more) of the data are below the RDL, a meaningful result may still be obtained if the substance is one that could plausibly be released from the Hibernia platform in quantities likely to be detected nearby, and the distribution of observations is consistent with the hypothesis that the substance may have originated from the platform; or
- although a substance was rarely detected in the 2009 data, it has been detected in prior EEM surveys at Hibernia, and the absence of detection in 2009 may demonstrate recovery from an earlier effect.

Table 4.1 Summary of Detectable Chemical Data for the 2009 Hibernia EEM Sediment Stations

Parameters	RDL	Units	No. Samples	No. >RDL	Mean	SD	Median	Min	Max	ISQG	PEL
Weak Acid Metals											
Barium	5	mg/kg	36	22	10	9	7	<RDL	47	-	-
Total Metals											
Aluminum	10	mg/kg	36	36	5511	1607	5250	2300	10000	-	-
Arsenic	2	mg/kg	36	2	2	0	2	<RDL	3	7.24	41.6
Barium	5	mg/kg	36	36	192	223	135	60	1400	-	-
Cadmium	0.055	mg/kg	36	3	0.06	0.02	0.05	<RDL	0.16	0.7	4.2
Chromium	2	mg/kg	36	35	4	2	4	<RDL	15	52.3	160
Cobalt	1	mg/kg	36	3	1	0	1	<RDL	2	-	-

Parameters	RDL	Units	No. Samples	No. >RDL	Mean	SD	Median	Min	Max	ISQG	PEL
Copper	2	mg/kg	36	7	2	1	2	<RDL	4	18.7	108
Iron	50	mg/kg	36	36	1792	1005	1500	620	5400	-	-
Lead	0.5	mg/kg	36	36	2.5	0.9	2.3	1.3	5	30.2	112
Lithium	2	mg/kg	36	3	2	0	2	<RDL	3	-	-
Manganese	2	mg/kg	36	36	47	30	39	13	150	-	-
Mercury	0.01	mg/kg	36	1	0.01	0.0	0.01	<RDL	0.01	0.13	0.70
Nickel	2	mg/kg	36	5	2	1	2	<RDL	6	-	-
Strontium	5	mg/kg	36	36	80	144	30	16	620	-	-
Thallium	0.1	mg/kg	36	4	0.1	0	0.1	<RDL	0.1	-	-
Uranium	0.1	mg/kg	36	36	0.2	0.1	0.2	0.1	0.4	-	-
Vanadium	2	mg/kg	36	36	7	4	5	4	22	-	-
Zinc	5	mg/kg	36	25	6	2	6	<RDL	13	124	271
Particle Size Analysis											
Gravel	0.1	%	36	32	5.1	9.6	0.4	<RDL	36.8	-	-
Sand	0.1	%	36	36	94	9.9	98.9	61.8	99.7	-	-
Silt	0.1	%	36	36	0.5	0.4	0.4	0.1	2	-	-
Clay	0.1	%	36	36	0.4	0.3	0.4	0.1	1.6	-	-
TIC/TOC											
Total Carbon	0.2	mg/kg	36	36	4.1	9.2	0.6	0.5	41.2	-	-
TIC	0.2	mg/kg	36	13	3	7.3	0.2	<RDL	35	-	-
TOC	0.2	mg/kg	36	33	1.1	2.4	0.4	<RDL	13	-	-
Hydrocarbons											
>C10-C21	0.3	mg/kg	36	30	4.1	10	0.9	<RDL	58	-	-
>C21-<C32	0.3	mg/kg	36	36	0.9	0.7	0.6	0.3	3	-	-
Other											
Sulphide	0.2	µg/g	36	10	0.7	1.9	0.2	<RDL	11	-	-
Moisture	0.3	%	36	36	16.5	2.5	17	9.6	25	-	-
Ammonia-N	0.3	mg/kg	36	35	3.3	4.8	1.6	<RDL	24	-	-

Table 4.2 Sediment Chemistry Data Retained for Analysis

Compounds/Elements	Data Available 1994	Data Available 1998/1999/2000	Data Available 2002/2004/2007/2009
Hydrocarbons			
TPH (C6-C32)	No	No	Yes
TEH (C10-C21) Fuel Range	Yes*	Yes	Yes
TEH (C21-C32) Lube Range	Yes*	Yes	Yes
Total Metals			
Aluminum	No	Yes	Yes
Barium	Yes	Yes	Yes
Chromium	Yes	Yes	Yes
Iron	Yes	Yes	Yes
Lead	Yes	Yes	Yes
Manganese	No	Yes	Yes
Strontium	No	Yes	Yes
Uranium	No	Yes	Yes

Compounds/Elements	Data Available 1994	Data Available 1998/1999/2000	Data Available 2002/2004/2007/2009
Vanadium	No	Yes	Yes
Zinc	Yes	Yes	Yes
Carbon			
Inorganic Carbon (TIC)	Yes	Yes	Yes
Organic Carbon (TOC)	Yes	Yes	Yes
Weak Acid Leachable Metal			
Barium	No	Yes	Yes
Other			
Ammonia (as N)	No	No	Yes
Sulphide	No	No	Yes
* 1994 values were all below the RDL of 10 mg/kg. The 1998, 1999, 2000, 2002, and 2004 RDLs were 0.25 mg/kg and 0.3 mg/kg in 2007 and 2009.			

4.1.3 Data Organization for Exploratory Analysis

Some data have changed RDL from 1994 to 1998. For the two total extractable hydrocarbons (TEHs), C11-C20 (fuel range) and C21-C32 (lube range), the 1994 RDL was 10 mg/kg while the 1998 RDL and for subsequent years was 0.25 mg/kg (rounded off to 0.3 mg/kg in 2007 and 2009). This was due to a refinement in methodology to reflect client requirements, techniques and technological advances. Due to the major changes in the hydrocarbon detection limits, the 1994 hydrocarbon data were not subjected to statistical analyses.

Three replicate data values (statistically deemed to be sub-samples) were generally available at each of the sampling locations for 1994 to 2002 data. Some of the measured values were below the RDL. For the purpose of exploratory data analysis, a summary statistic (the mean concentration value representing each Station and Time sampled) was calculated for use in subsequent statistical analysis. Only one true replicate data value was collected for each sampling location in 2004, 2007 and 2009, except for some stations in which a duplicate sample was collected for Quality Assurance/Quality Control (QA/QC) purposes.

The data were used to generate 2-D surface plots using colour intensity to easily visualize spatial and temporal trends for the sediment chemistry EEM data to date. Two-dimensional surface plots were selected, since three variables can be displayed on one graph. The Y-axis represents the north-south distance in kilometres from the Hibernia platform, the X-axis represents the east-west distance in kilometres from the Hibernia platform, and the colour of the contour represents the concentration for a substance in sediments. The X- and Y-axis scales have been reduced to accommodate the reduction in the distance sampled in 2004, 2007 and 2009 to 6,000 m from Hibernia. The Hibernia platform is located at the centre in each of these plots (i.e., at 0 km on both the X- and Y-axes). The colour plots are arranged so that a full sequence of plots is presented, representing the data collected since 1994. For analytes (chemical parameters) added subsequent to 1994, only plots for the years that data were collected for the analyte are presented. If the spatial scale was too large to visually detect the concentration of the analyte, it was then reduced to 1 km in all directions of the Hibernia platform.

SYSTAT version 10 statistical computing software (SPSS Inc.) was used to produce all the figures and perform the statistical analyses. The coloured mosaic contours in the 2-D plots, which extend to the margins of the plot, are generated by interpolation of the station EEM sediment chemistry data using the method of Lodwick and Whittle (1970) combined with Linear

interpolation. This method is most factual and appropriate to present the data in a visual meaningful way with minimum distortion and for the purposes of the exploratory data analysis. Confidence intervals associated with the information in these plots is dependent on the spacing between stations (the greater the distance between stations, as on the edges of the plot, the greater the confidence interval).

Overall, the colored mosaic contours in the 2-D plots are easier to interpret and obtain an overview of the spatial variability for a given parameter sampled for the various years of the EEM Program. Further, the 2-D surface plots provide an indication for the approximate location of elevated chemical levels that is important to detect in the EEM programs and potential environmental effects from Hibernia over the years of sampling.

4.2 Sediment Chemistry Data Analyses

4.2.1 Sediment Chemistry Exploratory Data Analysis

The metals selected for analyses for the production phase EEM program (HMDC 1996) are presented in Table 4.3. In 1998, it was determined that the level of effort for the selected required parameters (as listed in Table 4.3) was the same as per the chemical analyses of a suite of 23 metals. Therefore, it was decided to conduct the suite of metals presented in Table 4.4. However, only those metals shown in Table 4.3 are used for the subsequent statistical analyses because of their relevance to Hibernia or are present in relatively high background concentrations far from the Hibernia platform and which could assist in the interpretation of sediment toxicity data.

Table 4.3 Hibernia Sediment Metal Parameters As Detailed in the Hibernia Production Phase EEM Program Design (HMDC 1996)

Variable	RDL	Units	Method of Analysis	Holding Conditions
Aluminum	10	mg/kg	ICP-MS	-20°C
Barium	5	mg/kg	ICP-MS	-20°C
Cadmium	0.3	mg/kg	ICP-MS	-20°C
Chromium	2	mg/kg	ICP-MS	-20°C
Copper	2	mg/kg	ICP-MS	-20°C
Iron	20	mg/kg	ICP-MS	-20°C
Lead	0.5	mg/kg	ICP-MS	-20°C
Lithium	5	mg/kg	ICP-MS	-20°C
Mercury	0.01	mg/kg	CVAA	-20°C
Zinc	2	mg/kg	ICP-MS	-20°C

Table 4.4 Hibernia Sediment Metal Parameters Conducted in 1998, 1999, 2000, 2002, 2004, 2007, and 2009

Variable	RDL for 2009	Units	Method of Analysis	Holding Conditions
Aluminum	10	mg/kg	ICP-MS	-20°C
Antimony	2	mg/kg	ICP-MS	-20°C
Arsenic	2	mg/kg	ICP-MS	-20°C
Barium	5	mg/kg	ICP-MS	-20°C

Variable	RDL for 2009	Units	Method of Analysis	Holding Conditions
Beryllium	2	mg/kg	ICP-MS	-20°C
Cadmium	0.05	mg/kg	ICP-MS	-20°C
Chromium	2	mg/kg	ICP-MS	-20°C
Cobalt	1	mg/kg	ICP-MS	-20°C
Copper	2	mg/kg	ICP-MS	-20°C
Iron	50	mg/kg	ICP-MS	-20°C
Lead	0.5	mg/kg	ICP-MS	-20°C
Manganese	2	mg/kg	ICP-MS	-20°C
Mercury	0.01	mg/kg	CVAA	-20°C
Molybdenum	2	mg/kg	ICP-MS	-20°C
Nickel	2	mg/kg	ICP-MS	-20°C
Selenium	2	mg/kg	ICP-MS	-20°C
Strontium	5	mg/kg	ICP-MS	-20°C
Thallium	0.1	mg/kg	ICP-MS	-20°C
Tin	2	mg/kg	ICP-MS	-20°C
Uranium	0.1	mg/kg	ICP-MS	-20°C
Vanadium	2	mg/kg	ICP-MS	-20°C
Zinc	5	mg/kg	ICP-MS	-20°C

The 2-D surface plots were examined visually to determine whether spatial and temporal trends in metal concentrations were observed. The data presented in this section are a description of the visual examination of the data for which no statistical significance had been determined.

4.2.1.1 Particle Size

Sediment grain size exerts a strong influence on the sediment mineralogy and trace element composition. Sands, particularly quartz sands, are often composed predominantly of silicon dioxide, and have relatively low concentrations of many other elements. Sands and gravels also have relatively low surface area, weak surface binding sites, and often tend to be relatively depleted with respect to heavy metal concentrations. In contrast, silts and clays are composed of a variety of minerals, including aluminosilicate minerals derived from the weathering of feldspars. They have a relatively high surface area and, as such, provide abundant surface binding sites for heavy metals, many of which would be leachable in weak acid.

Sediments throughout the study area are predominantly sand with the balance being made up of a mixture of gravel and lesser fractions of silt and clay. The sand content appears to have increased to the northeast and decreased towards the northwest of the Hibernia platform in 2007 and remained similar in 2009 (Figure 4.1), with an overall increase in gravel content since 2007 (Figure 4.2). A general trend for silt shows an increase since the year 2000 (Figure 4.3) with a similar spatial distribution in 2007 and 2009. Around Hibernia, and at a higher scale of 1 km in Figure 4.4, it can be seen that the silt content increased in 2004, which then decreased in 2007 and remained the same in 2009. The trend can be explained by the fact that during the 2004 EEM field program both drill rigs on Hibernia were drilling and discharging water-based muds (WBM's) and cuttings at the same time as the EEM program. The clay content on the other hand is about the same since 2000, and which has decreased from 1994 and even more in 2009 compared to 2007 (Figure 4.5).

Figure 4.1 Spatial and Temporal Variability of Sand Content in Hibernia Sediment

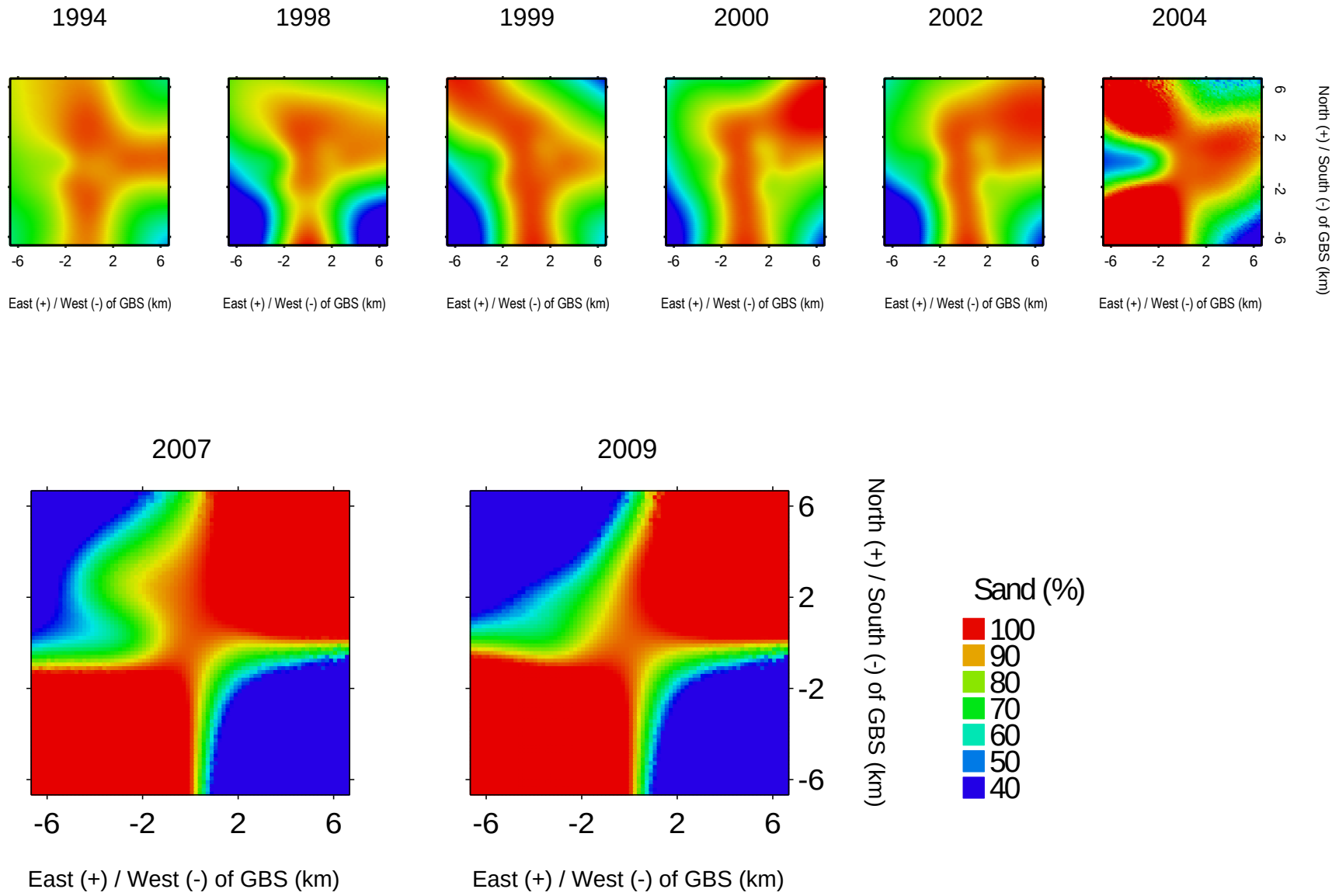


Figure 4.2 Spatial and Temporal Variability of Gravel Content in Hibernia Sediment

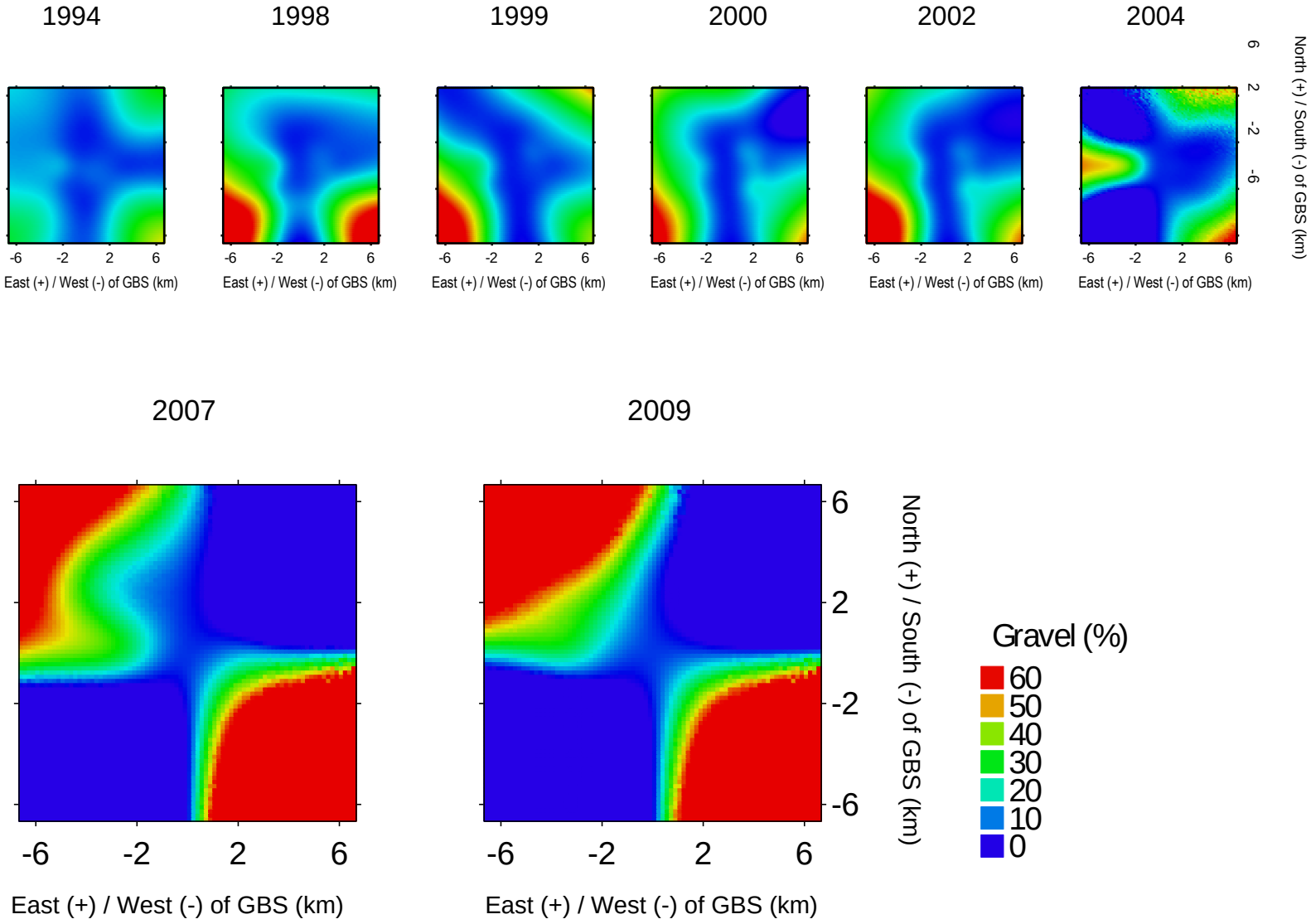


Figure 4.3 Spatial and Temporal Variability of Silt Content in Hibernia Sediment

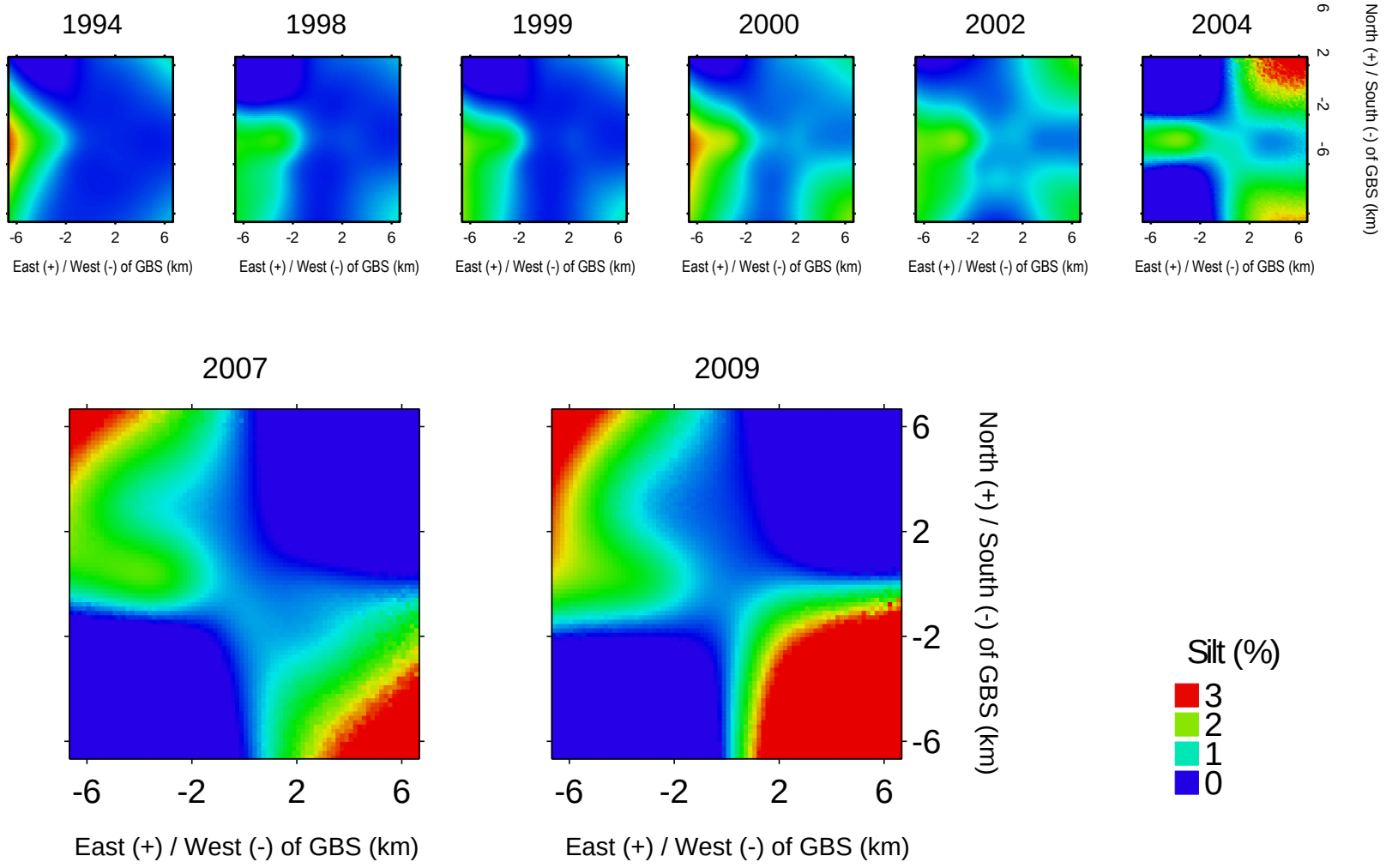


Figure 4.4 Spatial and Temporal Variability of Silt Content in Hibernia Sediment within 1 km of GBS

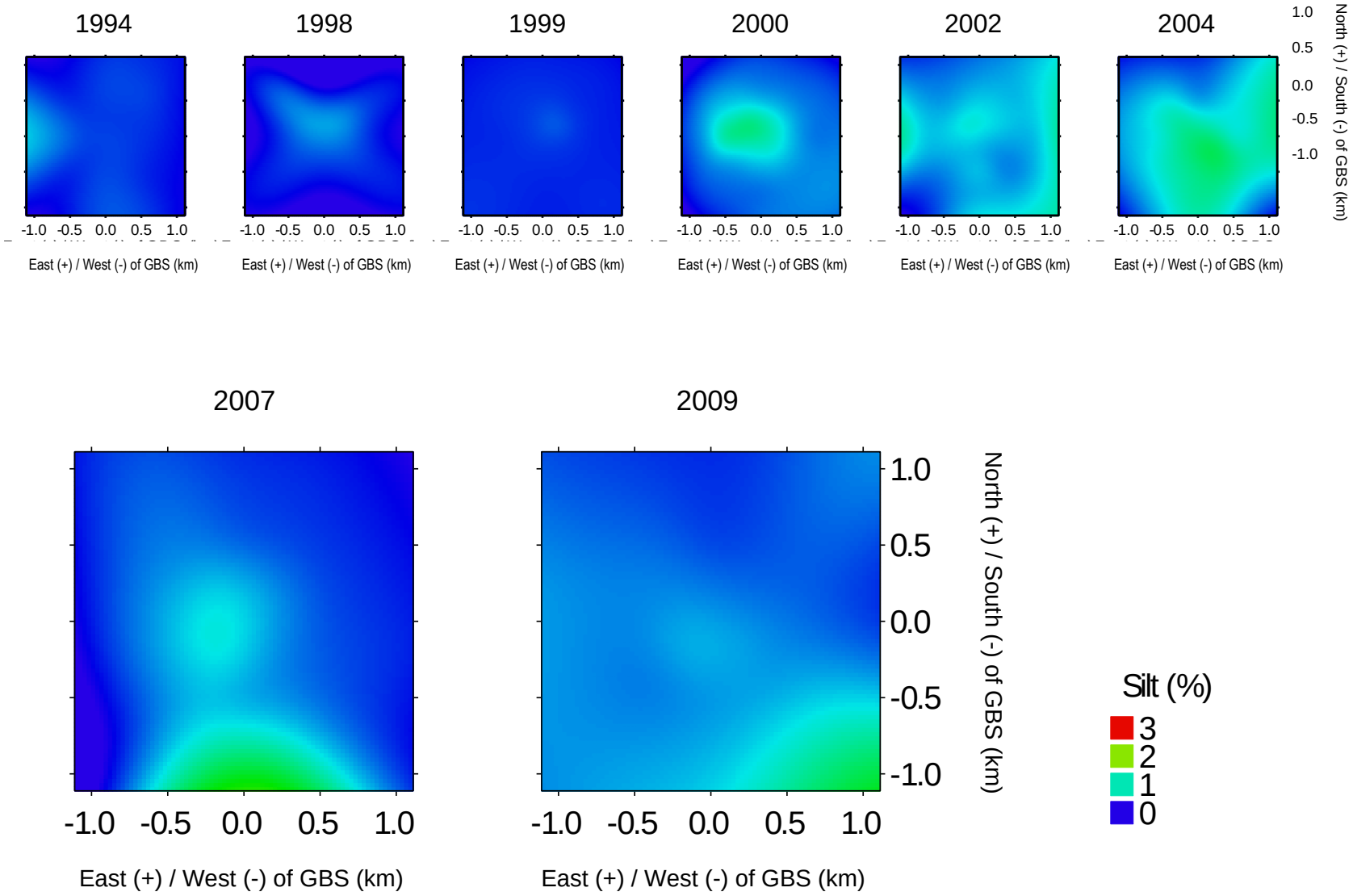
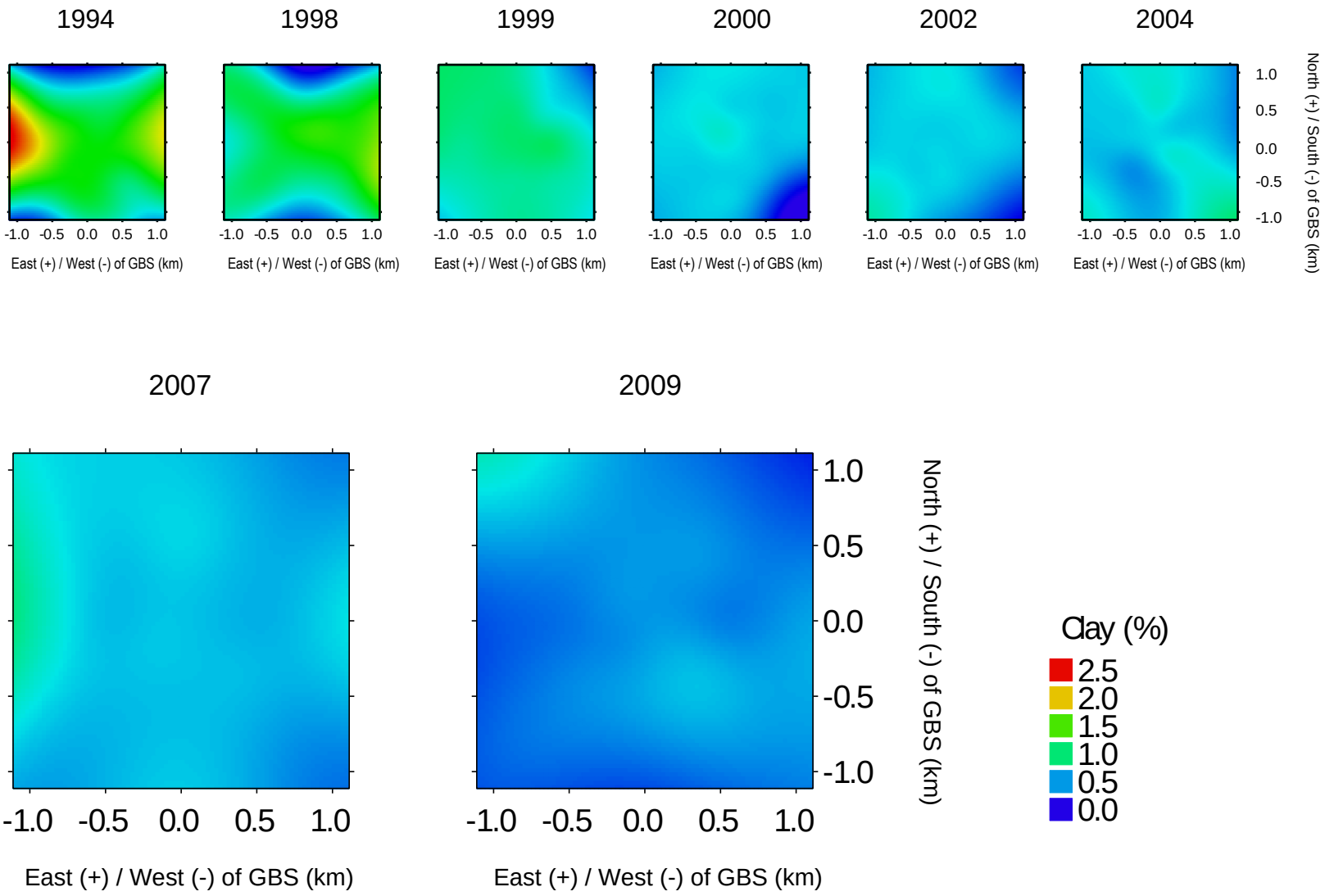


Figure 4.5 Spatial and Temporal Variability of Clay Content in Hibernia Sediment within 1 km of GBS



4.2.1.2 Total Metals

Total metal concentrations in sediment, most notably aluminum, were shown to have persistent spatial and temporal distributions (HMDC 2003a, 2005, 2009). Total metal concentrations generally correlate with trends in sediment grain size. The spatial distribution for most years shows that primarily the southwest and, to some extent, the northeast corners of the study area have relatively high total metal concentrations (Figure 4.6). In the case of aluminum, it is a major mineral-forming element, and a major component of feldspars and the weathered derivatives of feldspars. As a result, aluminum tends to be relatively enriched in gravels, silts and clays, and relatively depleted in sands that are composed of fine quartz. This is evident for 2009 where areas of higher aluminum concentrations are positively correlated with higher silt and gravel content (Figures 4.2 and 4.3), and negatively correlated with higher sand content (Figure 4.1). The correlation coefficient calculated on the 2007 sediment raw data between the aluminum concentration and the percent sand content yielded a relatively strong negative correlation coefficient of -0.65 (n=36). This is in agreement with the overall relationship observed for the 2009 sediment data, but which had a weaker negative correlation coefficient of -0.25 (n=36). This explains the dissimilarity in the 2D contour figure for aluminum (Figure 4.6) between 2007 and 2009 and yet appears similar for sand content (Figure 4.1). This may also be related to fewer data biasing the 2D contour figures further out from the GBS.

Similar relationships, with naturally occurring trends across the study area that appear to be related to sediment grain size and mineralogy, are evident for total metal concentrations for arsenic, cobalt, copper, iron, lead, nickel, strontium, thallium, uranium and vanadium in 2009. It should be noted that the discussion is not based on the analysis of the mineralogy of Hibernia sediments but rather, inferred from observations on the physical and chemical analysis of the sediment samples. Similar trends were also identified for some weak acid leachable elements (based upon the 1998-2004 data), including strontium, uranium and vanadium (HMDC 2003a), which have not been retained for analyses in the 2004, 2007 and 2009 EEM programs. Plots for total metals that were not expected to be potential or sensitive indicators of environmental contaminants of the Hibernia platform can be also found in Appendix C (i.e., chromium, manganese and zinc).

Some parameters have low natural variability, and are recognized to be associated with platform operations. These parameters, including barium and hydrocarbons, are discussed in the following sections.

4.2.1.3 Barium

Qualitative analysis of the 2-D contour plots at a scale of 1 km from Hibernia illustrates well the spatial and temporal trends for total barium (Figure 4.7). Total barium concentrations show that this parameter, which was uniformly low in 1994, increased in the vicinity of the Hibernia platform in 1999, and increased again substantially in 2000 (Figure 4.7). The 2002 results show a considerable decline in barium concentrations in the vicinity of the Hibernia platform, and an even further decline in 2007 to that comparable to low levels in 1999. In 2009, concentrations of total barium increased over 2007 and were similar to 2002. It should be noted that no inference is being made with respect to significant results based on statistics but rather, a description based solely on visual interpretation of the 2D contour figures. Examination of the weak-acid

extractable barium plots shows that the concentration of this parameter, which was uniformly low in 1998 (weak-acid extractable metals were not analyzed in the 1994 baseline survey), increased in the vicinity of the Hibernia platform in 1999, and increased again substantially in 2000 (Figure 4.8). The 2002 results show a decline in weak-acid extractable barium concentrations in the vicinity of the Hibernia platform, so that the 2002 results are comparable to the 1999 results. Concentrations increased again in 2004 (two rigs were drilling with WBM's and discharging WBM's at the time of the 2004 EEM field program), but to concentrations lower than those observed in 2000, and then decreased in 2007, to concentrations more similar to those in 1999 and less than in 2002. In 2009, weak-acid extractable barium levels are similar to those lower concentrations in 2007.

Hibernia began reinjecting SBM cuttings in 2001-2002 which reduced releases to sea. However, WBM and cuttings, which also contain barium, continue to be discharged.

4.2.1.4 Fuel Range Hydrocarbon (C10-C21)

A qualitative assessment of the fuel range hydrocarbon (C10-C21) results (Figure 4.9) illustrates that an accumulation of fuel range hydrocarbon occurred in the vicinity of the Hibernia platform beginning in 1999, becoming pronounced in 2000, and reverting to a low level and comparable to the 1999 results in 2002. Fuel range hydrocarbon concentrations in 2004, 2007 and 2009 decreased even more since 2002 to low levels comparable to 1998, and possibly to baseline levels of 1994 (the detection limit for fuel range hydrocarbons was higher in 1994 (<10 mg/kg) compared to 2004 (<0.25 mg/kg) and 2007 and 2009 (<0.3 mg/kg)).

4.2.1.5 Lube Range Hydrocarbon (C21-C32)

Lube range hydrocarbon (C21-C32) concentrations were observed in sediments around Hibernia in 1998 (Figure 4.10), and increased or remained constant until 2000. Thereafter in 2002, 2004, 2007 and 2009, the lube range hydrocarbons were generally much lower to non-detectable and lowest in 2009.

Figure 4.6 Spatial and Temporal Variability of Total Aluminum Content in Hibernia Sediment

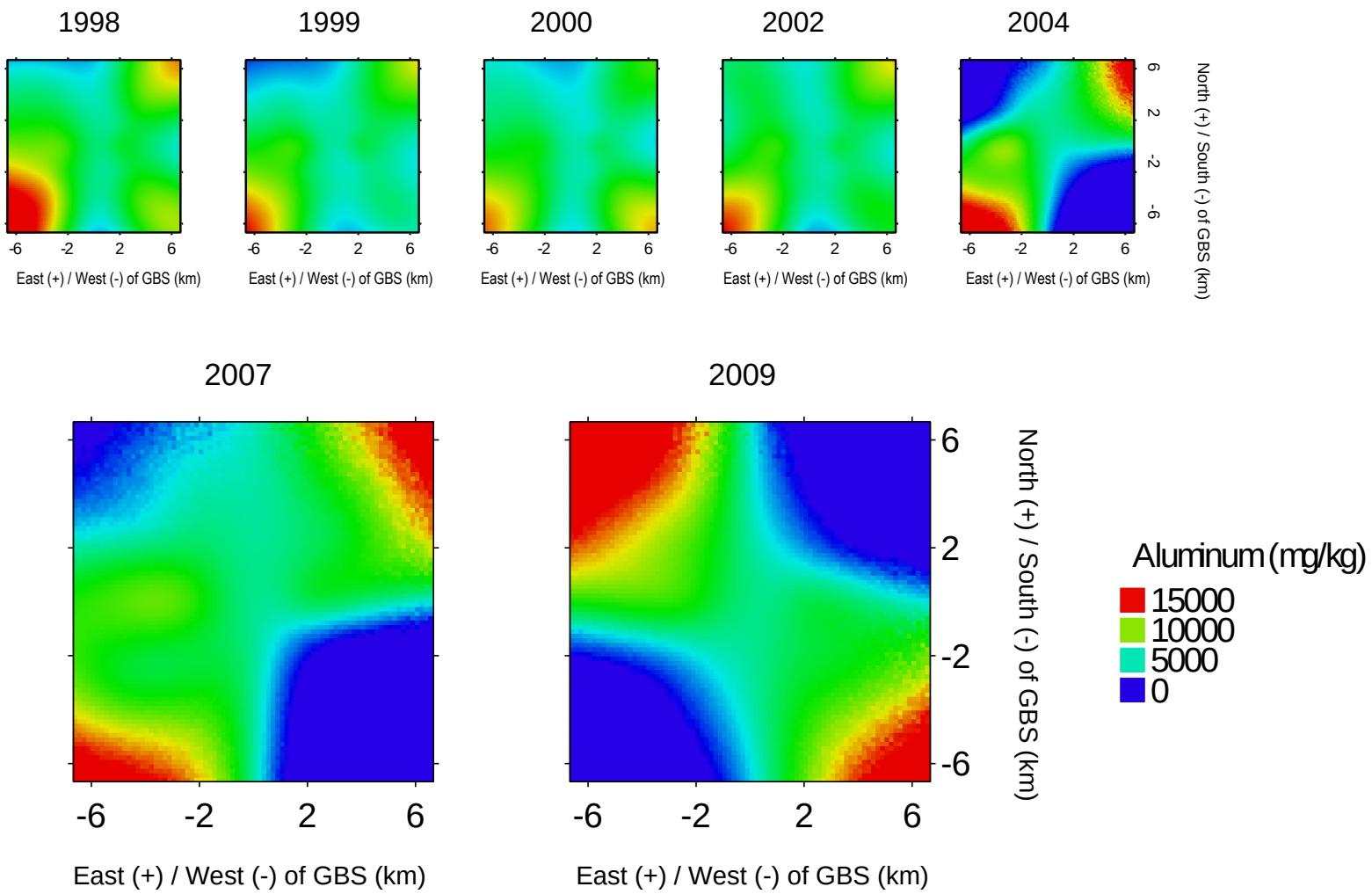


Figure 4.7 Spatial and Temporal Variability of Barium Total Metal Concentration in Hibernia Sediment within 1 km of GBS

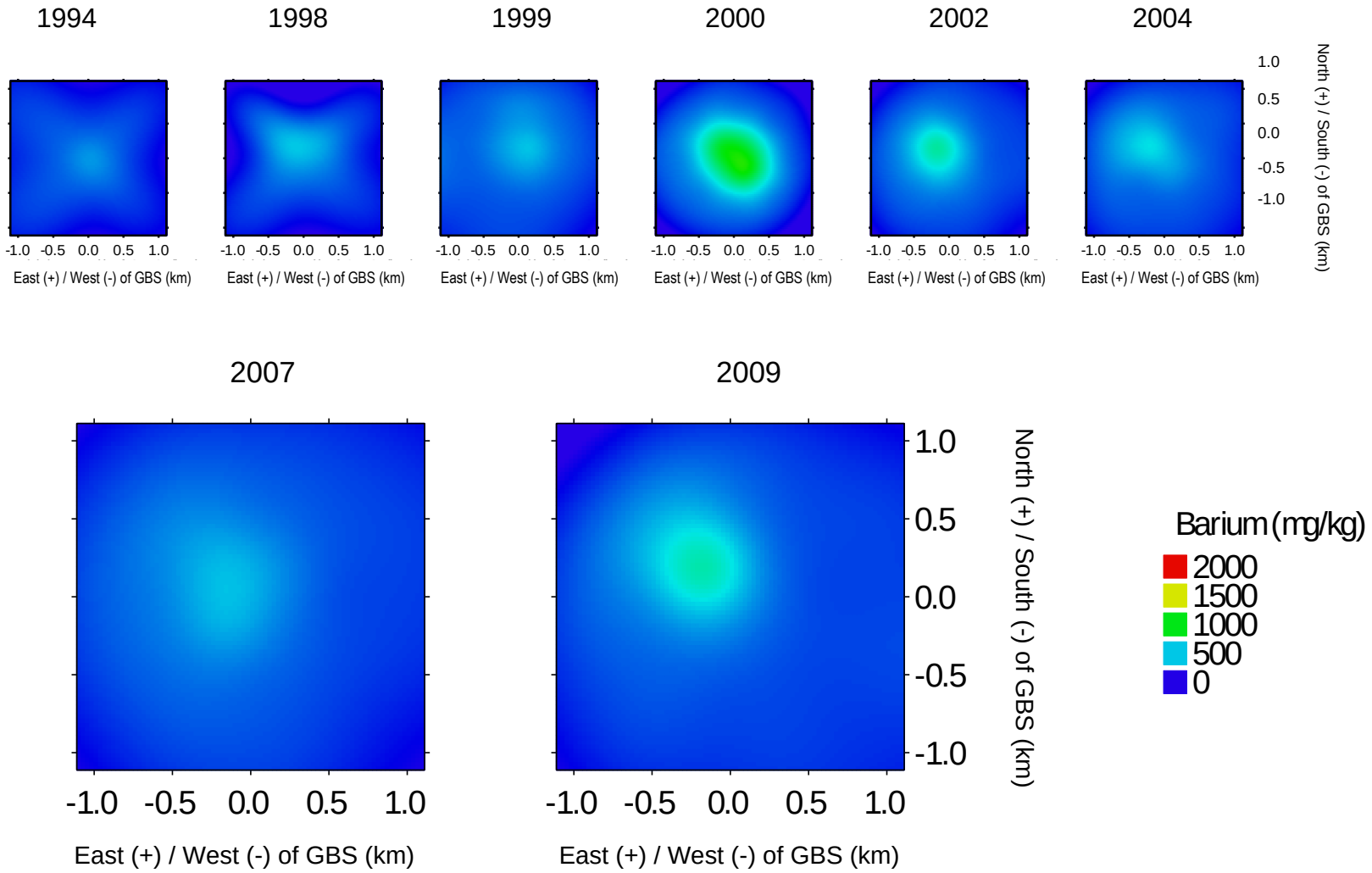


Figure 4.8 Spatial and Temporal Variability of Barium Concentration after Weak Acid Leachate Analysis in Hibernia Sediment within 1 km of GBS

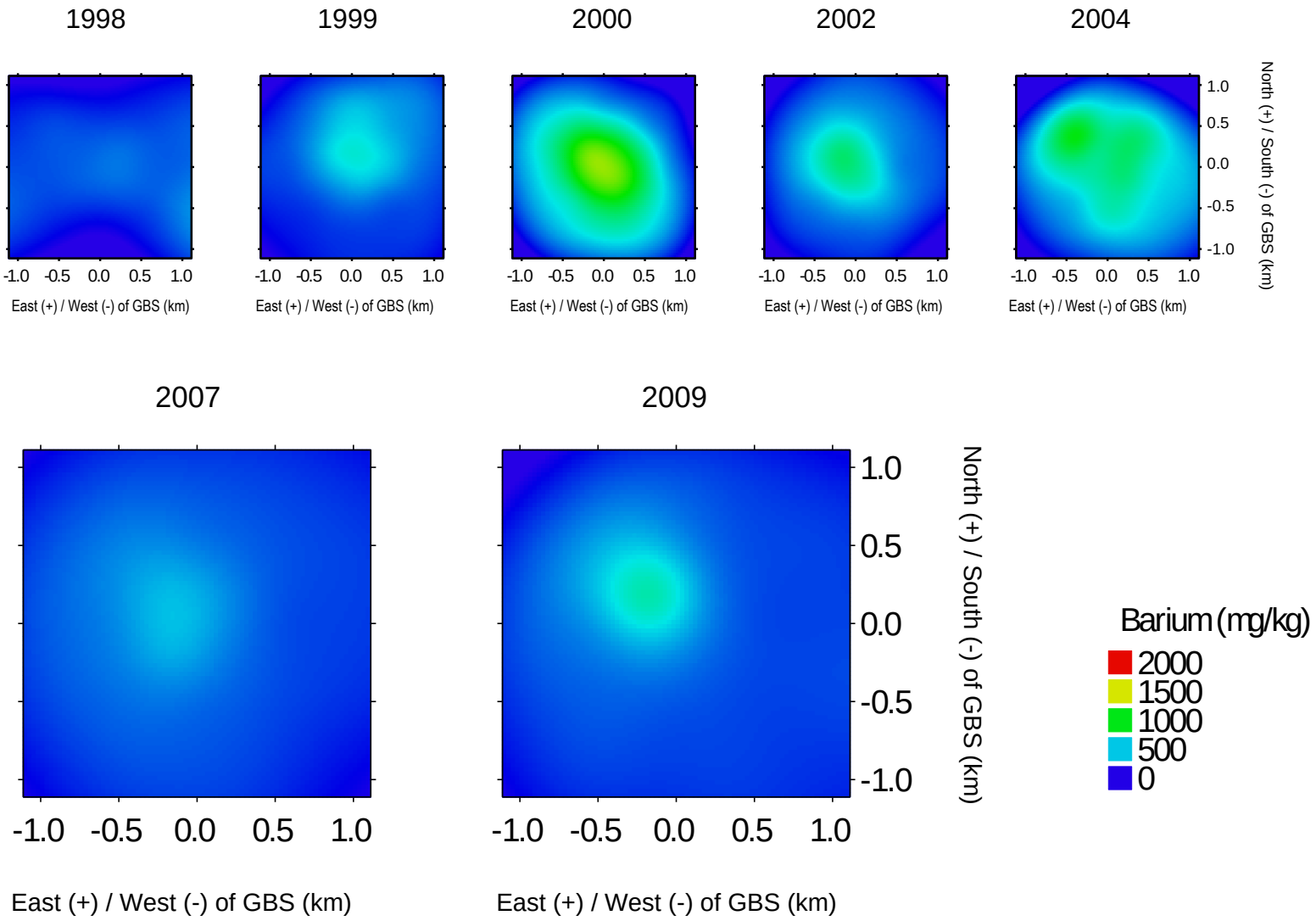


Figure 4.9 Spatial and Temporal Variability of Fuel Range Hydrocarbon (C10-C21) Concentration in Hibernia Sediment Within 1 km of GBS

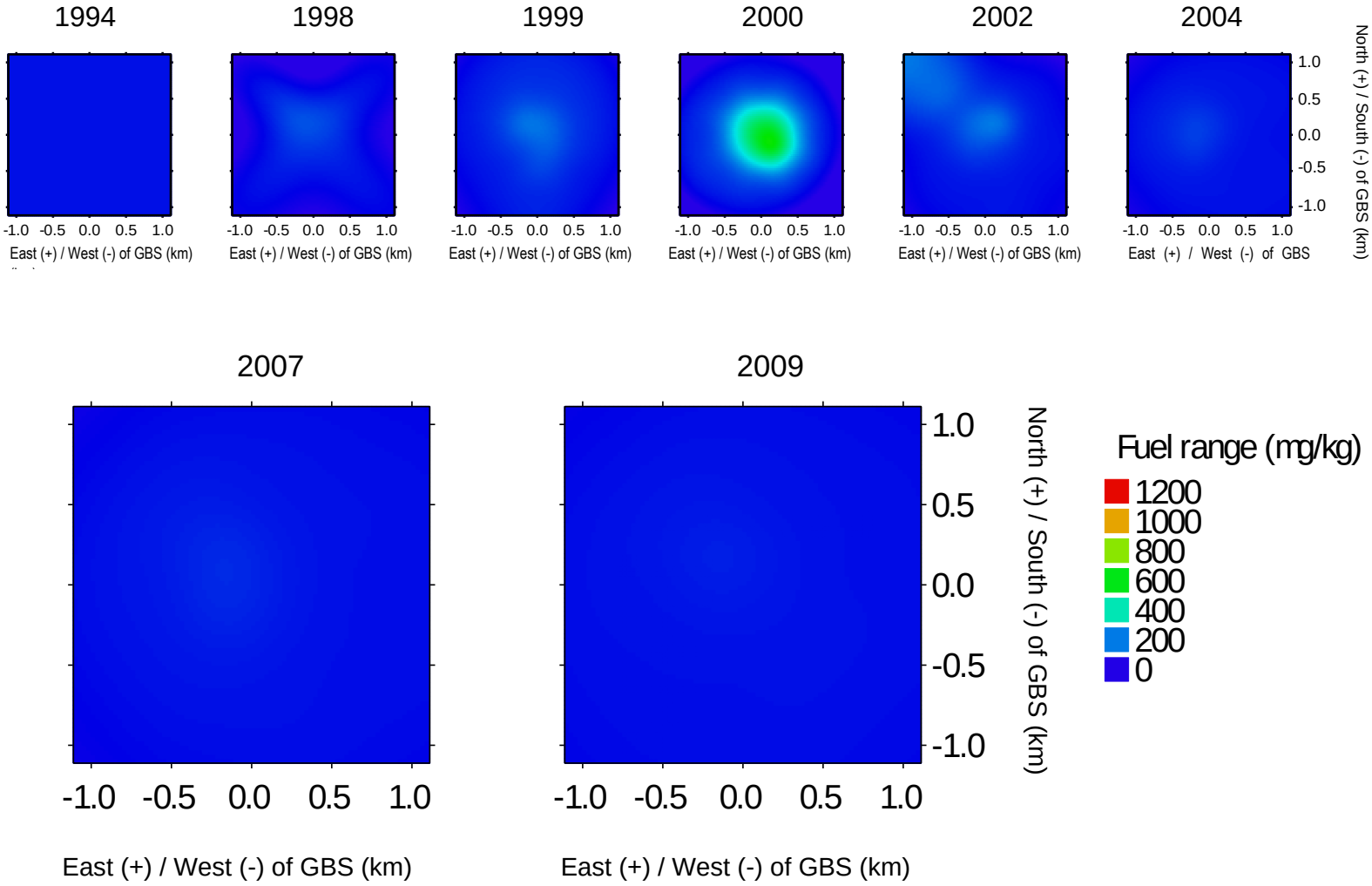
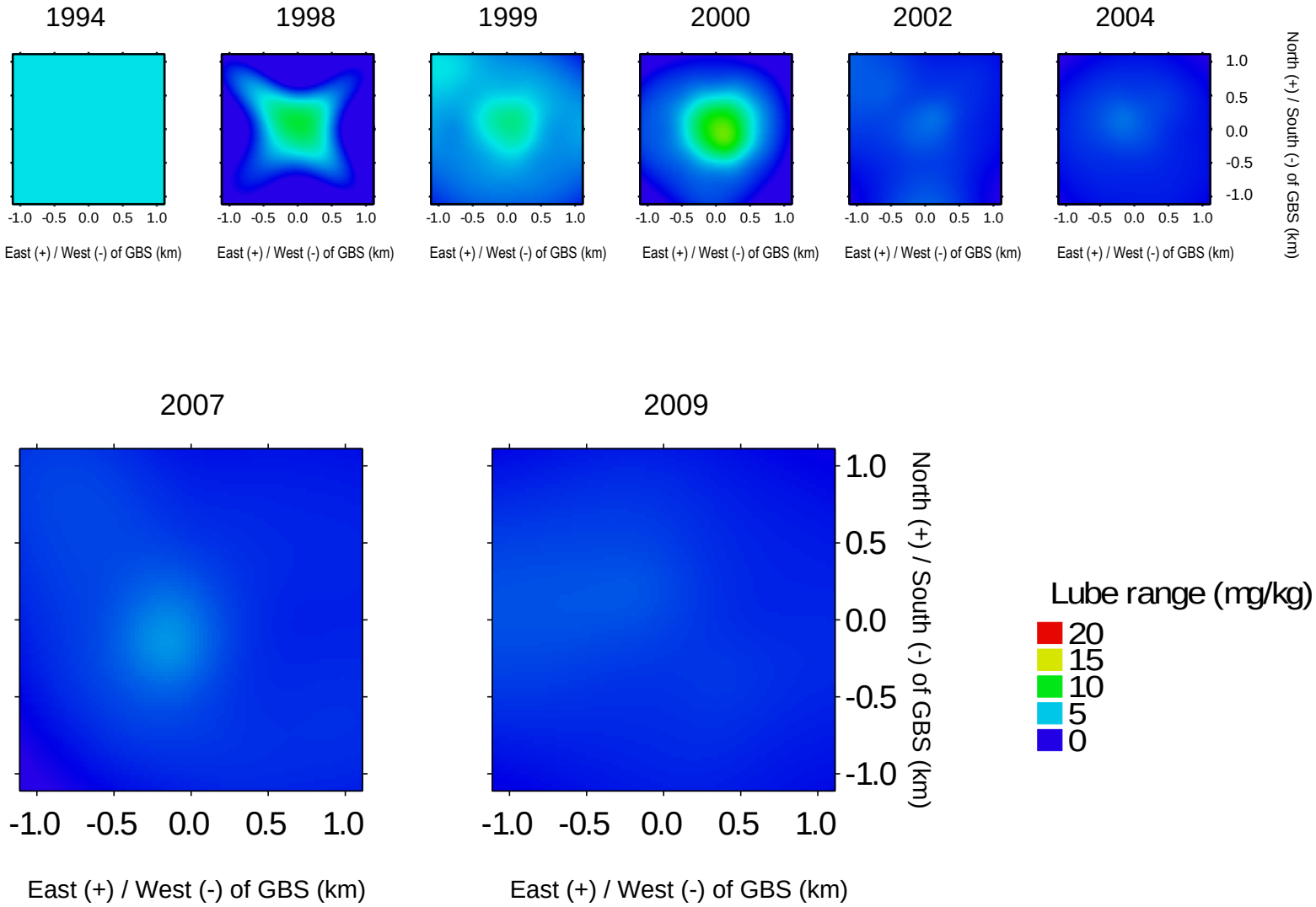


Figure 4.10 Spatial and Temporal Variability of Lube Range Hydrocarbon (C21-C32) Concentration in Hibernia Sediment within 1 km of GBS



5.0 HIBERNIA 2009 SEDIMENT CHEMISTRY STATISTICAL ANALYSES

The statistical approach used to evaluate the 2009 EEM data as compared to data from previous years has been structured to consider two levels of scale. This is explained in more detail in Appendix B. The first level of analyses is a screening for differences between Near-field (stations between 250 and 1,000 m from the platform), Mid-field (stations between 1,500 and 3,000 m from the platform), and Far-field (stations 6,000 m from the platform). The division of stations into Near-, Mid- and Far-field groupings for all the data is arbitrary, but has proven effective (HMDC 2003a, 2005, 2009) to reflect the varying spatial extent of contaminant predictions developed in the Hibernia production phase EEM program (HMDC 1996). The second statistical analysis focuses on contaminants within 1,000 m of the platform, by looking explicitly at contaminants along radii at distances of 250, 500 and 1,000 m. The 750-m distance stations from Hibernia were discontinued in 2004, and therefore are not included in the "Distance" factor for this statistical analysis; however, they are included in the "Field" factor for the years sampled before 2004. In both analyses scenarios, the analysis explicitly tests for variation in contaminant concentrations attributable to distance from the platform, either as Field (Near-, Mid- or Far-field distances) or explicitly as Distance (250, 500 and 1,000 m); the year of the EEM program (Year) and, most importantly, the potential interaction terms (Year x Field or Year x Distance).

5.1 Sediment Chemistry Year and Field/Distance Comparisons via ANOVA

Based on the results of the data analysis described above, the following parameters were subjected to statistical analysis using ANOVA: total barium; weak-acid extractable barium; fuel range hydrocarbon; and lube range hydrocarbon. These are considered to be useful indicators for EEM purposes because they should be present at low or non-detectable concentrations in the environment and they are components of drilling fluids, or could represent releases of hydrocarbon products. The results of these analyses are described in the subsequent sections.

Each parameter is evaluated in a two-step process. The first series is a field scale analysis, where the various distances were divided into Near-field, Mid-field and Far-field groups. All years for which data were available were processed. At each sampling station, if more than one sample was collected, the mean value for the available data for each year was taken as the best point estimate. Note that in this statistical analysis, the sampling station is the experimental unit (refer also to Appendix B). The use of individual samples collected at each station is, therefore, not valid, since these samples are not true replicates, but should be viewed as sub-samples. The statistical analysis seeks variation in contaminant concentrations that are attributable to Year, to Field, or to the Year x Field interaction. A result due to Year would suggest that most of the values for one or more years were different from observations for one or more other years. A result due to Field would suggest that the overall values observed in one or more Fields were different than values in other Fields. Although potentially indicative of major changes in the environment, neither of these two factors is likely to be a direct indicator of contamination as a result of Hibernia operations. The third factor, the Year x Field interaction, would indicate that one or more of the Fields was different from itself or other Fields, in one or more years. This

term is, therefore, the most likely to indicate environmental contamination that can be linked to Hibernia operations. The second step in the analysis is to examine the Near-field stations, and to look for contaminants attributable to Year, Distance (250, 500, or 1,000 m), or the Year x Distance interactions for the area (s) where contaminants are most likely to be observed. Again, it is the interaction term that is the most likely to identify environmental contaminants genuinely associated with operations of the Hibernia platform.

5.1.1 Total Barium

Total barium data are available for all years. Therefore, this analysis describes the 1994, 1998, 1999, 2000, 2002, 2004, 2007 and 2009 data. All data were log_{10}-transformed prior to analysis, and the analysis is based upon the mean value of the three field replicate samples collected at each station for the years from 1994 to 2002, and the value of the single sample collected at each station in 2004, 2007 and 2009.

To assess if the concentration of total barium in sediment may also vary with sediment grain size, using a grain size index as the parameter to summarize the content of gravel, sand, silt and clay in the sediment sample, the Pearson correlation coefficient was calculated for log-transformed barium and grain size index data. The grain size index is a value that ranges from 100 for a sediment sample that contains purely silts and clays, to 300 for only gravel and calculated as (% gravel x 3)+(% sand x 2)+((% silt + % clay) x 1). Analysis of the correlation coefficient was carried out to determine if barium concentrations correlate with grain size distribution of the sediment sample and if grain size should be considered as a covariate whereby an analysis of covariance (ANCOVA) would be more appropriate (to adjust for variations as a result of grain size) than ANOVA. The correlation coefficient of barium with grain size index for data for the Field factor is very low (r = 0.113), and also low for data for the Distance factor (r = -0.203). These results indicate that barium concentrations do not vary with sediment grain size. Therefore grain size does not need to be considered as a covariate and ANOVA is an appropriate technique for statistical analysis.

The total barium results are shown as ANOVA tables for Year, Field and Year x Field, and for Year, Distance and Year x Distance in Table 5.1. Where statistically significant differences (p<0.05) attributable to any of the experimental factors or interaction terms were indicated, the source of these differences was investigated using Tukey's HSD multiple comparison tests.

Table 5.1 Two-Factor ANOVA for Log_{10}-Transformed Total Barium Concentration in Hibernia Sediment

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	*p*
Year and Field (Near-, Mid-, Far-) Factors					
Year	0.359	7	0.051	1.009	0.424
Field	1.739	2	0.870	17.137	< 0.001
Year*Field	0.521	14	0.037	0.734	0.740
Error	16.697	329	0.051		
Year and Distance (in Near-Field – 250, 500, 1,000 m) Factors					
Year	1.706	7	0.244	11.441	< 0.001
Distance	3.928	2	1.964	92.177	< 0.001
Year*Distance	0.784	14	0.056	2.628	0.002
Error	2.578	121	0.021		

Grouping the sampling stations according to Near-, Mid- and Far-field, there was a significant (p<0.001) difference identified for Field, as was observed in previous EEM programs. The Year and Year x Field interactions were not significant (p=0.424 and p=0.740, respectively). This indicates that barium concentrations are not varying significantly for the years sampled from 1994 to 2009 and with respect to station locations grouped as Near-, Mid- and Far-field (i.e., the interaction term) to the Hibernia platform. Further investigation using Tukey's HSD test indicated that for the Field factor, Near-field stations have significantly higher total barium concentrations than Mid- or Far-field stations. The mean barium concentrations in Near-field sediments during 2009 were similar to 2007, 2004, 2002 and 1999 data, with these years being lower than in 2000 (though not significantly different).

Concentrating on the data for the Near-field stations, there were significant (p<0.001) differences identified for Year, Distance and the Year x Distance interaction. Further investigation using Tukey's HSD test indicated that total barium concentrations were elevated at the 250-m distance from Hibernia platform as compared to the other distances (500 and 1,000 m). Total barium concentrations were also higher at the 500-m distance than at the 1,000-m distance. Focusing on the stations within 1,000 m of the platform, the Year effect is due to total barium concentrations in sediments increasing progressively from 1994 to 2000. Total barium concentrations decreased significantly from 2000 to 2002, and decreased again in 2004. At 250-m, 500-m, and 1,000-m distances from the platform, total barium concentrations in 2009 are not significantly different from total barium baseline (1994) concentrations at these distances, respectively. The significant interaction term is caused by statistically significant increases in total barium concentrations at the 250-m and 500-m distances, particularly for the year 2000 concentrations, relative to other years, including 2009. However, the mean total barium concentrations at 250 m in 2009 remained elevated (510 mg/kg) in comparison with both the 500 m (169 mg/kg) and 1,000 m (139 mg/kg) distances.

These results indicate that significant increases in total barium concentrations have been observed in the Near-field area, with the most pronounced increases being observed at the 250- and 500-m distances, particularly in 2000. Based upon the weight of evidence principles outlined above, these difference are considered to be associated with discharges from the Hibernia Project. Overall results for 2009 are comparable to the concentrations observed in baseline 1994 conditions and before the Hibernia production phase.

5.1.2 Weak Acid Leachable Barium

Data are not available for weak-acid leachable barium in 1994. Therefore, this analysis describes data collected for 1998, 1999, 2000, 2002, 2004, 2007 and 2009. All data were $\log_{10}$-transformed prior to analysis, and the analysis is based upon the mean value of the three primary samples collected at each station from 1998 to 2002, and the value of the single sample collected at each station in 2004, 2007 and 2009.

The Pearson correlation coefficient of weak-acid leachable barium with grain size index for log-transformed data was calculated to determine if grain size index should be used as a covariate in the statistical analysis. The correlation coefficient for the Field factor data (coarse scale analysis) is very low (r = -0.136), and also low for data for the Distance factor (r = -0.158). These results indicate that weak-acid leachable barium concentrations do not vary with

sediment grain size. Therefore grain size does not need to be considered as a covariate and ANOVA is an appropriate technique for statistical analysis.

The weak-acid leachable barium results are shown as ANOVA tables for Year, Field and Year x Field; and for Year, Distance and Year x Distance in Table 5.2. Where statistically significant differences attributable to experimental factors or interaction terms were indicated, the source of these differences was investigated using Tukey's HSD multiple comparison tests.

Table 5.2 Two-Factor ANOVA for Log_{10}-Transformed Weak-acid Leachable Barium Concentration in Hibernia Sediment

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	*p*
Year and Field (Near-, Mid-, Far-) Factors					
Year	2.964	6	0.494	8.916	< 0.001
Field	11.745	2	5.872	105.983	< 0.001
Year*Field	1.045	12	0.087	1.572	0.099
Error	15.902	287	0.055		
Year and Distance (in Near-Field – 250, 500, 1,000 m) Factors					
Year	3.270	6	0.545	14.307	< 0.001
Distance	5.088	2	2.544	66.774	< 0.001
Year*Distance	0.904	12	0.075	1.977	0.033
Error	4.267	112	0.038		

Grouping the sampling stations according to Near-, Mid- and Far-field, there were significant (p<0.001) differences identified for Year and Field. Further investigation using Tukey's HSD test indicated that Near-field stations have significantly higher weak-acid leachable barium concentrations than for Mid- or Far-field stations. The Year effect is due to higher weak-acid leachable barium concentrations in sediments collected in 2000 and in 2004, when compared to other years (see also Figure 4.8). The analysis shows that the weak-acid leachable barium concentrations were higher overall in 2000 than in 1998, 1999, 2002, 2007 or 2009.

With respect to the data for the Near-field stations only, there were significant (p<0.001) differences identified for Year and Distance, as well as a significant difference for the Year x Distance interaction. Further investigation using Tukey's HSD test indicated that overall weak-acid leachable barium concentrations are significantly higher at the 250-m distance from Hibernia platform than at 500 or 1,000 m. Weak-acid leachable barium concentrations are also higher overall at the 500-m distance than at the 1,000-m distance. Again, considering only stations within 1,000 m of the platform, the Year effect is due to weak-acid leachable barium concentrations in sediments increasing from 1998 to 2000, then decreasing in 2002 relative to 2000. The concentration of weak-acid leachable barium in the Near-field in 2009 was overall lower than in 2000, 2002 or 2004, and was similar to 1999. The significant interaction term is caused by increases in weak-acid leachable barium concentrations at the 250-, 500- and 1,000-m distance in 2000, relative to 1998, 1999, 2002, 2007 and 2009. There was a significant decrease in the weak-acid leachable barium concentration at the 500-m and 1,000-m distances in 2009 compared to 2004 and 2000, and are similar to concentrations observed in 2007. Notwithstanding, the mean weak-acid leachable barium concentrations at 250 m in 2009 are

higher (19.9 mg/kg) in comparison to the 500 m (10.9 mg/kg) and 1,000 m (5.2 mg/kg) distances.

Taken overall, these results indicate that significant increases in weak-acid leachable barium concentrations have been observed in the Near-field area, with the most pronounced increase observed in 2000. Improvement occurred in 2002 in that results were comparable to the values observed in 1999, but with weak-acid leachable barium concentrations increasing again in 2004 at the 500-m and 1,000-m distances. Results in 2009 are similar to 2007 results, where concentrations of weak-acid leachable barium are generally comparable to those observed in 1999. Concentrations remain elevated in proximity to the Hibernia platform. Based upon the weight of evidence principles outlined above, these differences in weak-acid leachable barium concentrations are considered to be associated with discharges from the Hibernia Project.

5.1.3 Fuel Range Hydrocarbon (C10-C21)

Data are not available for fuel range hydrocarbon in 1994 in a format comparable to subsequent years, due to elevated detection limits in 1994. This analysis describes only the 1998, 1999, 2000, 2002, 2004, 2007 and 2009 data. All data were $\log_{10}$-transformed prior to analysis, and the analysis is based upon the mean value of the three primary samples collected at each station from 1998 to 2002, and the value of the single sample collected at each station in 2004, 2007 and 2009.

To assess if sediment grain size is a covariate and should be used in the statistical analysis of fuel range hydrocarbons, the Pearson correlation coefficient was calculated on log-transformed fuel range hydrocarbon and grain size index data. The correlation coefficient for the Field factor data was found to be low (r = -0.219), and also low for data for the Distance factor (r = -0.258). These results indicate that fuel range hydrocarbon concentrations do not vary with sediment grain size. Therefore sediment grain size does not need to be considered as a covariate for fuel range hydrocarbon and ANOVA is an appropriate technique for statistical analysis.

The fuel range hydrocarbon results are shown as ANOVA tables for Year, Field and Year x Field, and for Year, Distance and Year x Distance in Table 5.3. Where statistically significant differences attributable to any of the experimental factors or interaction terms were indicated, the source of these differences was investigated using Tukey's HSD multiple comparison tests.

Table 5.3 Two-Factor ANOVA for Log_{10}-Transformed Fuel Range Hydrocarbon (C10-C21) Concentration in Hibernia Sediment

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	*p*
Year and Field (Near-, Mid-, Far-) Factors					
Year	11.242	6	1.874	11.196	< 0.001
Field	28.862	2	14.431	86.231	< 0.001
Year x Field	4.084	12	0.340	2.034	0.021
Error	48.030	287	0.167		

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	*p*
Year and Distance (in Near Field – 250, 500, 1,000 m) Factors					
Year	22.314	6	3.719	40.087	< 0.001
Distance	20.563	2	10.282	110.827	< 0.001
Year x Distance	1.386	12	0.116	1.245	0.262
Error	10.390	112	0.093		

Grouping the sampling stations according to Near-, Mid- and Far-field, there were significant (p<0.001) differences identified for Year and Field. The Year x Field interaction term was statistically significant (p=0.021). Near-field stations have significantly higher fuel range hydrocarbon concentrations than Mid- or Far-field stations, and the Mid-field station results are significantly elevated relative to the Far-field results. The Year effect is due to higher fuel range hydrocarbon concentrations in sediments for 1999 and 2000, when compared to concentrations in sediment for 1998, 2002, 2004, 2007 or 2009. Further investigation using Tukey's HSD test indicated that the significant interaction term is caused by higher fuel range hydrocarbon concentrations in Mid-field sediments for the 1999 and 2000 data, when compared with the same areas in other years. This same trend (1999 and 2000) was more pronounced in the Near-field. The concentration of fuel range hydrocarbon in the Near-field in 2009 was not significantly different from 2007 in all fields and was significantly less than in the Near-field from 1998 to 2002.

Focusing on the Near-field stations, there were significant (p<0.001) differences identified for Distance and Year, but not the Distance x Year interaction (p=0.262). Further investigation using Tukey's HSD test indicated that overall fuel range hydrocarbon concentrations are significantly higher at the 250-m distance from Hibernia platform than at 500 or 1,000 m. Fuel range hydrocarbon concentrations are also higher overall at the 500-m distance than at the 1,000-m distance. Focusing on stations within 1,000 m of the platform, the Year effect is due to higher fuel range hydrocarbon concentrations in sediments for 1999 and 2000 relative to 1998, 2002, 2004, 2007 and 2009. Fuel range hydrocarbon concentrations also increased year over year between 1998 and 2000. The fuel range hydrocarbon concentration decreased significantly in 2002, relative to the 2000 and 1999 concentrations, and decreased significantly again in 2004, 2007 and subsequently again in 2009. The concentrations of fuel range hydrocarbon were significantly lower in 2009 compared to all years with the exception of 2007. In 2009, the mean fuel range hydrocarbon concentrations at 250 m remained higher overall (22.6 mg/kg) in comparison with both the 500 m (3.2 mg/kg) and 1,000 (1.0 mg/kg) distances.

These results indicate that significant increases in fuel range hydrocarbon concentrations have been observed in the Near- and Mid-field areas, relative to the Far-field, and particularly at the 250-m distance relative to other distances. The most pronounced increases in fuel range hydrocarbon concentrations were observed in 2000. Improvement is evident in that fuel range hydrocarbon concentrations have been significantly decreasing since 2000, and in 2009 were not significantly different from values observed in 1998. However, fuel range hydrocarbons remain elevated at 250 m when compared to 500 m and 1,000 m in 2009. Based upon the weight of evidence principles outlined above, these differences observed for fuel range hydrocarbons are considered to be associated with discharges from the Hibernia Project.

5.1.4 Lube Range Hydrocarbon (C21-C32)

Data are not available for lube range hydrocarbon in 1994 in a format comparable to subsequent years, due to elevated detection limits in 1994. Therefore, this analysis describes only the 1998, 1999, 2000, 2002, 2004, 2007 and 2009 data. All data were log_{10}-transformed prior to analysis, and the analysis is based upon the mean value of the three primary samples collected at each station from 1998 to 2002, and the value of the single sample collected at each station in 2004, 2007 and 2009.

To assess if sediment grain size is a covariate and should be used in the statistical analysis of lube range hydrocarbons, the Pearson correlation coefficient was calculated on log-transformed lube range hydrocarbon and grain size index data. The correlation coefficient for the Field factor data was found to be very low (r= 0.046), and also low for data for the Distance factor (r = -0.201). These results indicate that lube range hydrocarbon concentrations do not vary with sediment grain. Therefore sediment grain size does not need to be considered as a covariate for lube range hydrocarbon and ANOVA is an appropriate technique for statistical analysis.

The lube range hydrocarbon results are shown as ANOVA tables for Year, Field and Year x Field, and for Year, Distance and Year x Distance in Table 5.4. Where statistically significant differences attributable to any of the experimental factors or interaction terms were indicated, the source of these differences was investigated using Tukey's HSD multiple comparison tests.

Table 5.4 Two-Factor ANOVA for Log_{10}-Transformed Lube Range Hydrocarbon (C21-C32) Concentration in Hibernia Sediment

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	*p*
Year and Field (Near-, Mid-, Far-) Factors					
Year	4.263	6	0.711	18.117	< 0.001
Field	0.747	2	0.373	9.520	< 0.001
Year x Field	0.477	12	0.040	1.013	0.436
Error	11.257	287	0.039		
Year and Distance (in Near-Field – 250, 500, 1,000 m) Factors					
Year	3.990	6	0.665	47.502	< 0.001
Distance	3.246	2	1.623	115.938	< 0.001
Year x Distance	1.167	12	0.097	6.948	< 0.001
Error	1.568	112	0.014		

Grouping the sampling stations according to Near-, Mid- and Far-field, there were statistically significant (p<0.001) differences identified for Year and Field. The Year x Field interaction term was not statistically significant (p=0.436). Further investigation using Tukey's HSD test indicated that Near-field stations have significantly elevated lube range hydrocarbon concentrations when compared to Mid- or Far-field stations. The Year effect is due to elevated lube range hydrocarbon concentrations in sediments collected in 1999, when compared to other years. The analysis also shows that the lube range hydrocarbon concentrations were significantly lower in 2002, 2004, 2007 and 2009, as compared to 1999. The concentration of lube range hydrocarbon in 2009 is not significantly different from that in 2000, 2002, 2004 or 2007.

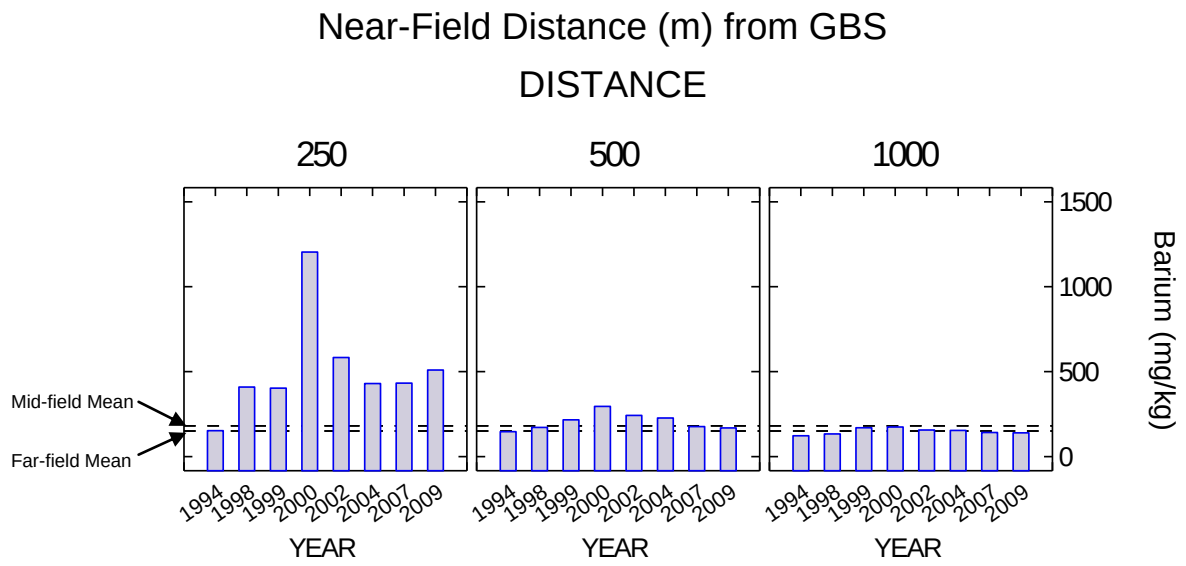
Focusing on the stations within 1,000 m of the platform, the Year effect is due to higher lube range hydrocarbon concentrations in sediments in 1999 than were observed in any other year. Lube range hydrocarbon concentrations increased significantly to peak in 1999, relative to 1998. The lube range hydrocarbon concentration trended downward and was significantly lower in 2000 when compared with 1999 (but not 1998), and again declined significantly in 2002, 2004, 2007 and 2009 (the lowest level observed was in 2004). The significant Distance x Year interaction term is caused by several factors; however, the most important factor is a significant decline in lube range hydrocarbon concentrations observed within 250 m, 500 m and 1,000 m of the platform during 2002, 2004, 2007 and 2009, relative to levels within 250 m of the platform and to some extent at the 500-m and 1,000-m distances for other years. There was no significant difference in lube range hydrocarbon concentrations in 2009 in the Near- and Mid-fields compared to 2002, 2004 and 2007 for a given distance from the Hibernia platform. In 2009, however, the mean lube range hydrocarbon concentrations at 250 m remain elevated (1.3 mg/kg) in comparison with both the 500 m (1.0 mg/kg) and 1,000 (0.7 mg/kg) distances, though not statistically significant.

These results indicate that statistically significant increases in lube range hydrocarbon concentrations have been observed in the Near-field area, with the most pronounced increase being observed in the 1999 concentrations. Improvement is evident in that results for 2009, and since 2002, are comparable to or below the concentrations observed for all previous years (excluding 1994 due to elevated detection limits). Concentrations of lube range hydrocarbons at 250 m distance in 2009 remained elevated when compared to concentrations at 500 m and 1,000 m. Based upon the weight of evidence principles outlined above, these differences observed for lube range hydrocarbons are considered to be associated with discharges from the Hibernia Project.

5.1.5 Summary

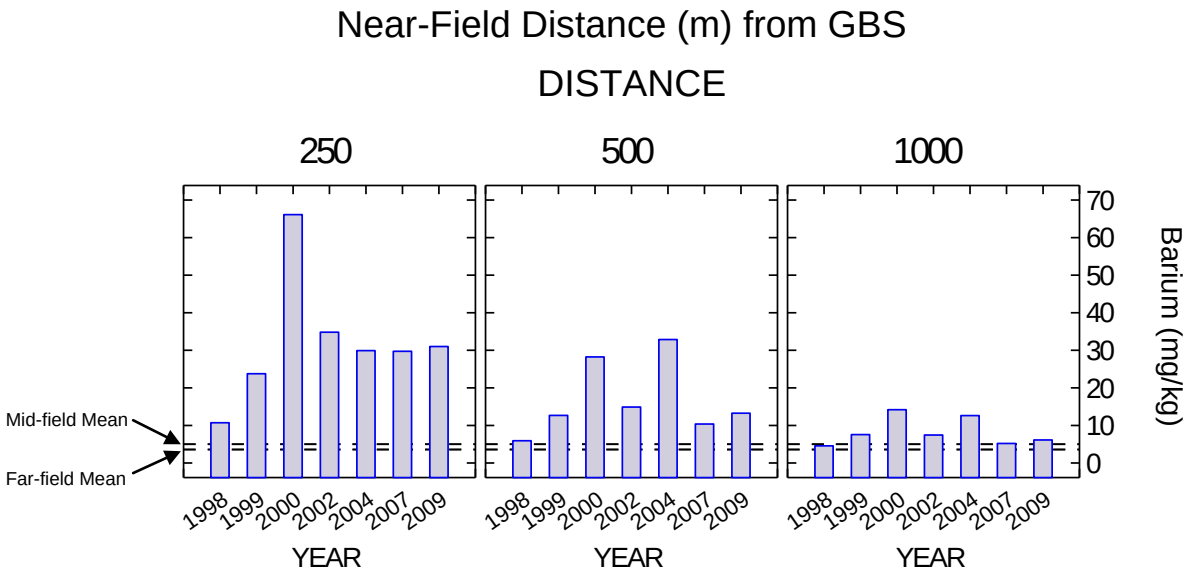
From the data and statistical analyses presented in the sections above, it can be summarized that overall 2009 was similar to 2007 and an improvement over 1999 to 2000. A declining trend has been observed with respect to sediment contamination around the platform since 2000, when a peak was experienced in the concentrations of total barium, weak-acid leachable barium, and fuel and lube range hydrocarbons. These trends are clearly visible in Figures 5.1 to 5.4.

Figure 5.1 Trend of Total Barium Concentrations at Near-Field Distances to the Hibernia Platform from 1994 to 2009



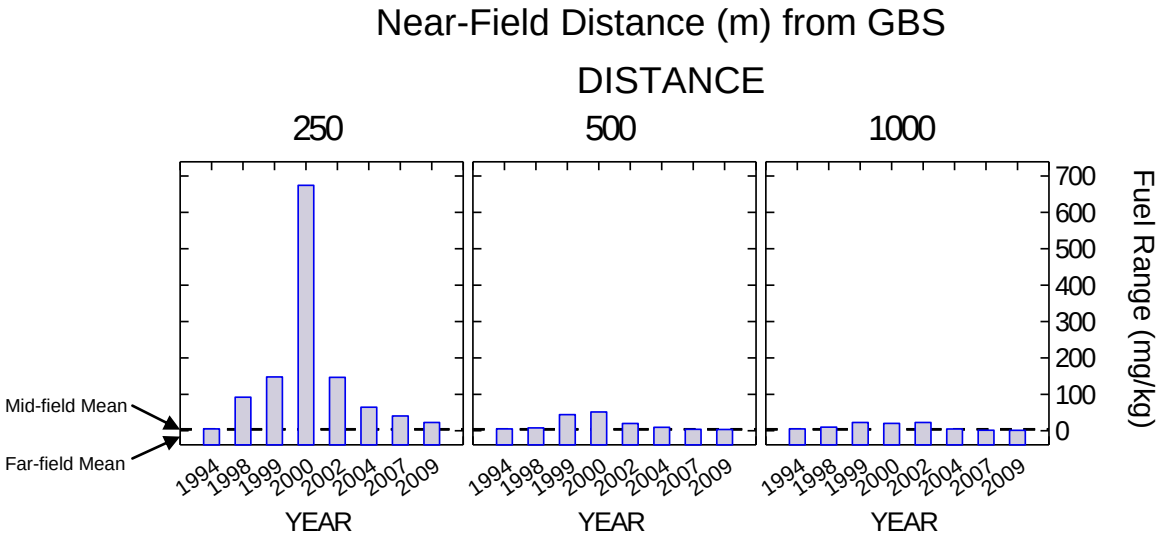
Note: The mean concentration for the Mid-Field and Far-Field for all years combined is also shown in the figure with a dashed line.

Figure 5.2 Trend of Weak-Acid Leachable Barium Concentrations at Near-Field Distances to the Hibernia Platform from 1994 to 2009



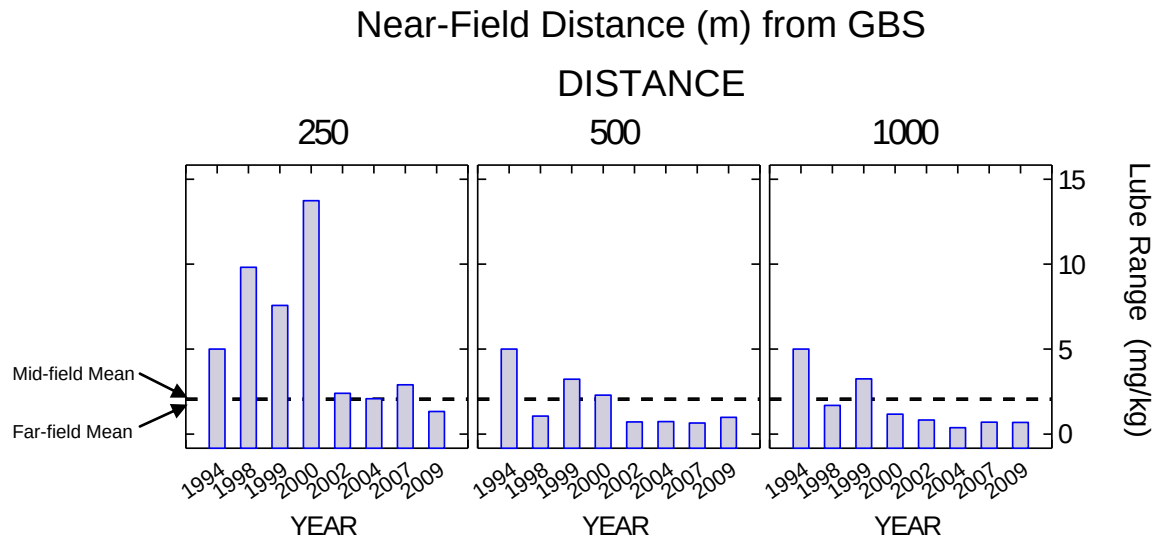
Note: The mean concentration for the Mid-Field and Far-Field for all years combined is also shown in the figure with a dashed line.

Figure 5.3 Trend of Fuel Range Hydrocarbons at Near-Field Distances to the Hibernia Platform from 1994 to 2009



Note: The mean concentration for the Mid-Field and Far-Field for all years combined is also shown in the figure with a dashed line. Mean concentrations include 1994 data that were all below the RDL of 10 mg/kg and which were set equal to 5 mg/kg for the calculation of the mean.

Figure 5.4 Trend of Lube Range Hydrocarbons at Near-Field Distances to the Hibernia Platform from 1994 to 2009



Note: The mean concentration for the Mid-Field and Far-Field for all years combined is also shown in the figure with a dashed line. Mean concentrations include 1994 data that were all below the RDL of 10 mg/kg and which were set equal to 5 mg/kg for the calculation of the mean.

There are still trends observed in 2009 in the Near-field area where the concentrations of these analytes at the 250-m distance remained elevated when compared to concentrations at 500 m and 1,000 m. These differences are considered to be associated with historic discharges from the Hibernia Project as the remaining cutting piles may be mobilized over time. Although there is a slight predominant current regime to the southwest at Hibernia, the rotary character of currents around the Hibernia platform (Seaconsult 1994) and storm-induced ocean currents likely have a greater effect than the predominant current. The sediment chemistry EEM data for 1998, 1999, 2000, 2002, 2004, 2007 and 2009 would seem to indicate that such is the case.

The radials that exhibited the highest concentrations of hydrocarbons have changed from radial 8 (1998 and 1999) to radial 4 (2000) to radial 2 (2002) to radial 6 (2004), to radial 8 (2007 and 2009) which suggests a clockwise rotation may be occurring around the Hibernia platform. A similar pattern of concentrations has been observed for barium.

Muschenheim and Milligan (1996) have noted that a near-seabed velocity in excess of 20 cm/s was sufficient to re-suspend drilling cuttings. The implication of this information and its applicability to the Hibernia site is not necessarily clear cut. Hibernia bottom current speeds are generally between 5 to 14 cm/s, with currents greater than 23 cm/s occurring approximately 8 percent of the time (Seaconsult 1994). Regardless, this indicates that for 8 percent of the time, even heavy particulate matter associated with the drilling discharges can be transported due to bottom current velocities. Furthermore, the bottom current velocities are such that transport of fine particulates may well occur on a regular basis at the Hibernia site.

It should be noted that the elevated contaminant concentration observed within the Near-field are restricted to within 250 m. Concentrations at 500 m and 1,000 m are more similar to concentrations in the Mid- and Far-fields that are at a distance greater than 1,000 m from the Hibernia platform and are approaching background levels (with the exception of those parameters associated with WBMs (e.g., weak-acid leachable barium)).

The shale chutes used for the discharge of cuttings are located on radials 2 and 8. Using TPH as an indicator of cuttings, the observed 2009 values at 250 m (expressed as mg/kg) are radial 2 = 18.1, radial 4 = 4, radial 6 = 13.1 and radial 8 = 60.5. Of all of the likely sources of hydrocarbons (cuttings, produced water, storage displacement water, platform drainage water, process area water, and drillage area drainage water) it is reasonable to conclude that cuttings is the primary source of observed hydrocarbons in sediment. A review of sediment GC scans for the 250 m stations indicate that elevated levels of hydrocarbons are primarily a result of retained synthetic oil (pure drill) on cuttings. A comparison of the GC scans from the 250-m stations with the GC scan for “pure drill” reveals that the largest component of hydrocarbons at the 250-m stations is related to the “pure drill”.

A summary of statistically significant results for metals and hydrocarbons (in sediment) for all EEM programs is included in Table 5.5. The interpretation of statistical significance is based on attenuation or a statistically significant difference between Near-field and Mid-field or Far-field for a specific parameter during the year of the EEM Study.

For Hydrocarbons, attenuation or a statistically significant difference between Near-field, Mid-field or Far-field was detected for both fuel and lube range hydrocarbons during all EEM study years (with near-field stations having the highest concentrations). For metals, total barium concentration was statistically higher in the Near-field for all years. Weak acid leachable barium was statistically higher in the Near-field for all years except 1999.

Table 5.5 Summary of Statistically Significant Results for Metals and Hydrocarbons in Sediment for all EEM Years

Parameter	Year						
	1998	1999	2000	2002	2004	2007	2009
Hydrocarbons							
TEH	Yes	Yes	Yes	No Data	No Data	No Data	No Data
Fuel	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lube	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Metals							
Total Barium	Yes	Yes	Yes	Yes	Yes	Yes	Yes
WAM Barium	Yes	No	Yes	Yes	Yes	Yes	Yes
Iron	Yes	No	No	No	No	No	No
WAM Iron	Yes	No	No	No	No	No	No Data
WAM Aluminum	Yes	No	No	No	No	No	No Data

Note: Statistical significance is based on attenuation or a statistically significant difference between Near-field and Mid-field or Far-field.

6.0 HIBERNIA 2009 SEDIMENT TOXICITY PROGRAM

A summary of results for the 2009 sediment toxicity data is presented in Table 6.1. Complete Year 2009 sediment toxicity reports are provided in *Hibernia Production Phase Environmental Effects Monitoring Program – Year Seven Volume II* (HMDC 2010).

In 2009, as in 2000, 2002, 2004 and 2007, the results of the Microtox bacterial luminescence assay determined which further analyses (i.e., amphipod survival and juvenile polychaete toxicity tests) would be required. Where any site had Microtox results that had IC50 values of less than or equal to 40,000 mg/L (which is considered to be a toxic response for Hibernia sediments), the amphipod survival and juvenile polychaete assays were completed. In addition, all stations within 500 m of the Hibernia platform and reference stations were subjected to the amphipod survival and juvenile polychaete assays. The 2009 sediment toxicity data presented in Table 6.1 are based on actual results from single toxicity tests without replication. The majority of Microtox tests on 2009 sediment had results that indicated an IC50 value of greater than 197,000 (as was the case in 2004 and 2007) which is a higher IC50 value than in 2002 and prior to this time (> 98,684 mg/L). In 2004, the Microtox analysis was changed from the small volume tests to the large volume test thereby resulting in an increased initial dilution. All the 2009 Microtox test data in Table 6.1 are considered detected values for the toxicity results (as well as for other years of the EEM program), which include the upper bound IC50 value of >197,000 mg/L that indicates a non-toxic response for the test.

Table 6.1 Year 2009 Sediment Toxicity Test Data – Sample Interpretation of Toxic Results

Station ID	Microtox (mg/L)	Amphipod Survival (%)	Juvenile Polychaete Survival (%)	Juvenile Polychaete Growth (mg/worm)
1-500	> 197,000	96	88	19.59
1-1000	> 197,000			
1-2000	> 197,000			
1-3000	> 197,000			
1-6000	34,020	87	64	28.33
1-16000	> 197,000	85	100	18.92
1-16000 (field dup)	> 197,000	86	92	27.24
2-250	> 197,000	81	56	41.58
2-500	> 197,000	93	88	39.27
2-1000	> 197,000			
3-500	12,570	88	72	15.03
3-1000	> 197,000			
3-2000	3,429	98	92	27.78
3-3000	> 197,000			

Station ID	Microtox (mg/L)	Amphipod Survival (%)	Juvenile Polychaete Survival (%)	Juvenile Polychaete Growth (mg/worm)
3-6000	> 197,000			
4-250	85,260	89	96	38.29
4-500	> 197,000	98	76	29.05
4-1000	24,720	88	68	12.37
5-500	> 197,000	91	76	35.06
5-1000	> 197,000			
5-2000	> 197,000			
5-3000	> 197,000			
5-6000	> 197,000			
6-250	> 197,000	91	88	36.38
6-500	> 197,000	88	64	20.58
6-1000	> 197,000			
7-500	> 197,000	90	76	47.95
7-1000	> 197,000			
7-2000	1,904	52	24	7.43
7-3000	1,973	65	16	16.35
7-6000	1,376	92	76	25.95
7-16000	> 197,000	83	88	77.76
7-16000 (field dup)	> 197,000	90	92	61.1
8-250	> 197,000	90	92	16.81
8-500	> 197,000	88	72	46.75
8-1000	> 197,000			

6.1 Microtox Results

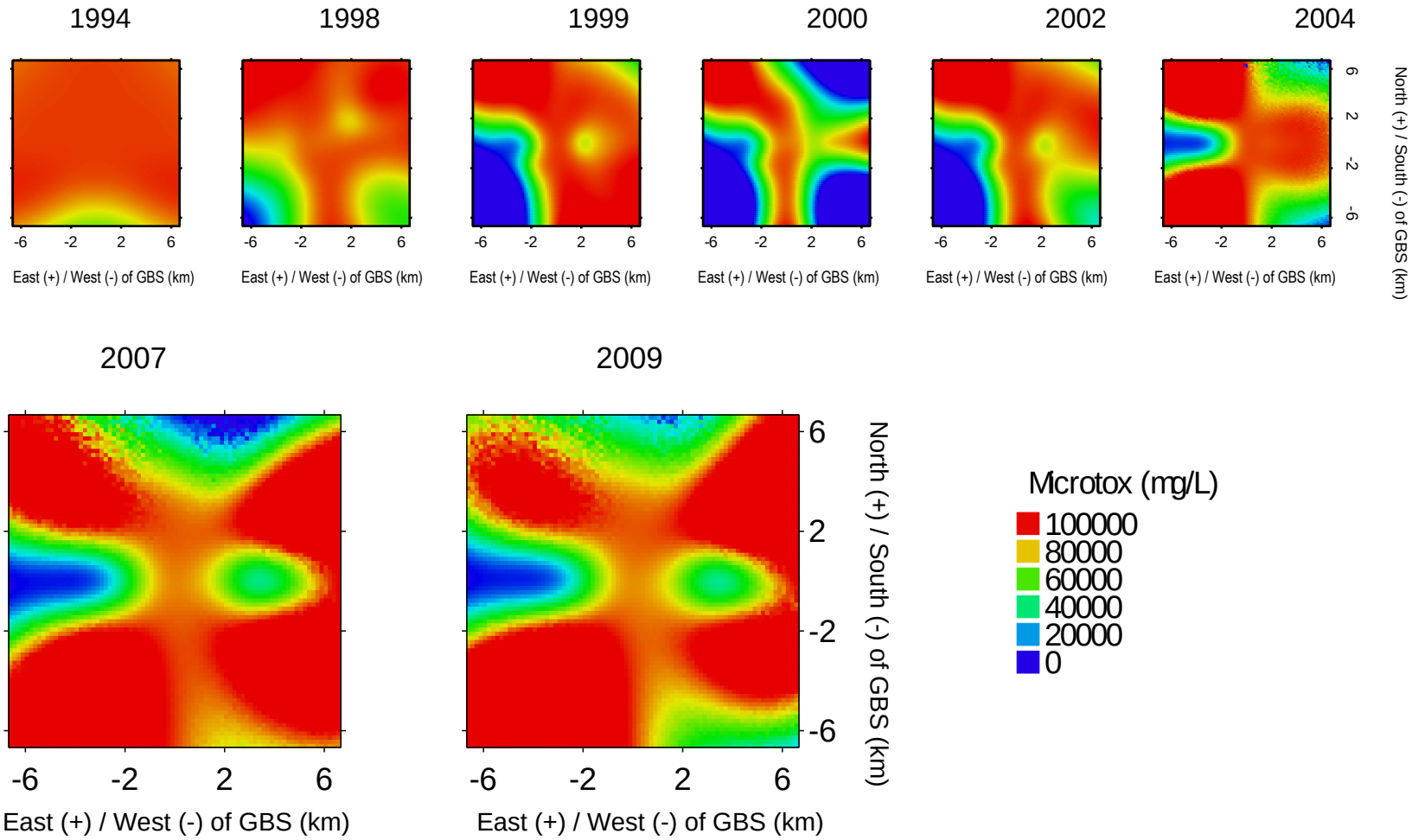
Of a total of 36 Microtox tests conducted in 2009 on samples from 34 stations (Table 6.1), seven responses, and the same number of responses and station locations as those in 2007, were considered toxic. This is based on Microtox IC50 values of less than or equal to 40,000 mg/L and the interpretative guidelines developed for the Hibernia project, which took into account natural toxic responses observed during the baseline studies. Three of the seven toxic responses were along radial 7 (west gradient), at stations located between 2,000 and 6,000 m from the Hibernia platform, where toxic responses were also observed in 2002, 2004 and 2007. Similar to results from 2007, there were toxic responses observed in 2009 along radial 3 at the 500 and 2,000 m, radial 1 at 6,000 m and radial 4 at 1,000 m.

The spatial and temporal trends are shown in Figure 6.1 using 2-D surface contour plots of Microtox sediment toxicity results for the Hibernia site in 1994, 1998, 1999, 2000, 2002, 2004, 2007 and 2009. Since 2004, the reported Microtox test results have been adjusted in order to keep the maximum values (i.e., 197,000 mg/L since 2004 and 98,684 mg/L for previous years) on a consistent reporting basis. It can be seen in Figure 6.1 that Microtox toxicity responses were limited in 1994 to a single response at station 5-6,000 (radial 5 at 6,000 m). Microtox responses increased in 1998 along the southwest corner and eastern edge. Microtox responses

increased for these areas in 1999 to the maximum observed Microtox responses in 2000. Microtox responses subsequently decreased in 2002 and even more so in 2004 to return to levels similar to the levels observed in 1998 and 1999. Microtox values in 2007 and 2009 are very similar and show a slight increase compared to 2004 at stations located north and west of the Hibernia platform. Microtox values at the Hibernia platform in the centre of the plots remain above the toxic response limit of 40,000 mg/L (Figure 6.1). It is noteworthy that the pattern of the observed Microtox responses (i.e., toxic responses), for the period between 1998 and 2004, resembles the spatial distribution of sediment grain size (see also Figures 4.1 to 4.4). This resemblance weakens for the 2007 and 2009 data except for along radial 7 (to the west quadrant) and suggests that other factors, likely natural, may be responsible for the Microtox response.

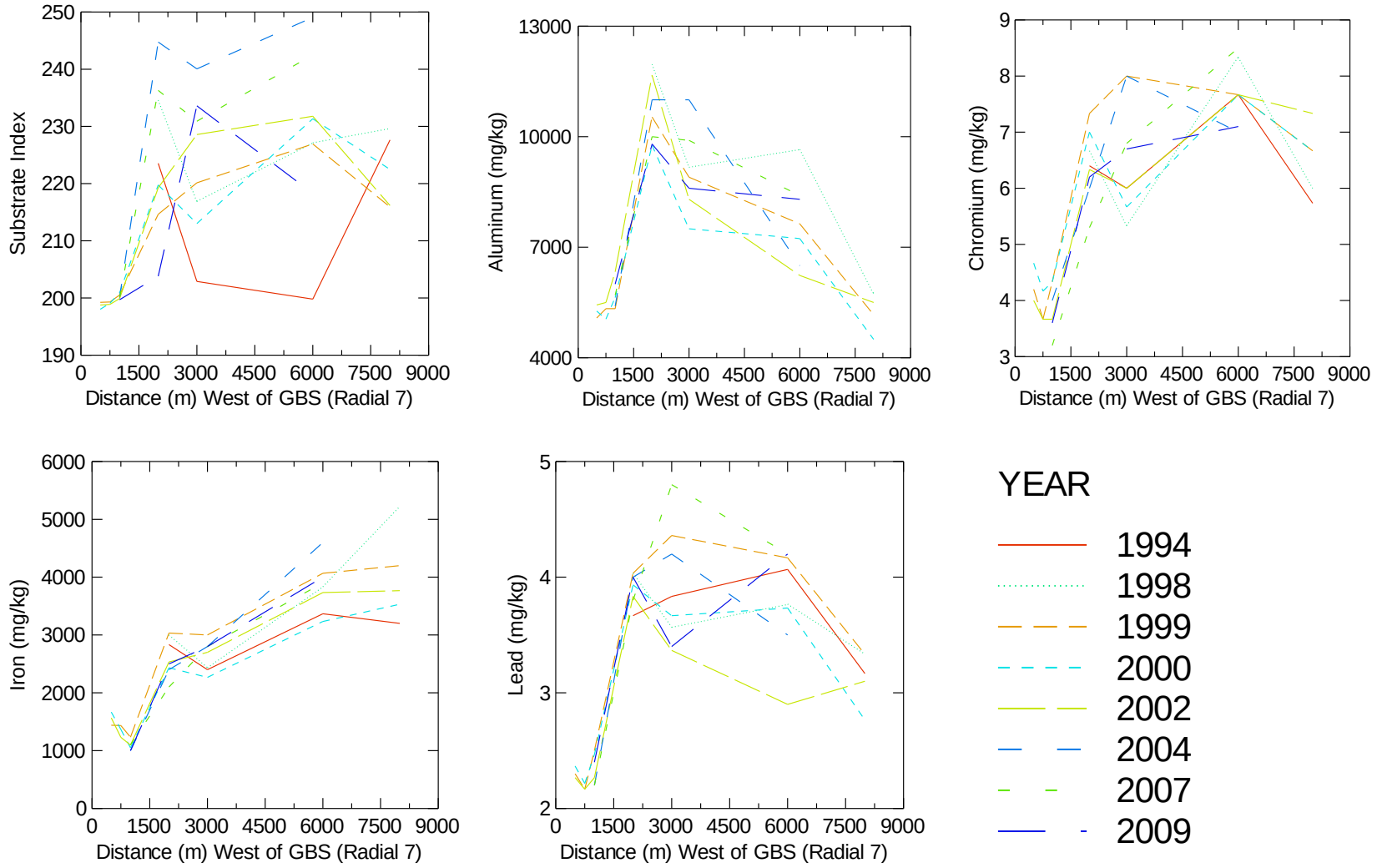
Some of the stronger relationships for sediment quality specifically along radial 7 for all years of the EEM program are shown in Figure 6.2. These include sediment grain size index (presented as a substrate index that integrates the sediment gravel, sand and silt plus clay content of a sample into a single index value ranging from 100 for purely silts and clays to 300 for sediment composed of only gravel) and the concentration of aluminum, chromium, iron, lead, manganese, strontium, uranium, vanadium, and total inorganic carbon in the sediment (moisture content and ammonia concentrations also revealed a similar trend but are not provided in Figure 6.2). The concentrations of these sediment quality parameters are generally much lower close to the Hibernia platform and which increase substantially from approximately 2,000 m to 6,000 m away from Hibernia on radial 7 for almost all years from 1994 to 2009. The higher metal and inorganic carbon concentrations also appear to be associated with coarser sediment between that of sand and gravel (substrate index value generally greater than 200). Further, the Microtox toxicity values along radial 7 and shown in Figure 6.3 indicate an inverse relationship when compared to sediment quality concentrations and sediment coarseness (Figure 6.2). That is, the sediment between 2,000 m and 6,000 m from Hibernia on radial 7 is generally coarser, contains higher metal concentrations, and exhibits a more toxic Microtox response. In addition, the higher toxicity responses (i.e., lower Microtox IC50 toxicity values) within this area are not affected by environmental contaminants from Hibernia as observed by, for example, the lower fuel range hydrocarbon concentrations farther from GBS on radial 7 (Figure 6.3). In fact, sediment from stations closer to the GBS generally contained higher fuel range hydrocarbon concentrations and had a lower toxicity response to the Microtox test. The analysis for statistical significance and correlations among factors that may likely contribute to the observed toxicity responses is presented in the following sections.

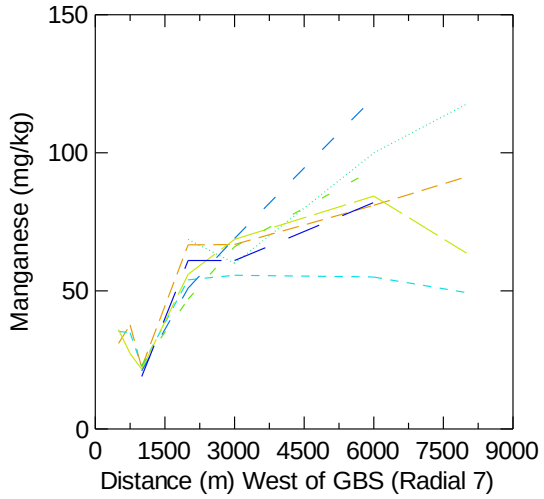
Figure 6.1 Spatial and Temporal Variability of Microtox IC50 Toxicity Values in Hibernia Sediment

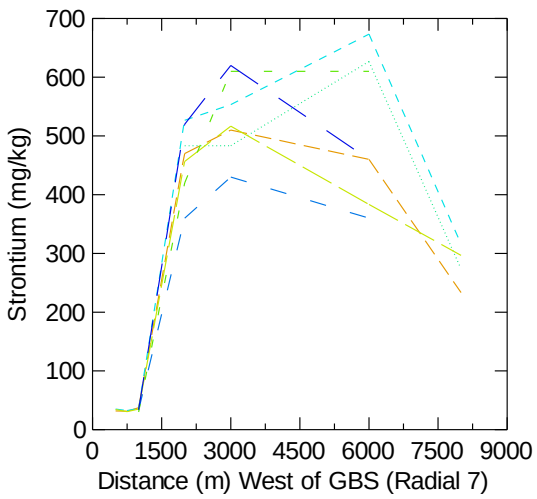


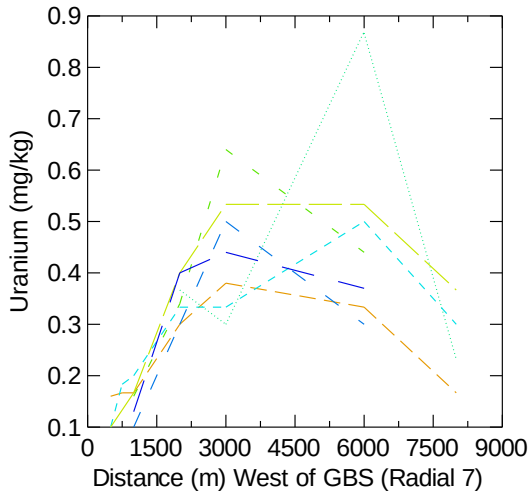
(Microtox values <40,000 mg/L are considered toxic on the basis of interpretative guidelines developed for the Hibernia Project)

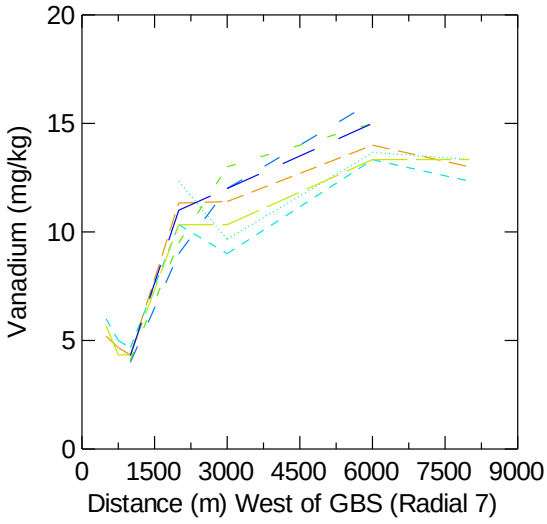
Figure 6.2 Sediment Characteristics and Chemical Concentrations measured along Radial 7

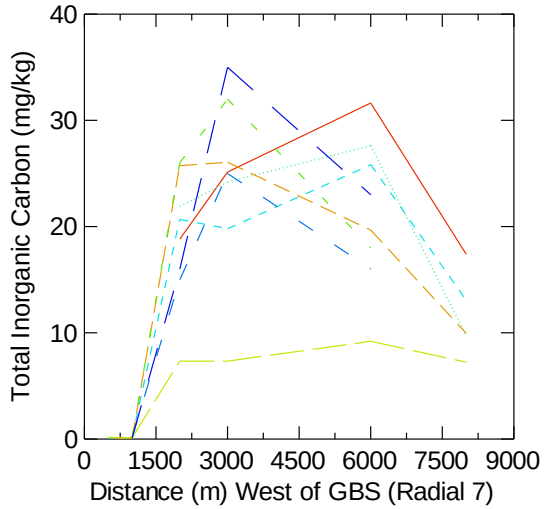



Manganese (mg/kg)
150
100
50
0
0 1500 3000 4500 6000 7500 9000
Distance (m) West of GBS (Radial 7)


Strontium (mg/kg)
700
600
500
400
300
200
100
0
0 1500 3000 4500 6000 7500 9000
Distance (m) West of GBS (Radial 7)


Uranium (mg/kg)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0 1500 3000 4500 6000 7500 9000
Distance (m) West of GBS (Radial 7)


Vanadium (mg/kg)
20
15
10
5
0
0 1500 3000 4500 6000 7500 9000
Distance (m) West of GBS (Radial 7)


Total Inorganic Carbon (mg/kg)
40
30
20
10
0
0 1500 3000 4500 6000 7500 9000
Distance (m) West of GBS (Radial 7)

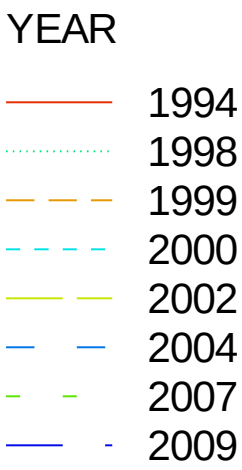
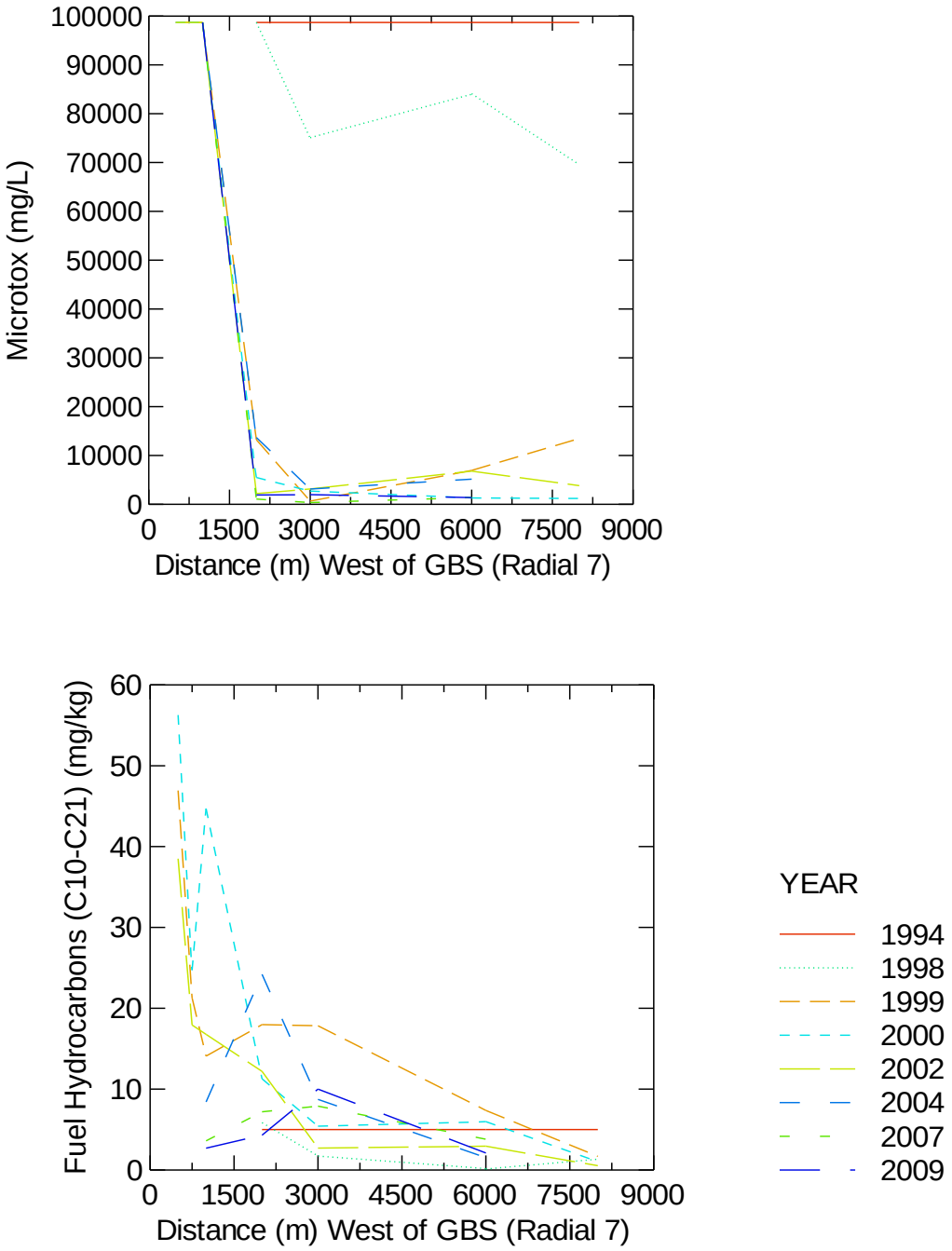

YEAR
1994
1998
1999
2000
2002
2004
2007
2009

Figure 6.3 Microtox IC50 Toxicity Values and Fuel Range Hydrocarbons in Hibernia Sediment Measured Along Radial 7



6.1.1 ANOVA for Microtox Test Results

An ANOVA for Microtox tests on log-transformed IC50 values was performed first at a gross spatial scale to assess potential environmental effects from Hibernia. The ANOVA results showed that there was a statistically significant (p=0.011) interaction effect (Year x Field), which is the important term to examine even if the Year and Field factors were statistically significant at p<0.001 (Table 6.2). Tukey's HSD multiple comparison tests for the interaction term indicated that Microtox IC50 values in the Near-field for all years were greater (i,e., indicating a less toxic response) than the value in 2000 in the Far-field. There were no significant differences between values of Microtox IC50 for all years in the Near-field. The only significant difference between Microtox IC50 values in the Far-field were values in 2000 being significantly lower (i.e., indicating a more toxic response) than all values in 1994 and in the Near-field for all years of the EEM Program (i.e., the 2000 Far-field had overall the most toxic response of all fields and for all years). Mid-field values were not significantly different between years but were significantly lower (i.e., indicating a more toxic response) in 2007 than values recorded in the Near-fields for 2007 and 2009. The Microtox values for 2009 were similar to 2007 results in the Near- and Far-fields and higher (though not significantly different) in the Mid-field. The above results indicate that, in general, the Far-field in 2000 had the most toxic response, followed by the Mid-field in 2007. The least toxic response was observed in 2004 in the Near-field among all the fields.

The fine-scale Microtox ANOVA analysis to assess the Distance factor within the Near-field at the Hibernia platform for all years (Year factor) identified no statistically significant differences for the Distance or interaction effects (i.e., Year x Distance, Table 6.2). The Year effect, however, was significant (p<0.001). Tukey's HSD multiple comparison tests revealed that the Microtox IC50 values overall in 2004 were significantly higher (i.e., indicating a less toxic response within the Near-field) than for all other years except for 2007 and 2009. Microtox IC50 values for 2009 were only significantly higher than values in 2000 and were not significantly different than any other year.

The above statistical results suggest that the Microtox results and toxic responses are not attributed to being in close proximity to the Hibernia platform. The Pearson correlation coefficient was calculated to investigate if sediment grain size, using the substrate index, is a covariate and correlates with the Microtox tests. A correlation coefficient of -0.621 (sample size n=352) was obtained and which suggests that sediment with a higher substrate index (i.e., coarser sediment containing more sand and gravel content) is overall likely to have a lower Microtox IC50 value (i.e., a more toxic response). The substrate index in the Near-field and within 1000 m of Hibernia had a much lower correlation coefficient of -0.375 (n=146) with Microtox tests. Other potential sediment quality parameters as covariates and factors that likely contribute to the variance in the Microtox tests are further investigated in Section 6.4.

Table 6.2 Two-Factor ANOVA for Log_{10}-Transformed Microtox Toxicity of Hibernia Sediment

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	p
Year and Field (Near-, Mid-, Far-) Factors					
Year	4.492	7	0.642	3.494	0.001
Field	9.199	2	4.600	25.046	<0.001
Year x Field	5.424	14	0.387	2.110	0.011
Error	60.234	328	0.184		
Year and Distance (in Near Field – 250, 500, 1,000 m) Factors					
Year	2.193	7	0.313	7.606	<0.001
Distance	0.049	2	0.025	0.595	0.553
Year x Distance	0.365	14	0.026	0.632	0.834
Error	5.026	122	0.041		

6.2 Amphipod Test Results

The amphipod survival test results indicated that station 7-2000 and 7-3000 were toxic. Radial 7 had the lowest survival rates at 52 percent and 65 percent at 2,000 m and 3,000 m, respectively. This lower amphipod survival rate is within the same area of higher Microtox toxicity responses noted above for radial 7 and likely a result of the same natural factors. All other 2009 amphipod survival tests had survival rates of 81 percent or greater.

Amphipod survival tests conducted in all previous EEM programs have been non-toxic with the exception of a single station in the 2000 EEM, when hydrocarbon levels peaked at the 250 m stations. This response was predicted as TPH approached or exceeded 1000 ppm. This response did not reoccur in 2004, 2007 or 2009 as TPH levels decreased. The occurrence of this single response that correlated with sediment containment levels validates the use of the amphipod bioassay as a measure of sediment toxicity.

6.3 Juvenile Polychaete Test Results

The juvenile polychaete survival test results indicated that station 7-2000 and 7-3000 were also toxic. All 2009 polychaete survival tests had survival rates of 56 percent or greater with the exception of radial 7 which had the lowest survival rates at 24 percent and 16 percent at 2,000 m and 3,000 m, respectively. The juvenile polychaete growth test results for 2009 varied from a low of 7.43 mg/worm to a high of 77.76 mg/worm, with a mean (±SD) value of 31.34 (±16.79 mg/worm). The low value was recorded at a distance of 2,000 m on radial 7, whereas the high value was recorded at a distance of 16,000 m on radial 7. For the 13 stations located within 500 m of the Hibernia platform, the average juvenile polychaete growth result was 30.46 mg/worm. This is not significantly different from the overall average of 31.34 mg/worm for all test results, or the average of 32.32 mg/worm for the seven stations that were sampled between 2,000 m and 16,000 m from GBS.

6.4 Multivariate Analysis of Toxicity Test Results

Further investigation of the Microtox results obtained during 2009, as well as for all data collected between 2002 and 2009, was carried out to assess if there may be a relationship

between inhibition of microbial luminescence and any other measured environmental parameters. Years prior to 2002 were not included in this analysis, as several key parameters were not measured prior to that date.

6.4.1 Multivariate Analysis of 2009 Toxicity Test Results

The 2009 sediment quality data were available and consistently detectable for aluminum, barium, weak acid extractable barium, chromium, lead, manganese, strontium, vanadium, zinc, fuel-range hydrocarbons, lube-range hydrocarbons, total organic carbon, total inorganic carbon, sulphide, ammonia, sediment moisture content, and the sediment grain size index. Data were log-transformed to ensure normality, and that the variances were reasonably commensurate, and a Principal Components Analysis (with Varimax rotations) was applied to the data. Four factors were retained, as for the 2009 data (HMDC 2009), representing the original data as follows:

- Factor 1: fuel-range hydrocarbon, weak acid extractable barium, total barium, sulphide, lube-range hydrocarbon and chromium;
- Factor 2: total inorganic carbon, strontium, grain size index, ammonia, and total organic carbon;
- Factor 3: moisture and aluminum; and
- Factor 4: manganese, vanadium, zinc, and chromium (loading equivalent to that for Factor 1).

These four Factors represented 86.0 % of the variance in the original data set, but lead was not strongly captured by any one factor.

Pearson correlation coefficients were used to examine how Factors 1, 2, 3 and 4 were correlated with the four log-transformed sediment toxicity variables (i.e., Microtox, amphipod survival, polychaete survival and polychaete growth). Correlation coefficients exceeding an arbitrary cut-off value of 0.5 were identified as follows:

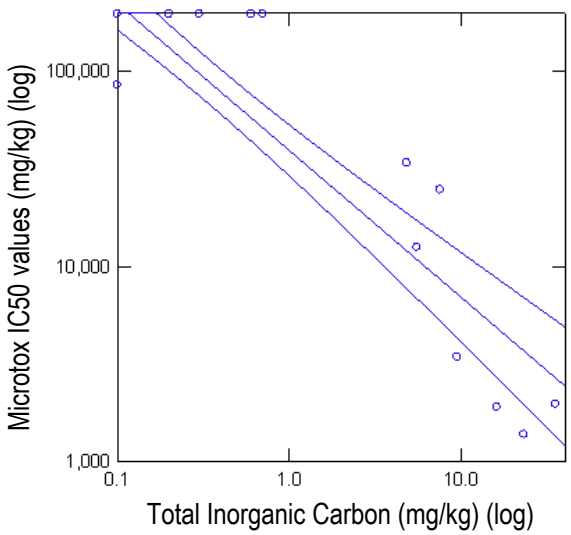
- The Microtox test results were correlated with Factor 2 ($r = -0.907$);
- The polychaete growth test results were correlated with polychaete survival ($r=0.526$), amphipod survival ($r = 0.565$), and Factor 3 ($r= -0.504$);
- The polychaete survival test results were correlated with polychaete growth ($r=0.526$) and amphipod survival ($r=0.830$); and
- The amphipod survival test results were correlated with the polychaete growth ($r = 0.565$) and polychaete survival test results ($r=0.830$).

Of these results, the outstanding correlation is between the Microtox test results and Factor 2, which represents some factor or combination of factors correlated with sediment grain size, inorganic and organic carbon, ammonia and strontium concentrations. These results were the same as for 2007 data (HMDC 2009).

Stepwise multiple linear regression was used to further explore relationships between the Microtox test results and the sediment quality parameters correlated with Factor 2. Unlike in

2007 where the result that was the best and simplest model involved the sediment grain size index, in 2009 total inorganic carbon (r = 0.973) was the best sediment quality parameter to indicate toxic responses (strontium concentration was second best parameter with r=0.960). Although not indicative of a causal relationship, this result suggests that the Microtox test results in 2009 are responding in some fundamental way to some aspect of sediment quality that is related to total inorganic carbon. The relationship between Microtox test results and total inorganic carbon in sediment for the 2009 data only is shown in Figure 6.4. The underlying importance to note in the above statistical analyses, however, is that there was no indication at any level that the Microtox test results were correlated in any way with the presence of hydrocarbons or barium, which are indicators of activities at the Hibernia platform. This is further evident in the fact that the Microtox and sediment relationship tends to occur at distances away from the platform as opposed to areas near the platform (refer to Section 6.1). Similar findings and conclusions were also reached with only the 2007 Microtox test results (HMDC 2009).

Figure 6.4 The Relationship Between Microtox Test Results and Total Inorganic Carbon for 2009



6.4.2 Multivariate Analysis of 2002, 2004, 2007 and 2009 Toxicity Test Results

Data were also examined for the years 2002, 2004, 2007 and 2009, and a Principal Components Analysis (with Varimax rotations) was applied. Again, four factors were retained, representing the original data as follows:

- Factor 1: fuel-range hydrocarbon, total barium, sulphide, weak acid extractable barium and lube-range hydrocarbon;
- Factor 2: total inorganic carbon, grain size index, strontium, total organic carbon, and ammonia;
- Factor 3: manganese, vanadium, chromium, and zinc; and
- Factor 4: sediment moisture, aluminum, and lead.

These four Factors represented 79.5 percent of the variance in the original data set.

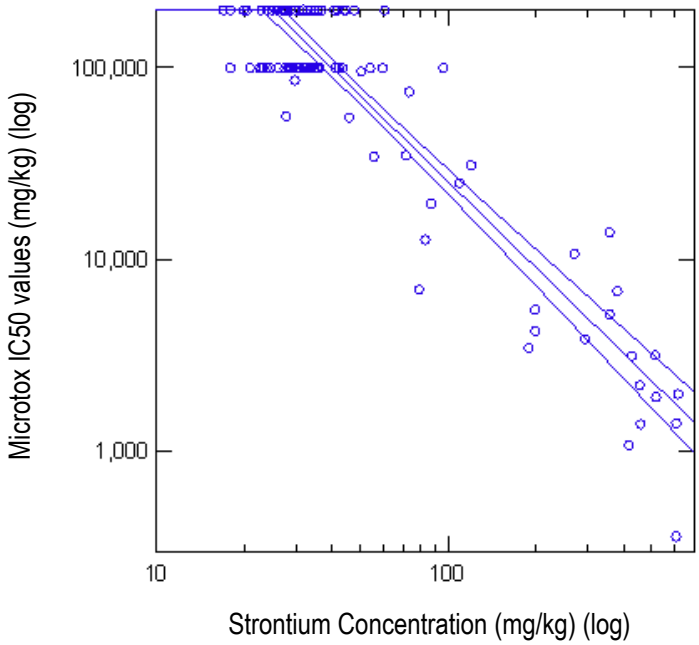
Pearson correlation coefficients were used to examine how Factors 1, 2, 3 and 4 were correlated with the four log-transformed sediment toxicity variables (i.e., Microtox, amphipod survival, polychaete survival and polychaete growth). Correlation coefficients exceeding an arbitrary cut-off value of 0.5 were identified as follows:

- The Microtox test results were correlated with Factor 2 (r = -0.907); and
- The polychaete survival test results were correlated with the amphipod survival test (r=0.983).

Stepwise multiple linear regression was used to further explore relationships between the Microtox test results and sediment quality, with the result that the best and simplest model involved the sediment strontium concentration (r = -0.922), as noted for the 2007 EEM program as well (HMDC 2009). Again, this is not indicative of a causal relationship, but the overall association between Factor 2 and the Microtox test results suggests that sediment toxicity is responding in some fundamental way to some aspect of sediment quality that is related to sediment grain size, and in particular in the sediments that contain significant fractions of gravel. Further, as for the 2009 data when analyzed separately, there was no indication at any level that the Microtox test results were correlated in any way with Factor 1, which was indicative of the presence of hydrocarbons or barium.

The relationship between Microtox test results and sediment strontium concentration for the 2002, 2004, 2007 and 2009 data is shown in Figure 6.5.

Figure 6.5 The Relationship Between Microtox Test Results and Sediment Strontium Concentrations for 2002, 2004, 2007, and 2009



7.0 HIBERNIA 2009 WATER COLUMN CHEMISTRY PROGRAM

A produced water column chemistry program was conducted as part of the 2009 Hibernia EEM Program. The primary purpose of the water column program is to determine estimates of dispersion factors and predict a zone of potential effects from available toxicity data.

Water samples were collected during the sediment cruise portion of the 2009 Hibernia EEM program in August 2009. Water samples were collected at 13 study area stations located approximately 33 to 200 m from the produced water outlet, as well as two reference stations located 16 km from the GBS along Radials 1 and 7 (Figure 7.1). The stations are defined as near-field (<55 m), mid-field (55 to 200 m) and far-field (16,000 m) from the GBS. In the 2009 program water samples were collected at three depths for each station, as determined by the examination of CTD profiles. Water samples were collected:

- 1 m below the surface;
- In the mixed layer where a change in the vertical profile of temperature, conductivity and dissolved oxygen of the water column was observed; and
- off the sea bottom for each station.

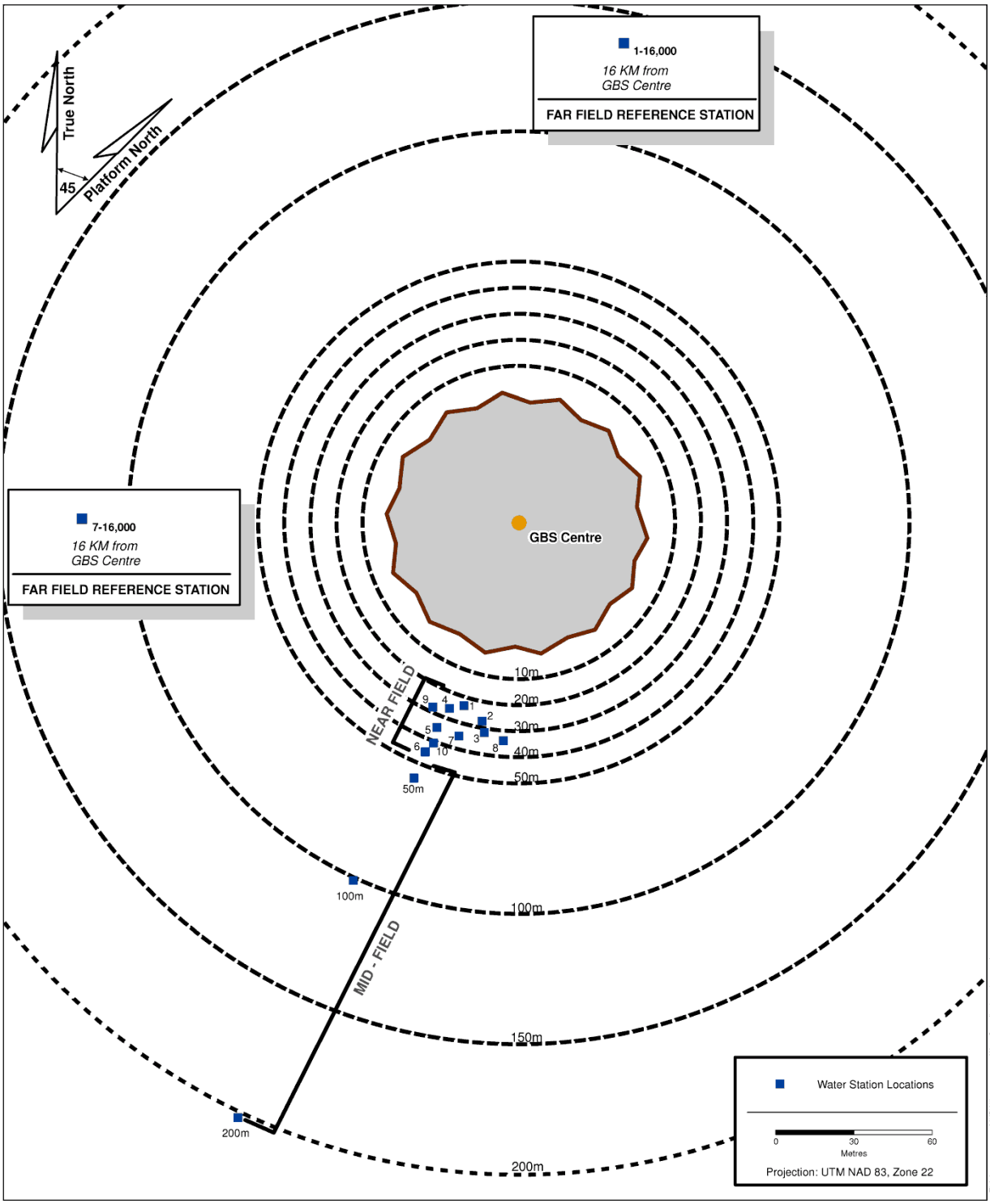
It is assumed that adjacent to the platform, the mixed layer where changes in the vertical profiles of temperature, conductivity and dissolved oxygen of the water column were observed contain the produced water plume. A total of 39 water samples were collected at Hibernia and six water samples were collected at the Reference Sites (1-16,000 and 7-16,000). Samples were analyzed for metals and hydrocarbons, including BTEX, fuel (C10-C21) and lube (C21-C32) range hydrocarbons, PAHs, total nitrogen, ammonia-nitrogen and total phosphorus, as in 2007. Total nitrogen, ammonia-nitrogen and total phosphorus that were added to the suite of analytes in the 2007 sea water chemistry program were retained for the 2009 program. The analysis of alkyl PAHs were added to the 2009 sea water chemistry program.

7.1 Conductivity, Temperature and Density Data

An examination of CTD (Seabird 25) profiles conducted during the 2009 water sampling program indicates that differences in temperature, conductivity, dissolved oxygen and salinity with depth is a useful tool in locating the Hibernia produced water plume. CTD data from casts conducted in the near-field were often different from casts conducted at the Reference Area (far-field) and at 50, 100 and 200 m (mid-field).

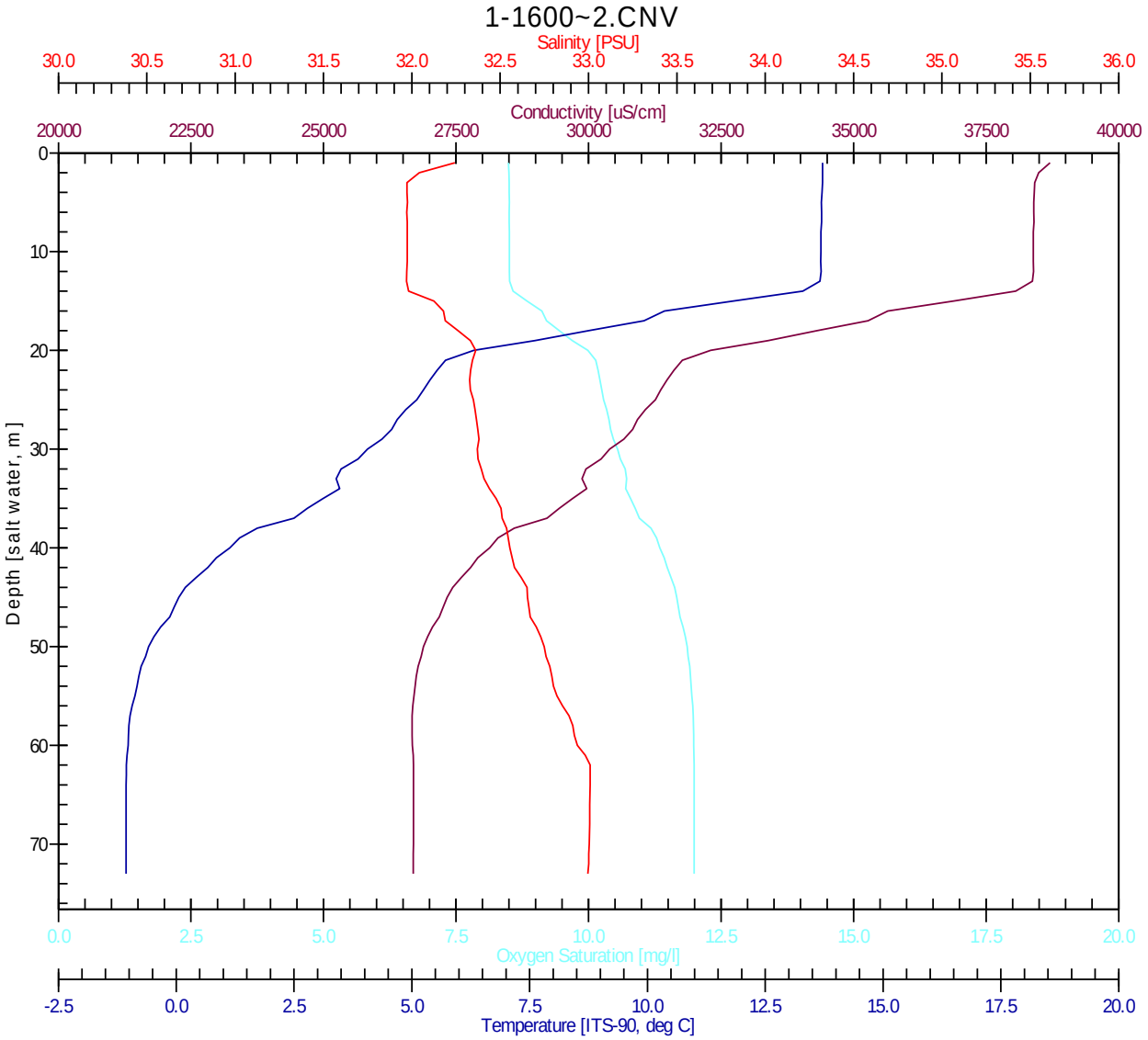
The 50, 100 and 200 m profiles were very similar to profiles conducted at the References Areas. Representative CTD casts conducted at the Reference Area and Near-field Area are illustrated in Figures 7.2 and 7.3, respectively. CTD casts for all stations are included in Volume II, Appendix K.

Figure 7.1 August 2009 Water Column Sampling Location Points



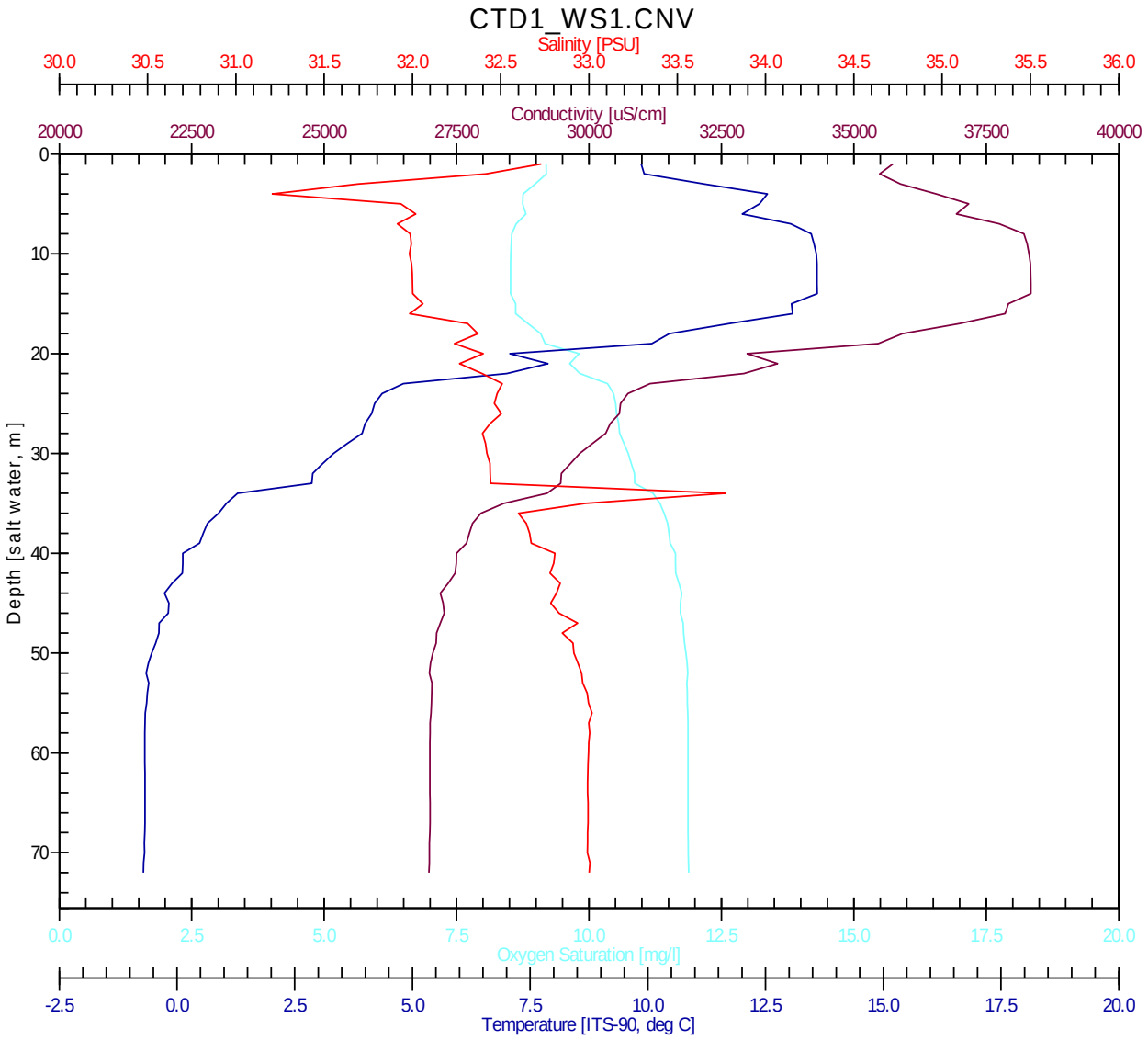
The reference station temperature (Figure 7.2) is constant at approximately 14° C for the top 16 m of the water column. A rapid drop to 1°C is observed between 16 to 40 m depth with temperature continuing to gradually decrease with depth until a temperature below 0° C is maintained from depths of 50 m to bottom. The conductivity profile mimics the temperature profile. Conductivity is steady at 38.5 mS/cm for the top 16 m, falling rapidly to 28 mS/cm between 16 to 50 m depth and gradually falling to 27 mS/cm at the bottom. The salinity profile decreases for the first 2 m then remains constant at 32 ppt from 2 m to 16 m. Salinity then increases to 33 ppt from 14 to 60 m depth and remains constant beyond 60 m depth. The oxygen levels are steady at 8.3 mg/L for the top 14 m, rapidly increasing to 10.5 mg/L between 14 to 22 m and gradually increasing to approximately 12 mg/L of dissolved oxygen at bottom.

Figure 7.2 Reference Station CTD Profile



The Near-field Area stations (except stations W50, W100 and W200) CTD profiles are different than the Reference Area profiles, particularly with respect to the top 16 m (Figure 7.3). Initially water temperatures increase from approximately 10°C (at the surface) to 13.5°C (at 10 - 16 m depth). This temperature increase is followed by a rapid decrease in temperature to approximately 1.5°C at 30 m depth. Temperature decreases gradually to – 0.5°C (at 50 m depth) and remains constant to near bottom.

Figure 7.3 Near-field Area (WS1) CTD Profile



The Near-field Area conductivity profiles mimic the temperature profiles. Initially, conductivity is variable, but generally show an increasing trend until a reading of approximately 38.5 mS/cm is attained (between 10-16 m depth). From 16 m to 26 m conductivity drops rapidly to approximately 30.5 mS/cm. A further decrease to 28 mS/cm occurs to a depth of 40 m, conductivity then remains constant to near bottom.

The salinity profile varies somewhat across stations but generally decreases from approximately 33.5 ppt at surface to below 32.0 ppt at depths of approximately 20 m. Salinity increases at depths greater than 20 m and reaches values in excess of 33.0 ppt near bottom.

Dissolved oxygen readings remain fairly constant (approximately 8.5 mg/L) from the surface to a depth of 20 m. Dissolved oxygen increases to approximately 10.5 mg/L at depths of 30 m and reaches a maximum of 12.0 mg/L near bottom.

Based on visual observations from the platform upwelling appears to be occurring in the vicinity of the sea water return and produced water outlets. It is hypothesized that this upwelling may be responsible for the variable temperature, conductivity, salinity and dissolved oxygen readings observed on the CTD profiles. Changes in CTD profiles are discernable for 6 of the 10 near-field stations. The CTD profiles for stations 2, 8, 9 and 10 exhibit changes in temperature, conductivity, salinity and dissolved oxygen, but to a lesser magnitude than other Near-field stations. The CTD profiles for stations W50, W100 and W200 are almost indistinguishable from the reference station CTD profiles (Refer to Volume II, Appendix K).

It is further hypothesized that the upwelling observed near the platform delivers a water mixture up from below the thermocline to the surface layers. The water that is brought up to the surface is hypothesized to be a water mixture composed of deep natural sea water, water released from the sea water return discharge and possibly some produced water discharge. This type of water mixture, if indeed is brought to the surface, would mix with natural surface water resulting in changes to the surface temperature, conductivity, salinity, and dissolved oxygen contents. The actual changes observed in temperature, conductivity, salinity and dissolved oxygen would be dependent upon the ratios of natural sea water, sea water return and produced water discharges that are contained within the upwelling mixture. The water layer from surface to 16 m depth is hypothesized to consist of a water mixture that includes natural sea water, water released from the sea water return and produced water outlet.

7.2 Water Column Chemistry

A summary of the chemical data for which analytes were detected in the 2009 water column samples is presented in Table 7.1 (the complete suite of chemicals that were analyzed in water samples is contained in Volume II, Appendix J). The RDL, the total number of samples analyzed, the number of samples that had values above the RDL and the associated mean, standard deviation, median and minimum and maximum numbers for each sampling area are provided in Table 7.1. The calculated descriptive statistics were based on samples with values above the RDL or equal to the RDL, if not detected analytically. The reason was to obtain a meaningful descriptive statistic (i.e., for the mean and median) to characterize the measured analyte and which would not be less than the RDL for that analyte.

7.2.1 Total Metals

In 2009, concentrations of arsenic, copper, and iron were above detectable levels in all water column samples collected from both Hibernia and reference locations. Chromium was marginally detected above the RDL in two of 39 samples at Hibernia and lead was also marginally detected in four of 39 samples at Hibernia. Chromium and lead were not detected at

the reference site, but zinc was marginally detected for only one sample at the RDL concentration and where it was not detected at Hibernia. Mercury was detected in one sample at the reference site and two samples at Hibernia. Cadmium, cobalt, manganese, and nickel were not detected in any samples from Hibernia or the reference locations. None of the measured metal concentrations in sea water samples collected from either Hibernia or reference locations exceeded the CCME marine water guidelines of Environment Canada (CCME 2003), with the exception of mercury at the reference site. The only detected water sample at the reference site had a mercury concentration of 0.042 µg/L which is above the CCME guideline of 0.016 µg/L.

7.2.2 Nutrients

Total nitrogen (Table 7.1) was detected in all water column samples collected from both Hibernia and reference locations with levels ranging from 0.04 to 0.23 mg/L. Ammonia-nitrogen was detected in all but one of the samples from Hibernia and all of the samples from the reference site (Table 7.1) with levels ranging from 0.06 to 0.15 mg/L. Total phosphorus (Table 7.1) was detected in less than half the samples at Hibernia and the reference sites with levels ranging from LRDL to 0.05 mg/L. Results for these nutrients were similar between both Hibernia and Reference Sites.

7.2.3 Hydrocarbons (TPH and PAH)

Water column samples at Hibernia contained mostly the lighter fraction of hydrocarbons (i.e., benzene, toluene, ethylbenzene and xylenes (BTEX; Table 7.1). Lube range hydrocarbons (C21-C32) were detected in only one water column sample from Hibernia, at a concentration equal to the RDL of 0.1 mg/L. Unlike water column stations < 55 m from Hibernia, none of the water column samples at stations > 55 m from Hibernia or the reference locations contained hydrocarbons, except for the one lube range hydrocarbon sample noted above and detected approximately 100 m from Hibernia (Table 7.2) at 13 m depth. Hydrocarbon concentrations (TPH plus BTEX) for water column stations and depths sampled during the 2009 EEM are presented in Table 7.2. Water column data collected during 2007 is provided in Appendix D.

Several concentrations of PAHs were detectable in water column samples from Hibernia, most notably: phenanthrene, at very low concentrations (mean of 0.02 µg/L compared to the RDL of 0.01 µg/L; Table 7.1); 1-methylnaphthalene (mean of 0.12 µg/L compared to the RDL of 0.05 µg/L; Table 7.1); and 2-methylnaphthalene (mean of 0.11 µg/L compared to the RDL of 0.05 µg/L; Table 7.1);

Other PAHs detected more than once in the Hibernia water column, all at very low concentrations and less frequently than phenanthrene, include fluorene and naphthalene. Fluorene and naphthalene were detected in some water column samples (6 and 5 out of 39 samples, respectively) at concentrations marginally above the RDL of 0.01 and 0.2 µg/L, respectively (Table 7.1).

Analysis of alkylated PAHs was conducted for the first time in 2009 for the Hibernia EEM sea water chemistry program. Fluorene, phenanthrene and C2 class of alkyl naphthalenes were the only alkyl PAHs detected and at a concentration above the RDL (Table 7.1). These alkyl PAHs were only detected at Hibernia and generally < 50 m from the produced water outlet (refer to Appendix J (Vol. II) for raw data).

Table 7.1 Summary Statistics for 2009 Sea Water Chemistry Data for Parameters with Concentrations above RDL

Analyte	RDL	Units	No. of Samples	No. > RDL	Mean	SD	Median	Min.	Max.	No. of Samples	No. > RDL	Mean	SD	Median	Min.	Max.	CCME*
			Hibernia Site							Reference Site							
METALS																	
Arsenic	0.1	µg/L	39	39	0.6	0.3	0.4	0.2	1.2	6	6	0.3	0.1	0.3	0.2	0.5	12.5
Barium	1	µg/L	39	39	6.1	2.8	4	3	10	3	3	5.3	4.9	5	2	9	n.a.
Chromium	0.5	µg/L	39	2	0.5	0.2	0.5	<RDL	1.5	6	0	-	-	-	<RDL	-	56
Copper	0.1	µg/L	39	39	0.3	0.1	0.3	0.2	0.5	6	6	0.3	0.1	0.3	0.2	0.3	n.a.
Iron	1	µg/L	39	39	3	1	3	2	6	6	6	3	1	3	2	4	n.a.
Lead	0.1	µg/L	39	9	0.1	0.0	0.1	<RDL	0.2	6	2	0.1	0.0	0.1	<RDL	0.10	n.a.
Mercury	0.01	µg/L	39	9	0.01	0.00	0.01	<RDL	0.015	6	1	0.02	0.01	0.01	<RDL	0.042	0.016
Strontium	10	µg/L	39	39	7667	221	7700	6870	8140	3	3	7763	99	7770	7690	7830	n.a.
Zinc	1	µg/L	39	0	-	-	-	<RDL	-	6	1	1	0	1	<RDL	1	n.a.
HYDROCARBONS																	
Benzene	0.001	mg/L	39	14	0.007	0.012	0.001	<RDL	0.046	6	0	-	-	-	<RDL	-	0.11
Toluene	0.001	mg/L	39	12	0.005	0.007	0.001	<RDL	0.028	6	0	-	-	-	<RDL	-	0.215
Ethylbenzene	0.001	mg/L	39	5	0.001	0.000	0.001	<RDL	0.002	6	0	-	-	-	<RDL	-	0.025
Xylenes	0.002	mg/L	39	7	0.003	0.002	0.002	<RDL	0.009	6	0	-	-	-	<RDL	-	n.a.
>C21-<C32	0.1	mg/L	39	1	0.1	0.0	0.1	<RDL	0.1	6	0	-	-	-	<RDL	-	n.a.
Modified TPH	0.1	mg/L	39	1	0.1	0.0	0.1	<RDL	0.1	6	0	-	-	-	<RDL	-	n.a.
PAHs																	
1-Methyl naphthalene	0.05	µg/L	39	10	0.12	0.16	0.05	<RDL	0.76	6	0	-	-	-	<RDL	-	n.a.
2-Methyl naphthalene	0.05	µg/L	39	10	0.11	0.14	0.05	<RDL	0.65	6	0	-	-	-	<RDL	-	n.a.
Benzo(b) fluranthene	0.01	µg/L	39	1	0.01	0.00	0.01	<RDL	0.04	6	0	-	-	-	<RDL	-	n.a.
Chrysene	0.01	µg/L	39	1	0.01	0.00	0.01	<RDL	0.02	6	0	-	-	-	<RDL	-	n.a.
Fluoranthene	0.01	µg/L	39	1	0.01	0.00	0.01	<RDL	0.02	6	0	-	-	-	<RDL	-	n.a.
Fluorene	0.01	µg/L	39	6	0.01	0.01	0.01	<RDL	0.04	6	0	-	-	-	<RDL	-	n.a.
Naphthalene	0.2	µg/L	39	5	0.2	0.1	0.2	<RDL	0.7	6	0	-	-	-	<RDL	-	1.4
Phenanthrene	0.01	µg/L	39	13	0.02	0.01	0.01	<RDL	0.06	6	0	-	-	-	<RDL	-	n.a.
Pyrene	0.01	µg/L	39	1	0.01	0.00	0.01	<RDL	0.02	6	0	-	-	-	<RDL	-	n.a.
Alkyl PAHs																	
Fluorene	0.01	µg/L	39	3	0.01	0.00	0.01	<RDL	0.03	6	0	-	-	-	<RDL	-	n.a.
Phenanthrene	0.01	µg/L	39	3	0.01	0.00	0.01	<RDL	0.02	6	0	-	-	-	<RDL	-	n.a.
C2-Napthalenes	0.1	µg/L	39	5	0.11	0.04	0.1	<RDL	0.3	6	0	-	-	-	<RDL	-	n.a.
NUTRIENTS																	
Total Nitrogen	0.02	mg/L	39	39	0.11	0.07	0.07	0.04	0.23	6	6	0.10	0.06	0.06	0.05	0.17	n.a.
Ammonia-N	0.05	mg/L	39	38	0.15	0.07	0.14	<RDL	0.33	6	6	0.11	0.04	0.12	0.06	0.15	n.a.
Total Phosphorus	0.02	mg/L	39	18	0.03	0.01	0.02	<RDL	0.05	6	2	0.03	0.01	0.02	<RDL	0.05	n.a.

Note: * Marine Water Quality Guidelines. Table 7.1 includes only those parameters with at least one value above RDL. Refer to Appendix J (Vol. II) for raw data associated with all parameters analyzed.

Table 7.2 Hydrocarbon Chemistry Data August 2009

Station	Distance from Outlet (m)	Depth from Surface (m)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylene (mg/L)	C6 - C10 (less BTEX) (mg/L)	>C10-C21 (mg/L)	>C21-<C32 (mg/L)	Hydrocarbons (BTEX plus TPH) (mg/L)
1	33	1	0.022	0.013	<0.001	0.004	<0.01	<0.05	<0.1	0.039
		14	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		68	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
2	39	1	0.046	0.028	0.002	0.009	<0.01	<0.05	<0.1	0.085
		18	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		70	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
3	44	1	0.031	0.018	0.001	0.006	<0.01	<0.05	<0.1	0.056
		12	0.008	0.005	<0.001	<0.002	<0.01	<0.05	<0.1	0.013
		68	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
4	35	1	0.033	0.020	0.001	0.007	<0.01	<0.05	<0.1	0.061
		14	0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	0.001
		68	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
5	44	1	0.008	0.005	<0.001	<0.002	<0.01	<0.05	<0.1	0.013
		12	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		72	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
6	54	1	0.004	0.002	<0.001	<0.002	<0.01	<0.05	<0.1	0.006
		12	0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	0.001
		70	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
7	45	1	0.039	0.024	0.001	0.007	<0.01	<0.05	<0.1	0.071
		10	0.034	0.021	0.001	0.007	<0.01	<0.05	<0.1	0.063
		70	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
8	48	1	0.014	0.009	<0.001	0.003	<0.01	<0.05	<0.1	0.026
		8	0.013	0.008	<0.001	<0.002	<0.01	<0.05	<0.1	0.021
		70	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
9	37	1	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		8	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		66	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
10	50	1	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		8	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		70	0.002	0.001	<0.001	<0.002	<0.01	<0.05	<0.1	0.003
W-50	65	1	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		13	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		67	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
W-100	110	1	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		13	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	0.1	0.1
		67	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND

Table 7.2 Hydrocarbon Chemistry Data August 2009 (continued)

Station	Distance from Outlet (m)	Depth from Surface (m)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylene (mg/L)	C6 - C10 (less BTEX) (mg/L)	>C10-C21 (mg/L)	>C21-<C32 (mg/L)	Hydrocarbons (BTEX plus TPH) (mg/L)
W-200	211	1	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		13	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		75	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
1-16000	**16000**	1	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		16	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		75	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
7-16000	**16000**	1	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		16	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND
		62	<0.001	<0.001	<0.001	<0.002	<0.01	<0.05	<0.1	ND

7.3 Dispersion Factors

7.3.1 Modelled Zone of Influence

The Hibernia produced water dispersion model (Lorax Environmental 2004) predicted that the produced water stream behaviour would be substantially affected by the seawater return stream. The Hibernia model results vary depending upon the amount and velocity of the produced water flow in conjunction with the amount and velocity of the seawater return stream flow, as well as the resulting plume direction at a given point in time. As a result of the interaction between the produced water and seawater return flow, the dispersion model predicts a 100:1 to 3,000:1 dilution ratio within 50 m from the platform, depending upon the flow rates and direction of the plume. The maximum dilution rate occurs in the south quadrant and the minimum dilution occurs in the northwest quadrant's. There were four cases of produced water discharges that were modelled:

- Case 1 is for produced water at a rate of 16,000 m3/d, with a initial near-field dilution of 10:1;
- Case 2 is for produced water at a rate of 27,000 m3/d, with a initial near-field dilution of 40:1;
- Case 3 is for produced water at a rate of 40,000 m3/d and a temperature of 72°C, with a near-field dilution of 40:1; and
- Case 4 is for produced water at a rate of 40,000 m3/d and a temperature of 63°C, with a near-field dilution of 60:1.

For the purposes of this report, the produced water flow rate of 27,000 m^3/d will be used. The 27,000 m^3/d model data will be used because it is the closest modelled discharge rate to the maximum observed produced water flow of 24,032 m^3d (August 05, 2009). At a produced water flow rate of 27,000 m^3/d the dilution ratios are:

- 0 to 50 m 150:1 to 1,600:1
- 50 to 100 m 1,816:1 to 8,198:1
- 100 to 250 m 22,900:1 to 74,250:1
- 250 to 1,250 m dilution ratios exceed 11,000:1 beyond 750 m

The model (Lorax Environmental 2004) results indicate that the produced water plume may reach trapping depths between approximately 50 and 75 m (from surface). The range is directly attributable to the differences in flow rate and, hence, the vertical momentum contained in the jets corresponding to minimum and maximum discharge volume.

Beyond 6 to 20 m below the discharge point (the point where the two plumes mix and which is located at 40 m below the water surface), the behaviour of the combined seawater return and produced water plume will be governed primarily by the seawater return plume due, in part, to its greater volume, momentum and physical size. Initial dilution of the produced water will take place prior to it impinging on the seawater return plume, after which, further dilution will take place inside the combined plume.

The effluent is transported away from the GBS in a narrow plume approximately 100 to 500 m wide depending on the ambient conditions. The position of the plume can be variable and can double back on the platform. The dilution ratio increases along the plume, dependent upon the strength and direction of the currents.

The produced water modelling results are considered to be conservative in that the dilution of the produced water within the seawater return does not take into account the turbulent kinetic energy contained in the produced water flow. This residual energy should produce greater dilutions than predicted, as it is not possible to estimate the effect of residual energy with confidence. In addition, other mechanisms besides dilution (adsorption on particulates, evaporation, biodegradation, etc.) that act to reduce pollutant concentrations in the water column were not considered in the modelling study.

It is recognized and accepted that produced water can be toxic at its discharge point, although this will vary within fields and among fields. However, the potential effects upon the receiving environment are limited to immediately adjacent to the discharge point. The dilution ratios as described in the Lorax Environmental (2004) model for the produced water upon discharge into the receiving environment that most closely approximate the current discharge regime is case 2 at a rate of 27,000 m^3/d. The model output for the minimum dilution based on maximum effluent concentration (for 27,000 m^3/d) over a 10 day simulation period is illustrated in Figure 7.4.

The dilution effects of the receiving environment on produced water concentrations can be significant. The produced water concentrations that can be expected in the receiving environment based on the dilution factors as modelled by Lorax Environmental (2004) are presented in Table 7.3.

Table 7.3 Produced Water Concentrations in Receiving Environment Based on Discharge Rate of 27,000 m^3/day

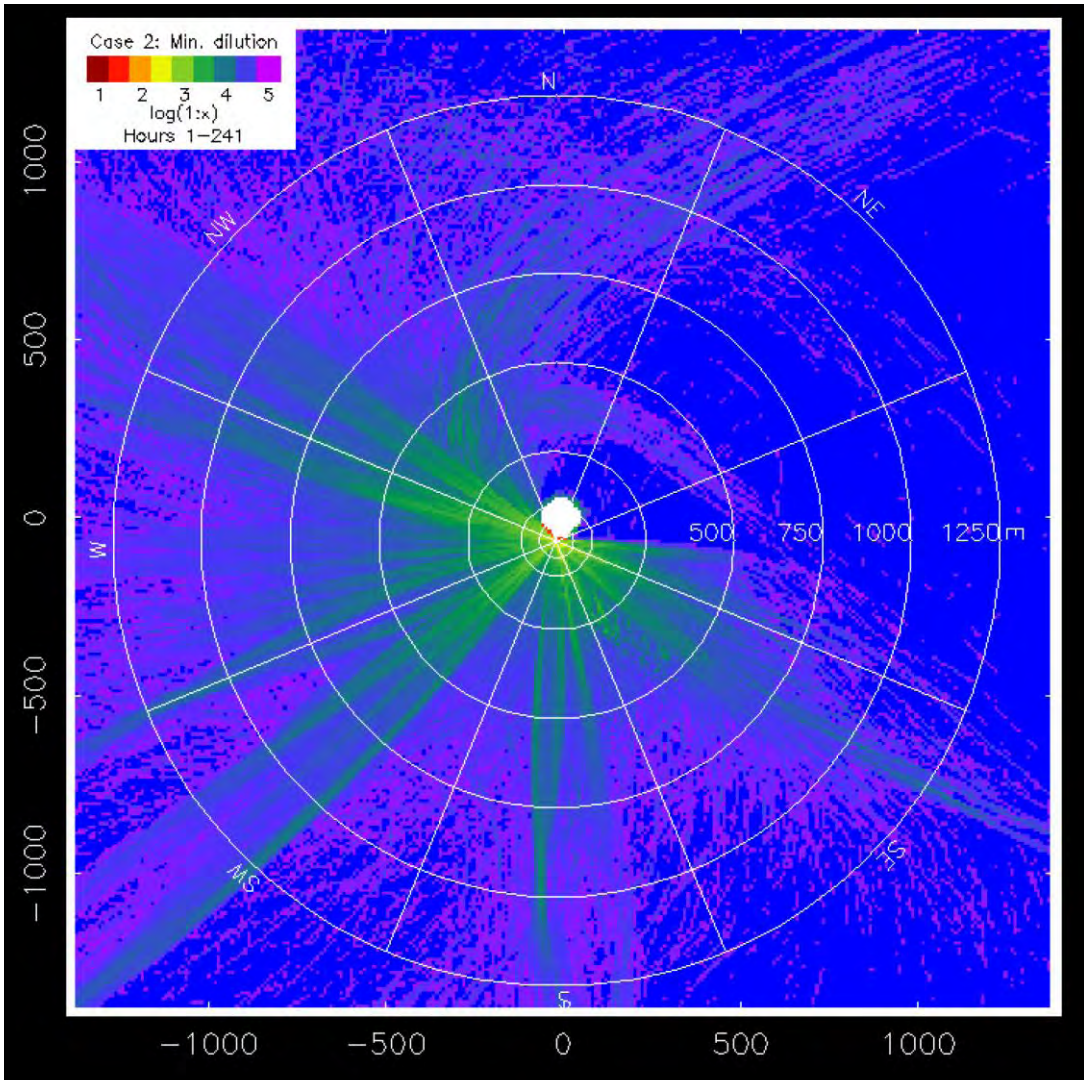
Produced Water Location	Produced Water Concentrations %
End of Pipe (Used for toxicity tests)	100
Initial Dilution in Receiving Environment	2.5
0-50 M	0.06 to 0.7
50-100 M	0.01 to 0.06
100-250 M	0.001 to 0.004
250-1250 M	<0.009
Note a: The dilution rate for 250-1250 m distance is highly variable and is dependent upon case.	

7.3.2 Field Validation

Raw produced water sampling on the Hibernia platform was conducted concurrently with the 2009 water column sampling program. Parameter concentrations from produced water (as determined by platform sampling) were used along with parameter concentrations in the water column sample locations to calculate dispersion factors and percent concentrations for each sample station. Dispersion factors and percent concentration for select parameters were calculated for the 2009 water column data (Table 7.4). The selection of parameters for the determination of dilution factors and percent concentration was based on summary statistics

included in Table 7.1. The parameters selected were relatively common in Hibernia site samples and absent from reference site samples. Iron and manganese were also selected due to their relatively high concentration in produced water. Sample locations where the minimum dilution (maximum concentration) of parameters occurred are indicated by bold in Table 7.4. Predicted and field validated results for TPH and BTEX concentrations are illustrated in Figures 7.5 and 7.6, respectively.

Figure 7.4 Minimum Dilution Based on Maximum Effluent Concentration in Each PTDM Cell Over the 10-day Simulation Period



Note: X-Axis indicates Distance (m) East or West of Hibernia GBS
Y-Axis indicates Distance (m) North or South of Hibernia GBS
Legend – colour indicates dilution factors

Dilution factors were calculated for selected parameters (Table 7.4) analyzed during the 2009 water column program. The dilution factor for parameters with values above detection limits in the near-field ranged from a dilution factor of 139 (at the 45 m sampling station) to greater than 14,400 (at the 33 m sampling station). The concentration of produced water was also calculated

for selected parameters. The calculated concentration of produced water ranged from < 0.01 percent at the 33 m station (depths 14 and 62 m) to 0.72 percent at the 45 m station (depth 1 m). The calculated produced water concentrations correspond with the predicted produced water concentrations of 0.06 to 0.7 percent that was expected to occur (Table 7.3) based on the modeled discharge rate of 27,000 m^3/day. Although all results in the near-field were within the predictions of the produced water model (Table 7.3) an anomalous result occurred for lube range hydrocarbons (>C21-<C32) in the midfield (100 m station) at 13 m depth. Lube range hydrocarbons were detected at the RDL of 0.1 mg/L which corresponds to a dilution of 27:1 and a produced water concentration of 3.7% which is well above the predicted produced water concentration of 0.01 to 0.06 percent (Table 7.3). No evidence of a produced water plume was apparent from the CTD cast at this station (Appendix K, Volume II) and lube range hydrocarbon concentrations were below RDL for all other stations (including near-field stations).

Table 7.4 Dispersion Factors for Selected Parameters Associated with the 2009 Water Column Program

Station Name	Distance from outlet	Iron (Fe)		Manganese (Mn)		Benzene		Toluene		Ethylbenzene		Xylene (Total)		>C10-C21 Hydrocarbons		>C21-<C32 Hydrocarbons		1-Methylnaphthalene		2-Methylnaphthalene		Fluorene		Naphthalene		Phenanthrene		C2 Naphthalene	
		Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %
1-1	33.00	831.67	0.12	>412	<0.24	654.55	0.15	684.62	0.15	>600	<0.167	700.00	0.14	>144	<0.694	>27	<3.7	421.20	0.24	590.00	0.17	610.00	0.16	>590.5	<0.17	455.00	0.22	>1110	<0.09
1-2	33.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	0.01	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
1-3	33.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	910.00	0.11	>1110	<0.09
2-1	39.00	499.00	0.20	**412.00**	**0.24**	**313.04**	**0.32**	**317.86**	**0.31**	**300.00**	**0.33**	**311.11**	**0.32**	>144	<0.694	>27	<3.7	457.83	0.22	934.17	0.11	**305.00**	**0.33**	>590.5	<0.17	364.00	0.27	>1110	<0.09
2-2	39.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
2-3	39.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	910.00	0.11	>1110	<0.09
3-1	44.00	831.67	0.12	>412	<0.24	464.52	0.22	494.44	0.20	600.00	0.17	466.67	0.21	>144	<0.694	>27	<3.7	239.32	0.42	302.97	0.33	406.67	0.25	295.25	0.34	455.00	0.22	693.8	0.14
3-2	44.00	831.67	0.12	>412	<0.24	1800.00	0.06	1780.00	0.06	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	1053.00	0.09	1245.56	0.08	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
3-3	44.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	455.00	0.22	>1110	<0.09
4-1	35.00	499.00	0.20	>412	<0.24	436.36	0.23	445.00	0.22	600.00	0.17	400.00	0.25	>144	<0.694	>27	<3.7	195.00	0.51	238.51	0.42	406.67	0.25	**168.71**	**0.59**	364.00	0.27	**358.1**	**0.28**
4-2	35.00	831.67	0.12	>412	<0.24	14400.00	0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
4-3	35.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
5-1	44.00	499.00	0.20	>412	<0.24	1800.00	0.06	1780.00	0.06	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	877.50	0.11	1121.00	0.09	>1220	<0.08	>590.5	<0.17	910.00	0.11	>1110	<0.09
5-2	44.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
5-3	44.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
6-1	54.00	831.67	0.12	>412	<0.24	3600.00	0.03	4450.00	0.02	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	2106.00	0.05	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
6-2	54.00	831.67	0.12	>412	<0.24	14400.00	0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
6-3	54.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	1220.00	0.08	>590.5	<0.17	455.00	0.22	>1110	<0.09
7-1	45.00	**415.83**	**0.24**	**412.00**	**0.24**	369.23	0.27	370.83	0.27	600.00	0.17	400.00	0.25	>144	<0.694	>27	<3.7	**138.55**	**0.72**	**172.46**	**0.58**	**305.00**	**0.33**	196.83	0.51	**303.33**	**0.33**	616.7	0.16
7-2	45.00	623.75	0.16	>412	<0.24	423.53	0.24	423.81	0.24	600.00	0.17	400.00	0.25	>144	<0.694	>27	<3.7	214.90	0.47	260.70	0.38	406.67	0.25	236.20	0.42	455.00	0.22	528.6	0.19
7-3	45.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
8-1	48.00	831.67	0.12	>412	<0.24	1028.57	0.10	988.89	0.10	>600	<0.167	933.33	0.11	>144	<0.694	>27	<3.7	457.83	0.22	560.50	0.18	1220.00	0.08	393.67	0.25	910.00	0.11	>1110	<0.09
8-2	48.00	831.67	0.12	>412	<0.24	1107.69	0.09	1112.50	0.09	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	554.21	0.18	700.63	0.14	1220.00	0.08	590.50	0.17	910.00	0.11	528.6	0.19
8-2 QA/QC	48.00	831.67	0.12	>412	<0.24	1200.00	0.08	1271.43	0.08	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	554.21	0.18	700.63	0.14	1220.00	0.08	>590.5	<0.17	910.00	0.11	>1110	<0.09
8-3	48.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
9-1	37.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
9-2	37.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
9-3	37.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
10-1	50.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
10-2	50.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
10-3	50.00	1247.50	0.08	>412	<0.24	7200.00	0.01	8900.00	0.01	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
W-50-1	65.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
W-50-2	65.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
W-50-3	65.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
W-100-1	110.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
W-100-2	110.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	**27.00**	**3.70**	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09

Table 7.4 Dispersion Factors for Selected Parameters Associated with the 2009 Water Column Program (continued)

Station Name	Distance from outlet	Iron (Fe)		Manganese (Mn)		Benzene		Toluene		Ethylbenzene		Xylene (Total)		>C10-C21 Hydrocarbons		>C21-<C32 Hydrocarbons		1-Methylnaphthalene		2-Methylnaphthalene		Fluorene		Naphthalene		Phenanthrene		C2 Naphthalene	
		Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %	Dilution	Conc. %
W-100-3	110.00	623.75	0.16	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
W-200-1	211.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
W-200-2	211.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
W-200-3	211.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
1-16000-1	16043.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
1-16000-2	16043.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
1-16000-3	16043.00	623.75	0.16	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
7-16000-1	15978.00	1247.50	0.08	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
7-16000-2	15978.00	623.75	0.16	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
7-16000-3	15978.00	831.67	0.12	>412	<0.24	>14400	<0.01	>8900	<0.011	>600	<0.167	>1400	<0.071	>144	<0.694	>27	<3.7	>2106	<0.047	>2242	<0.045	>1220	<0.08	>590.5	<0.17	>1820	<0.045	>1110	<0.09
Minimum		415.83	0.08	412.00	<0.24	313.04	<0.01	317.86	<0.011	300.00	<0.167	311.11	<0.071	>144	0.00	27.00	<3.70	138.55	<0.047	172.46	<0.045	305.00	<0.08	168.71	<0.17	303.33	<0.045	358.10	<0.09
Maximum		1247.50	0.24	>412	0.24	>144000	0.32	>8900	0.31	>600	0.33	>1400	0.32	>144	0.00	>27.00	3.70	>2106	0.72	>2242	0.58	>1220	0.33	>590.5	0.59	>1820	0.33	>1110	0.14

Note: > or < means data were below the RDL (detection limited). Minimum dilutions and maximum concentrations are bolded.

Figure 7.5 Hydrocarbon Levels (TPH) Associated with Produced Water (Model Predictions Versus Field Validation)

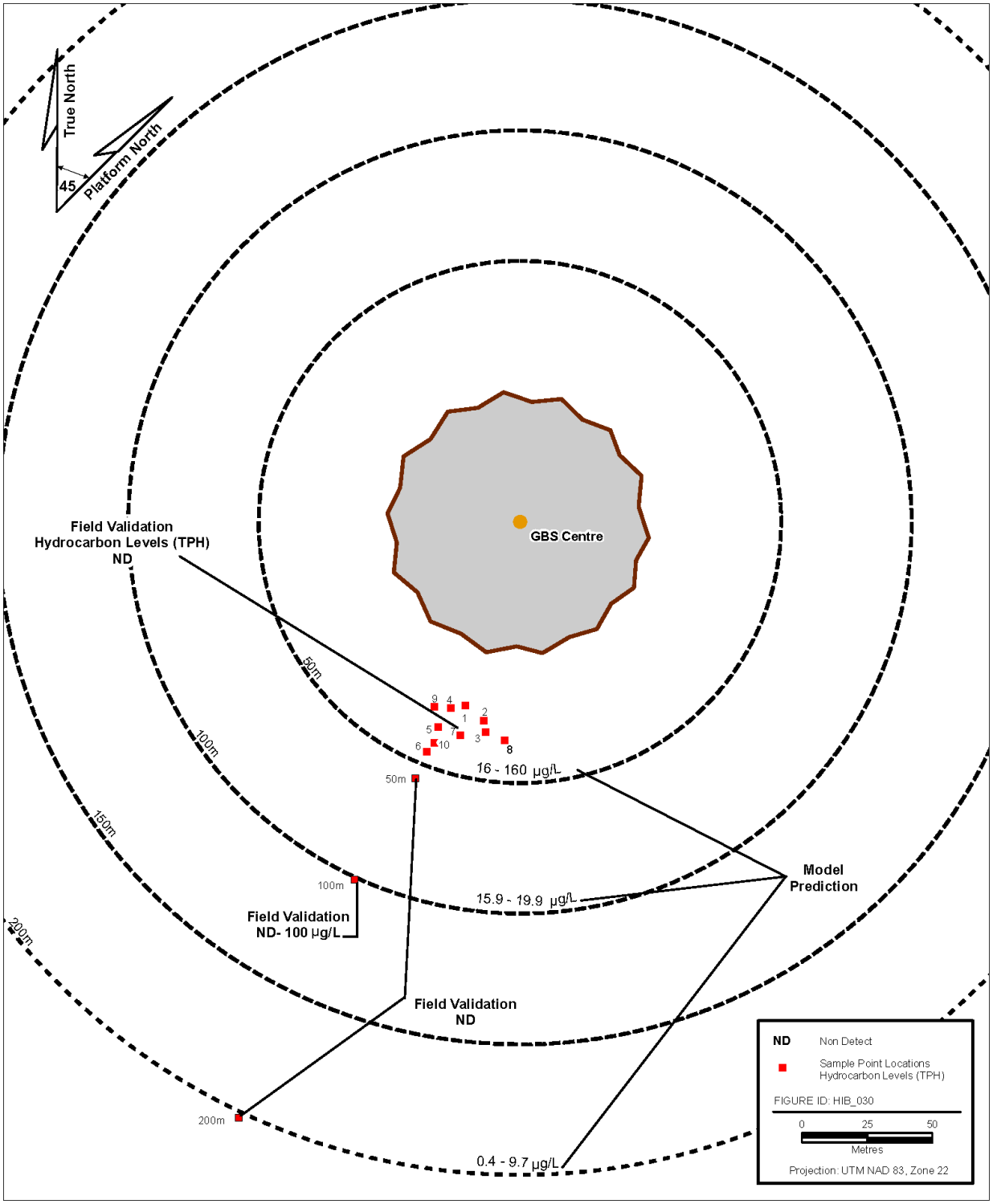
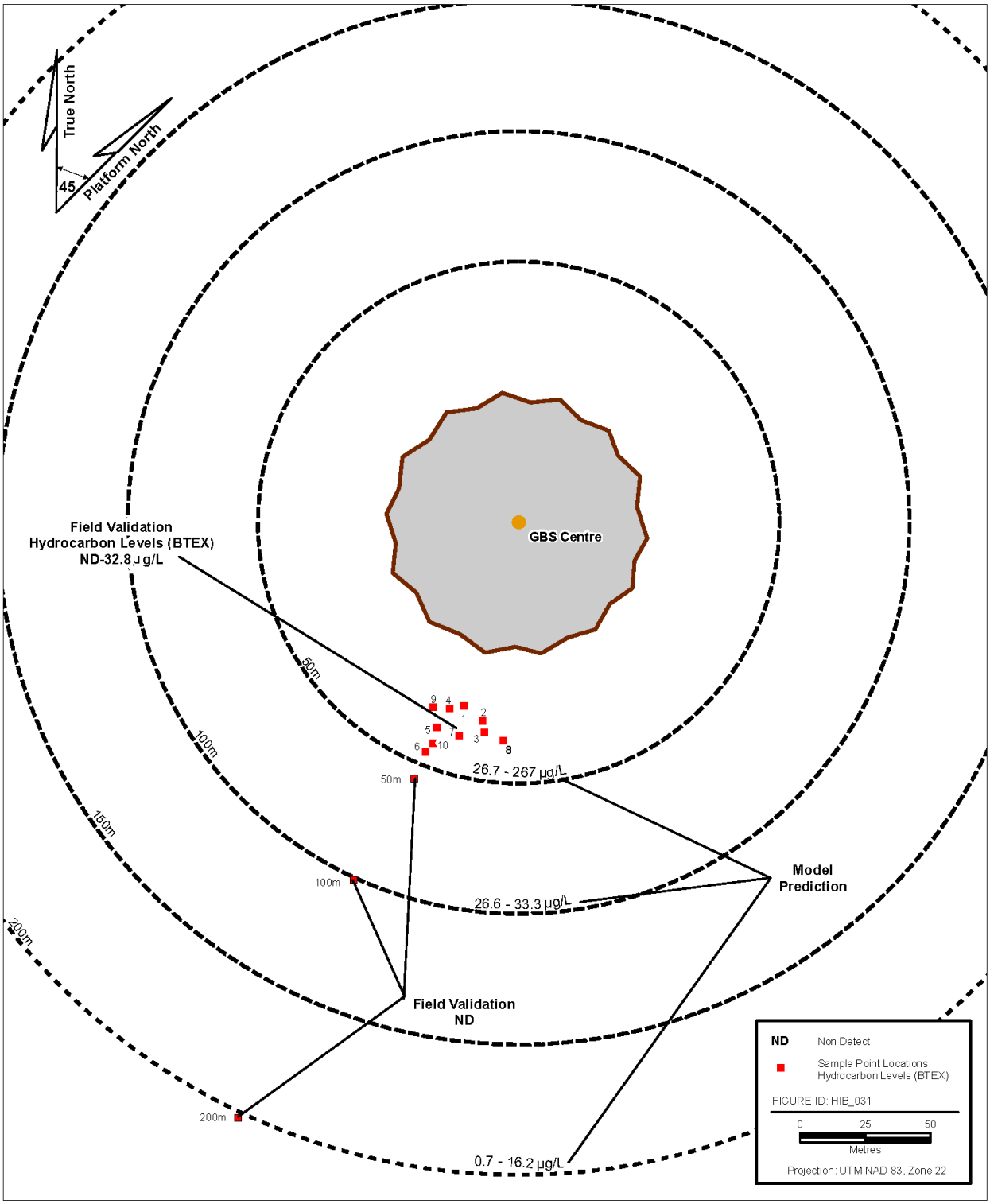


Figure 7.6 Hydrocarbon Levels (BTEX) Associated with Produced Water (Model Predictions Versus Field Validation)



7.4 Produced Water Toxicity

A suite of toxicity analyses are conducted annually on produced water as per the requirements under the OWTG (2002). The 2009 samples for toxicity analyses were collected in August 2009 concurrent with sampling for the produced water program. Results are provided in Table 7.5 below. The toxicity data range for 2004 to 2008 is also provided to demonstrate the variability in produced water data and toxicity.

Samples of produced water collected for use in toxicity tests are collected from end of pipe. Therefore the organisms used in toxicity tests are subjected to significantly higher produced water concentrations for greater duration than they would be subjected to in the receiving environment around the Hibernia platform (Table 7.4). Based on actual Hibernia toxicity test data collected in August 2009 (Table 7.5) coupled with the expected receiving environment produced water concentrations based on the produced water model (Lorax Environmental 2004), and the produced water concentrations as calculated in Section 7.3.2, any potential zone of effects that may exist is likely to be limited to immediately adjacent the discharge point.

Based on the dilution factors determined in Table 7.4 (with the exception of the anomalous results at the 100 m station), the actual produced water concentrations range from <0.01 to 0.72 percent at the 33 m and 45 m stations. When compared to toxicity data in Table 7.5 it is clear that concentrations required to achieve toxicity do not extend beyond approximately 33 to 45 m.

Table 7.5 Produced Water Toxicity Data

Toxicity Test Parameter		Units	Range 2004 to 2008	August 2009
Microtox EC50		%	1.7 – 11.4	4.07
Sea Urchin Fertilization	IC25	%	10.3 – 56	>100
	IC50	%	14.7 - >100	>100
	NOEC	%	6.25 – 30	> 100
	LOEC	%	12.5 – 100	>100
Silverside Growth	IC25	%	26.8 – 55.3	27
	IC50	%	33 – 67.4	34.4
	NOEC	%	12.5 – 50	25
	LOEC	%	25 – 100	30
Silversides Survival	LC50	%	31.5 – 41.5	41.3
	NOEC	%	25	25
	LOEC	%	50	50

All the toxicity testing data collected on Hibernia's produced water to date either as a requirement of the Environmental Protection Plan or in support of research (Payne pers. comm.; Lee pers. comm.) has been in agreement with the findings found within current scientific knowledge and literature (Neff 1987; Terrens and Tait 1993; Jacobs and Marquenie 1991).

The limited extent of the potential zone of effects is further substantiated given the inconsistency between the duration of exposures applied in laboratory toxicity tests and actual exposures in the receiving environment. The potential exposure times for free floating organisms to produced water are likely to be in the order of minutes or hours rather than days as are the norm in

laboratory based tests. Because the exposure time of free floating organisms to the produced water plumes are of limited duration, coupled with rapid produced water dilutions, their actual exposure to elevated produced water concentrations is likely to be below established no effect thresholds for chemical stressors associated with produced water (Johnsen *et al.* 2004). Therefore the presence of a chemical stressor in produced water by itself does not constitute evidence of an effect. A dose-response relationship must be established between the external chemical stressor concentrations, internal levels of tissue chemical stressor concentrations and the actual adverse effects.

8.0 HIBERNIA 2009 COMMERCIAL FISH PROGRAM

8.1 Fish Catches

In 2009, the commercial fish program was conducted aboard the Ocean Choice vessel *Aqviq* using a standard commercial otter trawl with a 154 mm codend. Previous EEM programs were conducted aboard Canadian Coast Guard vessels using a Campellan 1800 trawl with a 44 mm codend. In 2009, as in previous EEM years, otter trawl tows were conducted for 15 minutes at a speed of three knots. A total of 14 otter trawl tows were conducted in 2009 (seven within a 2 km radius of the GBS and seven at a reference area located approximately 50 km northwest of the GBS. Otter trawl transect locations are illustrated in Figure 8.1. Catch data collected during the commercial fish program have been compiled and presented as an estimate of catch per unit effort (CPUE), which was measured as the number of individuals caught per tow. Length groupings of American plaice by sex have also been compiled.

The CPUE for all fish species caught at the Hibernia site during the 2009 EEM program is illustrated in Figure 8.2. The CPUE for each species is plotted for individual otter trawl tows and for all tows combined. In total, 6 species were caught in the seven tows conducted at the Hibernia site. The highest overall CPUE occurred for American plaice, at 62 individuals per tow. The CPUE for American plaice ranged from 42 individuals per tow (GBS--04) to 83 individuals per tow (GBS-06). The next highest overall CPUE occurred for snow crab (12 individuals per tow), followed by invertebrates (2.1 individuals per tow). Other species exhibited lower CPUE and a more sporadic occurrence, as evidenced by their absence from several tows.

The CPUE for all fish species caught at the reference site in 2009 is illustrated in Figure 8.3. In total, 11 species were caught in seven tows conducted at the site. The highest overall CPUE occurred for American plaice at 28.4 individuals/tow. The CPUE for American plaice ranged from 18 individuals/tow (Ref-02) to 50 individuals/tow (Ref-05). The next highest overall CPUE occurred for snow crab (11.7 individuals per tow), followed by sand lance (9.9 individuals per tow) and invertebrates (7.1 individuals per tow).

Catches of American plaice only, at the Hibernia area and reference area are illustrated in Figures 8.4 and 8.5 respectively. At the Hibernia area catches of American plaice ranged from 42 per tow at GBS-04 to 83 per tow at GBS-06. At the reference area catches ranged from 18 per tow at Ref-02 to 50 per tow at Ref-05.

Figure 8.1 Otter Trawl Transect Locations

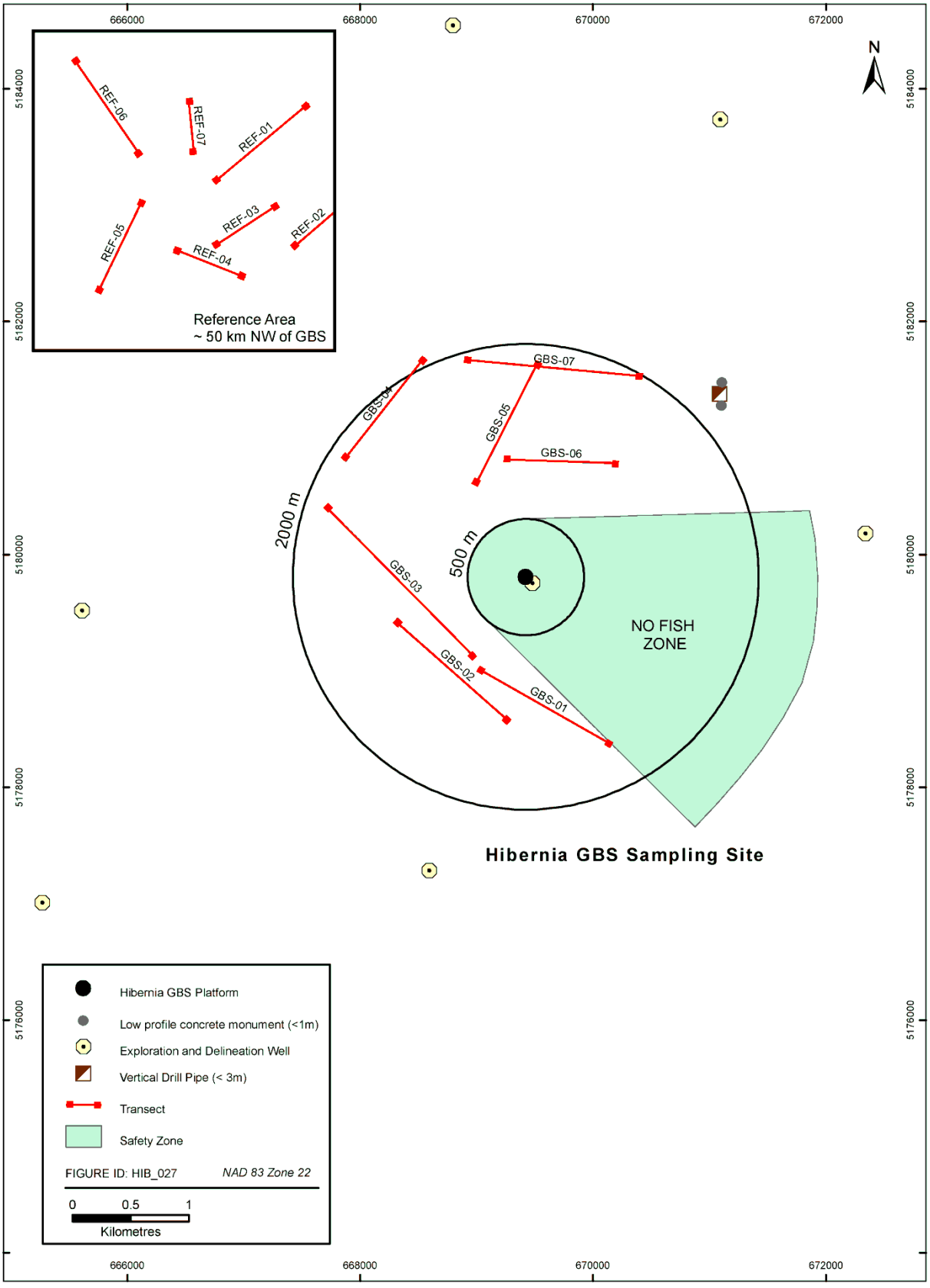


Figure 8.2 2009 Hibernia Area Otter Trawl Fish Catches – All Species

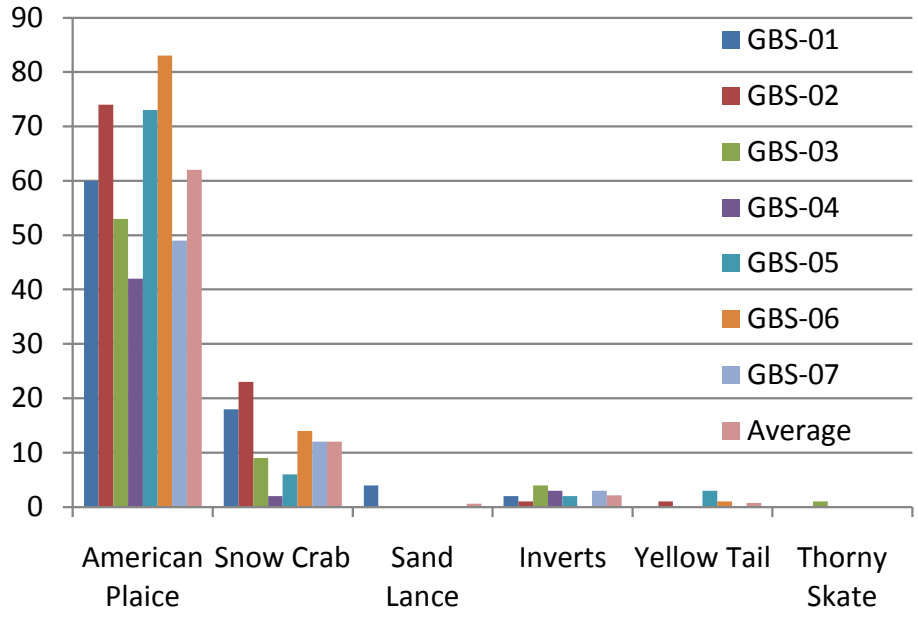


Figure 8.3 2009 Reference Area Otter Trawl Fish Catches – All Species

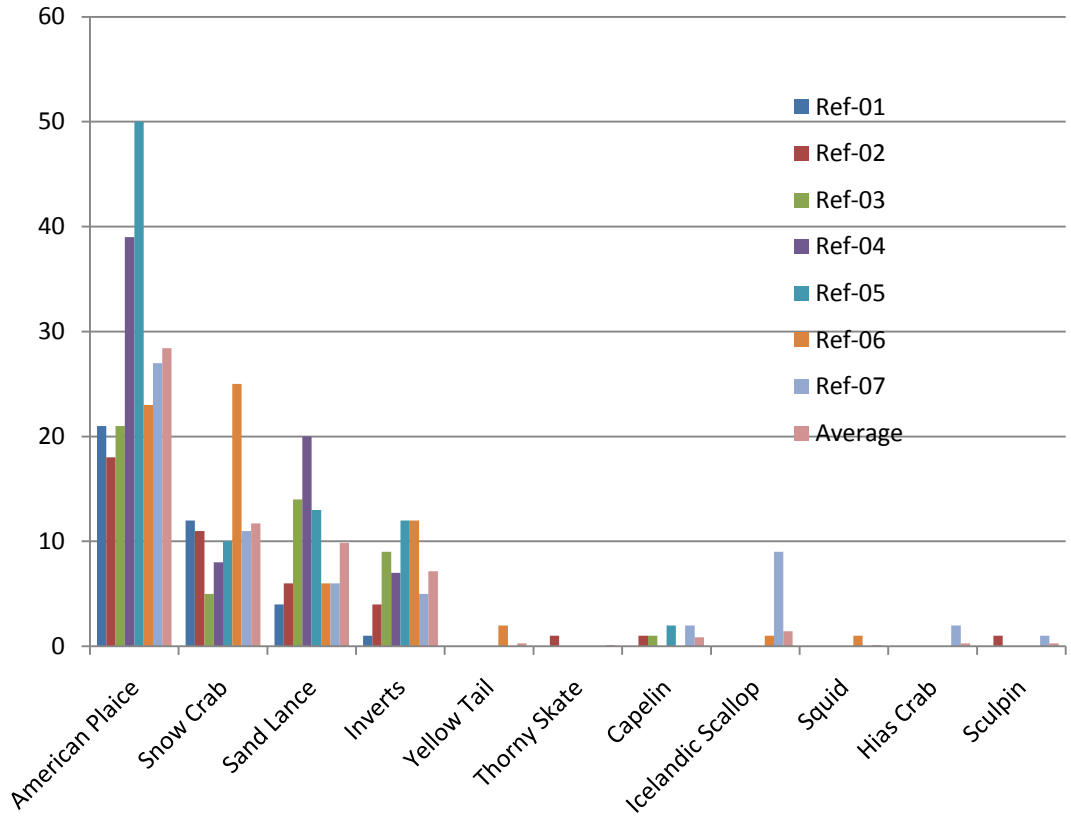


Figure 8.4 American Plaice Catches by Trawl at Hibernia Area

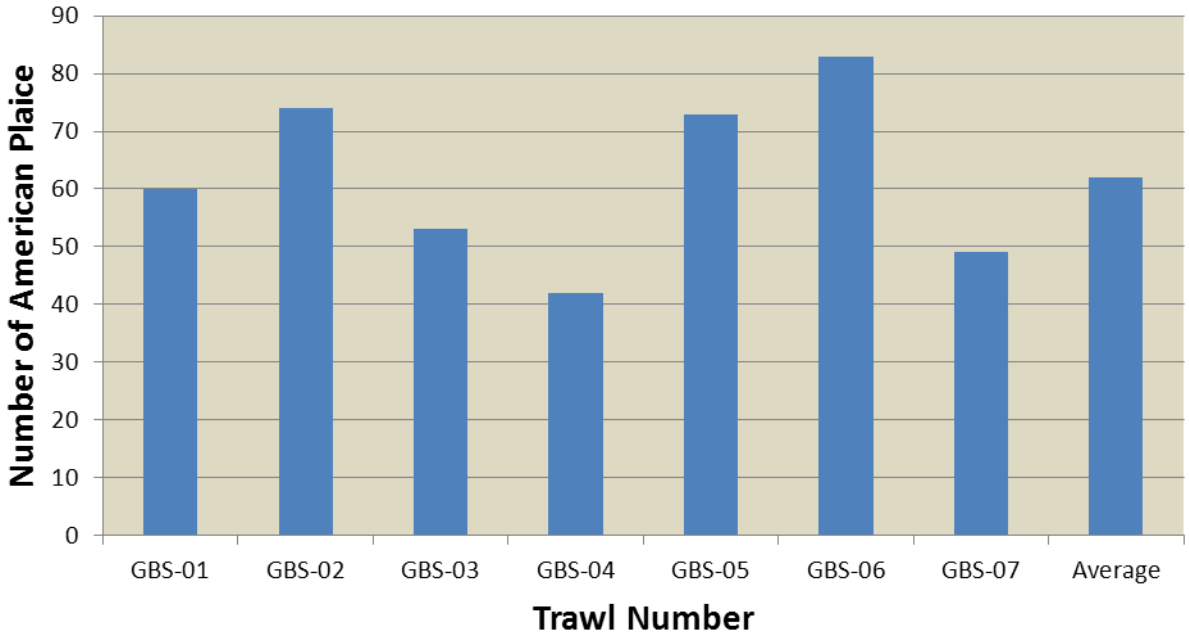
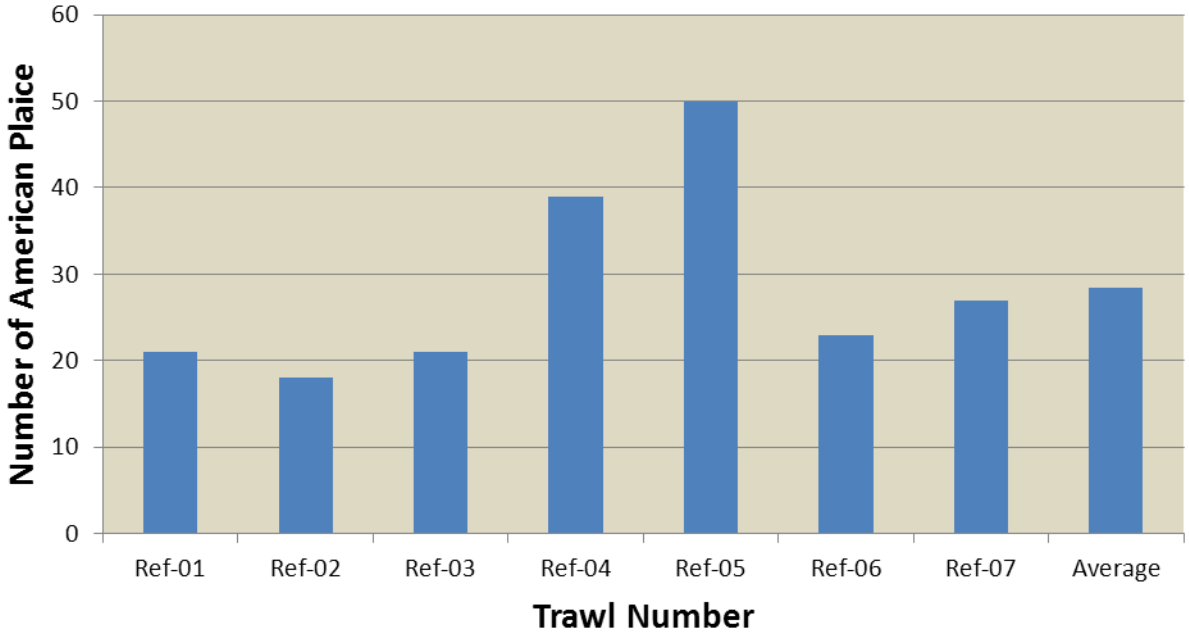


Figure 8.5 American Plaice Catches by Trawl at the Reference Area



The length-frequency distribution of male and female American plaice caught at the Hibernia area and reference area is illustrated in Figures 8.6 and 8.7. A total of 633 American plaice were caught during the program (434 American plaice at the Hibernia area and 199 at the reference area). At each area 50 American plaice were sampled as part of the biological component of the EEM program. All other American plaice in surplus of 50 were released alive and are not included in the length-frequency distribution.

American plaice sampled at the Hibernia area are comprised of 64% females and 36% males. American plaice in the larger length classes are predominantly female with only one male fish (6%) measuring greater than 40 cm. American plaice in the smaller length classes are predominantly male with only six percent of female fish measuring less than 40 cm.

American plaice sampled at the reference area are comprised of 62% females and 38% males. Fish larger than 39 cm in length are predominantly female, while fish less than 39 cm are predominantly male.

Figure 8.6 Length Groupings by Sex of American Plaice Sampled at the Hibernia Area (2009)

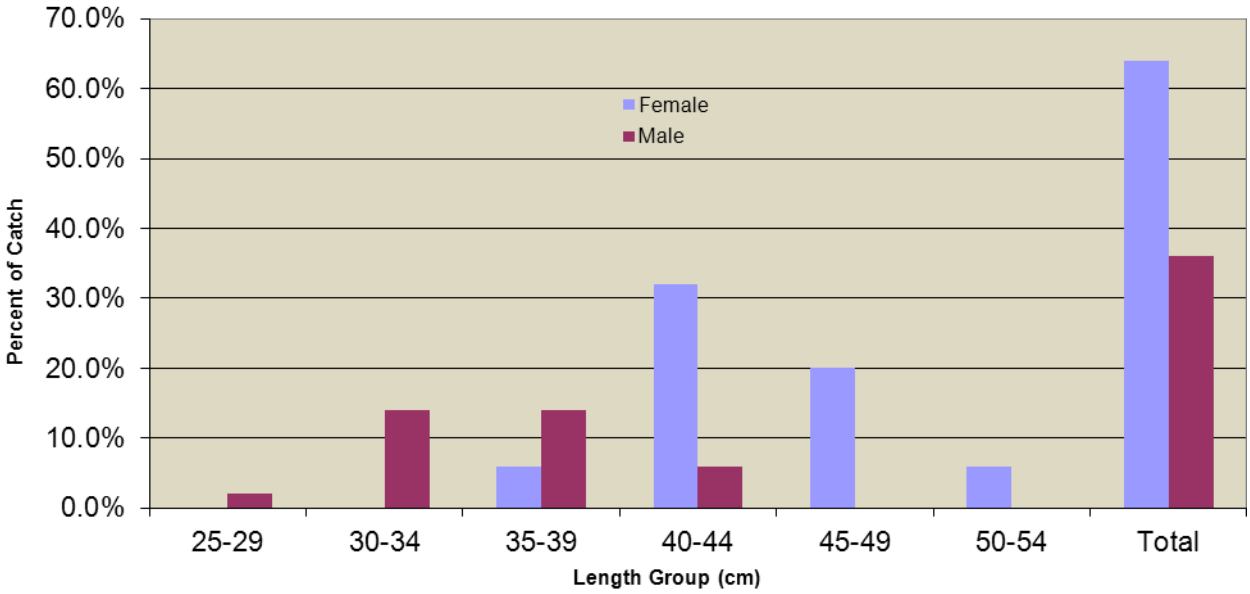
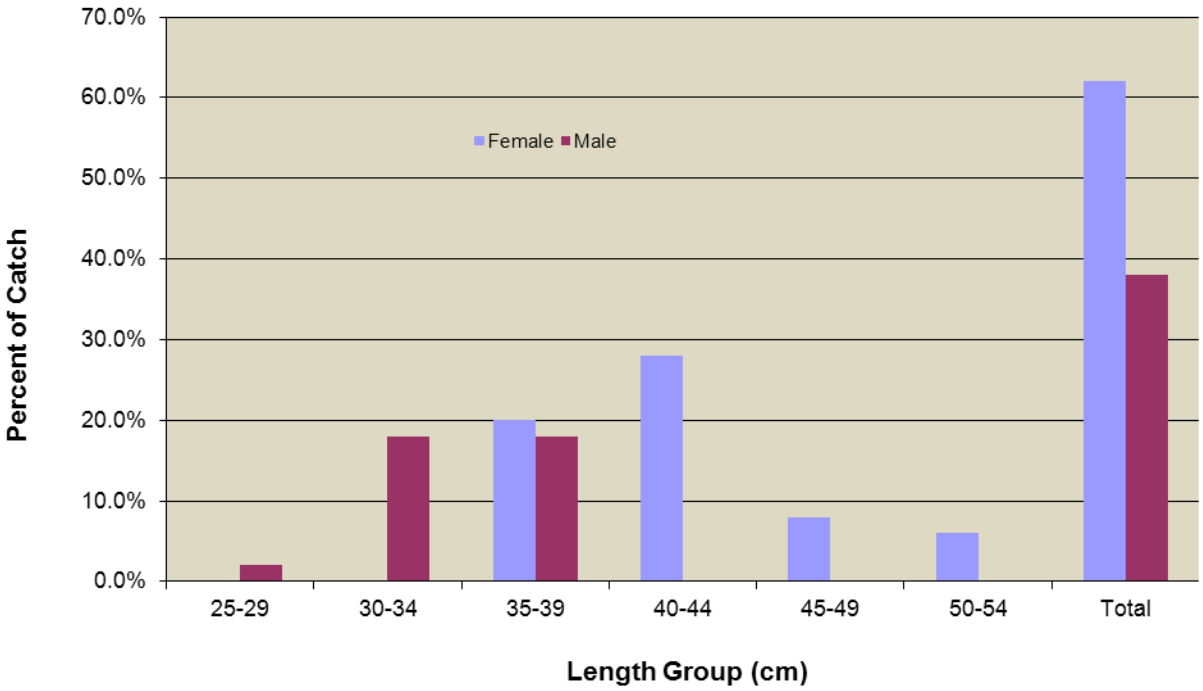


Figure 8.7 Length Groupings by Sex of American Plaice Caught at the Reference Area (2009)



8.2 Chemical Profiles for American Plaice Tissues

Fish muscle tissue and liver samples were obtained from American plaice collected in 2009 at the Hibernia (exposure) area and a reference site, as in earlier Hibernia EEM programs. Ten samples for each tissue type were collected from the exposure and reference sites, and were analyzed for metals, fuel (C10-C21) and lube (C21-C32) range hydrocarbons, PAHs and alkyl PAHs (only 9 liver samples were analyzed for the reference site due to insufficient sample volume as a result of sample splitting between fish health and chemistry requirements).

A summary of the chemical data for analytes that were detected in the 2009 Hibernia EEM Biological Sampling Program is presented in Table 8.1. The complete suite of chemicals that were analyzed in fish tissues is contained in Volume II, Appendix L. The RDL, the total number of samples analyzed, the number of samples that had values above the RDL and the associated mean, standard deviation, median and minimum and maximum numbers for each sampling area and tissue type are provided in Table 8.1. The calculated descriptive statistics were based on samples with values above the RDL or equal to the RDL, if not detected analytically. The reason was to obtain a meaningful descriptive statistic (i.e., for the mean and median) to characterize the measured analyte and which would not be less than the RDL for that analyte.

Table 8.1 Summary Statistics of American Plaice Liver and Muscle Tissue 2009 Data

Analyte	RDL	Units	No. of Samples	No. > RDL	Mean	SD	Median	Min	Max	No. of Samples	No. > RDL	Mean	SD	Median	Min	Max
			Hibernia Liver							Reference Liver						
Arsenic	0.5	mg/kg	10	10	20.2	14.1	13.8	3.6	42.9	9	9	27.9	15.7	26.9	11.3	61.6
Cadmium	0.05	mg/kg	10	10	0.97	0.33	0.98	0.34	1.40	9	9	1.18	0.31	1.09	0.79	1.65
Cobalt	0.2	mg/kg	10	1	0.2	0.0	0.2	<RDL	0.3	9	1	0.2	0.0	0.2	<RDL	0.2
Copper	0.5	mg/kg	10	10	12.1	6.9	10.6	4.7	27.5	9	9	9.7	2.7	8.7	6.7	15.1
Iron	15	mg/kg	10	10	97	44	93	46	187	9	9	110	58	79	37	215
Manganese	0.5	mg/kg	10	10	1.0	0.4	0.9	0.7	2.0	9	7	0.7	0.1	0.7	<RDL	0.9
Mercury	0.01	mg/kg	10	10	0.05	0.01	0.05	0.03	0.07	9	9	0.05	0.03	0.04	0.03	0.11
Selenium	0.5	mg/kg	10	10	3.0	0.7	3.1	2.0	4.1	9	8	2.9	1.1	2.8	<RDL	4.5
Silver	0.12	mg/kg	10	5	0.20	0.13	0.13	<RDL	0.48	9	3	0.13	0.03	0.12	<RDL	0.22
Strontium	1.5	mg/kg	10	1	1.5	0.0	1.5	<RDL	1.6	9	0	-	-	-	<RDL	-
Vanadium	0.5	mg/kg	10	0	-	-	-	<RDL	-	9	1	0.5	0.0	0.5	<RDL	0.6
Zinc	1.5	mg/kg	10	10	37.4	5.2	37.8	30.2	48.2	9	9	36.6	5.0	34.4	31.8	47.2
>C10-C21 (Fuel Range)	15	mg/kg	10	10	39	9	38	22	52	10	10	45	17	37	25	82
>C21-C32 (Lube Range)	15	mg/kg	10	10	138	40	140	87	200	10	10	144	59	125	82	260
Polycyclic Aromatic Hydrocarbons																
1-Methylnaphthalene	0.05	mg/kg	10	1	0.07	0.07	0.05	<RDL	0.28	10	0	-	-	-	<RDL	-
2-Methylnaphthalene	0.05	mg/kg	10	5	0.08	0.04	0.07	<RDL	0.14	10	5	0.09	0.08	0.07	<RDL	0.30
Chrysene	0.05	mg/kg	10	0	-	-	-	<RDL	-	10	1	0.05	0.01	0.05	<RDL	0.09
Fluoranthene	0.05	mg/kg	10	3	0.11	0.11	0.05	<RDL	0.35	10	2	0.06	0.02	0.05	<RDL	0.12
Fluorene	0.05	mg/kg	10	0	-	-	-	<RDL	-	10	1	0.07	0.05	0.05	<RDL	0.21
Naphthalene	0.05	mg/kg	10	3	0.06	0.01	0.05	<RDL	0.08	10	1	0.05	0.01	0.05	<RDL	0.07
Alkyl Polycyclic Aromatic Hydrocarbons																
1-Methylnapthalene	0.01	mg/kg	10	1	0.01	0.00	0.01	<RDL	0.01	9	0	-	-	-	<RDL	-
2-Methylnapthalene	0.01	mg/kg	10	3	0.01	0.00	0.01	<RDL	0.02	9	0	-	-	-	<RDL	-
			Hibernia Muscle							Reference Muscle						
Arsenic	0.5	mg/kg	10	10	2.8	0.9	2.7	1.8	4.5	10	10	2.8	0.8	3.0	1.5	3.8
Mercury	0.01	mg/Kg	10	10	0.10	0.04	0.09	0.06	0.18	10	9	0.09	0.05	0.09	<RDL	0.17
Selenium	0.5	mg/Kg	10	3	0.5	0.1	0.5	<RDL	0.7	10	1	0.5	0.0	0.5	<RDL	0.6
Zinc	1.5	mg/Kg	10	10	3.7	0.3	3.7	3.1	4.1	10	10	3.4	0.3	3.4	3.0	3.9
Fat, Crude	0.5	%(w)	10	10	1.2	0.6	1.0	0.6	2.6	10	10	1.2	0.7	0.9	0.6	2.8

8.3 Data Screening of Biological Tissue Data

Statistical tests (ANOVA) were performed on the biological tissue data to evaluate whether there were significant differences in the chemical concentrations present in liver or muscle tissues of American plaice, between fish collected in the vicinity of the Hibernia platform and those collected at a reference site. Only the data collected in 1998, 1999, 2000, 2002, 2004, 2007 and 2009 were used for the analysis, since the data collected in 1994 did not include sufficient fish to support the statistical analysis.

The first step of analysis was to screen the data and select for further analysis only those substances that were detected in several samples. If data for 1998, 1999, 2000, 2002, 2004, 2007 and 2009 were routinely below the RDL, then no further analysis was conducted. Based upon this screening (Table 8.1), selected substances were retained for statistical analysis of muscle and liver tissues. In the one or two cases where data values below the RDL were present for a substance that was the subject of further statistical evaluation, the "non-detectable" observation was replaced in the data set by one-half the RDL (refer also to Appendix B for rationale).

8.3.1 Total Metals Results for Biological Tissues

8.3.1.1 Liver

In the 2009 biological data, as in previous years, concentrations of arsenic, cadmium, copper, iron, manganese, mercury, selenium and zinc concentrations were above detectable levels in all the American plaice liver tissues in both Reference and Hibernia fish. For selenium and manganese, all but one and two samples, respectively, of liver tissue from the Reference fish were above detectable levels while all samples in the tissues from the Hibernia fish were above detectable levels. Strontium and vanadium were detected in one liver tissue sample each at Hibernia and at the Reference site, respectively.

8.3.1.2 Muscle

Concentrations of arsenic and zinc were above detectable levels in all the American plaice muscle tissues in both Reference and Hibernia fish in 2009, and for other years. Concentrations of mercury were above detectable levels in all the American plaice muscle tissues in Hibernia fish and all but one of the Reference fish.

8.3.2 Hydrocarbons (PAH and TPH) Results for Biological Tissues

Fish tissue contains abundant naturally-occurring oils that could be extracted and quantified by the same analytical techniques that are used to quantify petroleum hydrocarbon concentrations. Therefore, the presence of a substance identified as lube or fuel range hydrocarbon in fish tissue at either the Hibernia or Reference sites does not necessarily indicate that the hydrocarbons originate from petroleum. Aliphatic and aromatic hydrocarbons such as lube and fuel range oils are readily digested and metabolized by vertebrates and are, in fact, not likely to be bioconcentrated in either liver or muscle tissues.

In addition to the analysis of PAHs, alkyl PAHs were analyzed in liver and muscle tissue samples for the first time in 2009 for the Hibernia EEM Biological Sampling Program.

8.3.2.1 Liver

Similar to the 2007 hydrocarbon (TPH) data in fish liver, the 2009 data from both the Reference and Hibernia sites were above the RDL, which was not the case prior to 2004. In 2009, both the fuel range (C10-C21) and the lube range hydrocarbons (C21-C32) were present above an RDL of 15 mg/kg in all livers from American plaice collected in both Hibernia and Reference sites. Unlike previous years, six PAHs were detected in samples at both the Hibernia and Reference sites. The PAH 2-Methylnaphthalene was detected most frequently in five liver samples from both the Hibernia and Reference sites. Chrysene and fluorene were only detected in one sample at the Reference site while 1-methylnaphthalene was detected in one sample at Hibernia. The analysis of alkyl PAHs detected only 1-methylnaphthalene and 2-methylnaphthalene at a concentration above the RDL in one and three liver samples of ten samples each, respectively, for Hibernia fish. Overall, the concentration of PAHs when detected was equal to or marginally above the RDL for both Hibernia and Reference site liver samples. Further, PAHs were detected for a low number of liver samples for both the Hibernia or Reference sites (Table 8.1). Therefore, no further statistical analysis of PAH data was considered necessary.

8.3.2.2 Muscle

Fuel range hydrocarbons were not detected above the RDL in any of the muscle samples, thus no further statistical analysis was conducted.

Lube range hydrocarbons were not detected above the RDL in any of the muscle samples from both the Hibernia or Reference sites in 2009. Thus no further statistical analyses were conducted.

Concentrations of PAHs were not detectable in muscle tissues during 2009, from either the Hibernia or Reference sites. In addition, all alkyl PAH concentrations in muscle tissue were below the detection limit. Therefore, no further statistical analysis of PAH data was considered necessary for muscle tissue of American plaice, as has been the case for previous EEM programs.

8.4 Statistical Analyses of Biological Tissues

A two-way ANOVA using the general linear model was used to determine whether statistically significant differences in chemical concentration were present in American plaice liver and muscle tissues caught near the Hibernia platform and from a reference site. The experimental factors or treatments that were statistically tested in the ANOVA include Year (1998, 1999, 2000, 2002, 2004, 2007, and 2009) and Area (Hibernia and Reference), as well as the Year × Area interaction term. Where statistically significant differences attributable to either of the experimental factors or interaction term were indicated in the ANOVA, the source of these differences was investigated using Tukey's HSD multiple comparison tests. All data were $\log_{10}$-transformed prior to analysis to ensure the assumptions of the ANOVA analyses were met.

8.4.1 Total Metals in Liver Tissues

The metals results for American plaice livers are shown as ANOVA tables for Year, Area and Year × Area in Tables 8.2 to 8.9. No statistically significant differences were found for Area or the Year × Area interaction term in the liver samples. It can, therefore, be concluded that there are no significant differences between liver metal concentrations in American plaice collected near the Hibernia platform when compared to the reference site, and the important Year × Area interaction term was also not significant, indicating no effect on American plaice livers from Hibernia since production.

There were statistically significant (p<0.001) differences attributable to Year for arsenic, cadmium, copper, iron, manganese, and zinc. Tukey's HSD multiple comparison tests indicate that the differences between "years" for these metals can generally be attributed to lower overall metal concentrations in liver as determined in 1998, than in other years. Further, arsenic, cadmium, copper and iron concentrations in 2009 were higher compared to other years, and were generally significantly different from 1998. Cadmium, copper and iron concentrations in 2009 liver samples were, in most cases, similar to other years, but were significantly higher when compared to 1998 samples. Manganese was similar to 2007 results in that concentrations were not significantly different than the lowest values in 2000. Results for zinc indicate that since 2000, the results are not significantly different from each other with 1999 having the highest level and 1998 the lowest level. There was no statistically significant difference in the ANOVA results for mercury or selenium. These differences are likely due to natural inter-annual or seasonal variation in metals concentrations generally. This interpretation can be further substantiated given liver metal concentrations in American plaice collected near the Hibernia platform were not significantly different when compared to the reference site for 2009, as for other years of the EEM programs.

Table 8.2 Two-Factor ANOVA for Log_{10}-Transformed Arsenic Concentration in Liver Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	7.304	6	1.217	19.132	< 0.001
Area	0.000	1	0.000	0.000	0.989
Year x Area	0.517	6	0.086	1.355	0.240
Error	6.617	104	0.064		

Table 8.3 Two-Factor ANOVA for Log_{10}-Transformed Cadmium Concentration in Liver Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	0.195	6	0.033	6.208	< 0.001
Area	0.019	1	0.019	3.560	0.062
Year x Area	0.009	6	0.002	0.297	0.937
Error	0.545	104	0.005		

Table 8.4 Two-Factor ANOVA for Log_{10}-Transformed Copper Concentration in Liver Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	2.189	6	0.365	10.495	< 0.001
Area	0.023	1	0.023	0.668	0.416
Year x Area	0.120	6	0.020	0.574	0.750
Error	3.616	104	0.035		

Table 8.5 Two-Factor ANOVA for Log_{10}-Transformed Iron Concentration in Liver Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	2.270	6	0.378	7.963	< 0.001
Area	0.045	1	0.045	0.957	0.330
Year x Area	0.181	6	0.030	0.635	0.702
Error	4.942	104	0.048		

Table 8.6 Two-Factor ANOVA for Log_{10}-Transformed Manganese Concentration in Liver Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	0.128	6	0.021	10.916	< 0.001
Area	0.002	1	0.002	1.105	0.296
Year x Area	0.007	6	0.001	0.624	0.711
Error	0.204	104	0.002		

Table 8.7 Two-Factor ANOVA for Log_{10}-Transformed Mercury Concentration in Liver Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	0.012	6	0.002	1.945	0.080
Area	0.001	1	0.001	0.560	0.456
Year x Area	0.004	6	0.001	0.628	0.708
Error	0.109	104	0.001		

Table 8.8 Two-Factor ANOVA for Log_{10}-Transformed Selenium Concentration in Liver Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	0.068	6	0.011	2.090	0.061
Area	0.000	1	0.000	0.049	0.825
Year x Area	0.046	6	0.008	1.415	0.216
Error	0.565	104	0.005		

Table 8.9 Two-Factor ANOVA for Log_{10}-Transformed Zinc Concentration in Liver Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	0.323	6	0.054	9.300	< 0.001
Area	0.004	1	0.004	0.688	0.409
Year x Area	0.068	6	0.011	1.957	0.079
Error	0.601	104	0.006		

8.4.2 Total Metals in Muscle Tissues

The ANOVA results for arsenic, mercury and zinc in muscle tissue of American plaice are shown in Tables 8.10 to 8.12, respectively. There was no statistically significant Area effect, or Year × Area interaction effect for arsenic or mercury concentrations in muscle tissue (Tables 8.10 and 8.11). There was a statistically significant Year effect attributed to higher arsenic concentrations in muscle tissue in 2004 as compared to 1998 and 1999 and which was not statistically different from all other years, including 2009. The concentration of mercury in muscle tissue from 2000 through 2009 was not significantly different. Mercury concentrations in muscle tissue from 1998 and 1999 were significantly lower than all other years.

Table 8.10 Two-Factor ANOVA for Log_{10}-Transformed Arsenic Concentration in Muscle Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	0.281	6	0.047	3.444	0.003
Area	0.011	1	0.011	0.801	0.373
Year x Area	0.075	6	0.013	0.924	0.480
Error	1.711	126	0.014		

Table 8.11 Two-Factor ANOVA for Log_{10}-Transformed Mercury Concentration in Muscle Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	0.020	6	0.003	18.309	< 0.001
Area	0.000	1	0.000	0.066	0.797
Year x Area	0.002	6	0.000	1.855	0.094
Error	0.023	126	0.000		

Table 8.12 Two-Factor ANOVA for Log_{10}-Transformed Zinc Concentration in Muscle Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	0.064	6	0.011	5.574	< 0.001
Area	0.006	1	0.006	2.931	0.089
Year x Area	0.030	6	0.005	2.615	0.020
Error	0.243	126	0.002		

The ANOVA results for zinc (Table 8.12) indicate that there is a statistically significant Year effect (p<0.001) and Year × Area effect (p=0.020). Tukey's HSD multiple comparison tests for the interaction effect indicate that 1999 zinc concentrations in American plaice muscle tissue from the Hibernia site while not significantly different from the 1999 reference site concentrations, had the highest value observed at the Hibernia site. The 2004 zinc concentrations in muscle tissues were higher than those observed in 1999 for the reference site muscle tissues. The 2009 zinc concentrations in muscle tissues at the reference site were not significantly different from the 2009 Hibernia muscle tissue concentrations. However the 2009 reference site muscle tissue concentrations for zinc were the lowest observed values to date and were significantly lower than all previous years at Hibernia except for the 2000 concentrations. The 2009 reference muscle zinc concentrations were significantly different than the 2004 reference muscle concentrations which were the highest observed concentrations to date. These results suggest zinc concentration variations observed are as a result of natural variation rather than from the Hibernia Production Platform.

8.4.3 Fuel Range Hydrocarbon (C10-C21) in Liver Tissues

The statistical analyses of fuel range hydrocarbons in American plaice livers was not conducted for all years of the EEM programs because only 2007 and 2009 values were consistently above the RDL for both the Reference and Hibernia sites. The ANOVA for fuel range hydrocarbons in American plaice livers revealed that there is a statistically significant (p<0.001) difference between 2007 and 2009, but no statistically significant difference for the Area or the more important Year × Area interaction effect (Table 8.13). Fuel range hydrocarbon concentrations in liver tissues collected in 2009 are overall statistically higher than those of 2007 liver tissues.

Table 8.13 Two-Factor ANOVA for Log_{10}-Transformed Fuel Range Hydrocarbon (C10-C21) Concentration in Liver Tissue of American Plaice in 2009

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	0.123	1	0.123	5.081	0.030
Area	0.003	1	0.003	0.125	0.726
Year x Area	0.008	1	0.008	0.333	0.567
Error	0.869	36	0.024		

8.4.4 Lube Range Hydrocarbon (C21-C32) in Liver Tissues

The ANOVA for lube range hydrocarbons in American plaice livers revealed that there is a statistically significant (p<0.001) difference between years, but no statistically significant Area or Year × Area interaction (Table 8.14). Tukey's HSD multiple comparison tests indicate that the overall source of the "Year" differences can be attributed to the fact that 2002, 2004, 2007 and 2009 lube range hydrocarbon concentrations in livers (for both the Hibernia and Reference areas) are significantly elevated as compared to other years. In addition, 2002 and 2009 lube range hydrocarbon concentrations were significantly higher than in both 2004 and 2007 for both Hibernia and Reference sites. Lube range hydrocarbon concentrations in American plaice livers were not significantly different in 1998, 1999 and 2000. The ANOVA for lube range hydrocarbons in muscle tissue was not conducted because all samples had values below the RDL of 15 mg/kg for both Hibernia and Reference sites.

Table 8.14 Two-Factor ANOVA for Log_{10}-Transformed Lube Range Hydrocarbon (C21-C32) Concentration in Liver Tissue of American Plaice

Year and Area (Hibernia, Reference) Factors					
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F-Ratio**	***p***
Year	28.255	6	4.709	208.010	< 0.001
Area	0.000	1	0.000	0.000	0.989
Year x Area	0.130	6	0.022	0.957	0.458
Error	2.377	105	0.023		

Even though American plaice livers contained higher lube range hydrocarbons in 2002, 2004, 2007 and 2009, there is no evidence that these livers contained petroleum-derived hydrocarbons originating from the Hibernia platform; since the "Year" effect applied to fish caught at both the Hibernia and reference sites. These differences may be due to natural inter-annual or seasonal variation.

8.4.5 Summary

On the basis of the data and statistical analyses presented in the sections above, it can be concluded that for the statistically significant differences observed for the metals arsenic, cadmium, copper, iron, manganese, mercury, selenium, zinc and fuel and lube range hydrocarbons measured in livers from American plaice, were attributed to differences between

the sampling years and not to a difference between Hibernia and reference site samples. This is evident in Figure 8.8 where the concentration of metals and lube range hydrocarbons in liver tissue are very similar between Hibernia and the reference site for the different years of the EEM program. Further, an inter-annual difference can be noted with lower values in 1998 and a peak in 2002.

Figure 8.9 summarizes the mean concentration of arsenic, mercury and zinc metals consistently detected in muscle tissue of American plaice for all years of the EEM program. Statistical analyses of arsenic, mercury and zinc concentrations in muscle tissue revealed that there were significant differences between Hibernia and the reference site for zinc and for the years 1998, 1999, 2004, and 2009. Zinc in 1999 Hibernia muscle samples had generally higher concentrations, whereas the reference site samples in 2004 had statistically significantly higher concentrations than those of Hibernia. Mercury in 1998 and 1999 muscle tissue was statistically lower than 2000, 2002, 2004, 2007, and 2009 muscle tissue. Mercury concentrations in Hibernia fish muscle in 1999 were not significantly different from 2002 and 2007 samples, but were statistically different for 2004 and 2009 samples. It should be noted that the statistical differences observed for metals were attributed to inter-annual differences, as for liver tissue, with the results not attributed to discharges associated with the Hibernia platform.

A summary of statistically significant results for metals and hydrocarbons (in liver and muscle tissue) for all EEM programs is included in Table 8.15. The interpretation of statistical significance is based on a statistically significant result occurring between the Hibernia Area and Reference Area for a specific parameter during the year of the EEM Study.

Table 8.15 illustrates that mercury concentrations in American plaice liver were statistically different (Hibernia versus Reference) in 1998, with the Reference Area having the highest concentration. No other statistically significant results have occurred with regard to American plaice livers.

For American plaice muscle, mercury concentrations were statistically higher at the Reference Area in 1998. Arsenic concentrations were statistically higher at the Hibernia Area in 1999 and zinc concentrations were statistically higher at the Hibernia Area in 1999 and 2002. No statistical difference in hydrocarbon concentrations (Hibernia versus Reference) has occurred in any EEM year including 2009.

Figure 8.8 Mean Metals and Lube Range Hydrocarbon Concentrations in Fish Liver Tissue near the Hibernia Platform and Reference sites from 1998 to 2009

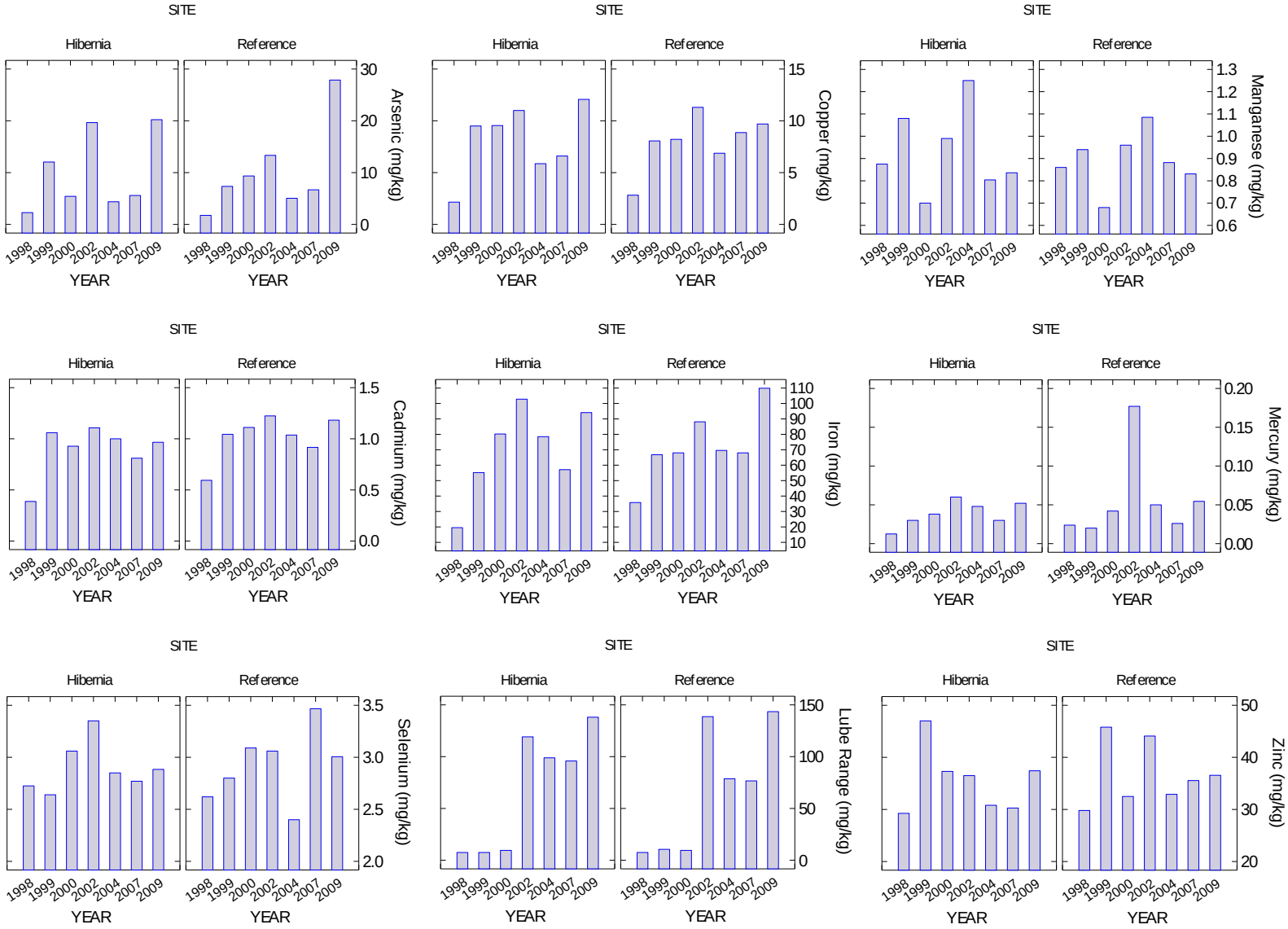


Figure 8.9 Mean Metal Concentrations in Fish Muscle Tissue near the Hibernia Platform and Reference sites from 1998 to 2009

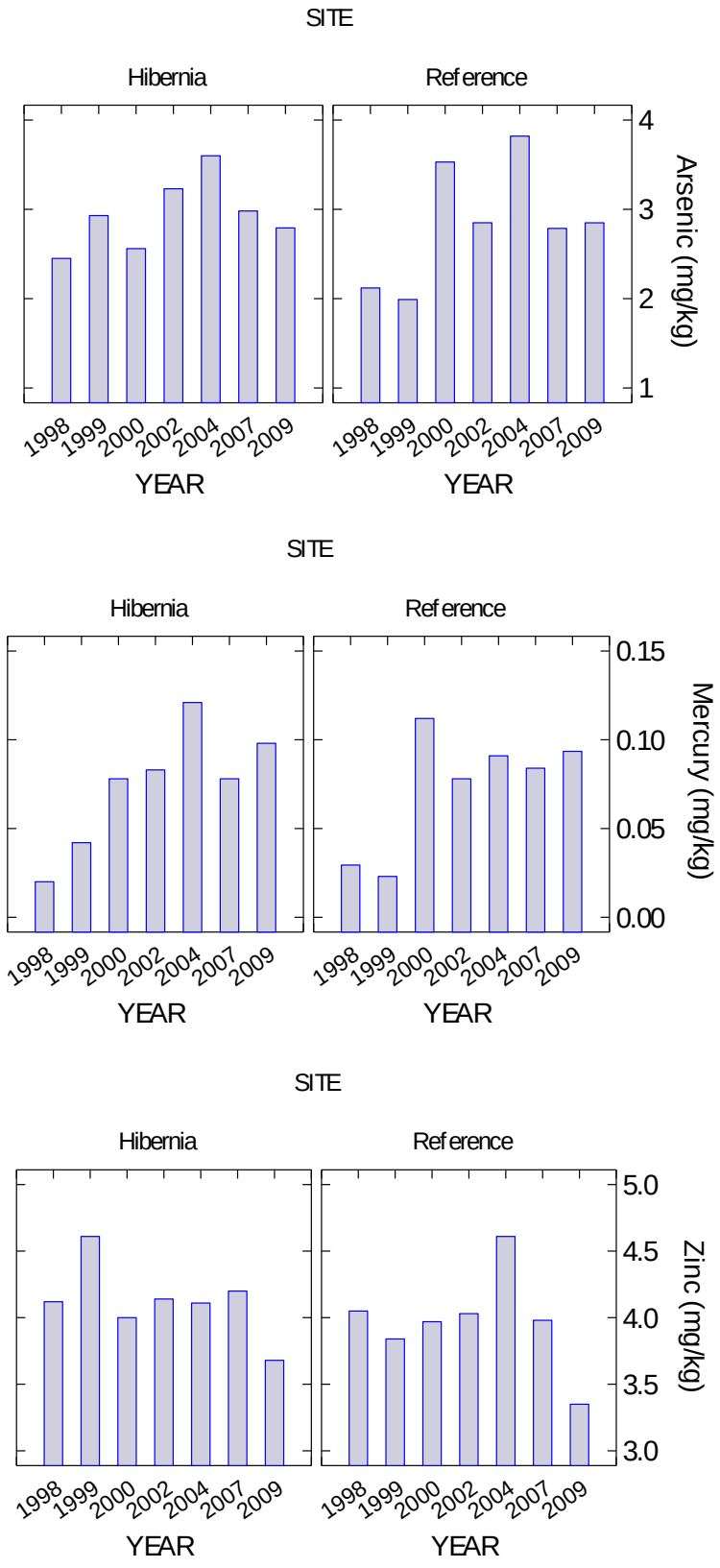


Table 8.15 Summary of Statistically Significant Results for Metals and Hydrocarbons in American Plaice Liver and Muscle Tissue for All EEM Years

Parameter	Year						
	1998	1999	2000	2002	2004	2007	2009
Liver							
Mercury	Yes (reference higher)	No	No	No	No	No	No
Hydrocarbon (Fuel)	No	No	No	No	No	No	No
Hydrocarbon (Lube)	No	No	No	No	No	No	No
Muscle							
Mercury	Yes (reference higher)	No	No	No	No	No	No
Arsenic	No	Yes	No	No	No	No	No
Zinc	No	Yes	No	Yes	No	No	No
Hydrocarbon (Fuel)	No	No	No	No	No	No	No
Hydrocarbon (Lube)	No	No	No	No	No	No	No
Note: Statistical significance is based on a statistically significant difference between Study and Reference Areas during the year of the EEM and not inter-annual differences.							

8.5 Taint

No significant difference was noted between American plaice collected in the Study and Reference Areas in the hedonic scaling test, but panellists for the triangle test were successful in discriminating 14 out of 24 samples. This result for the triangle test is significant at $\alpha = 0.05$ (Appendix E) and indicate that panellists could identify a difference between American plaice collected in the Study Area and those collected in the Reference Area.

ANOVA statistics for hedonic scaling are provided in Table 8.16 and a frequency histogram of results is provided in Figure 8.10. These results show no significant taste difference between Areas.

Incidental comments provided by panellists during the hedonic scaling and triangle test are included in Table 8.17 and 8.18. Comments associated with the triangle test are included in Table 8.17 and are presented under four headings. RA correctly identified as odd sample, RA incorrectly identified as odd sample, SA correctly identified as odd sample and SA incorrectly identified as odd sample.

Comments associated with the hedonic scaling test are included in the upper portion of the table. Comments from the hedonic scaling test are presented under two headings (panellist who preferred RA Samples and panellists who preferred SA Samples). From ancillary comments (Tables 8.17 and 8.18) there were no consistent comments identifying abnormal or foreign odour or taste. The majority of the comments associated with both the triangle test and hedonic scaling refer to flavour or taste.

Table 8.16 ANOVA Table for Preference Evaluation by Hedonic Scaling

Anova: Single Factor						
SUMMARY						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Study	24	156	6.5	1.73913		
Ref	24	165	6.875	1.853261		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	1.6875	1	1.6875	0.939486	0.337479	4.051749
Within Groups	82.625	46	1.796196			
Total	84.3125	47				

Figure 8.10 2009 Frequency Histogram for Hedonic Scaling Sensory Evaluation

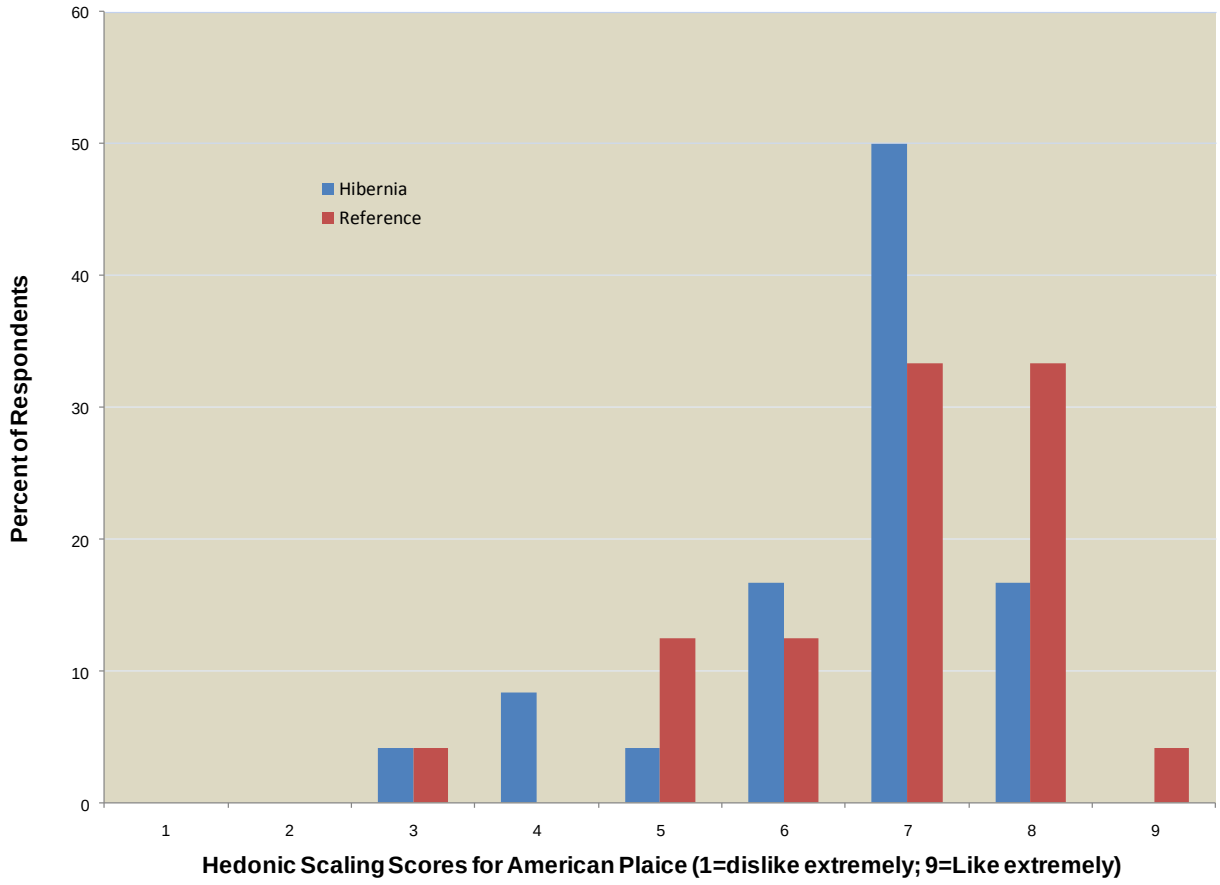


Table 8.17 2009 Comments from Triangle Test

Reference Area (RA)	Study Area (SA)
Correctly Identified as Odd Sample	**Correctly Identified as Odd Sample**
500 (SA) bland, 763 (SA) bland 756 (RA), little sweeter and more of an odour. Not a lot of difference between the samples.	Sample 686 (SA) is most preferred flavour. The other two were fairly flat in flavour.
500 (SA) mild fish smell, slightly sweet. 763 (SA) mild fish smell, slightly sweet. 756 (RA) mild fish smell, slightly sweet, but different mouth feel.	686 (SA) couldn't put my finger on it, but this one had a stronger flavour, not as appealing as the other two.
	686 (SA) had a much stronger "bad fish" taste. 591 (RA) and 230 (RA) had very little after taste.
	686 (SA) doesn't small as fishy as the other two. 686 (SA) does have a different taste than the other two. 591 (RA) and 230 (RA) taste bland.
	Very difficult to taste any difference. I made a guess, but they tasted the same to me.
	446 (SA) slightly less bland i.e., more flavour. Other than that, all good.
Incorrectly Identified as Odd Sample	**Incorrectly Identified as Odd Sample**
591 (RA) had a better taste and odour. Did not like the other two.	Not much difference.
452 (RA) has a stronger odour than the other two samples. 619 (RA) had the most flavour, 452 (RA) most bland.	Differed very slightly. 143 (SA) was a little more bland than the other two.
	Difficult to distinguish. 143 (SA) tasted somewhat different, but not enough to really tell.
	Very similar, hard to tell.
	143 (SA) had a slightly different odour than the others; the taste was a little off as well.
	I didn't find a large difference in them.
	Very subtle difference if any. Not fully confident in choice however, sample 500 (SA) seemed to have a slight odour difference. Also texture in sample 500 (SA) seemed a little drier, chewy, stringy. As opposed to 763(SA) and 756 (RA) being a little moister and succulent.

Table 8.18 2009 Comments from Hedonic Scaling Tests

Preferred Reference Area (RA)	Preferred Study Area (SA)
804 (SA) more moist than 879 (RA).	Bland taste on both.
879 (RA) doesn't seem as fishy.	No observable difference.
No observable difference.	After taste I found to be strong on both samples. Sample 260 (SA) had a little fishy smell.
After taste I found to be strong on both samples. Sample 260 (SA) had a little fishy smell.	There was no discernable difference in taste. 260 (SA) had a better odour than 812 (RA).
260 (SA) has a more fishy odour and at first taste is more fishy than 812 (RA).	Slightly better taste on sample 961 (SA).

Preferred Reference Area (RA)	Preferred Study Area (SA)
812 (RA) had a much better taste. 260 (SA) tasted like the fish was a bit old. The odour of 812 (RA) was much less than 260 (SA).	Sample 961 (SA) has a more pleasant flavour than sample 615 (RA).
Seems very similar in taste and smell.	Both samples tasted equal.
Nicer flavour to 615 (RA). I liked 961 (SA) as well, but not as much as 615 (RA).	102 (RA) was a little bland.
961 (SA) had a stronger odour than 615 (RA).	No detectable difference.
Both samples tasted equal.	Both are pretty good, could not determine which is better.
Both were pretty good, but 102 (RA) had a better odour and slightly better taste.	
Sample 731 (SA) had a slight different flavour.	
No detectable difference.	
Both are pretty good, could not determine which is better.	

A summary of results for the triangle test and hedonic scaling test (1995 to 2009) is presented in Table 8.19. The 2009 triangle test result was the only statistically significant result detected in any EEM year. The 2009 triangle test result coupled with the hedonic scaling test and ancillary comments noted for both taste tests indicated that while panellists were able to detect a difference between the samples, the difference is not due to discharges associated with the Hibernia platform. Therefore, taint was not detected for American plaice.

Table 8.19 Summary of Significant Results for Taint Testing (Triangle Test and Hedonic Scaling Test) All EEM Years

Year	Triangle Test		Hedonic Scaling
	# Correctly Identified	Significant Result	Significant Result
1995	8/24	No	No
1998	10/24	No	No
1999	5/24	No	No
2000	11/24	No	No
2002	11/24	No	No
2004	9/24	No	No
2007	9/24	No	No
2009	14/24	Yes	No

9.0 HIBERNIA 2009 FISH HEALTH PROGRAM

One hundred American plaice were examined for early warning effects on fish health. Fifty fish were sampled in the vicinity of the Hibernia development area (Study Area) and 50 fish at approximately 50 km northwest of the rig (Reference Area). The complete 2009 Oceans Report is included in Appendix F.

9.1 Biological Characteristics and Condition of Fish

Information on biological characteristics (sex, maturity, size, and age) as well as fish condition is valuable for interpreting the results of early warning effects on fish health. Biological characteristics and fish condition were analyzed separately for each sex.

Thirty three females and 17 males were collected in the Study Area while 31 females and 19 males were collected in the Reference Area. Female:Male (F:M) ratios were not significantly different between the two Areas (p=0.835; Fisher exact Test).

Maturity stages of male and female fish were defined according to procedures used by DFO (Appendix F, Volume II) and results, expressed as frequencies (percentages) of maturity stages, were compared between the Reference and Study Areas with the Fisher exact test.

All males but one were mature and no significant differences in frequencies of various maturity stages were observed between the two Areas (Table 9.1).

Table 9.1 Frequencies (%) of Maturity Stages of Male American Plaice from the 2009 Hibernia Survey

	N	Immature M-100 [a]	Maturing to spawn this year M-140 [a]	Partly spent M-150 [a]	Spent this year M-160+M-170 [a]	Maturing for next year M-180 [a]
Reference Area	19	5.3	15.8	68.4	10.5	0.0
Study Area	17	0.0	23.6	52.9	23.5	0.0
p Value [b]		1.000	0.684	0.495	0.391	1.000

[a] Maturity stages were defined according to procedures used by DFO (Appendix F, Volume II)
[b] p Value obtained with the Fisher exact test

All females were mature, except for three in the Study Area (Table 9.2), and no significant inter-site differences in frequencies of various maturity stages were observed.

Table 9.2 Frequencies (%) of Maturity Stages of Female American Plaice from the 2009 Hibernia Survey

	N	Immature F-500 [a]	Maturing to spawn this year F-520 to F-540 [a]	Partly spent F-550 [a]	Spent this year F-560+F-570 [a]	Maturing for next year F-580 [a]
Reference Area	31	0.0	32.3	16.1	48.4	3.2
Study Area	33	9.1	21.2	12.1	57.6	0.0
p Value [b]		0.239	0.400	0.729	0.617	0.484

[a] Maturity stages were defined according to procedures used by DFO (Appendix F, Volume II)
[b] p Value obtained with the Fisher exact test

Length, total and gutted body weight, liver and gonad weight and age were compared between the Reference and Study Areas using the Unpaired t-test or the Mann-Whitney Rank Sum test, when the groups were not normally distributed.

Fish condition, which can be defined as a state of physical fitness, was assessed by calculating different condition indices (Dutil *et al.*, 1995) such as (a) condition index expressed as Fulton's condition factor and calculated as 100 x body weight/length^3 based on gutted weight (b) hepato-somatic index (HSI) calculated as 100 x liver weight/gutted weight and (c) gonado-somatic index (GSI) calculated as 100 x gonad weight/gutted weight. Since these condition indices are commonly used, they are presented for general interest with comparisons between the two Areas being carried out by the Unpaired t-test. However, since use of these indices assumes that body weight is proportional to the cube of length, and liver and gonad weights are linearly related to gutted weight (which is not always the case), log-log regressions of body gutted weight on length, and liver and gonad weight on body gutted weight were also tested by analysis of covariance (ANCOVA). When ANCOVA revealed equality of regression slopes between areas, comparisons were made and adjusted means calculated.

Males

Information on biological characteristics and condition of male fish (all maturity stages pooled) from the Reference and Study Areas are summarized in Table 9.3. Data are expressed as means and standard deviations. The complete data set is provided in Volume II, Appendix O.

There were no significant differences in any of the parameters measured in male fish from the two Areas.

Table 9.3 Biological Characteristics and Condition Indices of Male American Plaice (all Maturity Stages Pooled) from 2009 Hibernia Survey

	Reference Area	Study Area	p Value [d]
Fish number	19	17	
Length (cm)	34.5 ± 2.6	35.0 ± 3.7	0.612
Total body weight (g)	359 ± 90	377 ± 136	0.682
Gutted body weight (g)	314 ± 82	342 ± 121	0.419
Liver weight (g)	2.6 ± 1.8	3.3 ± 2.0	0.288

	Reference Area	Study Area	p Value [d]
Gonad weight (g)	3.8 ± 4.8	4.0 ± 4.9	0.911
Age (year)	8.3 ± 0.8	7.8 ± 1.8	0.348
Fulton's condition factor [a]	0.748 ± 0.072	0.763 ± 0.110	0.632
Hepato-somatic index [b]	0.815 ± 0.538	0.941 ± 0.436	0.217
Gonado-somatic index [c]	1.164 ± 1.247	1.134 ± 1.349	0.949

All data are expressed as mean of raw values ± standard deviation
[a] Calculated as 100 x gutted body weight/length ^{3}
[b] Calculated as 100 x liver weight/gutted body weight
[c] Calculated as 100 x gonad weight/gutted body weight
[d] p Value obtained with the Mann-Whitney Rank Sum test

With respect to adjusted means (Table 9.4), gutted body weight relative to the covariate length as well as liver and gonad weight relative to the covariate gutted weight did not differ significantly between the two Areas after ANCOVA analysis.

Table 9.4 Adjusted Means of Male American Plaice (all Maturity Stages Pooled) from the 2009 Hibernia Survey

Variable	Covariate	Adjusted Means		p Value [a]
		Reference Area	Study Area	
Gutted weight	Length	309	311	0.877
Liver weight	Gutted weight	2.1	2.6	0.212
Gonad weight	Gutted weight	1.8	1.8	0.943

Adjusted means are predictive mean variable at overall mean covariate
[a] p Value obtained after ANCOVA analysis of log-log regression of variable on covariate

Females

Information on biological characteristics and condition of female fish (all maturity stages pooled) from the Reference and Study Areas are summarised in Table 9.5. Data are expressed as means and standard deviations and were compared between Areas using the Unpaired t-test or the Mann-Whitney Rank Sum test. The complete data set is provided in Volume II, Appendix O.

Table 9.5 Biological Characteristics and Condition Indices of Female American Plaice (all Maturity Stages Pooled) from 2009 Hibernia Survey

	Reference Area	Study Area	p Value [d]
Fish number	31	33	
Length (cm)	41.9 ± 4.3	43.4 ± 4.0	0.166
Total body weight (g)	675 ± 230	769 ± 250	0.060
Gutted body weight (g)	568 ± 228	662 ± 231	*0.030**
Liver weight (g)	5.9 ± 3.6	8.9 ± 4.1	*0.002**
Gonad weight (g)	60.3 ± 61.5	60.2 ± 83.2	0.629
Age (year)	10.3 ± 1.7	10.5 ± 1.8	0.508
Fulton's condition factor [a]	0.740 ± 0.100	0.792 ± 0.080	*0.002**
Hepato-somatic index [b]	1.012 ± 0.508	1.351 ± 0.516	*0.004**
Gonado-somatic index [c]	10.317 ± 8.907	8.047 ± 8.556	0.189

All data are expressed as mean of raw values ± standard deviation
[a] Calculated as 100 x gutted body weight/length ^{3}
[b] Calculated as 100 x liver weight/gutted body weight
[c] Calculated as 100 x gonad weight/gutted body weight
[d] p Value obtained with the Mann-Whitney Rank Sum test
* Significantly different ($p<0.05$)

There were no significant differences in length, gonad weight, age or gonado-somatic index in female fish from the two Areas. However, significant differences were observed for gutted body weight (p=0.030) and liver weight (p=0.002) as well as for Fulton's condition factor (0.002) and hepato-somatic index (p=0.004), with fish from the Study Area displaying higher values for all these parameters.

With respect to adjusted means by ANCOVA, there was a significant difference between Reference and Study Areas in gutted weight on length (p=0.028) with a higher value observed in females from the Study Area (Table 9.6). However, for liver and gonad weight on gutted body weight, since the condition of equality of slopes between the two Areas was not met, comparisons of adjusted means were not suitable for ANCOVA.

Table 9.6 Adjusted Means of Female American Plaice (all Maturity Stages Pooled) from the 2009 Hibernia Survey

Variable	Covariate	Adjusted Means		*p* Value [a]
		Reference Area	Study Area	
Gutted weight	Length	562	598	*0.028 **
Liver weight	Gutted weight	ns	ns	ns
Gonad weight	Gutted weight	ns	ns	ns

Adjusted means are predictive mean variable at overall mean covariate
ns = not suitable for comparison due to inequality of slopes
[a] *p* Value obtained after ANCOVA analysis of log-log regression of variable on covariate
* Significantly different ($p<0.05$)

9.2 Gross Pathology

One fish from the Study Area exhibited nematode worms on its liver and another had its intestine protruding from the anus while one fish from the Reference Area had pale gills. No other external or internal lesions/abnormalities were observed on the 100 fish examined.

9.3 Haematology

Blood smears were examined for various types of cells. The red blood cells of all fish appeared to be normal in size and shape. Coloration was also similar indicating a similar degree of haemoglobinization.

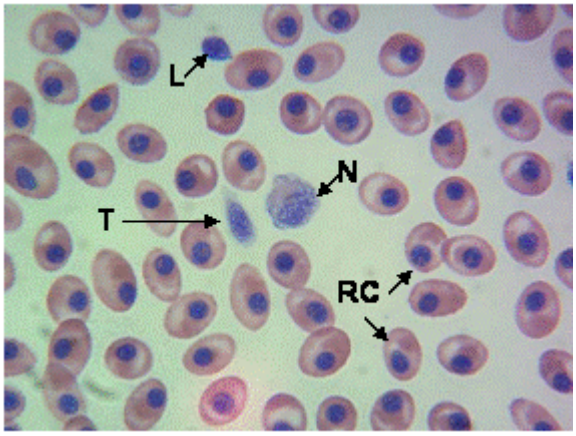


Photo 2 Representative blood smear

A differential cell count of lymphocytes, neutrophils and thrombocytes was carried out on a total of 95 fish. Blood smears of 1 fish from the Reference Area and 4 fish from the Study area were not suitable for cell counting. For the other blood smears, 200 cells were counted per fish and the results were expressed as mean percentage ± standard deviation of each cell type for each Area (Table 9.7). The complete data set on the different cells examined is provided in Volume II, Appendix P and a representative photograph of a blood smear is included in Photo 2.

Table 9.7 Frequencies of Blood Cell Types in American Plaice from the 2009 Hibernia Survey

	Reference Area N = 49	Study Area N = 46	p Value [a]
Lymphocytes	82.9 ± 26.4	82.2 ± 28.4	0.186
Neutrophils	0.5 ± 0.6	0.5 ± 0.8	0.748
Thrombocytes	16.6 ± 2.7	17.3 ± 2.8	0.231

All data are expressed as mean percentage ± standard deviation of each type of cell on 200 white blood cells counted
[a] *p* Value obtained with Mann-Whitney Rank Sum test after arcsin square root transformation of percentages

Inter-area comparisons were carried out with the Mann-Whitney Rank Sum test analysis after arcsin square root transformation of percentages. There were no significant differences between areas in the percentages of the 3 types of blood cells examined.

9.4 Mixed Function Oxygenase (MFO) Activity

Results of MFO enzyme activity, measured as EROD, in the liver of male and female American plaice (all maturity stages pooled) from the Reference and Study Areas are summarised in Figures 9.1 and 9.2. The complete data set is provided in Volume II, Appendix Q.

There was no significant difference in EROD activity levels in males between the two Areas (*p*=0.924; Unpaired t-test). However, the difference in enzyme activity was marginally significant in females (all maturity stages pooled) with an activity 1.8 fold higher in fish from the Study Area (*p*=0.058; Mann-Whitney Rank Sum test).

Figure 9.1 EROD Activity in the Liver of Male American Plaice (All Maturity Stages Combined)

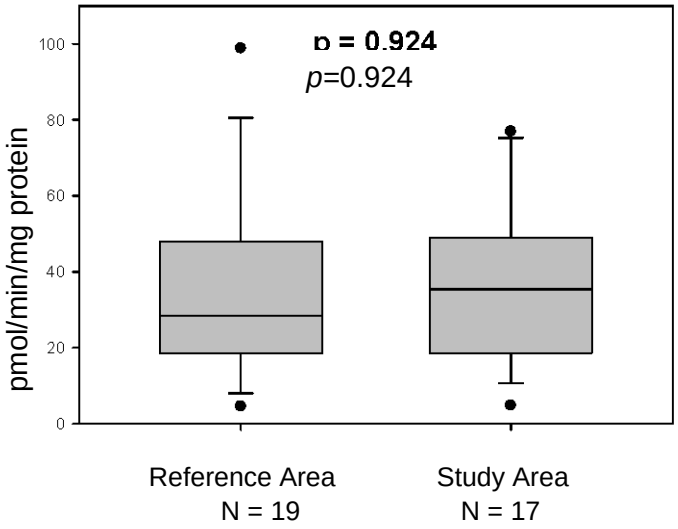
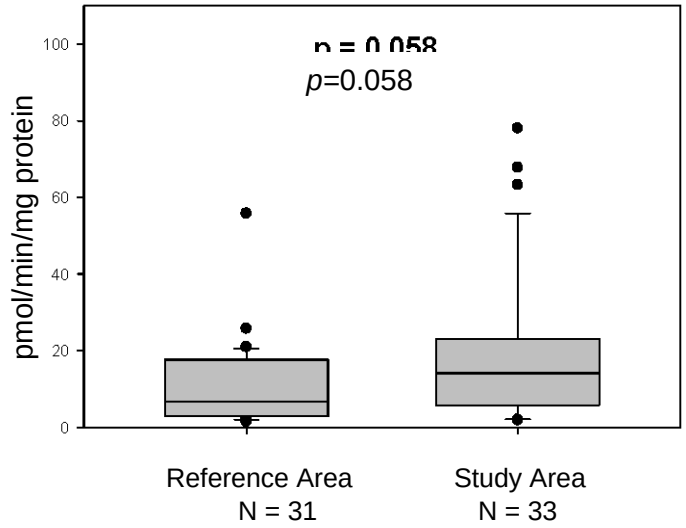


Figure 9.2 EROD Activity in the Liver of Female American Plaice (All Maturity Stages Combined)



Data plotted are median (horizontal line in box), 25th and 75th percentiles (bottom and top of box) and 10th and 90th percentiles (error bars). Data points beyond the 5th and 95th percentiles are also displayed.

Since EROD activity can vary with the stage of sexual maturity in female fish (e.g. Whyte *et al.*, 2000), comparisons were also carried out separately for various stages including: mature (all females minus immature females), maturing to spawn this year (including stages F-510 to

F-540), partially spent (Stage F-550) and spent of the year (including stages F-560 and F-570), Comparisons are provided in Table 9.8.

Table 9.8 Hepatic EROD Activity in Various Maturity Stages of Female American Plaice from the 2009 Hibernia Survey

	Reference Area	Study Area	*p*-Value [a]
All Females (Stages F-500 to F-580)	(31) 10.4 ± 10.9	(33) 19.0 ± 19.6	0.058
Mature females (all females minus immature females)	(31) 10.4 ± 10.9	(30) 15.3 ± 14.8	0.168
Maturing to spawn (stages F-510 to F-540 pooled)	(10) 5.0 ± 3.8	(7) 4.8 ± 5.8	0.464
Partially spent (stage F-550)	(5) 5.6 ± 7.1	(4) 10.6 ± 10.6	0.190
Spent females (stages F-560 and F-570 pooled)	(15) 21.6 ± 14.8	(19) 31.3 ± 20.8	0.510

All data are expressed as mean of raw values ± standard deviation
Maturity stages were defined according to procedures used by DFO (Appendix F, Volume II)
() = Number of fish analyzed
[a] *p* Value obtained with the Mann-Whitney Rank Sum test

EROD enzyme activities in mature (p=0.168), maturing to spawn (p=0.464), partially spent (p=0.190) and spent (p=0.510) females were not significantly different between the two Areas. However, it is noted that the number of fish in the partially spent category was low for carrying out comparative analysis.

9.5 Histopathology

9.5.1 Liver Histopathology

Detailed histopathological studies were carried out on liver tissues of American plaice with observations on various lesions that have been commonly associated with chemical toxicity. These included nuclear pleomorphism, megalocytic hepatosis, basophilic, eosinophilic and clear cell foci, fibrillar inclusions, fibrosis, carcinoma, cholangioma, cholangiofibrosis, proliferation of macrophage aggregates and hydropic vacuolation. Any other observations were also recorded and included hepatocellular vacuolation and parasitic infestation of the biliary system. Lesions were recorded for each fish as present or absent (Volume II, Appendix R), except for macrophage aggregation which was rated on a relative scale from 0 to 7.

Results were expressed as percentage of fish affected by each type of lesion/observation (or prevalence of lesion) in the Reference and Study Areas (Table 9.9). A representative photograph of a normal liver structure is included in (Photo 3).

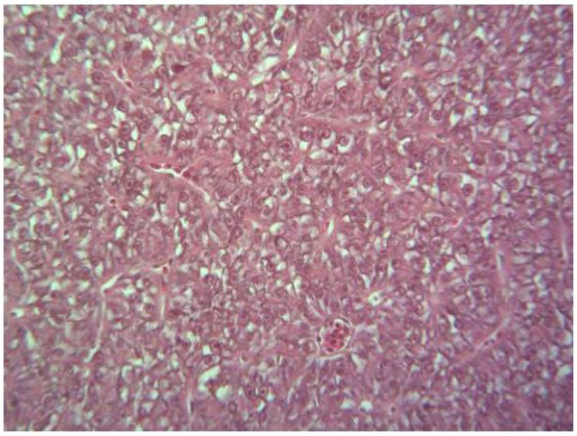


Photo 3 Normal Liver Structure

Fifty fish from the Study Area and 49 fish from the Reference Area were examined. There were no observation of lesions that have been commonly associated with chemical toxicity.

The frequencies of macrophage aggregates in livers of fish from the various Areas were low (0-2 rating on a relative scale from 0-7) and no cases of moderate to high aggregation (4 or higher on the relative scale) that are considered as a proliferation of macrophage aggregates were observed.

With respect to other observations, a patchy distribution of hepatocellular vacuolation (Photo 4), not associated with degenerative changes, was observed in 6.1 % of fish from the Reference Area and in 2% of fish from the Study Area. The difference in incidences between the two Areas was not significant (p=0.362; Fisher's Exact Test). An infestation of the biliary system with a myxosporean parasite, possibly *Myxidium sp*., was also observed in 4.1 % of fish from the Reference Area and in 18.0 % of fish from the Study Area. The infestation did not appear to result in any other pathological changes in hepatic tissues. Inter-site difference in incidence of parasitic infestation was marginally significant (p=0.051; Fisher's Exact Test).

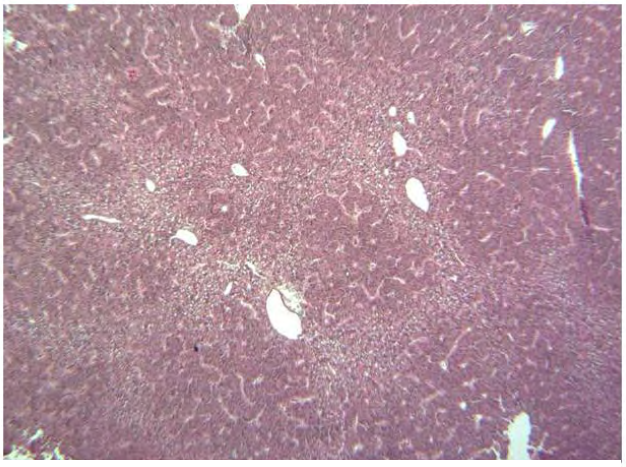


Photo 4 Patchy Hepatocellur Vacuolation

Table 9.9 Number of American Plaice with Specific types of Hepatic Lesions and Prevalence of Lesions in the 2009 Hibernia Survey

Lesions	Reference Area (N = 49)		Study Area (N = 50)	
	Fish affected	Prevalence % [a]	Fish affected	Prevalence % [a]
Nuclear pleomorphism	0	0	0	0
Megalocytic hepatosis	0	0	0	0
Fibrillar inclusions	0	0	0	0
Eosinophilic foci	0	0	0	0
Basophilic foci	0	0	0	0
Clear cell foci	0	0	0	0
Carcinoma	0	0	0	0
Cholangioma	0	0	0	0
Cholangiofibrosis	0	0	0	0
Proliferation of macrophage aggregation [b]	0	0	0	0
Hydropic vacuolation	0	0	0	0
Hepatocellular vacuolation	3	6.1	1	2.0
Parasitic infestation of biliary system	2	4.1	9	18.0

[a] Percentage of fish affected
[b] Defined as scores greater than 3 on a 0-7 relative scale.

The observations on hepatocellular vacuolation and parasitism are of general interest but the absence of liver lesions that have been commonly associated with chemical toxicity are more relevant from an EEM perspective.

9.5.2 Gill Histopathology

Gill sections were examined for the presence of various gill lesions commonly associated with chemical toxicity. These included epithelial lifting, basal, distal and tip hyperplasia, fusion, telangiectasis and severe oedema.

For each fish, lamellar counts were performed on four filaments, when possible, and are presented as the percentage of secondary lamellae affected by each type of lesion in relation to the total number of secondary lamellae counted (Volume II, Appendix S). One fish from the Reference Area (which exhibited pale gills during necropsy) displayed extensive proliferation of ovoid and pale staining cells, or X-cells, in the interlamellar spaces of secondary lamellae (Photo 5) and tissue structure was altered to such an extent that it was not possible to count the secondary lamellae. Accurate counts were also not possible for another fish from the Reference Area due to inadequate orientation of the primary lamellae. Lamellar counts were thus carried out on gill tissues of 48 fish from the Reference Area and 50 fish from the Study Area. A representative picture of normal gill secondary lamellae is provided in Photo 6.

When lesions were observed, they were small and limited to a few secondary lamellae among the large number of lamellae examined (between 352 and 928 lamellae per fish). There were no cases of epithelial lifting in fish from either site and the percentages of lamellae affected by the other lesions were very low, all were less than 4%, except for one fish from the Reference Area with 8.4% of lamellae exhibiting basal hyperplasia as well as 11.3% of lamellae exhibiting fusion (Photo 7). The degree of oedema, which was recorded on a 0-3 relative scale, was quite low in both Areas.

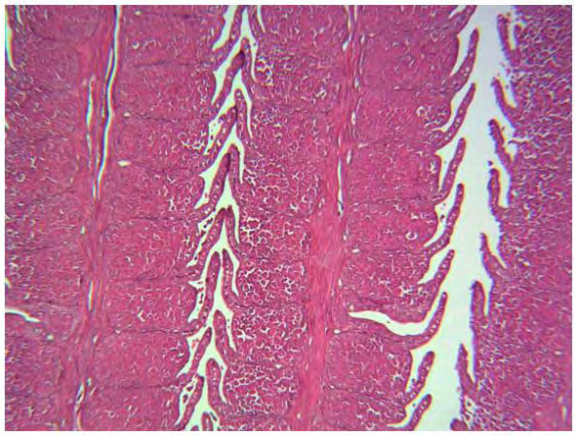


Photo 5 Extensive Proliferation of X-cells between Gill Secondary Lamellae (H&E x63)

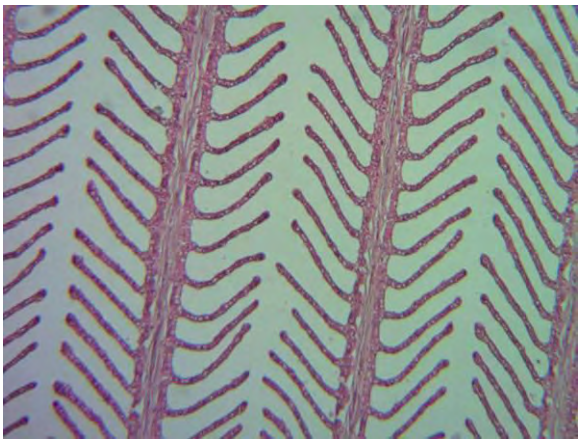


Photo 6 Normal Gill Secondary Lamellae (H&E x63)

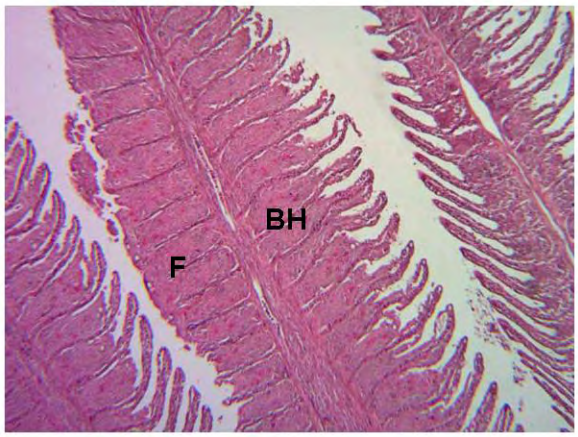


Photo 7 Basal Hyperplasia (BH) and Fusion (F) of Gill Secondary Lamellae (H&E x63)

Results, expressed as means ± standard deviations of percentage of secondary lamellae affected by lesions, are summarized in Table 9.10.

Table 9.10 Percentages of Secondary Lamellae Affected by Lesions and Rating of Oedema Condition in the Gill Tissues of American Plaice from the 2009 Hibernia Survey

	Reference Area n = 48	Study Area n = 50	*p* Value [c]
Epithelial lifting [a]	0	0	1.000
Basal hyperplasia 1 [a]	0.22 ± 1.21	0.04 ± 0.13	0.278
Basal hyperplasia 2 [a]	0.02 ± 0.09	0.03 ± 0.16	0.621
Distal hyperplasia [a]	0.16 ± 0.64	0.09 ± 0.22	0.161
Tip hyperplasia [a]	0.09 ± 0.36	0.13 ± 0.33	0.255
Fusion [a]	0.36 ± 1.68	0.20 ± 0.49	0.471
Telangiectasis [a]	0.01 ± 0.03	0.01 ± 0.05	0.992
Oedema condition [b]	0.833 ± 0.907	0.864 ± 0.805	0.669

All data are means ± standard deviations
[a] Mean percentage of lamellae presenting the lesion
[b] Mean of rating on a relative 0-3 scale
[c] *p* Value obtained with the Mann-Whitney Rank Sum test on arcsine square root-transformed percentages of the lesions or on ranking of oedema

Overall, less than 0.4% of the secondary lamellae were affected by the various lesions and no significant differences between the Study and Reference Areas were observed after the Mann-Whitney Rank Sum test on arcsine square root-transformed percentages of the lesions or on ranking of oedema.

9.6 Summary

A summary of statistical significant results for fish health indices is provided in Table 9.11. The interpretation of statistical significance is based on a statistical significant difference between samples caught at the Hibernia site and a reference site. Any of the indicators that had a statistically significant difference detected between the Hibernia and reference location has been attributed to natural variability with no discernable pattern detected.

Table 9.11 Summary of Statistically Significant Results for Fish Health Indicators in American Plaice

Indicators	2002	Comments	2004	2007	Comments	2009	
Fulton's condition factor	No		No	No		No	
Hepato-somatic index	Yes	Males only. Higher HSI in fish from the Study Area. Likely natural variation.	No	No		Yes	Females only. Higher FCF in fish from Study Area, likely natural variation.
Gonado-somatic index	Yes	Males only. Lower GSI in fish from the Study Area. Likely natural variation.	No	Yes	Females only. Higher GSI in fish from the Study Area. Likely natural variation.	Yes	Females only. Higher HSI in fish from Study Area, likely natural variation.
Gross Pathology	No		No	No		No	
Haematology	No		No data	Yes	Fewer lymphocytes and more thrombocytes in fish from the Study Area. Likely natural variation.	No	
MFO	Yes	Females only. Slightly lower activity in fish from the Study Area. Likely natural variation.	No	No		Yes	Marginally significant in females only. Higher activity in Study Area, likely natural variation.
Liver Histopathology	No		No	Yes	Fibrillar inclusions in a single fish from the Study Area. Very low prevalence, likely natural.	Yes	Incidence of parasitic infestation was marginally significant. Higher in Study Area, likely natural variation.
Gill Histopathology	Yes	Distal hyperplasia slightly lower in fish from the Study Area. Likely natural variation.	No	No		No	

10.0 DISCUSSION AND INTERPRETATION

The purpose of an EEM program is to identify and quantify the change (spatially and temporally) to the surrounding environment relative to project operations, in this case, those specifically related to the operation of the Hibernia platform. The goals and objectives of any EEM program are to:

- characterize the extent and magnitude of the observed contaminants;
- verify model predictions;
- provide an early warning sign of potential biological effects;
- provide management information with which to influence operational practices or implement additional mitigative measures;
- provide the basis for technological improvements and advances; and
- provide a scientific basis for improved regulatory environmental management.

The term contamination recognized by GESAMP (1993) has been used to refer to raised levels of a chemical compared with background levels. Based on this definition, two contaminants were detected at the Hibernia site, TPH and barium. Both of the contaminants are major constituents of drilling muds and produced water. In the case of drilling muds, TPH reflects the residual synthetic drilling fluid retained on cuttings. Other chemical constituents that may be associated with drilling muds, such as silver, arsenic, cadmium, copper, chromium, nickel, lead, strontium, vanadium, zinc and mercury, have not been detectable at statistically significant levels above baseline and, as such, are not considered to be contaminants.

The demonstration of contaminants does not necessarily indicate a “biological effect”. GESAMP (1993) recognizes the difference between contamination, the detection of contaminants, as defined by statistical significance from the baseline, and pollution, the “effects” of the contamination that are normally detected or measured in biota.

The discussion of the Hibernia EEM data will focus on TPH and barium for sediment chemistry data, sediment toxicity data, biological chemistry data, fish health indicators and taint study results. Other data such as particle size profile, carbon data (TIC/TOC) and other ancillary information will be discussed in the context of an interpretative tool for other data where appropriate.

10.1 Sediment Chemistry

10.1.1 Hydrocarbons

Sediment samples were analyzed for TPH fractions and a suite of PAHs and Alkyl PAHs. All PAH data were below the detection limit of 0.01 mg/kg and all alkyl PAH data were below the detection limit of 0.01 or 0.05 mg/kg, depending on the parameter. The TPH data consisted of three hydrocarbon fractions, C6-C10, >C10-C20 (the fuel range fraction) and >C21-C32 (the lube range fraction). Prior to the 2002 EEM Program, hydrocarbons were analyzed as TEH. The

fraction range for TEH was C11-C32, with C11-C20 representing the fuel range fraction and C21-C32 representing the lube range fraction.

The range of observed hydrocarbon results for Hibernia EEM programs since the baseline program in 1994 to date are provided in Table 10.1. As evident from Table 10.1, hydrocarbon methodologies were refined between 1994, the Baseline EEM program, and 1998, the first Production Phase EEM program. This resulted in improved detection limits that are demonstrated by the 1998 hydrocarbon minimum data limits (<0.5 and <0.25 mg/kg in 1998, as compared to <30.2 and <10 mg/kg in 1994).

Table 10.1 Hydrocarbon Data Summary for 1994 to 2009

Hydrocarbon Concentrations (mg/kg)	Years							
	1994	1998	1999	2000	2002	2004	2007	2009
TEH (C11-C32)	<30.2	<0.5 - 363	<0.5 - 472	<0.5 - 4170	*	*	*	*
C11-C20 (Fuel Range)	<10	<0.25 - 341	<0.25 - 454	<0.25 - 4100	*	*	*	*
C21-C32 (Lube Range)	<10	<0.25 - 24.1	<0.25 - 30.5	<0.25 - 69.5	*	*	*	*
TPH (C6-C32)	*	*	*	*	<3 - 798	<3 - 134	<3 - 81	0.4 - 60.5
C10-C21 (Fuel Range)	*	*	*	*	<0.25 - 793	<0.25 - 131	<3 - 78	<0.3 - 58
C21-C32 (Lube Range)	*	*	*	*	<0.25 - 6.43	<0.25 - 4.2	<3 - 7.1	<0.3 - 3.0

* The hydrocarbon fractions measured changed in 2002 to TPH. Hydrocarbon measurements pre-2002 where measured as TEH.

The highest hydrocarbon concentrations have been found at the 250-m stations, the stations closest to the Hibernia platform. The lowest hydrocarbon concentrations were found at the control stations (16,000 m), specifically along radial 7 for 1998-2000 data, with the lowest concentrations in 2002 at the control station (16,000 m) along radial 1. In 1998 and 1999, the highest concentrations were found at the 250-m station along radial 8. In 2000, the highest hydrocarbon concentrations were found at the 250-m station along radial 4. In 2002, the highest concentrations were found at the 250-m station along radial 2. In 2004, the highest concentrations were found at the 250-m station along radial 6. In 2007 and 2009 the highest concentrations were found at the 250-m station along radial 8.

The 2009 Hibernia EEM statistical analyses, like the 2002, 2004 and 2007 statistical analyses indicate that there was an overall decrease in hydrocarbon concentration levels when compared to the 2000 data. Results of the 2004, 2007 and 2009 hydrocarbon data are comparable to or below concentrations observed in 1998. The decreases in hydrocarbon concentrations are linked to reinjection of synthetic based drill cuttings, solids and muds. Concurrent with the reinjection of drill cuttings, solids and muds, produced water discharges increased significantly achieving a maximum discharge of 24,032 m^3 during this period. The increase in produced water discharge and associated hydrocarbons did not impact sediment hydrocarbon concentrations in a discernible manner.

10.1.2 Barium

The range of observed barium results for Hibernia EEM programs since the baseline program in 1994 to date are provided in Table 10.2.

Table 10.2 Barium Data Summary for 1994 to 2009

Barium Fractions (mg/kg)	Years							
	1994	1998	1999	2000	2002	2004	2007	2009
Barium (Total)	60 – 3,000*	< 5 - 1,400	< 5 - 1,000	< 5 - 3,100	56 - 1,400	57 – 780	56 – 600	60 – 1,400
Barium (Weak acid Leachable)	**	< 5 - 18	< 5 - 42	< 5 - 92	< 5 - 56	<5 - 82	<5 - 39	<5 - 47
* One sample located where Hibernia platform is currently located. Next highest value was 450.								
** Not conducted in 1994 during baseline EEM program.								

The highest total barium concentrations have been found at the 250-m stations, which are the stations closest to the Hibernia platform. The lowest total barium concentrations were found in the Far-field stations varying yearly from between stations at 6,000 m to the control stations (16,000 m). The 2004 and 2007 total barium concentrations are comparable to 1998 and 1999 concentrations. In 2009, concentrations of barium at the 250 m stations increased over 2007 concentrations and are similar to 2002 concentrations.

Improvement was noted in 2002 weak acid leachable barium concentrations that were comparable to 1999 levels. However, weak acid leachable barium concentrations increased in 2004 likely attributable to both drilling rigs discharging at the same time. In 2007, weak acid leachable barium concentrations once again decreased and are comparable to 1999 levels. In 2009, weak acid barium concentrations are similar to 2007 concentrations.

10.1.3 Hydrocarbon and Barium Data for International Fields

An overview of existing international TPH and barium data (Table 10.3) are presented to place the Hibernia results in context with the international data collected from a variety of field studies in the North Sea and Gulf of Mexico on the effects of SBMs. SBM is a non-aqueous system that uses a non-water soluble base fluid as the continuous phase, with brine emulsified and dispersed within the mud (IBP SHE Technical Committee 1999). Non-aqueous muds include diesel, mineral, low-toxicity minerals oils (LTMOs) and synthetic-based muds. SBMs possess virtually no PAHs and have a variety of specific chemistries that are designed to optimize drilling and environmental performance. Esters, ethers, acetals, paraffins, olefins and alkanes are examples of SBMs. The drilling fluid used at Hibernia, PureDrill IA-35, is an alkane synthetic-base fluid (Petro-Canada Technical Bulletin).

Table 10.3 International and Hibernia Data for Hydrocarbon and Barium in Sediments

Well Location	Synthetic Mud Type	Distance from Source (m)	TPH (mg/kg)	Barium (mg/kg)
Gulf of Mexico (NPI-895) (Candler *et al.* 1995)	PAO	50 200 2000	134,428 80 to 1,460 24	47,437 540 to 5,641 no data
Dutch Continental Shelf (K14-13) (Daan and Mulder 1996)	Ester	200	54 to 161	
Norway (Gyda 2/1-9) (Bakke *et al.* 1995)	Ether	100 to 200	236	
Norway (Tordis) (Gjøs *et al.* 1991)	PAO	500	8,920	
Norway (U/a 2/7-29) (Vik *et al.* 1996)	Acetal	200	1,000 to 2,368	
Hibernia (2000 Data, Hydrocarbon Data as TEH)	Alkane	250 500 2,000	472 to 1086 26 to 90 5 to 13	728 to 1,700 177 to 447 133 to 423
Hibernia (2002 Data, Hydrocarbon Data as TPH)	Alkane	250 500 2,000	< 3 to 798 < 3 to 58.9 < 3 to 22.7	150 to 1,300 130 to 540 110 to 590
Hibernia (2004 Data, Hydrocarbon Data as TPH)	Alkane	250 500 2,000	14.4 to 134 < 3 to 29.8 < 3 to 25.7	250 to 780 150 to 390 120 to 420
Hibernia 2007 Data Hydrocarbon Data as TPH	Alkane	250 500 2,000	10.7 – 80.6 0.3 – 16.0 <0.3 – 19.4	210 – 600 91 – 300 120 – 510
Hibernia 2009 Data Hydrocarbon Data as TPH	Alkane	250 500 2,000	4.0 – 60.5 0.9 – 11.4 0.8 – 6.8	180 – 1,400 110 – 310 110 - 400

It must be kept in mind while reviewing this information that these studies are not directly comparable. Biodegradation of base fluids and dispersion mechanisms for contaminants are directly influenced by factors such as mud type, seafloor water temperature, bottom currents, bioturbation, depth, sediment geochemistry, benthic boundary transport mechanisms and other factors. These factors vary greatly between the North Sea, the Gulf of Mexico and the Grand Banks, but the data serve to place the Hibernia results in context with other offshore oil developments. Another consideration that must be acknowledged when examining data from other studies is the field maturation and the volume of drill cuttings and solids discharged. The drill fluids used in these older fields may range from diesel-based muds, to oil-based muds (OBMs), to LTMOs to SBMs. Whenever possible, results are cited from sample locations comparable to the Hibernia sample locations with respect to distance from the contaminant source.

The first well drilled in the Gulf of Mexico using a SBM (PAO) was an exploratory well that was completed in June 1992 (Candler *et al.* 1995). Samples collected in June 1992 had TPH values ranging from 24 mg/kg (at 2,000 m from source) to 134,428 (at 50 m from source). Samples at the 200-m station from the NPI-895 well had TPH values ranging from 80 to 1,460 mg/kg.

In 1996 and 1997, as part of the first Norwegian regional monitoring program, 1,200 sediment sample stations were sampled, with approximately 4,000 samples analyzed for hydrocarbons and heavy metals (Bryne 2000). The TPH values ranged from 1 to 24,400 mg/kg, depending upon distance from point source.

Daan and Mulder (1996) found TPH values at 200 m ranged from 54 to 161 mg/kg on the Dutch Continental shelf for K14-13 site (ester SBM). Bakke *et al.* (1995) found 236 mg/kg at 100- to 200-m stations for Gyda 2/1-9 site (ether SBM), while Gjøs *et al.* (1991) reported TPH values of 8,920 mg/kg at 500-m stations for the Tordis site (PAO SBM). Johansen, as cited in Vik *et al.* (1996), found TPH values for Ula 2/7-29 (acetal SBM) varied between 1,000 to 2,368 mg/kg at the 200-m stations.

A review of the data indicates that the TPH values obtained for the 2009 Hibernia EEM program are less than those for other field studies from various international locations. The international results are used to place the Hibernia TPH concentrations in context are based on SBMs that are olefins, esters, or acetals, while the base fluid used at Hibernia is an iso-alkane. The ultimate fate of these drilling muds will vary depending upon a variety of factors, including biodegradation, environmental factors such as temperature, currents and depth, and sediment transport mechanisms.

Candler *et al.* (1995) indicated that barium levels for the exploratory NPI-895 well (PAO SBM) in the Gulf of Mexico (Table 10.3) ranged from 540 mg/kg at 25 m to 47,437 mg/kg at 50 m. Barium concentrations for the 200-m stations ranged from 542 to 5,641 mg/kg. Platform discharges near offshore oil and gas production platforms in the Santa Maria basin in California resulted in increased barium concentrations of 10 to 40 percent in the sediments and 200 to 300 percent in suspended materials (Steinhauer *et al.* 1994).

Barium concentrations, when analyzed in an appropriate manner, are a good indicator to identify sediment transport routes and potential geographical “sinks” for drilling discharges. They also help distinguish drilling waste contamination from other contaminant sources, such as produced water or fishing activities (Kaiser and Spencer 1996). This is particularly important for oil production platforms, where discharged cuttings are dispersed by strong tidal or wave-induced currents. Statistically significant increases in barium concentrations up to 65 km downstream were detected from exploratory wells on the Georges Banks (Neff *et al.* 1989a). Finally, barium may be the most enduring marker of SBMs that have base fluids (i.e., esters, PAOs, etc.), many of which are readily biodegradable (Hartley 1996).

The spatial distribution of barium contamination for many offshore production platforms is not well characterized due to limited scientific literature available for SBMs. Neff *et al.* (1989b) indicated that barium contamination could be detected up to 65,000 m and Olsgård and Gray (1995) indicated barium contamination was detectable up to 6,000 m. The major differences in reported spatial distribution of barium contamination may be due in part to concerns with barium analytical methods identified by Hartley (1996), the transport of barium contamination outside of study areas, or a combination of the two.

Modelling conducted for the Santa Maria basin drilling discharges predicted that 50 percent of the total mass of the drilling muds would be transported out of the study area (Phillips *et al.* 1998). The transport of the muds was considered reasonable given the predominant fine grain size of drilling muds and vigorous currents. The study area was limited to within 1,000 m of three production platforms within the Santa Maria basin. The model predicted that only the coarser fractions would be deposited within the study area and the silt and clay-sized particles

would be widely dispersed. The results of the Santa Maria study seem to validate the model predictions.

A review of the data indicates that the barium values obtained for the 2009 Hibernia EEM program are comparable to the ranges (low end) cited from other international locations.

10.1.4 Canadian Sediment Quality Guidelines

Canadian Sediment Chemistry Guidelines for the Protection of Aquatic Life have been developed by the Water Quality Guidelines Task Group of the CCME. These guidelines are numerical limits recommended to support and maintain aquatic life associated with sea bed sediments (CCME 2003). A comparison of the Hibernia EEM sediment chemistry data with the Marine Interim Sediment Quality Guidelines (ISQG) is provided in Table 10.4. All Hibernia sediment chemistry concentrations are lower than both the probable effects levels (PEL) and the ISQG.

The PEL is the concentration of a chemical above which adverse biological effects are expected to occur frequently (CCME 2003). The interim sediment quality guidelines (ISQG) are derived when data sets are available but limited, and the information gaps are clearly outlined which is the lesser of the CCME requirements. If the minimum data set requirements are met for the approach of the National Status and Trends Program, the ISQGs are derived using weight of evidence from the available toxicological information. The ISQG's may become full Sediment Quality Guidelines (SQG) once the scientific community have addressed the data gaps and when the ISQGs can be linked to specific sediment types, characteristics of the sediments or overlying water column. In other words, once a relationship is demonstrated to exist and is predictable under field conditions.

The development of the ISQGs relies on the compilation and analyses of all available North American data collected from various locations and for a variety of species that are normally associated with sediment (CCME 2003). Minimum toxicological data requirements are established thereby ensuring that the ISQGs are supported by weight of evidence linking chemical concentrations to biological effects. For each chemical a functional threshold level (TEL) is calculated to consistently determine a range of sediment concentrations for which no adverse biological effects are never or almost never observed. If the uncertainty associated with the TEL is high, a safety factor may be applied to the TEL. Otherwise the TEL is considered to be representative of the concentration below which adverse biological effects are not anticipated.

Safety factors are commonly used in the development of environmental quality guidelines (CCME 2003). The incorporation of safety factors is intended to achieve a more stringent estimate of sediment associated chemical concentrations that will not cause harm to aquatic organisms associated with the sediments over indefinite exposure periods. The size of the safety factor will reflect the types and uncertainties that are addressed by the data set. Thus the larger a safety factor, the larger the uncertainty is with respect to a particular data set. The uncertainties with data sets often involve differences within species due to age, life cycle stage, sex, and genetic variability of the organism; differences among species, differences in measured toxicity of a chemical due to end-point sensitivity; interpretation of data set endpoints;

factors controlling the bioavailability of the chemical; as well as the extrapolation of laboratory results to field conditions.

The Canadian ISQGs and PELs are flexible interpretative tools for evaluating the toxicological significance of sediment chemistry data. The resulting guidelines are intended as scientific benchmarks for the evaluation, protection and enhancement of sediment quality. As benchmarks, the guidelines assist in evaluating the significance of sediment chemical data, thereby allowing for the evaluation of the effectiveness of proposed management strategies in protecting the aquatic environment. Sediment chemical concentrations below the ISQGs are not expected to be associated with any adverse biological effects. Therefore, no adverse biological effects from the chemicals listed in Table 10.4 are expected to be associated with the Hibernia sediment.

Table 10.4 Comparison of 2009 Hibernia EEM Sediment Chemistry Data with Marine Sediment Quality Guideline

Parameter	Hibernia Sediment Chemistry Ranges (mg/kg)	Canadian Interim Sediment Quality Guidelines*	
		ISQG (mg/kg) PAH's in μg/kg	PEL (mg/kg) PAH's in μg/kg
Metals			
Arsenic	<2 – 3	7.24	41.6
Cadmium	<0.15 - 0.16	0.7	4.2
Chromium	<2 – 15	52.3	160
Copper	<2 – 4	18.7	108
Lead	1.3 – 5	30.2	112
Zinc	<5 - 13	124	271
Mercury	<0.01-0.01	0.13	0.70
PAHs			
Acenaphthene	<0.05	6.71	88.9
Acenaphthylene	<0.05	5.87	128
Anthracene	<0.05	46.9	245
Benz(a)anthracene	<0.05	74.8	693
Benzo(a)pyrene	<0.05	88.8	763
Chrysene	<0.05	108	846
Dibenz(a,h)anthracene	<0.05	6.22	135
Fluoranthene	<0.05	113	1,494
Fluorene	<0.05	21.2	144
2-Methylnaphthalene	<0.05	20.2	201
Naphthalene	<0.05	34.6	391
Phenathrene	<0.05	86.7	544
Pyrene	<0.05	153	1,398
Source: CCME 2003.			

10.1.5 2009 Sediment Chemistry Data Implications

The 2009 Hibernia EEM sediment chemistry data indicate that the hydrocarbon concentrations have returned to levels observed in 1998 and possibly to baseline levels of 1994. Total barium concentrations are at levels similar to 2002 while weak acid leachable barium concentrations are at levels similar to 1999. Hibernia commenced partial re-injection of SBM drill cuttings in 2000 (just prior to the 2000 EEM program) and the re-injection of cuttings has resulted in substantial improvement in sediment contaminant concentrations. In the third quarter of 2002, the cuttings reinjection (CRI) installation was completed which enabled approximately 95 percent SBM cuttings re-injection, and this operational change resulted in further decreases in the observed sediment contaminant levels around the Hibernia platform. The improvement in the sediment contaminant concentrations has continued as drilling waste discharges were significantly reduced and produced water discharge rates (since 2002) significantly increased.

Olsgård and Gray's (1995) examination of Norwegian EEM data concluded that there was a continuous increase in the total area affected by contamination, even several years after cessation of drilling waste discharges. This indicates factors other than drilling waste loading play an important role in the fate and effects of contaminants. These factors include, but are not limited to, mud type, particle size of the cuttings, hydrographic conditions, sediment characteristics and the rate of biodegradion of the drilling muds. These factors will most likely influence the spatial distribution and the rate of improvement in sediment contaminant levels around the Hibernia platform. Nevertheless, based on the 2009 sediment chemistry data and the operational changes instituted on the Hibernia platform, continuous improvements in the contaminant levels attributable to SBM drill cuttings and solids discharges are expected.

10.2 Sediment Toxicity

A suite of bioassays using both lethal and sublethal (growth, biomass, and light production) endpoints was employed to measure the potential toxicity of Hibernia sediments (i.e., to measure the effect of contamination). The bioassays used were the 10-day marine amphipod assay using *Rhepoxynius abronius* (mortality), the 20-day juvenile polychaete using *Neanthes arenaceodentata* (growth and biomass), and the Microtox solid phase using *vibrio fisheri* (light production). The amphipod and juvenile polycheate bioassays were two of the primary solid phase bioassays used for sediment toxicity assessments along the West Coast of North America (Anderson *et al.* 1998).

The amphipod and juvenile polychaete bioassays were conducted as per established protocols using recommended species that are appropriate to the sediment physio-chemical characteristics. These bioassays use indicator organisms that are not native to the Newfoundland region. The non-native species are used because the protocols dictate the species that can be used (Environment Canada 1998). The juvenile polychaete bioassay protocol was developed using non-native species because at the program initiation, no Canadian test method was available. Environment Canada has subsequently developed a test method for polychaete worms using the polychaete species *Polydora cornuta* (Environment Canada 2001). However, since all previous EEM programs used the polychaete species *Neanthes arenaceodentata*, Stantec recommends the continued use the *Neanthes* species for

direct comparability. Regardless, these species are still ecologically relevant to the Grand Banks, as they function as surrogate species to Grand Bank amphipod and polychaete species.

10.2.1 Amphipod Bioassays

The amphipod (a marine crustacean) bioassay using *Rhepoxynius abronius* has been demonstrated to be an effective tool to aid in the characterization of contaminated sediments (Swartz *et al.* 1991). Amphipods are among the most sensitive of benthic infauna to contaminants (Swartz *et al.* 1986); polychaetes (a marine worm) dominate benthic habitats and are key ecological constituents of benthic assemblages (Anderson *et al.* 1998).

Photo 8 Amphipod Bioassay in Progress

The determination of a toxic/non-toxic response for the amphipod bioassay (Photo 8) is based on toxicity test interpretative guidelines developed by Environment Canada (Environment Canada 1996). The amphipod survival test results are considered toxic when the endpoint (mortality) is 30 percent lower and statistically significantly different than the negative control (Whidbey Island sediment). Alternatively, reference stations (1-16,000 and 7-16,000) can be used and a 20 percent reduction target is used instead of the 30 percent target.

The amphipod survival bioassay had two responses in 2009 that were classified as toxic based on the criteria established by Environment Canada using the control sediment (Whilbey Island) and the reference station sediment (1-16,000 and 7-16,000). The two toxic responses occurred at stations 7-2000 and 7-3000 (Photo 9 and 10) with survival rates of 52 and 65 percent, respectively.

The only previously observed amphipod toxic response for the Hibernia EEM programs occurred in 2000. Station 4-250 in 2000 had the highest levels of barium and hydrocarbons (1,086 mg/kg) observed to date and were considered to be toxic as compared to the Hibernia reference stations (1-16,000 and 7-16,000). The literature provides examples of benthic organisms existing in contaminated sediments with total hydrocarbons in the 1,000 to 10,000 mg/kg range (Steinhauer and Imamura 1990). It has been reported that 1,000 mg/kg of TPH from synthetic muds was required before benthic community structure was affected (Candler *et al.* 1995). The 4-250 station in 2000 was the first demonstrated occurrence of habitat impairment observed for the Hibernia EEM program. However, the possibility of amphipod failures was considered and the null hypothesis was designed to reflect this possibility (H_0 No.1 stating that **"**Operational discharges from the Hibernia platform will not result in major biological effects (as measured by the amphipod survival assay) beyond the predicted impact zone of a 1,000-m radius around the production platform"). The 2002, 2004 and 2007 data have shown an improvement, with no toxic amphipod responses. This was anticipated, as there has been a decrease in sediment contaminant concentrations observed around the Hibernia platform, with concentrations returning to 1998 and 1999 concentrations.

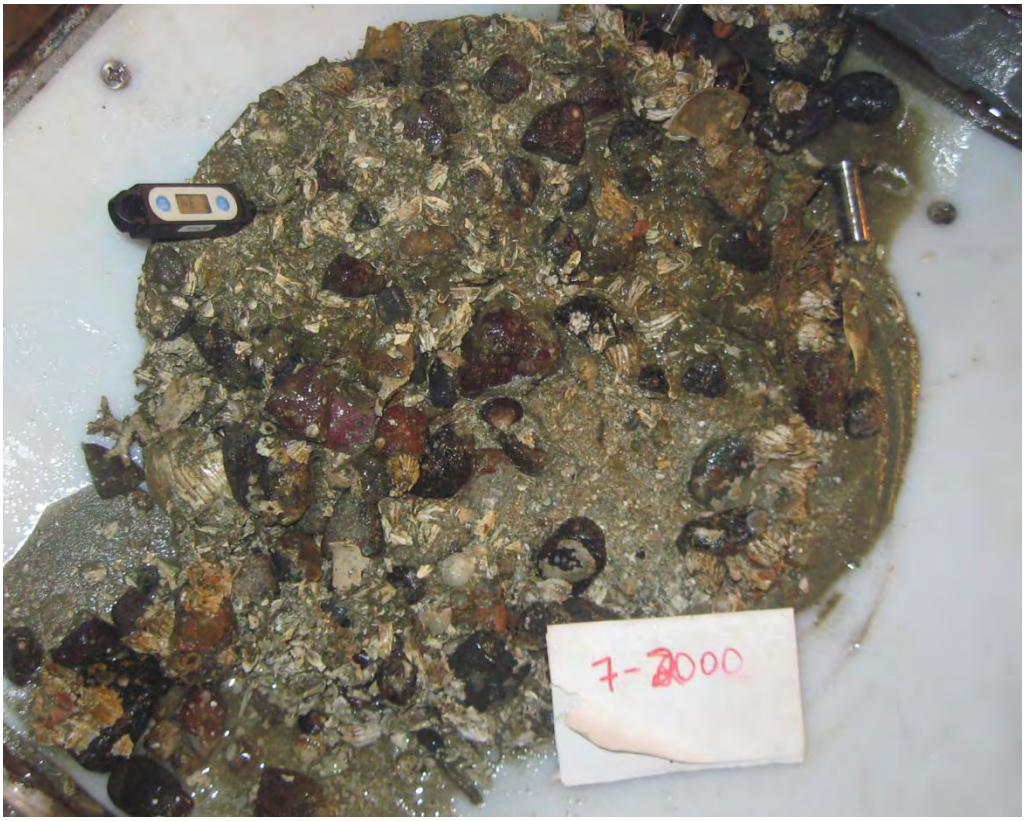


Photo 9 Sediment Type at Station 7-2000

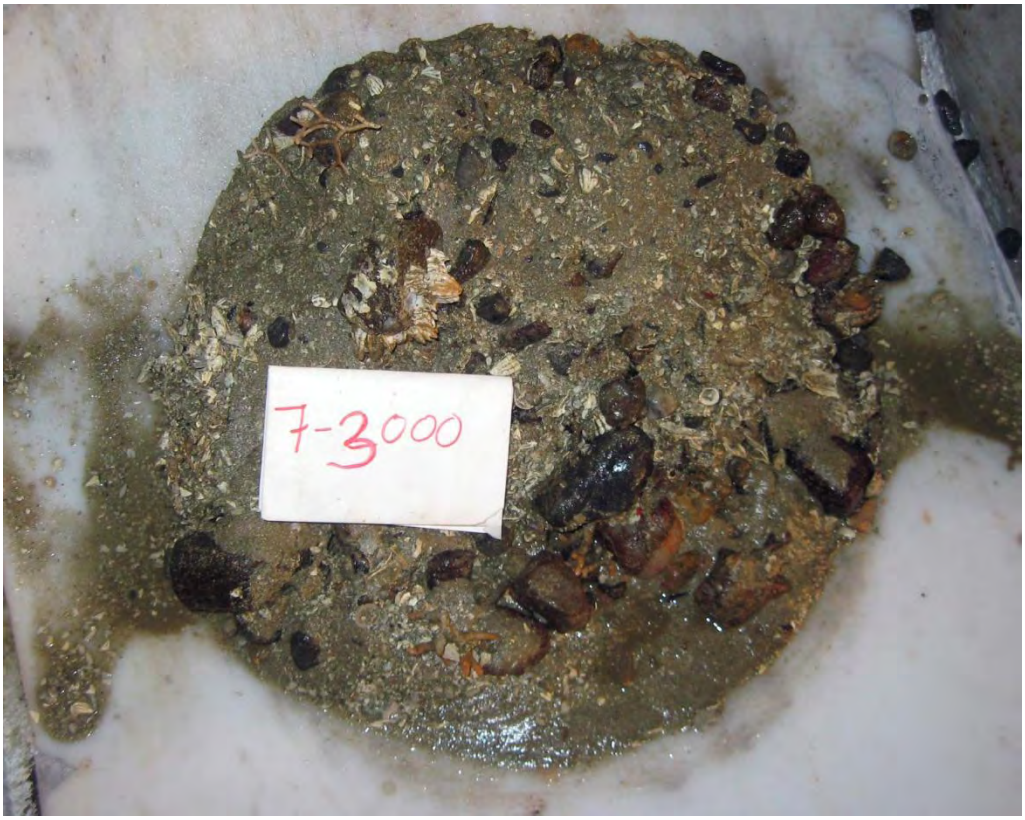


Photo 10 Sediment Type at Station 7-3000

The 2009 toxic responses unlike the 2000 toxic responses cannot be linked to Hibernia discharges. The BTEX compounds were below RDLs for 7-2000 and 7-3000). TPH and metal concentrations were similar to recent years and were at levels not expected to produce a response. Thus there is no readily discernable reason for the observed toxic responses at stations 7-2000 and 7-3000. It is important to note that these stations are also the stations which have traditionally had Microtox responses.

10.2.2 Juvenile Polychaete Bioassays

Using a similar toxicity interpretation guideline used for the amphipod bioassay (HMDC 1999) the juvenile polychaete bioassay would be considered toxic when the endpoints (biomass and growth) are 30 percent lower and statistically significantly different than the negative control (silica sand). Alternatively, reference stations (1-16,000 and 7-16,000) can be used and a 20 percent reduction target is used instead of the 30 percent target.

Photo 11 Polychaete Worms at Day 20 Prior to Dry Weight Determination

The result of the 2009 juvenile polychaete lethality tests (Photo 11) based on the criteria established results in three stations classified as toxic. The three responses occurred at stations 2-250, 7-2000 and 7-3000 with survival rates of 56, 24 and 16, respectively. The remainder of survival rates ranged from 64 to 96 percent. There was no significant difference in the average juvenile polychaete growth result for stations within 500 m of the platform (32.20 mg/worm) when compared to the overall average for all stations (30.42 mg/worm) or to the average growth at stations located between 2,000 m and 16,000 m of the GBS (32.42 mg/worm).

The two toxic responses observed at station 7-2000 and 7-3000 (Photo 9 and 10) in 2009 cannot be linked to Hibernia discharges. As noted in Section 10.2.1, the chemical contaminant concentrations are at levels not expected to produce a response and as such there is no readily discernable reason for the observed toxic responses at stations 7-2000 and 7-3000. The toxic response observed at 2-250 (Photo 12) is equally puzzling as the surviving polychaete worms exhibited one of the highest growth rates with a mean growth rate (mg/worm) not that different than at the reference stations (41.58 mg/worm for 2-250 as compared to 46.51 mg/worm at reference stations). Based on these results it is possible that some of the juvenile polychaete worms may have been “weaker” for some reason despite health criteria being achieved.

Photo 12 Sediment Type at Station 2-250

The data for the juvenile polychaete bioassays are extremely variable and interpretations must be

applied with caution. This is demonstrated by the fact that in three of the four 250 m, 25 stations had higher growth rates than two of the four 16,000 m station. Anderson *et al.* (1998) examined the results of 341 split samples from the west coast of North America that were subjected to amphipod and juvenile polychaete bioassays. A review of the data indicated that there is a high between-replicate variability associated with the juvenile polychaete bioassay survival, biomass and growth endpoints that limited the statistical power of the tests. This high between-replicate variability has also been observed for the Hibernia EEM test data.

Several sources of variability are inherent in the test design, including initial worm weight, which may contribute to the between-replicate variability (Anderson *et al.* 1998). Minimizing several sources of variability, including food type, feeding regime, organism history and handling, organism culture and acclimation, and analyst experience may help reduce between-replicate variability. Anderson *et al.* (1998) noted considerable between-replicate variation occurred in the size of the worms at the end of the bioassays, which was also noted in the Hibernia samples. The reason this occurred was not clear, because feeding rates were the same for replicates and samples (Anderson *et al.* 1998).

10.2.3 Microtox Bioassays

The bacterial bioassay (Microtox) toxic/non-toxic interpretation guideline established for Hibernia sediment samples was that an IC50 response of <40,000 mg/L is considered to be toxic. This value accounted for baseline observations that produced Microtox responses, as well as the Hibernia sediment particle size profile. In 2002, Environment Canada published a new reference method for Solid Phase Microtox Testing (Photo 13). The new reference method (Environment Canada 2002) contains new interim guidelines for assessing Microtox toxicity.

Photo 13 Microtox Analyzer (m 500)

All of the Hibernia data was analyzed based on the original value of <40,000 mg/L to determine a toxic/non-toxic response, as the <40,000 mg/L value was derived using similar principles that are the basis of the new Environment Canada (2002) interim guidelines for solid phase Microtox. Therefore, it is felt that the current value of <40,000 mg/L toxic/non-toxic interpretation guideline is still relevant and applicable.

Historically, three sediment profiles occurred at the Hibernia site. These sediment profiles were described on the basis of their particle size indices (particle size profiles 4.1 to 4.4). The primary sediment profile is dominated by sand (>98 percent) and accounts for approximately 70 to 75 percent of the Hibernia EEM stations. The secondary sediment is a mixed sediment profile comprised of approximately 15 to 45 percent gravel, 40 to 74 percent sand and 10 to 15 percent fines (silt/clay). Approximately 15 percent of the Hibernia EEM stations fit this profile. The remaining 10 percent of the Hibernia EEM stations had sediment profiles that were transitional between the primary and secondary stations types. The transitional stations were comprised of approximately 75 to 98 percent sand, ≤10 percent gravel and <2 percent fines (silt/clay).

In 2009, 68 percent of the stations were of the primary sediment type, 20 percent were of the secondary sediment type and 12 percent were transitional. An important difference is that in 2009, the secondary sediment type had a lower level of fines than had previously been associated with this sediment type.

The 2009 Hibernia EEM program had eight Microtox responses at stations 1-6,000, 3-500, 3-2,000, 4-1,000, 7-2,000, 7-3,000 and 7-6,000. All the 2009 Microtox responses occurred at stations with secondary sediment profile and is consistent with previous EEM programs for which Microtox responses were observed at stations with either secondary or transitional sediment profiles. In addition to the secondary or transitional sediment profiles, higher levels of strontium, TIC, TOC and ammonia characterize the 2009 stations for which Microtox responses were observed. This is consistent with previous Hibernia EEM programs.

Photo 14 **Secondary Hibernia Sediment Type (Station 7-6000)**

Photo 15 **Primary Sediment Type (Station 3 -3000)**

The elevated metals, strontium (TIC/TOC) and particle size profile at the secondary sediment type as illustrated by Station 7-6000 (Photo 14) has been observed for all sampling sessions, including the baseline studies. A second, but important difference, associated with the secondary sediment type, has been the presence of shell fragments (based on visual observations of the sediment grabs and not substantiated by analysis). Elevated metals are quite common for areas that have a high content of shell fragments (J. Ray, pers. comm.), particularly strontium (Maxxam Analytics, pers. comm.). The dominant sediment type encountered during the Hibernia EEM is illustrated by Station 3-3000 (Photo 15).

Many trace metals are associated with calcium and are incorporated into the shell during development. This accounts for the elevated metals at the secondary sediment types and may account for elevated inorganic carbon. The soft tissues that once would have been associated with the shells may account for the elevated carbon levels.

The 2004 Microtox toxic responses (IC50 responses of <40,000 mg/L) were observed in sediments with more than 1.5 percent silt and clay fraction. Conversely, none of the samples from sediments with less than 1.5 percent silt and clay were classified as toxic (IC50 responses of >40,000 mg/L) using the Microtox test. Similar results were noted in the 1999, 2000 and 2002 toxicity data, but the relationship did not hold for 1994, 1998, 2007 and 2009 data (Figure 4.14). No causal relationship could be ascertained linking Microtox responses with Hibernia platform discharges.

10.2.4 ROV Environmental Survey

A remotely operated vehicle (ROV) environmental seabed survey was undertaken on June 20, 2010 at Stations 7-2000 and 7-3000. The goal of this survey was to provide a visual inspection of the seabed at these locales to aid in the interpretation of the toxic responses observed during the 2009 EEM program. The ROV survey consisted of the examination of the seabed within 50 m of the target coordinates with straight-line transects crossing the 50 m radius to ensure full visual coverage was achieved. The survey report (Pro-Dive Marine Services 2010) contains a DVD of the entire survey, which is provided in Appendix G.

The ROV survey indicated that both stations 7-2000 and 7-3000 (at a depth of approximately 80 m), was comprised of small to large cobble, with sporadic large boulders and minimal amount of visible sand and fines. Much of the cobble is colonized by barnacles, various algal species and other macrobenthic organisms. Based on a review of the video, a thriving macrobenthic environment was observed at these stations and included crabs (both *Hyas* species and *Chionocetes opilo*), ascidians, barnacles, tunicates, sea urchins (*Stongylocentrocus droebrachiensis*), a variety of large sea stars, sea sponges, shrimp, jellyfish, corals, and variety of algal species (Photo 16). Several different shell types were observed along the bottom (Iceland and sea scallop, propeller clam, other clams shells and whelk shells), many of which were empty. A couple of cod, a sculpin, and several unidentified fish were also observed.

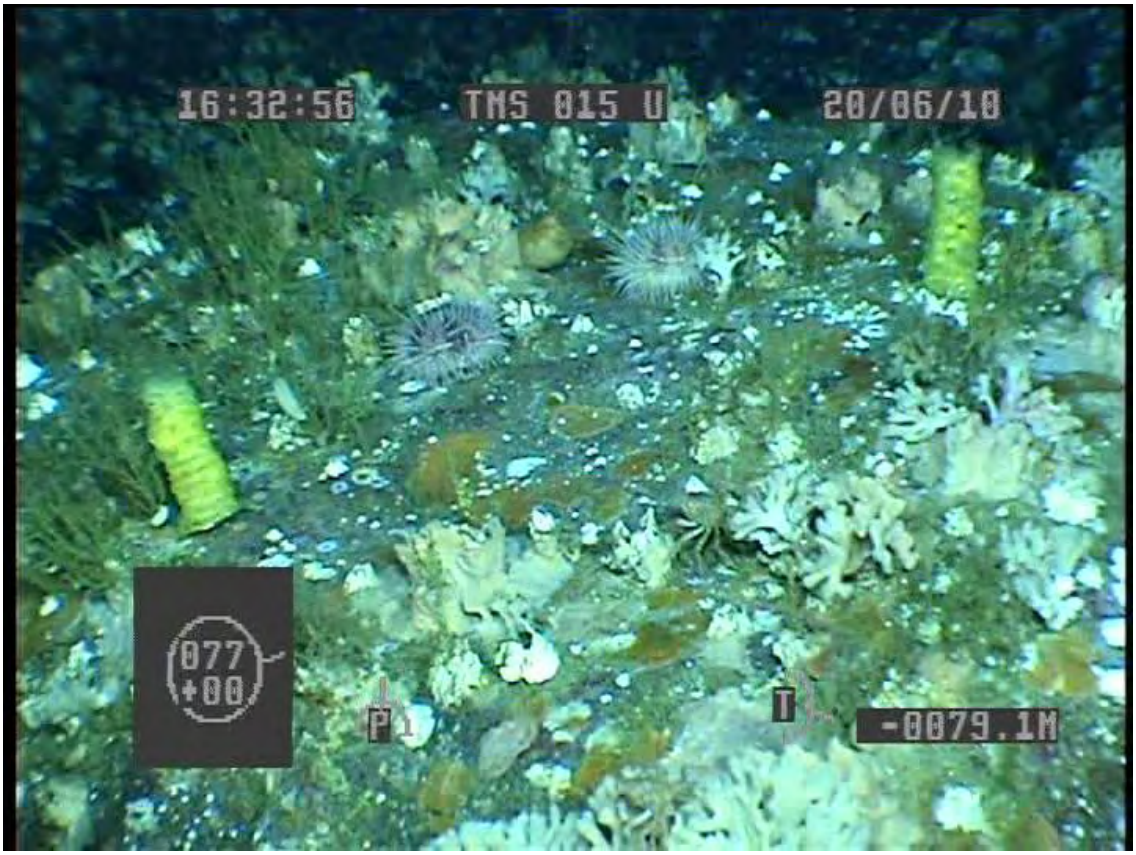


Photo 16 Boulder with an Example of Biota Found at Stations 7-2000 and 7-3000.

While it is recognized that the ROV survey occurred approximately 10 months after the 2009 EEM Program, there was no visually identifiable reason for the toxic responses observed at station 7-2000 and 7-3000 during the 2009 EEM. This coupled with the fact that the chemical contaminant concentrations measured for stations 7-2000 and 7-3000 were at levels not expected to produce a toxic response, there is no readily discernable reason for the observed toxic responses and no causal link to Hibernia discharges could be established.

10.3 Water Column Quality Program

The presence of increased trace metal, hydrocarbon and PAH concentrations in proximity to the Hibernia platform is considered to be related to the discharge of produced water. However, the low measured concentrations and the very limited spatial extent of these observations leads to the conclusion that a zone of potential effects is localized near the produced water discharge area (within 50 m or less from the discharge point).

BTEX hydrocarbon levels during the 2009 water program were found to range from ND to 32.8 µg/L within 50 m of the produced water outlet. BTEX levels from samples collected at 50 m and beyond were non-detectable. TPH levels were non-detectable at all near-field (50 m from produced water outlet) sample stations. However, an anomalous result occurred for the mid-depth sample at the 100 m sample station where lube range hydrocarbons were detected at the RDL of 0.1 mg/L. Lube range hydrocarbon concentrations were below RDL for all other stations including near-field stations.

The Hibernia produced water modelling indicated that dilutions ratios ranging from 1:150 to 1:1,600 would occur by 50 m from the discharge point (Lorax Environmental 2004). Based on the dilution rates cited in the model, TPH levels ranging between 16 to 160 µg/L would be expected to occur at a distance of 50 m from the produced water discharge point (Figure 7.5).

Thus, the Hibernia produced water model has been shown to be valid for the near-field stations, since the actual field validation concentrations (August 2009) for the hydrocarbon levels (TPH and BTEX) are lower than those predicted in the model.

The Lorax produced water model predicts produced water concentrations of 0.01 to 0.06 percent at 100 m. All parameters listed in Table 7.4 are non-detectable at the 100 m station with the exception of iron and lube range hydrocarbons. Iron concentrations are practically identical to concentrations observed at the reference stations, and can be assumed to represent ambient sea water concentrations. The detection of lube range hydrocarbons at 100 m results in a calculated produced water concentration of 3.7 percent; well above the predicted 0.01 to 0.06 percent concentration. Given the lack of an identifiable produced water plume at 100 m (as illustrated by the CTD cast in Volume II, Appendix K) and the absence of detectable concentrations of lube range hydrocarbons in the near-field; this result is considered to be an anomaly and does not constitute invalidation of the Lorax model.

The Lorax produced water model results are considered to be conservative in that the dilution of produced water within the seawater return does not take into account the turbulent kinetic energy contained in the produced water flow. This residual energy should produce greater dilutions than predicted, as it is not possible to estimate the effect of residual energy with confidence. In addition, other mechanisms besides dilution (adsorption on particulates,

evaporation, biodegradation, etc.) that act to reduce pollutant concentrations in the water column were not considered in the modelling study.

10.3.1 Produced Water

The two primary components of produced water that are of environmental concern are the low molecular weight aromatic hydrocarbons particularly naphthalene and the BTEX fraction of TPH analysis, particularly benzene (Berry and Wells 2004; Neff 2002). BTEX is soluble in seawater and highly toxic to marine organisms. However, there is minimal exposure risk to marine organisms given the rapid loss due to evaporation, adsorption and sedimentation, biodegradation and photolysis (Johnsen *et al.* 2004). PAHs are less soluble but more persistent in the environment (Holdway and Heggie 2000). The associated toxicity to marine organisms are primarily related to benzene and naphthalene (Brand *et al.* 1989, as cited in Holdway and Heggie 2000).

Naphthalene fractions are rapidly degraded in the water column (Johnsen *et al.* 2004). Low-molecular weight PAHs are the dominant fraction of produced water; these fractions degrade more readily than the high-molecular PAH fractions, which generally have a more specific toxicological nature, potentially interacting with cellular protein and DNA (Neff 2002; Johnsen at al. 2004). However, their concentrations in a produced water plume are very low due to the rapid dilution following discharge, and are rarely at levels high enough to cause toxic effects in marine plants and animals (Neff 2002; Johnsen *et al.* 2004). Modelling and field sampling conducted for HMDC confirm this finding as illustrated in Figures 7.5 and 7.6.

It is recognized that produced water can be toxic at its discharge point, although this will vary within fields and among fields. However, the potential effects upon the receiving environment are limited to immediately adjacent to the discharge point. The dilution effects of the receiving environment on produced water concentrations can be significant. The produced water concentrations that can be expected in the receiving environment from Hibernia discharges based on the dilution factors as modelled by Lorax Environmental (2004) are presented in Table 7.3.

Produced water will be rapidly diluted upon release to the receiving environment. The predicted produced water concentration based on results of modeling of a discharge rate of 27,000 m^3/day (Lorax 2004) should result in produced water concentrations of 0.06 to 0.7 percent within 50 m of source. As noted in Section 7.3.2, these predicted concentrations correspond well with field based concentrations of <0.01 to 0.72 percent, which were calculated based on the estimate of dilution factors reflected in Table 7.4. At these concentrations the produced water will not be toxic to marine organisms except immediately adjacent to source, if at all.

Dispersion modelling studies conducted world-wide all predict a rapid dilution in the range of 30- to 100-fold within the first tens of metres of the discharge point, followed by slower dilution rates (Terrens and Tait 1993; Brandsma and Smith 1996; Neff 2002). The factors that influence the actual plume dynamics upon discharge are discharge rates, ambient current speed, tidal factors, wind-driven surface current, turbulent mixing regimes, water column stratification, water depth, density of the plume, chemical composition, and discharge pipe diameter. These factors

result in variable produced water plume concentrations that vary over space and time. Adding to this produced water plume variability is the fact that Hibernia produced water plume dynamics are strongly influenced by the seawater return discharge. Nevertheless, dilution rates observed for Hibernia Produced Water discharges are similar to dilution rates that have been found internationally.

Field validation studies of produced water discharge dispersion have verified that dilution is rapid (Neff 2002). Field validation studies conducted worldwide have used a variety of means to monitor produced water plume dilutions ranging from tracer dyes, radium, hydrocarbon concentrations, BTEX compounds, polycyclic aromatic hydrocarbons (PAH) components, mussels, semi-permeable membrane devices (SPMDs), and trace metals (specifically barium, iron and manganese). A summary of these studies are presented in Table 10.5.

Table 10.5 Produced Water Field Validation Data

Study	Method	Conclusion
Continental Shelf Associates 1993	radium 226	426-fold dilution at 5 m 1,065-fold dilution at 50 m
Smith *et al.* 1994	dye tracer	100-fold dilution at 10 m 1,000-fold dilution at 103 m
Somerville *et al.* 1987	dye tracer	100-fold dilution at 50 m 2,800-fold dilution at 1,000 m
Brooks *et al.* 1980	benzene	150,000-fold dilution at discharge
Terrens and Tait 1996	BTEX/PAHs	Varied depending upon fractions Ranged from 2,000- to 53,000-fold dilution
Holdway and Heggie 1998	benzene and toluene	10,000-fold dilution at 1,000 m
Rabalais *et al.* 1991; 1992	Barium	Elevated levels found in sediment out to 1,000 m of discharge
US Department of Energy 1997	benzene	Various fields: 41- to 260-fold dilution at 5 m 150- to 3,400-fold dilution at 100 m 4,900- to undetectable at 2,000 m
HMDC EEM Report (2009)	TPH	139 to >14,400 < 50 m from the platform
Source: as reported in Neff (2002) except HMDC (2009).		

The most sensitive of the toxicity tests conducted on Hibernia's produced water was the bacterial bioluminescent assay, Microtox (Table 7.5). The closest and safest (given the sea state at time of sampling) 2009 water column sampling location was 33 m from source. Based on the toxicity data and calculated produced water concentrations (Table 7.3) a potential zone of effects is not expected beyond 33 m and should not produce toxic responses. The Microtox EC50 value for Hibernia produced water at the time of the water quality program was 4.07%. This is above the produced water concentrations which are calculated in Table 7.4. Thus, any toxic responses should be limited to immediately adjacent to the source and less than 33 m.

In addition to toxicity testing conducted as per the OWTG (2002) J. Payne (pers. comm.) exposed crab larvae (20 per dilution) for a 24 hour exposure period to Hibernia produced water at dilutions of 50, 25, 10, 5 and 0 percent. There was 100 percent survival of the crab larvae for all dilutions, indicating that the Hibernia produced water was not acutely lethal to crab larvae at the exposure concentrations tested.

The scientific literature indicates that toxicity of produced water is related to the produced water's chemical composition and varies widely, ranging from non-toxic to toxic. The causative agents of the observed toxicity in produced waters are not known. However, it has been theorized that the toxic responses may be related to extremely high dissolved solids (salinity) concentrations, altered ratios of seawater ions, elevated ammonia (Moffitt *et al.* 1992), hydrocarbons, hydrogen sulphide and volatile compounds (Sauer *et al.* 1992). Research has indicated that crustaceans are usually more sensitive to produced water effects than fish (Neff 1987; Terrens and Tait 1993; Jacobs and Marquenie 1991), with mysiids appearing to be as sensitive as or more sensitive than other species. Given the rapid rate of dilution and dispersion of most produced waters upon discharge to the receiving waters, most produced waters would be expected to have minimal adverse biological effects in the environment.

Research has indicated that aqueous soluble hydrocarbons specifically benzene, toluene, ethylbenzene and xylenes (BTEX) as well as low molecular weight-polycyclic aromatic hydrocarbons, particularly naphthalene are the substances of concern associated with produced water (Berry and Wells 2004). Berry and Wells (2004) conducted an integrated model for exposure assessment of produced water on the Sable Island Bank. The integrated model showed that soluble benzene and naphthalene fractions of the aqueous soluble hydrocarbons achieved chronic no-effect concentrations at distances of 1.0 m from discharge point.

Exposure in an environmental context is defined as the contact between a stressor (for produced water, primary concern would be aqueous soluble hydrocarbons) and a receptor (biological organisms). The characterization of exposure is the relationship and magnitude that a stressor exerts on a receptor. Toxicity tests are a surrogate by which exposure can be characterized. One particular measure of exposure that is of particular interest is the no-effect scenario that are measured by the comparison of stressor levels that indicate no significant adverse effects as compared to controls, commonly referred to as the no-observed effects concentrations (NOEC).

The data in Table 10.6 provides additional evidence that any toxic responses would be limited to immediately adjacent the discharge point. The aqueous soluble hydrocarbon concentrations from the 2009 water column program were significantly lower than the NOEC concentrations indicating that the produced water had been significantly diluted and was not toxic 33 m from the discharge point.

Table 10.6 Aqueous Soluble Hydrocarbon Concentrations and Experimentally Derived NOEC Data[(a)]

Chemical	NOEC (µg/L)[(a)]	2009 Water Column Concentrations (µg/L)	2009 Produced Water Concentrations (µg/L)
Benzene	700	1 – 46	14,400
Toluene	5,000	1 – 28	8,900
Naphthalene	620	0.2 – 0.7	118.1
Anthracene	710	<0.01	<0.1
Phenathrene	4.6	0.01 – 0.06	18.2
[(a)] Berry and Wells 2004			

Although, the concentrations of the key stressors (noted in Table 10.6) for end of pipe produced water were significantly higher than all the NOEC concentrations except for Anthracene and Naphthalene, it is demonstrated above that chronic no-effect concentrations are achieved very close (within 33 m) to the Hibernia platform (discharge point) which is consistent with the scientific literature.

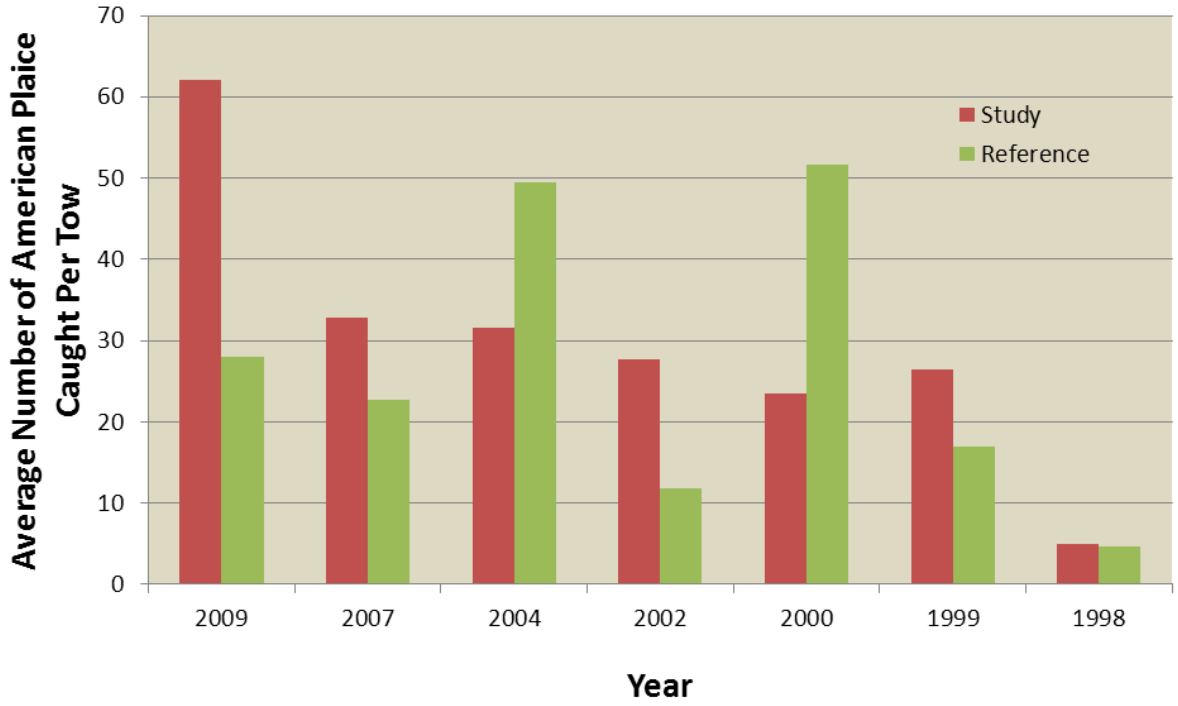
10.4 Fish Catches

Over the course of environmental effects monitoring at Hibernia (1998-2009), a number of important methodology changes have been implemented during the commercial fish programs. These changes affect the comparability of data over time, especially the catch per unit of effort data for smaller bodied species. A summary of methodology changes are provided below.

The 1999 biological program was changed from late fall/early winter to late spring/early summer in an effort to improve catch rates of American plaice. Improved catch rates were observed and therefore the change was made permanent. Also, during the 2004 and 2007 biological programs, the cod end liner was removed in an effort to reduce the amount of time associated with the processing of non-target species. The cod end liner was used in all previous biological programs and its removal in 2004 and 2007 likely reduced the catch rate of smaller-bodied species. In 2009, a commercial otter trawl with a codend mesh size of 152 mm was used. It is likely that this further reduced the catch rate of smaller bodied organisms. For this reason the comparison of CPUE over time is limited to American plaice only.

An indication of change in CPUE over time (1998 to 2009) for American plaice at the Hibernia Area and the Reference Area is illustrated in Figures 10.1. CPUE is expressed as the average number of American plaice caught per otter trawl tow per year.

Figure 10.1 Average Catch of American Plaice per Otter Trawl Tow at the Hibernia Area (All EEM Years)



The state of maturity at length for female and male American plaice at the Hibernia and Reference Areas are presented in Table 10.7. A graphic representation of the data is presented in Figures 10.2 and 10.3. A total of 434 American plaice were caught at the Hibernia Area, with 50 fish sampled for state of maturity. Of the 50 fish sampled, 3 were immature, 11 were maturing and 36 were spent. Of 32 females sampled at the Hibernia Area, 3 were immature, 7 were maturing and 22 were spent. Of 18 males caught at the Hibernia Area, 4 were maturing and 14 were spent. All immature females are less than 44 cm in length; no immature males were sampled.

A total of 199 American plaice were caught at the Reference Area with 50 fish sampled for state of maturity. Of the 50 fish sampled, 12 were immature, 14 were maturing and 24 were spent. Of 31 females caught at the Reference Area, 11 were immature, 11 were maturing and 9 were spent. Of the 19 males, 1 was immature, 3 were maturing and 15 were spent. All immature females were less than 44 cm, the immature male was less than 34 cm.

Table 10.7 State of Maturity at Length for Female and Male American Plaice at the Hibernia and Reference Areas

Sex and state of maturity	Hibernia Area							Reference area						
	Length Class							Length Class						
	25-29	30-34	35-39	40-44	45-49	50-54	Tot	25-29	30-34	35-39	40-44	45-49	50-54	Tot
Female														
Immature	0	0	2	1	0	0	3	0	0	0	11	0	0	11
Maturing	0	0	0	4	1	2	7	0	0	5	3	1	2	11
Spent	0	0	1	11	9	1	22	0	0	5	0	3	1	9
Total Female	0	0	3	16	10	3	32	0	0	10	14	4	3	31
Male							0							
Immature	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Maturing	1	2	1	0	0	0	4	0	2	1	0	0	0	3
Spent	0	5	6	3	0	0	14	1	6	8	0	0	0	15
Total Male	1	7	7	3	0	0	18	1	9	9	0	0	0	19
Total Fish	1	7	10	19	10	3	50	1	9	19	14	4	3	50
Maturity codes:														
Female:								Male:						
Immature includes DFO maturity code 500.								Maturing includes DFO maturity code 140, 180 and 190.						
Maturing includes DFO maturity code 520, 530,540 and 580.								Spent includes DFO maturity code 150, 160 and 170.						
Spent includes DFO maturity code 550, 560 and 570.														
Note: A description of DFO maturity codes is included in Appendix F, Volume II.														

Figure 10.2 State of Maturity at Length at the Hibernia Area

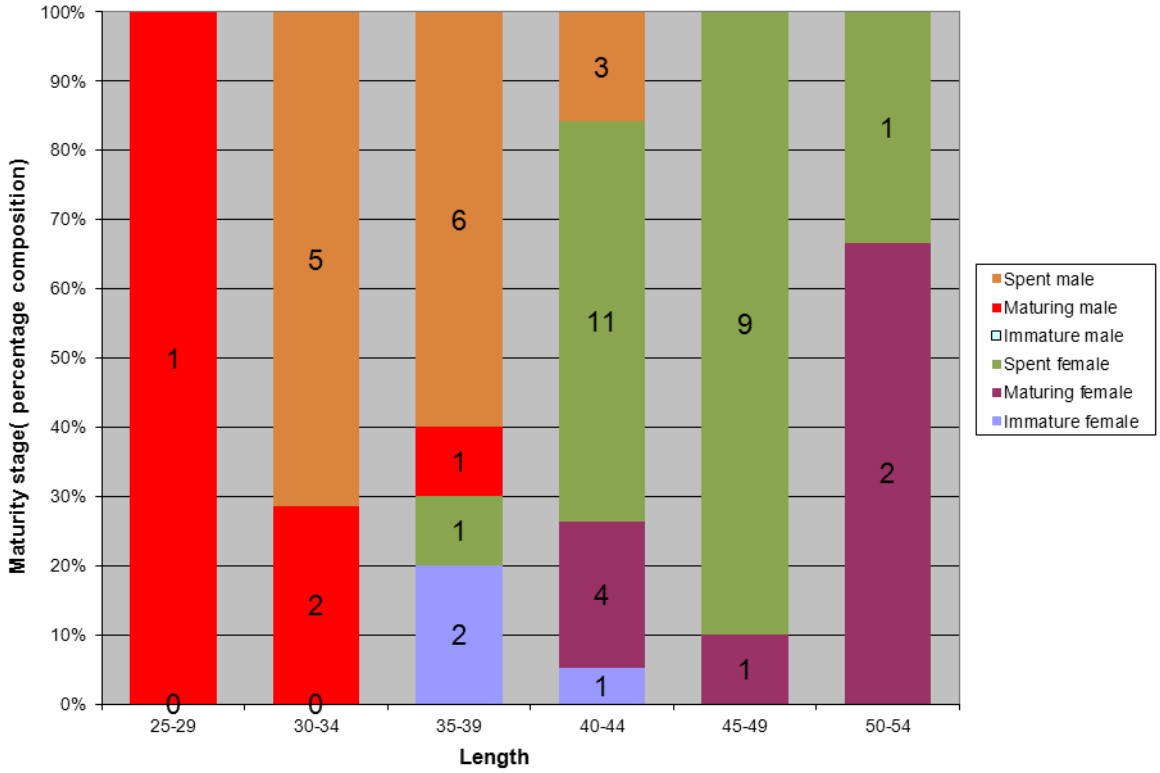
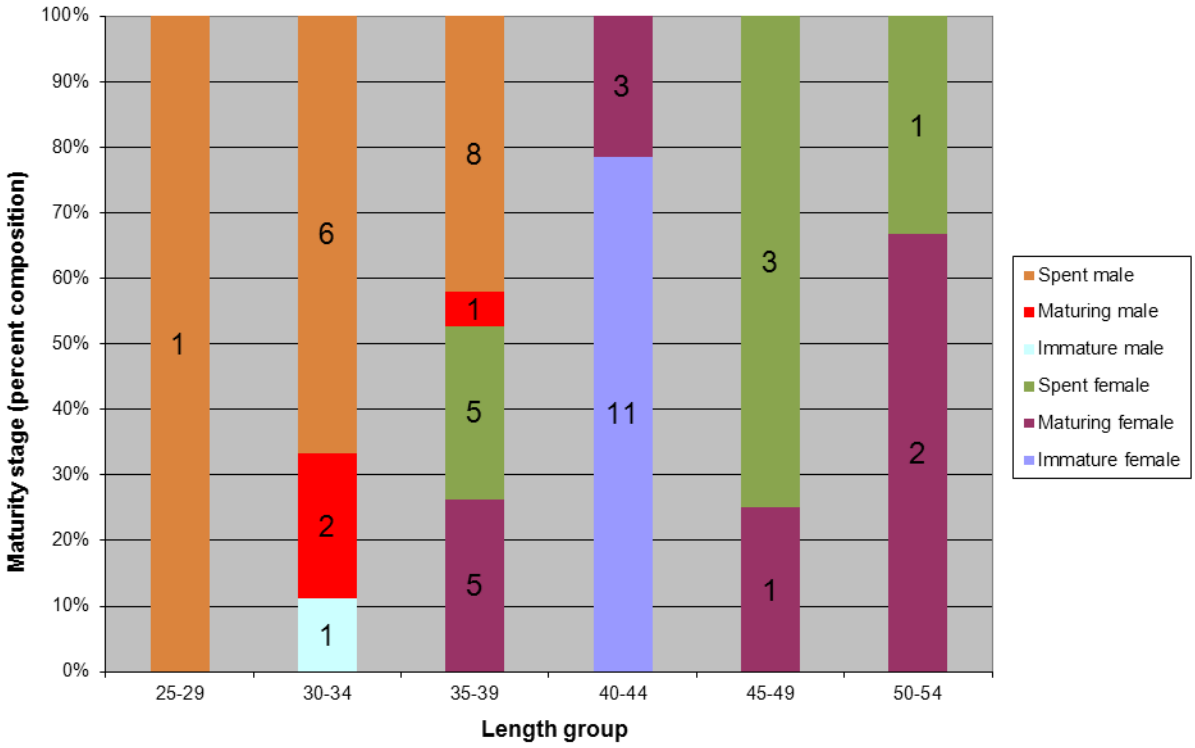


Figure 10.3 State of Maturity at Length at the Reference Area



10.5 American Plaice Chemical Profiles in Tissues

American plaice liver and muscle tissues were analyzed for a suite of metals, PAHs, alkyl PAHs and TPHs. A factor that must be considered is the 1999 and subsequent biological collection programs were conducted in June-July, whereas previous programs were conducted in November-December. The change in the collection time of the biological specimens may result in confounding factors, which are tied to seasonality and not Hibernia activities per se. The change in collection time was based on a recommendation following the 1998 EEM program, to enable the collection of a larger sample size of biological specimens for analyses. The change in the collection time from November/December to June was effective, resulting in an increased catch success, and has been permanently adopted.

10.5.1 Hydrocarbons Concentrations in Biological Tissues

As for past EEM programs, all hydrocarbon data for fish muscle were below the RDL for all Hibernia Area and Reference Area samples.

10.5.1.1 Fuel Range Hydrocarbons

In 2009, as in 2007, all the fuel range hydrocarbon data in fish liver were above the RDL, which was not the case in previous years (concentrations of fuel range hydrocarbons were statistically higher in 2009 than 2007). However, no statistically significant difference for fuel range hydrocarbons in liver were determined between Reference Area and Hibernia Area samples in 2009. Since the statistical difference applied to fish caught at both the Hibernia Area and Reference Area, the increase in concentrations are likely natural in origin and do not reflect bioaccumulation of petroleum derived hydrocarbons.

10.5.1.2 Lube Range Hydrocarbons

Lube range hydrocarbon concentrations were significantly higher in 2009 than in 2004 and 2007. However, the statistically higher concentration applied to both Hibernia Area and Reference Area samples, indicating that the higher concentrations are likely natural in origin.

10.5.1.3 PAH and Alkyl PAH

In 2009, six PAHs were detected in liver samples from both the Hibernia and Reference sites. This represents the first time PAHs were detected in any EEM program. The PAH 2-methylnaphthalene was the most commonly detected PAH (detected in five livers in both the Hibernia Area and Reference Area) indicating it is likely natural in origin.

10.5.1.4 Metal Concentrations in Biological Tissues

Year effects for increased or decreased metal concentrations in both American plaice livers and muscles have been observed with no pattern emerging. The annual differences are likely due to natural variability.

10.6 Taint

The triangle test data for 2009 indicates that panellist could identify a difference between the Hibernia Area and Reference Area samples. However, ancillary comments by panellist did not identify any abnormal / foreign odour or taste, confirming that American plaice samples do not have hydrocarbon tainting. This was confirmed by the Hedonic scaling test.

10.7 Fish Health

Cellular and sub-cellular bioindicator responses along with observations on visible lesions on skin and internal organs are valuable monitoring tools for identifying adverse health conditions in animals in advance of population level responses. As such, they can provide early warning of potential health effects and aid in identifying their nature, scope and cause (see reviews by Payne *et al.*, 1987; Peakall, 1992; Society of Environmental Toxicology and Chemistry (SETAC) Special Publication Series 1992; Adams, 2002; Tillitt and Papoulias, 2003; Schlenk *et al.*, 2008). They are of special value in this regard for use in EEM programs around development sites on important fishing grounds in the open ocean where population level effects or for instance site-induced changes in various condition indices could be very difficult to resolve in the absence of major impacts (Mathieu *et al.*, 2005). They can also be a valuable scientific tool for addressing public concerns of a real or perceptual nature about the scope of potential impacts on fish stocks of commercial importance. However, it is recognized that as for fish growth, fish organ condition, or for instance benthic community structure, bioindicator endpoints can display some natural variability and the focus should be on the prevalence of observations and recurrences or trends over time.

Various bioindicator studies were carried out on American plaice taken in the vicinity of the Hibernia development area (Study Area) as well as approximately 50 km Northwest of the rig (Reference Area). Blood smears of fish from each area were examined for changes in red blood cell morphology and staining characteristics as well as changes in different types of white blood cells. EROD activity was determined in male and female liver tissues. Pathological studies were carried out and included observations on gross pathology of fish as well as detailed histopathological studies on liver and gill tissues. Histopathological studies included observations on 13 different indices in liver and 7 in gills. The biochemical and pathological indices studied were generally in line with those used in many local and national monitoring programs and specifically endorsed by the London-Paris Commission (Stagg, 1998). A health effect indicator approach has also more recently been endorsed by the European Union for use in assessing effects on fish health in such programs as the CITY FISH Program (Mierke *et al.*, 2002) as well as by the US Geological Survey for their Biomonitoring of Environmental Status and Trends (BEST) Program (Schmitt and Dethloff, 2000; Schmitt *et al.*, 2004). The latter program also includes a haematology component (Jenkins, 2003 and 2004). A variety of health effect indicators have also recently been employed in major monitoring programs carried out in the North Sea and the Baltic Sea (Hylland *et al.*, 2006; Lehtonen and Schiedek, 2006).

Haematological changes can provide insight into potential contaminant mediated effects on immune function and resistance to disease (e.g. Weeks *et al.*, 1992; Jenkins, 2003; Tort *et al.*, 2004), while induction of MFO enzymes is known to be a sensitive response to selected organic contaminants of environmental concern including petroleum hydrocarbons (e.g. Payne *et al.*,

1987). Pathology can provide information on the potentially injurious and cumulative effects of various contaminants, e.g. petroleum hydrocarbons, metals, etc. (e.g. Evans, 1987; Kiceniuk and Khan, 1987; Hinton *et al.*, 1992; Moore *et al.*, 1996; Myers *et al.*, 1998; Stentiford *et al.*, 2003). Moreover, both general pathology and histopathology have been used extensively in monitoring programs (e.g. Murchelano, 1990; Myers *et al.*, 1994; Baumann and Harshbarger, 1995; Moore *et al.*, 1996; Myers *et al.*, 1998; Stagg, 1998; Hinck *et al.*, 2006).

Biological characteristics and fish condition were also recorded.

10.7.1 Biological Characteristics and Condition of Fish

Information on fish biological characteristics and condition is valuable for interpreting results of bioindicator studies (Levine *et al.*, 1995; Barton *et al.*, 2002). Although a limited number of fish are being analyzed from a population perspective (Dutil *et al.* 1995), such data could also provide a level of information for assessing major effects on animal condition.

American plaice were sorted by sex and by maturity stages as defined by DFO. The female:male ratio did not differ between the Reference and Study Areas and no significant inter-site differences in the frequencies of the various maturity stages in male and female fish were observed between the Hibernia and Reference Areas.

All biological characteristics and condition indices were similar in male fish from the two Areas.

With respect to females, length, gonad weight, age and gonado-somatic were similar in fish from the two Areas. However, gutted body weight, liver weight, Fulton's condition factor and hepato-somatic index were significantly higher in fish from the Study Area.

Condition factor can vary naturally with feeding and reproductive status of fish (Dutil *et al.*, 1995; Maddock and Burton, 1999; Barton *et al.*, 2002) and Morgan (2003) has provided data indicating that condition in American plaice on the Grand Banks can vary in fish collected at sites in close proximity. But, condition factor can also vary in either direction outside the normal range in response to chemical exposure (e.g. Schmitt and Dethloff, 2000).

As for fish condition factor, the relative size of liver can vary naturally with feeding and reproductive status of fish (Dutil *et al.*, 1995; Maddock and Burton, 1999; Barton *et al.*, 2002), but also with various stressors including contaminants. Elevated HSI have been observed in fish at sites contaminated with pulp mill effluents (Hodson *et al.*, 1992; Munkittrick *et al.*, 1992; Servos *et al.*, 1992; Leblanc *et al.*, 1997; Billiard and Khan, 2003; Sepulveda *et al.*, 2004), industrial effluents (Fabacher and Baumann, 1985; Arcand-Hoy and Metcalfe, 1999; Orlando *et al.*, 1999), mixed pollution (Poels *et al.*, 1980; Sloof *et al.*, 1983; Everaarts *et al.*, 1993), sewage sludge and treatment works (Secombes *et al.*, 1995; Lye *et al.*, 1997; Corsi *et al.*, 2003) and metals (Mauk and Brown, 2001; Ozmen *et al.*, 2006).

Overall, the slight differences in condition indices observed in female American plaice between sites have to be interpreted cautiously as the size of samples was small for such population level comparisons (Dutil *et al.*, 1995). Such differences are likely due to natural factors such as nutritional and reproductive status. However, there is a body of literature from field studies indicating that a contaminant-related link cannot be ruled out.

10.7.2 Mixed Function Oxygenase (MFO) Activity

Since basal levels of MFO enzymes can vary seasonally between males and females of the same species (e.g. Walton *et al.*, 1983; Mathieu *et al.*, 1991; Whyte *et al.*, 2000), results were analyzed separately for each sex.

There was no significant difference in EROD activity in males between the two Areas. However, the difference in enzyme activity was marginally significant (p=0.058) in females when all maturity stages were pooled with a 1.8-fold higher level in fish from the Study Area. Since reproductive state can influence enzyme activity in female fish (e.g. Whyte *et al.*, 2000), a inter-area comparison of enzyme activity was also carried out on females of different maturity stages. This included mature (all maturity stages except immature), maturing to spawn, partially spent and spent females. Although the number of fish in the partially spent category was quite low for comparisons, there were no significant differences in enzyme levels for the various categories between the Reference and Study Areas. This indicates that the difference observed for all females (immature and mature pooled) was likely due to the presence of immature females that were only found in the Study Area. Immature fish of various species have been reported to have higher EROD activity than mature female fish (e.g. Whyte *et al.*, 2000). A trend towards higher levels of EROD in immature female American plaice has also been observed in other surveys on the Grand Banks.

10.7.3 Pathology

All fish were assessed during necropsy for any external or internal abnormalities on the skin or fins or on internal organs (gill, liver, gonads, digestive tract, musculature and spleen). Abnormalities were rarely encountered in the two Areas: one fish from the Study area exhibited nematode worms on its liver and another had its intestine protruding from the anus (likely due to trawling pressure) while one fish from the Study Area exhibited pale gills which were confirmed by microscopy to be X-cell lesions. Liver nematodes and anal protrusions are occasionally observed in American plaice on the Grand Banks (personal communication DFO). Likewise, a low frequency of X-cells has been reported in American plaice on the Grand Banks (Mathieu *et al.*, 2005 and 2010).

Haematology, including the analysis of red and white blood cells, has potential to assess the overall health of fish as well as to indicate immunological effects that may be important in disease susceptibility. Payne *et al.* (2005) have noted changes in white blood cells (in a 50% difference range) in cunner chronically exposed to relatively high levels of produced water under laboratory conditions. Alteration of some immunological parameters has also been observed in codfish chronically exposed for several weeks to produced water from the Grand Banks (Perez Casanova, 2009).

There were no apparent qualitative differences in morphology or staining characteristics of red blood cells and no significant differences in differential white blood cell counts in fish from the two Areas.

With respect to liver histopathology, there were no cases of hepatic lesions that have been associated with chemical toxicity in field and laboratory studies (e.g. Myers and Fournie, 2002; ICES, 2004). These included nuclear pleomorphism, megalocytic hepatosis, basophilic,

eosinophilic and clear cell foci, fibrillar inclusions, fibrosis, carcinoma, cholangioma, cholangiofibrosis, proliferation of macrophage aggregates and hydropic vacuolation.

As noted in previous years, a few other hepatic conditions were observed. A “patchy distribution” of hepatocellular vacuolation, not associated with degenerative changes, was observed in similar proportions of fish from each Area and is likely linked to gonadal maturation (Timashova, 1981; Bodammer and Murchelano, 1990; Couillard *et al.*, 1997). Also, an infestation of the biliary system with a myxosporean parasite was found in a number of fish from both Areas, but with a higher proportion (18%) observed in the Study Area (p=0.051). However, similar and higher prevalence of parasitic infestation (up to 58%) has been commonly observed in other surveys on the Grand Banks including at Reference and Development Areas. It is noted that, as reported in past surveys, the presence of parasites in the biliary system did not appear to result in any other pathological changes in hepatic tissues.

Observations on parasitism and hepatocellular vacuolation are of value in relation to providing general information on their presence in the survey area. However, it is important to note from an EEM perspective that liver lesions more commonly associated with chemical toxicity were absent.

With respect to gills, microstructural changes which have been associated with chemical toxicity (e.g. Mallat, 1985) such as epithelial lifting, hyperplasia, telangiectasis and lamellar fusion were absent or found at very low frequencies (less than 0.5%) in both Areas. The degree of severity of oedema was quite low in the 2 Areas. Also, X-cells, that have recently been shown to be caused by a protozoan parasite (Miwa *et al.*, 2004), were observed in one fish from the Reference Area. A few cases of X-cells have been reported in American plaice in other surveys on the Grand Banks (Mathieu *et al.*, 2005 and 2010).

As for the liver histopathological indices, it is of interest to note from an EEM perspective the absence or extremely low occurrence in the survey area of gill lesions associated with chemical toxicity.

10.8 Integrative Assessment

The data indicates that hydrocarbon fractions in the sediment have returned to or below the 1998 levels. The 2009 total barium concentrations increased over 2007 concentrations and are similar to 2002 concentrations (it should be noted that while the 2D contour (Figure 4.7) visually presents the total barium concentrations to be apparently higher in 2009 compared to 2007, statistical analysis of the data indicated no significant differences). Weak acid leachable barium levels are similar to 2007 concentrations and are comparable to the 1998 levels. The weak acid leachable levels had decreased in 2002 with an increase observed in 2004 and decreased again in 2007. The rise in weak acid leachable barium levels in 2004 is thought to be associated with EEM sampling occurring while both drill rigs were discharging WBM’s and cuttings at the same time. The overall improvement in the sediment quality observed at Hibernia is directly attributed to the reinjection of drill cuttings, solids and muds that commenced with partial reinjection in 2000 and had reached greater than 95 percent reinjection in September 2002. The improvement in sediment quality has coincided with a significant increase in produced water. Figure 10.4 presents the relationship between discharge volumes of produced water and

synthetic based mud cuttings as compared to the average TPH concentrations observed at the 250 meter stations for program years 1998 through 2009. The data has been log transformed due to the differing measurement scales that would be required to present the data in one graph.

Figure 10.4 Mean TPH Values (mg/L) at 250 Meter Stations Compared to Produced Water (m^3) and SBM (tonnes) Discharges

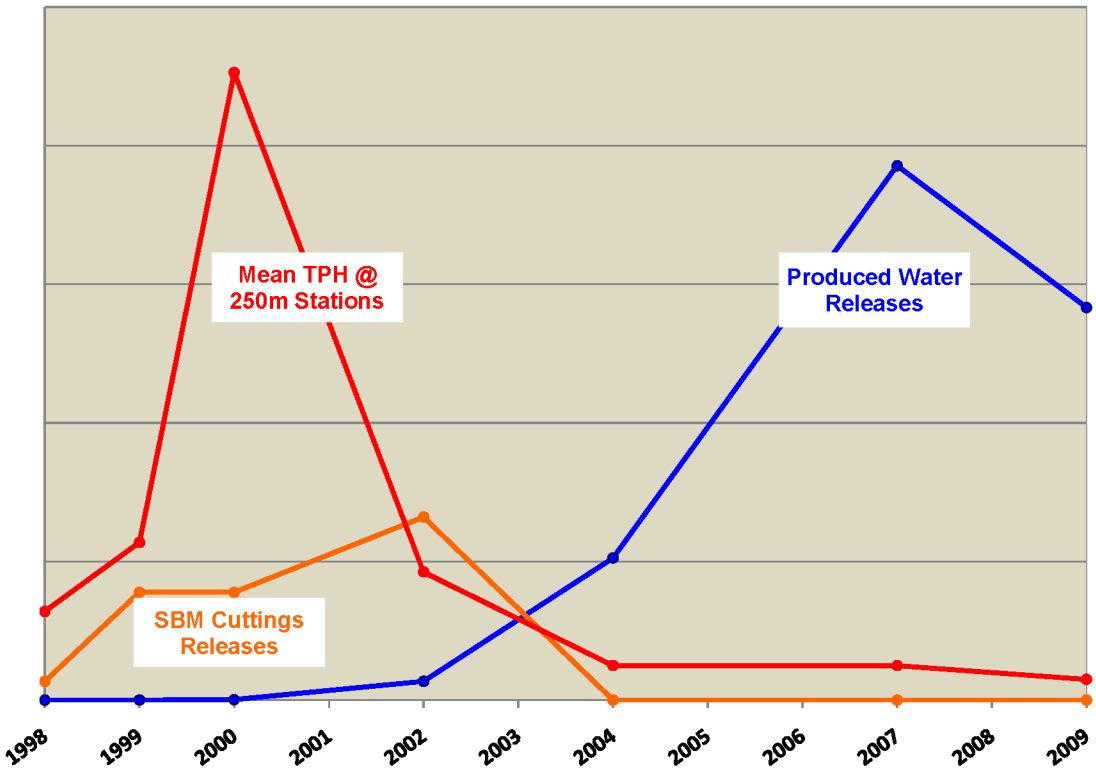


Figure 10.4 clearly demonstrates that the observed improvement in sediment quality is directly related to the reduction in SBM discharge. As expected, the increased discharge of produced water has not resulted in detectable contaminants in the sediment at or beyond 250 meters. Dilution ratios as predicted by the Hibernia produced water model (Lorax Environmental 2004) are 1:100 to 1:3000 within 50 meters of the discharge point. Dilutions of 1:10,000 are predicted to be achieved beyond 750 meters. Therefore the ability to detect contaminants associated with produced water discharges may be limited to immediately adjacent to the discharge point. This was not unanticipated as most dispersion modelling studies conducted worldwide have predicted that a rapid dilution in the range of 30- to 100-fold would occur within the first tens of metres of the discharge point (Terrens and Tait 1993; Brandsma and Smith 1996; Neff 2002). These model results have been verified by field validation studies conducted in 2004, 2007 and 2009. The Hibernia water column programs conducted at Hibernia concurs with other dispersion studies and also validates the Hibernia produced water model results.

The original drill cuttings piles are a potential source for the measureable TPH observed at 250 m in 2009 (as well as other years). The drilling cuttings piles have slowly been remobilized

since SBM discharges have ceased. This may contribute to the TPH values at 250 m. The TPH values at 250 m stations have been decreasing since SBM discharges have ceased. The 250 m stations were the stations which have historically had the highest TPH values. Past EEM programs have examined and presented GC-FID chromatographs that indicated the TPH values at the 250 m stations were due to SBMs. The period of time since reinjection commenced may be insufficient for these stations to reach near background due to remobilization and/or hydrocarbon degradation. These factors are reasons for measurable TPH levels at 250 m stations.

The amount of scientific literature publicly available with respect to the biological effects of SBMs is limited. Based on the available information, barium associated with drilling muds has limited bioavailability (Steinhauer and Imamura 1990) and has limited effects on marine organisms. The available information indicates that TPH values in excess of 1,000 mg/kg would be required before benthic communities would be affected (Candler *et al.* 1995). The TPH levels have decreased in 2002, 2004, 2007 and 2009. The highest TPH level in 2009 was reported at Station 8-250 (60.5 mg/kg). In 2000, one station had TEH levels greater than 1,000 mg/kg and this station, as expected, produced a toxic response in the amphipod test.

In 2009, two toxic responses were observed at mid-field stations (7-2000 and 7-3000) with no causal link to Hibernia discharges established. An ROV survey was conducted June 20, 2010 that demonstrated a thriving macrobenthic community exists at these two stations and there was no readily discernable reason for the observed toxic response. It is interesting to note that the two observed toxic responses in 2009 occurred at stations for which toxic Microtox responses have been observed since 1999. While no causal relationship could be established linking the toxic responses to Hibernia discharges, continued close observation of stations along radial 7 is warranted.

This information indicates that biological effects would not be predicted to occur with the observed barium and TPH concentrations presently reported for the Hibernia Area in 2002, 2004, 2007 and 2009 and, with the exception of the single observed amphipod toxic response in 2000 and the 2009 toxic responses described above, the same can be stated for the 1998, 1999 and 2000 EEM programs.

The most likely biological effects would be the burial of sessile megabenthos and macrobenthos. The literature reports major deleterious effects, which include burial, have been observed within 500 m from platforms (Davies *et al.* 1989). Although noise created by natural variability often makes it difficult to attribute community changes to SBMs (Jensen *et al.* 1999), toxic effects on benthic communities include indirect chemical toxicity and toxic effects due to anoxia caused by organic loading and biodegradation (IBP SHE Technical Committee 1999).

Laboratory toxicity tests, biological chemical profiles, fish health indices and taint studies are the means by which the “biological effects” are investigated for the Hibernia EEM program. There were toxic responses recorded for seven Microtox bioassays. The Microtox toxic responses in 2009 do not correspond to distance from the Hibernia platform or with contaminant concentrations of TPH and/or barium levels. Rather, the majority of Microtox responses occur at Hibernia stations known to have a unique particle size profile and elevated levels of aluminum, chromium, iron, lead, manganese, strontium, uranium, vanadium, total inorganic carbon and

ammonia). The Microtox responses that have occurred along radial 7 during the various EEM programs are included in Table 10.8.

Table 10.8 Microtox Responses Observed at Secondary Sediment Types

Station No.	Microtox Response (mg/L)							
	1994	1998	1999	2000	2002	2004	2007	2009
7-2,000	>98,600	>98,600	13,218	5,483	1, 933	13,767	1,069	1,904
7-3,000	>98,600	75,100	655	2,701	2, 817	3,117	358	1,973
7-6,000	>98,600	84,100	6,940	1,297	6, 128	5,156	1,390	1,376

The stations exhibiting a Microtox response are characterized by large amounts of shell fragments, indicating that the observed Microtox responses may be due to chemicals associated with natural decompositional processes, such as ammonia and sulphides. Although there is a preponderance of evidence for sulphur toxicity in liquid phase toxicity, it is often not considered in sediment toxicity. Under anoxic conditions, much of sulphur found in sediments from both natural and anthropogenic sources (Brouwer and Murphy 1995) will be in the form of reduced sulphur (hydrogen sulphide, metal sulphides, polysulphides, thiosulphides and elemental sulphur). Ancillary testing on the whole sediment for ammonia, sulphides and sulphur was conducted and ammonia was found to be one of the factors that correlate with the positive Microtox responses.

Regardless of the problems associated with interpreting Microtox data, the Microtox test is one of the most effective tools for screening the presence of toxic compounds in sediments (Tay *et al.* 1992). Tay *et al.* (1992) ranked toxicity tests based on their sensitivities to sediment contamination in the Halifax Harbour study as Microtox solvent extract = Microtox solid phase > amphipod (*R. abronius*) > Microtox pore water > amphipod (*C. volutator*) = juvenile polychaetes (*Neanthes* sp.). Becker *et al.* (1990) concluded that the Microtox bioassay was considered the most sensitive of three bioassays (amphipod using *R. abronius* and oyster larvae bioassay using *Crassostrea gigas* were the other two) because it successfully identified the highest percentage of altered benthic community assemblages.

The observed Microtox responses, with the exception of the stations along radial 7 and 1-6000, 3-500, 3-2000 and 4-1000, have returned to baseline responses with a stimulatory effect above control responses observed for stations consisting of the primary sediment type. The Microtox responses observed at the seven stations may be due to a variety of causal factors rather than one causal factor. One cannot be sure that an apparent explanatory variable is not a proxy for a variable that was causal, but not measured (Olsgård and Gray 1995) or, in this case, that there is some factor not associated with the Hibernia platform responsible for the observed deterioration in the Microtox responses. There is evidence that indicates samples with greater than 1.5 percent silt and clay fractions are more likely to produce a toxic Microtox response. This relationship has been observed for the 1999, 2000, 2002 and 2004 data, but not for the 1994, 1998, 2007 and 2009 data. Elevated levels of strontium, TIC, TOC and ammonia as well as secondary or transitional sediment profiles have been correlated with stations that had Microtox responses.

Two toxic response for juvenile polychaete survival and growth were observed at station 7-2000 and 7-3000. These are the same stations for which toxic responses in the amphipod bioassay were observed. This would suggest that something unusual had occurred at these mid-field stations in 2009. Sediment contaminants levels are similar or lower than previous years and no causal linkage can be established to the Hibernia discharges. However, attention should be paid to these stations to ensure no trend develops in future years.

A toxic response was also noted at station 2-250 for juvenile polychaete survival. This did not hold true for the growth assay. The growth rate at 4-250 was the second highest growth rate observed for study area samples. This suggests that some of the polychaetes may have been "weaker" or more susceptible to contaminants or other unknown factors.

The 2004 and 2007 juvenile polychaete data did not indicate that the observed enrichment effect close to the Hibernia platform that occurred for the 2000 and 2002 continued. The data analyses indicated that differences between years (primarily attributed to enhanced growth in 2000 and 2002) but not between distances. Caution must be applied to the interpretation of juvenile polychaete data due to the high degree of variability associated with this bioassay.

Eighty-six percent of variance in the collected data (sediment quality) could be explained by four factors (see Section 6.4.1). These factors were analyzed to determine what correlation they had with the sediment toxicity assays. None of the sediment toxicity assays correlated with factor 1 which consisted of hydrocarbons, barium, sulfide and chromium. Factor 1 represents the contaminants most closely linked to Hibernia discharges and as such these results indicates that no causal relationship between the 2009 toxicity test results and Hibernia operational discharges could be demonstrated.

Polychaete growth correlated with polychaete survival and Factor 3 (moisture and aluminum). Aluminum has remained similar throughout the years and is representative of background levels and natural variability. This would indicate that the polychaete toxicity results may be a result of some naturally occurring factor. Amphipod survival correlates with the polychaete results.

The fish health indicators as measured in American plaice indicate that the present health status of American plaice collected at the Hibernia Area is similar to that at the Reference Area.

10.9 Hypotheses

The EEM program was designed to detect any change in the environment due to the operation of the Hibernia platform. In order to make the detection of change more effective, a series of hypotheses were developed (HMDC 1995). The monitoring hypotheses developed for the Hibernia Production Phase EEM program design were based on biological endpoints. Sediment chemistry and biota body burden data are the determinants used to try to determine the cause or "effect" for the rejection of a null hypothesis in favour of an alternative hypothesis.

The hypotheses for the Hibernia Production Phase EEM Program are:

H_0 No.1 **=** Operational discharges from the Hibernia platform will not result in major biological effects (as measured by the amphipod survival assay) beyond the predicted impact zone of a 1,000-m radius around the production platform.

H_A No.1 **=** Operational discharges from the Hibernia platform will result in major biological effects (as measured by the amphipod survival assay) beyond the predicted impact zone of a 1,000-m radius around the production platform.

H_0 No.2 **=** Operational discharges from the Hibernia platform will not result in minor biological effects (as measured by Microtox and/or juvenile polychaete growth assays) beyond 4,000 m.

H_A No. 2 **=** Operational discharges from the Hibernia platform will result in minor biological effects (as measured by Microtox and/or juvenile polychaete growth assays) beyond 4,000 m.

H_0 No.3 **=** Operational discharges from the Hibernia platform will not result in the taint (as measured by organoleptic evaluations) of fishery resources outside of the fishing exclusion zone.

H_A No.3 **=** Operational discharges from the Hibernia platform will result in the taint (as measured by organoleptic evaluations) of fishery resources outside of the fishing exclusion zone.

At first glance, the 1999, 2000, 2002, 2004, 2007 and 2009 Hibernia EEM data would have resulted in the rejection of the null hypothesis for Hypothesis No.2 due to Microtox responses observed beyond 4,000 m. However, no causal relationship to Hibernia operational discharges can be determined. Project-induced causal factors could not be established for the observed Microtox responses and therefore, H_0 No.2 cannot be rejected. Microtox responses do not correlate with TPH, barium or with distance from the Hibernia platform. The pattern of the observed Microtox responses for the period between 1998 and 2004 resembles the spatial distribution of sediment grain size. This resemblance weakens for the 2007 data except for along Radial 7 and suggests that other factors as well may be responsible for the Microtox response. The Microtox responses correlate with stations that have elevated strontium levels, TIC, TOC and ammonia as well as secondary transitional sediment profiles. Therefore, null hypothesis No. 2 cannot be rejected.

The null hypothesis for Hypothesis No. 1 cannot be rejected despite the two amphipod toxic responses at 7-2000 and 7-3000. No causal relationship to Hibernia operational discharges could be established and therefore H_0 No.1 cannot be rejected. Amphipod toxic responses do not correlate with TPH, barium or distance from the Hibernia platform (see Section 6.4.1).

10.10 Impact and Model Predictions

The EIS predictions (Mobil 1985) based on oil-based drill cuttings and solids, was that cuttings would occur to 200 m from the Hibernia platform. A maximum of 1 to 2 km^2 of sediments was predicted to be contaminated with greater than 10 mg/L hydrocarbons. The 2009 TPH data have detected hydrocarbon concentrations above 10 mg/L out to a distance of 3,000 m along radial 7, and out to a maximum of 500 m for all other radials. The EIS prediction regarding hydrocarbon levels was based on the use of OBMs, when in fact SBMs have been used at the Hibernia platform. The oil on the cuttings is chemically different than the oil type used for the model. In addition, the cuttings dispersion pattern for SBMs is different than for OBMs.

Therefore, direct comparisons between the behaviour of the SBMs in the field and the model expectations are not valid.

The Hibernia effluent fate and effects model (Seaconsult 1994) predicted there would be an almost circular deposition of finer fractions of drill mud components out to distance of 1,200 m. It concluded that it was possible that some of the sediments would be mobile under severe storm conditions, but that the long-term transport rate was likely to be low. Cuttings are expected to accumulate adjacent to the production platform. Lack of information at the time of modelling precluded the calculation of hydrocarbon values for the Hibernia model. The data to date indicate sediment transport is occurring and is most likely due to a variety of sediment transport mechanisms. The effects of storm induced bottom currents may have a greater impact on sediment transport than originally anticipated as evident by restrictions to the Hibernia sea water intake flow after storm events.

The model prediction that indicated an almost circular deposition of finer fractions around the production platform was correct. Deposition of contaminants has occurred around the Hibernia platform. Based on the data, the prediction that the finer fractions would be distributed out to a distance of 1,200 m was based on the use of OBMs and not SBMs. Due to behavioural differences between the two mud types, direct comparisons between model expectations for OBMs and field observation for SBMs are not valid. The use of SBMs was not modelled or discussed in the EIS as the development of SBMs was just being initiated. Current knowledge would indicate that the observed hydrocarbon concentrations above 10 mg/kg are associated with the finer sediment fractions and their movement from the point source is expected to be greater than anticipated. Changes to Hibernia's operations with advent of approximately 95 percent SBM cuttings re-injection (late September 2002) has resulted in continued improvement in the observed sediment quality in 2004, 2007 and 2009 Hibernia EEM programs.

The cuttings reinjection and thus major reductions in SBM drill cuttings and solids discharges occurred concurrently with major increases in produced water discharges. The continued improvement in sediment quality indicates that contaminants contained within the produced water discharges may be limited to immediately adjacent the discharge point. Information contained with the Hibernia EIS (Mobil 1985) indicated worst case dilutions of 1:1000 would be achieved within 1 km and dilutions of 1:10,000 with in 8 kms. Dilution ratios as predicted by the Hibernia produced water model (Lorax Environmental 2004) are 1:100 to 1:3000 within 50 meters from the discharge point depending upon flow rates. Dilutions of 1:10,000 are predicted to be achieved beyond 750 meters regardless of flow rates. Field results from the 2004, 2007 and 2009 water column program validated model predictions and confirmed dilution ratios ranging from 1:139 to 1:14,400 within 33 m of the produced water outlet in 2009. This concurs with dispersion modelling studies conducted world-wide that all predict a rapid dilution in the range of 30- to 100-fold within the first tens of metres of the discharge point (Terrens and Tait 1993; Brandsma and Smith 1996; Neff 2002). Therefore the model predictions in the EIS with respect to produced water are very conservative and will most likely be achieved much closer to the discharge points than those noted in the EIS (Mobil 1985).

11.0 CONCLUSIONS AND RECOMMENDATIONS

11.1 Conclusions

The overall conclusion of the 2009 EEM program is that all null hypotheses developed for the Hibernia EEM program have been accepted. Specific conclusions associated with various program components (sediment, water and biological) are outlined in the following sections.

11.1.1 Sediment

The sediment contaminant concentrations continue to decrease to background concentrations as compared to previous years' concentrations. Operational changes, namely cuttings reinjection, continue to have a direct effect on the observed sediment contaminant concentrations.

The improvement in the sediment contaminant concentrations continued as drilling waste discharges were significantly reduced and produced water significantly increased.

The sediment toxicity tests resulted in toxic amphipod and polychaete bioassay responses at mid-field stations 7-2000 and 7-3000. No causal relationship with Hibernia operational discharges could be established.

A polychaete toxic response for survival only (not growth) occurred at station 8-250 in 2009. No causal relationship with Hibernia operational discharges could be established. The juvenile polychaete growth results indicated that there were no significant observances between near and far field results with respect to growth. Previous enrichment effects observed are no longer apparent.

Fuel range hydrocarbons (C10-C21) and lube range hydrocarbons (C21-C32) were present in all livers of American plaice collected at both the Hibernia and Reference Areas. There were no statistical differences between the two.

The observed seven Microtox toxic responses do not correlate to distance from the Hibernia platform, nor with contaminant concentrations for TPH and barium levels. Between 1998 and 2004, Microtox responses resembled the spatial distribution of sediment grain size. This relationship has weakened for the 2007 and 2009 data except along Radial 7 and suggests that other factors, likely natural, may be responsible for the Microtox response. Microtox IC50 values in the mid-field were significantly lower (more toxic response) in 2007 than in 1994 for the same field, although the 2009 data were similar to all other previous years. Further, the mid-field Microtox values were significantly lower than those measured in the near-field in 2009. Thus, it is unlikely that these differences in the mid-field Microtox tests in 2009 are attributable to the Hibernia platform.

Elevated levels of Strontium, TIC, TOC, ammonia and particle size at secondary or transitional sediment profiles have been correlated with stations that had Microtox responses.

11.1.2 Water

Results of the Hibernia water column program confirms the Hibernia produced water dispersion model predictions that produced water will rapidly dilute to non-toxic levels within 50 meters of the discharge point. Predicted dilution ratios between 1:100 to 1:1,000 or better were achieved within 50 meters of the discharge point. Actual dilution factors ranged from 94:1 to > 13,505:1.

11.1.3 Biological

The triangle test analyses used for the assessment of taint found statistically significant difference between samples collected at the Reference and Hibernia Areas. Ancillary comments did not identify any abnormal or foreign odour or taste. This coupled with the results of the Hedonic scaling indicated that taste panel testing was unable to detect taint at either location.

The results of the fish health study indicate that the present health status of American plaice collected at the Hibernia Area is similar to that of the Reference Area. Hibernia operational discharges have not had a negative impact on the fish health indicators measured. Observed differences are interpreted to be associated with natural variation.

The single fibrillar inclusion observed during the 2007 EEM was not observed in 2009, indicating that the single observation reflected natural variability.

11.2 Recommendations

11.2.1 Sediment

The statistical analyses techniques used in 2009 for the Hibernia sediment chemistry analyses should be retained and used in subsequent programs as long as they remain appropriate and relevant. The continued use and relevance of detailed statistics for parameters that have returned and remain at baseline levels should be examined. The continued use of contour plots to present the relevant data is recommended.

Continued attention will be directed to stations along radials 3 and 7 due to their unique nature and the number of Microtox toxic responses observed at these stations throughout the history of the Hibernia EEM program. A detailed examination of the Microtox responses was undertaken in 2007 and 2009. Microtox responses for the period between 1998 and 2004 correlate to the spatial distribution of sediment grain size, but this resemblance weakens in 2007 and 2009 except for data along Radial 7 and suggest other factors may be responsible for the Microtox response. Microtox responses have also been found to correlate (in addition to sediment grain size) with elevated levels of strontium, TIC, TOC and ammonia levels.

In addition to the Microtox responses, the amphipod and polychaete responses at stations along Radial 7 indicated that continued attention will be directed at Radial 7. This is required to confirm that the 2009 responses are not the start of a trend and related to some other unknown factor. Statistical analyses was unable to correlate juvenile polychaete and amphipod responses with contaminants associated with Hibernia operational discharges.

11.2.2 Water

It is recommended that due to the fluid nature of the produced water plume that predetermined locations for water quality sampling within 50 m of the discharge point not be imposed, rather the sample locations for each program year be directed by the CTD profiles.

It is recommended that when and if logistically feasible, OWTG sampling (including toxicity testing) be conducted concurrently with the water column program.

11.2.3 Biological

Continued monitoring of the body burden hydrocarbon data is required due to the incidences of hydrocarbons in tissues for both the Hibernia and Reference Areas, particularly with respect to livers.

Biological tissues that produced hydrocarbon data should be further analyzed by GC-MS where possible in an attempt to determine the possible source of the hydrocarbon in the tissue. Due to the use of liver data for both fish health indices and body burden assessments, the use of liver tissue for additional analyses is impossible. Therefore, GC-MS analyses to determine potential source of hydrocarbon data should be limited to muscle tissue. However, it must be recognized that the species used in the Hibernia EEM program is a mobile finfish species (American plaice) and this may be a confounding factor in data analyses and interpretation of the data.

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APPENDIX A

Glossary of Acronyms

GLOSSARY OF ACRONYMS

2-D	Two-dimensional
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
bbl	Barrel
BOD	Biochemical oxygen demand
BTEX	Benzene, toluene, ethylbenzene and xylenes
CAPP	Canadian Association of Petroleum Producers
CCME	Canadian Council of Ministers of the Environment
C-NLOPB	Canada-Newfoundland and Labrador Offshore Petroleum Board
C-NSOPB	Canada Nova Scotia Offshore Petroleum Board
COD	Chemical oxygen demand
CPUE	Catch per unit effort
CTD	Conductivity, temperature and density
DFO	Department of Fisheries and Oceans Canada
EEM	Environmental effects monitoring
EIS	Environmental impact statement
EQL	Estimated quantification limits
EROD	Ethoxyresorufin O-deethylase
g	Gram
GESAMP	United Nations Group of Experts on Scientific Aspects of Marine Environmental Protection
GBS	Gravity-based structure
HMDC	Hibernia Management and Development and Company Limited
ICES	International Council for the Exploration of the Sea
ISQG	Interim Sediment Quality Guidelines

kg	Kilogram
km	Kilometre
L	Litre
LOQ	Limit of quantification
LTMO	Low-toxicity mineral oil
m	Metre
m^3	Cubic metre
MFO	Mixed function oxygenase
mg	Milligram
ml	Millilitre
mm	Millimetre
NEB	National Energy Board
NOEC	No observed effect concentration
NRC	National Research Council
OBM	Oil-based mud
OCMS	Offshore Chemical Management System
OCNS	Offshore Chemical Notification System
OWTG	Offshore Waste Treatment Guidelines
p	Statistical probability level
PAH	Polycyclic aromatic hydrocarbons
PAO	Poly-alpha olefin
PEL	Probable effects levels
ppm	Parts per million
PSEP	Puget Sound Estuary Program
QA/QC	Quality assurance/quality control

RDL	Reportable Detection Limited
SBF	Synthetic-based fluid
SBM	Synthetic-based mud
SD	Standard deviation
SETAC	Society of Environmental Toxicology and Chemistry
TEH	Total extractable hydrocarbons
TIC	Total inorganic carbon
TOC	Total organic carbon
TPH	Total petroleum hydrocarbons
WAM	Weak acid extractable metals
WBM	Water-based mud
µg	Microgram

APPENDIX B

Rationale and Approach to the Statistical Analysis: Hibernia 2009 EEM Sediment Chemistry Program

APPENDIX C

Spatial and Temporal Variability of Total Metal Concentrations in Sediment

APPENDIX D

2007 Hydrocarbon Data

Station	Distance from GBS Wall (m)	Depth (from surface) (m)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylenes (mg/L)	C_6-C_{10} (less BTEX) (mg/L)	>C_{10}-C_{21} (Fuel Range) (mg/L)	>C_{21}-C_{32} (Lube Range) (mg/L)	Hydrocarbons BTEX plus TPH (Note [a]) mg/L
1	16.5	-1	0.023	0.016	0.001	0.007	0.02	0.06	<0.1	0.177
		-9	0.012	0.009	< 0.001	0.004	0.01	<0.05	<0.1	0.111
		-11	0.008	0.007	< 0.001	0.003	< 0.01	<0.05	<0.1	0.099
		- 65	0.023	0.015	< 0.001	0.005	0.02	<0.05	<0.1	0.139
2	21.2	-1	0.003	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.087
		-14	0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.083
		-18	< 0.001	0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.083
		-70	0.005	0.004	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.091
3	25.4	-1	0.002	0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.085
		-8	0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.083
		-12	0.002	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.086
		-70	0.007	0.004	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.093
4	21	-1	0.032	0.02	0.001	0.007	0.02	<0.05	<0.1	0.155
		-7	0.033	0.021	0.001	0.009	0.02	<0.05	<0.1	0.159
		-9	0.029	0.02	0.001	0.007	0.02	<0.05	<0.1	0.152
		-70	0.004	0.003	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.089
5	36	-1	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-6	0.04	0.026	0.002	0.009	0.03	0.05	<0.1	0.207
		-14	0.053	0.034	0.002	0.012	0.03	<0.05	<0.1	0.206
		-70	0.038	0.024	0.001	0.009	0.02	<0.05	<0.1	0.167
6	39	-1	0.017	0.011	< 0.001	0.004	0.01	<0.05	<0.1	0.118
		-6	0.012	0.008	< 0.001	0.002	< 0.01	<0.05	<0.1	0.103
		-14	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-70	0.012	0.008	< 0.001	0.002	< 0.01	<0.05	<0.1	0.103
7	24	-1	0.004	0.003	< 0.001	< 0.002	0.01	<0.05	<0.1	0.094
		-10	0.003	0.002	< 0.001	< 0.002	0.01	<0.05	<0.1	0.092
		-15	< 0.001	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.084
		-70	0.008	0.005	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.095
8	20.8	-1	0.09	0.055	0.004	0.021	0.05	0.09	0.1	0.410
		-10	0.044	0.028	0.002	0.011	0.03	<0.05	<0.1	0.190
		-11	0.09	0.006	< 0.001	< 0.002	0.01	<0.05	<0.1	0.183
		-70	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
9	26	-1	0.002	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.086
		-10	0.002	0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.085
		-13	0.001	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.085
		-70	0.003	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.087

Station	Distance from GBS Wall (m)	Depth (from surface) (m)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylenes (mg/L)	C6-C10 (less BTEX) (mg/L)	>C10-C21 (Fuel Range) (mg/L)	>C21-C32 (Lube Range) (mg/L)	Hydrocarbons BTEX plus TPH (Note a) mg/L
10	23.5	-1	0.002	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.086
		-12	0.002	0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.085
		-14	0.002	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.086
		-70	0.003	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.087
11	26.6	-1	0.032	0.02	0.001	0.008	0.02	<0.05	<0.1	0.156
		-10	0.01	0.007	< 0.001	0.002	< 0.01	<0.05	<0.1	0.100
		-16	< 0.001	0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.083
		-70	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
Ref 1	16,000 (Radial 7)	-1	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-25	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-45	< 0.001	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.084
		-75	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
Ref 2	16,000 (Radial 1)	-1	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-15	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-25	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-80	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
EQL			**0.001**	**0.001**	**0.001**	**0.002**	**0.01**	**0.05**	**0.1**	**0.2**

Note [a]: Hydrocarbons calculation note < EQL for samples that have all values below EQL and provide an estimate in brackets that uses ½ EQL ,. Thus the hydrocarbon calculation provided is a liberal estimate of the hydrocarbon levels for the samples analysed.

APPENDIX E

Triangle Test Statistical Table

TRIANGLE TEST, DIFFERENCE ANALYSIS

Number of Tasters	Number of Correct Answers Necessary to Establish Level of Significance			Number of Tasters	Number of Correct Answers Necessary to Establish Level of Significance		
	5%	1%	0.1%		5%	1%	0.1%
7	5	6	7	57	27	29	31
8	6	7	8	58	27	29	32
9	6	7	8	59	27	30	32
10	7	8	9	60	28	30	33
11	7	8	9	61	28	30	33
12	8	9	10	62	28	31	33
13	8	9	10	63	29	31	34
14	9	10	11	64	29	32	34
15	9	10	12	65	30	32	35
16	10	11	12	66	30	32	35
17	10	11	13	67	30	33	36
18	10	12	13	68	31	33	36
19	11	12	14	69	31	34	36
20	11	13	14	70	32	34	37
21	12	13	15	71	32	34	37
22	12	14	15	72	32	35	38
23	13	14	16	73	33	35	38
24	13	14	16	74	33	36	39
25	13	15	17	75	34	36	39
26	14	15	17	76	34	36	39
27	14	16	18	77	34	37	40
28	15	16	14	78	35	37	40
29	15	17	19	79	35	38	41
30	16	17	19	80	35	38	41
31	16	18	19	81	36	38	41
32	16	18	20	82	36	39	42
33	17	19	20	83	37	39	42
34	17	19	21	84	37	40	43
35	18	19	21	85	37	40	43
36	18	20	22	86	38	40	44
37	18	20	22	87	38	41	44
38	19	21	23	88	39	41	44
39	19	21	23	89	39	42	45
40	20	22	24	90	39	42	45
41	20	22	24	91	40	42	46
42	21	22	25	92	40	43	46
43	21	23	25	93	40	43	46
44	21	23	25	94	41	44	47
45	22	24	26	95	41	44	47
46	22	24	26	96	42	44	48
47	23	25	27	97	42	45	48
48	23	25	27	98	42	45	49
49	23	25	28	99	43	46	49
50	24	26	28	100	43	46	49
51	24	26	29	200	80	84	89
52	25	27	29	300	117	122	127
53	25	27	29	400	152	158	165
54	25	27	30	500	188	194	202
55	26	28	30	1000	363	372	383
56	26	28	31	2000	709	722	737

Source: Larmond 1977.

APPENDIX F

Oceans Ltd. (2009) Report

APPENDIX A

Glossary of Acronyms

GLOSSARY OF ACRONYMS

2-D	Two-dimensional
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
bbl	Barrel
BOD	Biochemical oxygen demand
BTEX	Benzene, toluene, ethylbenzene and xylenes
CAPP	Canadian Association of Petroleum Producers
CCME	Canadian Council of Ministers of the Environment
C-NLOPB	Canada-Newfoundland and Labrador Offshore Petroleum Board
C-NSOPB	Canada Nova Scotia Offshore Petroleum Board
COD	Chemical oxygen demand
CPUE	Catch per unit effort
CTD	Conductivity, temperature and density
DFO	Department of Fisheries and Oceans Canada
EEM	Environmental effects monitoring
EIS	Environmental impact statement
EQL	Estimated quantification limits
EROD	Ethoxyresorufin O-deethylase
g	Gram
GESAMP	United Nations Group of Experts on Scientific Aspects of Marine Environmental Protection
GBS	Gravity-based structure
HMDC	Hibernia Management and Development and Company Limited
ICES	International Council for the Exploration of the Sea
ISQG	Interim Sediment Quality Guidelines

kg	Kilogram
km	Kilometre
L	Litre
LOQ	Limit of quantification
LTMO	Low-toxicity mineral oil
m	Metre
m^3	Cubic metre
MFO	Mixed function oxygenase
mg	Milligram
ml	Millilitre
mm	Millimetre
NEB	National Energy Board
NOEC	No observed effect concentration
NRC	National Research Council
OBM	Oil-based mud
OCMS	Offshore Chemical Management System
OCNS	Offshore Chemical Notification System
OWTG	Offshore Waste Treatment Guidelines
p	Statistical probability level
PAH	Polycyclic aromatic hydrocarbons
PAO	Poly-alpha olefin
PEL	Probable effects levels
ppm	Parts per million
PSEP	Puget Sound Estuary Program
QA/QC	Quality assurance/quality control

RDL	Reportable Detection Limited
SBF	Synthetic-based fluid
SBM	Synthetic-based mud
SD	Standard deviation
SETAC	Society of Environmental Toxicology and Chemistry
TEH	Total extractable hydrocarbons
TIC	Total inorganic carbon
TOC	Total organic carbon
TPH	Total petroleum hydrocarbons
WAM	Weak acid extractable metals
WBM	Water-based mud
µg	Microgram

APPENDIX B

Rationale and Approach to the Statistical Analysis: Hibernia 2009 EEM Sediment Chemistry Program

B.1 Sediment Program Review and Resulting EEM Program Changes

The statistical challenge that is presented by a monitoring program such as the Hibernia EEM program is not insignificant. The EEM program must be designed to identify small chemical changes in the sedimentary environment, against a background that is spatially variable, and may be varying over time. This is an important point to emphasize, since most classical statistical analysis is based upon the assumption that experimental units (in this case, sampling locations) are initially uniform in characteristics or quality (the preferred condition). Failing that, it may be determined that the experimental units can be put in order or grouped so that treatments can be applied to "blocks" of units. At the very least, when the experimental units are heterogeneous, it may be determined that treatments can be assigned randomly to the experimental units, so that the initial conditions do not bias the outcome of the experiment. However, in field monitoring designs, none of these preferred approaches may be possible to apply. The environment is spatially variable, and can be expected to change over time. The monitoring program is focused on a fixed point (the Hibernia Platform). As a result, it is likely that the inherent variability between stations, upon which statistical analysis depends, increases with each concentric step away from the platform, or that there may be pre-existing patchiness or gradients in environmental conditions throughout the study area.

The statistical analysis of sediment quality data collected as part of the Hibernia EEM program has evolved over time. During the baseline (1994) EEM program, the Hibernia Platform had not yet been put in place, and there were only limited potential environmental contaminants in the study area, related to exploration activities. As a result, data collected at that time were viewed as baseline data. Statistical comparisons were only possible between different locations (no time component yet existed) and, as a result, a high level of replication within stations was considered important. A modest degree of spatial separation between replicate samples collected near each sampling station was introduced in order to try to avoid pseudoreplication of the field data.

As subsequent EEM surveys were carried out, the statistical evaluation changed in order to consider differences over time, as well as spatially. In the analysis carried out following the 2002 EEM program, sampling stations were first divided into Near-field (0 to 1 km), Mid-field (1.5 to 3 km) and Far-field (>3 km) groups. The data for all years were then analyzed as a function of distance from the platform (Field), the year of the data (Time), and the Field X Time interaction. Since this approach includes analysis of the baseline data, as well as all other monitoring years, it is the Field X Time interaction term that is most likely to identify statistically significant environmental contaminants attributed to the Hibernia Platform operations.

After comparing Near-field, Mid-field, and Far-field for relatively "gross" spatial scales of contaminants, the analysis in 2002, 2004, 2007 and 2009 then proceeded to examine the data for the Near-field only, testing for contaminants due to distance (250, 500, 750 or 1,000 m from the platform), Time (the 1994, 1998, 1999, 2000, 2002, 2004, 2007 and 2009 surveys), and the Distance X Time interaction term. As before, it is the interaction term that is considered most likely to identify the potential environmental contaminants attributed to the activities at the Hibernia Platform. The distinction between this analysis and the previous analysis is that underlying environmental gradients that may confound the analysis are expected to be of minor

importance as long as the spatial scale is kept small. In addition, the sensitivity to detect contaminants may be higher (if the underlying natural variability or "noise" is smaller), and the magnitude of contaminants is expected to be greatest near the Hibernia Platform.

In its most basic form, statistical power in an analysis of variance depends on the desired level of Type I and Type II error (i.e., α and β), the inherent variability present in the data, and the number of degrees of freedom that are available to determine the mean square error. Since most of these factors are fixed, or at least cannot be manipulated by the investigator, it is the number of samples that determines the available number of degrees of freedom. The degrees of freedom in turn determine the critical "F" value that is used to judge whether a difference is statistically significant or not. With few degrees of freedom, the critical F value is large, and the statistical test has low power. With increasing sample size, the critical F value decreases towards a lower end point. The effect of sample sizes on critical F values is illustrated in Table B.1. Gains in statistical power that can be achieved by increasing the sample size and available degrees of freedom are largest with small sample sizes, and become negligible when the sample size and degrees of freedom are already large.

Table B.1 Critical F values for *p* (α) = 0.05

Error Degrees of Freedom	Treatment Degrees of Freedom			
	1	**2**	**4**	**10**
1	161	200	225	242
5	6.61	5.79	5.19	4.74
10	4.96	4.10	3.48	2.98
60	4.00	3.15	2.53	1.99
120	3.92	3.07	2.45	1.91
infinity	3.84	3.00	2.37	1.83

Since the statistical design that has been applied at Hibernia continues to carry all of the stations and years of sampling as if they were statistically independent samples, the number of degrees of freedom available becomes progressively very large. Replication within stations at any given time is unnecessary, since replication is available spatially at any given time and over time at any given station.

As an illustration, the degrees of freedom available in the analysis of barium data in 2007, which has progressively increased as a result of adding 2009, are summarized in Table B.2.

Table B.2 Degrees of Freedom Available for Sediment Statistical Analysis, 2007 Hibernia EEM Program

All Data Combined		Near-Field Data Only	
Source of Variation	**Degrees of Freedom**	**Source of Variation**	**Degrees of Freedom**
Time	6	**Time**	6
Field	2	**Distance**	2
Field X Time	12	**Distance X Time**	12
Experimental Error	300	**Experimental Error**	104

The message conveyed by Table B.2 is that the statistical power generated through the available degrees of freedom is already very large, particularly for the critical comparison of

Field X Time (12 and 300 degrees of freedom) or Distance X Time (12 and 104 degrees of freedom). The available degrees of freedom will continue to increase with subsequent rounds of EEM, and this will result in small gains in the available statistical power. However, given the statistical analysis that is carried out at Hibernia, the available degrees of freedom are large, and in effect this maximizes the statistical power that is available. Further gains in statistical power that are available from increasing sampling effort would be marginal.

The statistical analysis of Hibernia EEM data in 2002, 2004, and 2007 resulted in the detection of some environmental contaminants in the Near-field, but essentially no contaminants were identified that extended more than 1 km away from the platform (i.e., no contamination was identified in the Mid- or Far-field areas). Those contaminants that were identified in the Near-field area were most pronounced close to the Hibernia Platform (i.e., at the 250-m distance), and were approaching background at a distance of 1 km from the platform. Therefore, it was recommended that future rounds of EEM should continue to place emphasis on sampling in the Near-field.

In the past Hibernia EEM surveys, there has been a high level of emphasis placed upon replication. However, as has been illustrated above, replication within stations is meaningless in the analysis of environmental contaminants at Hibernia. Replication within stations is not necessary for the primary statistical analysis that will be carried out in future rounds of EEM.

B.2 Assumptions of ANOVA Statistical Analysis

In any statistical testing program where a large number of tests is carried out, there is a chance that random factors will occasionally lead to the conclusion that a statistically significant effect exists, where in fact the effect is not related to the project or activity being monitored. Using the conventional statistical probability level (p) of $\alpha=0.05$, this will occur in approximately one test in twenty. However, the analysis of environmental data can be more prone to false positives than standard laboratory tests. This is because some of the underlying assumptions of ANOVA are not necessarily met when analyzing environmental data. For example, it is conventionally assumed that treatments are applied randomly to experimental units (such as growing plots in an agricultural field) that are originally similar, and for which the inherent variability is homogeneous across treatments. Alternatively, if gradients (such as changes in soil type or fertility) are known to exist prior to the experiment, techniques such as statistical blocking can be used to account and correct for these gradients. In EEM studies, these assumptions are usually not met. Variations are expected to occur across the study area, but often not enough is known about the nature of these variations to allow for statistical corrections to be applied, or the pattern of natural variability may itself change over time. At Hibernia, the EEM program is tasked with monitoring the release of substances from a fixed point over time. The allocation of treatments (substances released from the platform) to experimental units (sampling stations) is therefore not truly a random process. The inherent variability among the sampling stations is not homogeneous across treatments, but tends to increase as distance from the sampling platform increases.

The use of repeated sampling over time also results in a technical violation of the assumptions of ANOVA, since observations at any single station sampled over time can be said to be auto-correlated. In effect, observations taken sequentially at the same location tend to be more

similar to each other, than observations from the previous and subsequent sampling periods than they should be if they were truly statistically independent samples. In spite of these difficulties, there is no better statistical technique to apply to the data than ANOVA. As long as it is recognized that a relatively high incidence of false positive observations can be expected, the technique remains an important tool for data analysis and interpretation. In order to aid in interpreting the importance of statistically significant differences, and to help screen out "false positive" results, four principles can be considered. These are the principles of:

- proximity (differences are more likely to be real if they are observed in close proximity to the source of the disturbance);
- adjacency (differences are more likely to be real if two or more adjacent locations are affected);
- multiplicity (differences are more likely to be real if two or more parameters are affected simultaneously); and
- duration (differences are more likely to be real if they persist over time).

Although each of these principles is evaluated independently, they can be taken cumulatively to establish a weight of evidence suggesting that observed statistically significant differences are, or are not, attributable to the project.

B.3 Statistical Analysis Methods for the 2009 Sediment Chemistry Program

As was done for the 2007 EEM program, the statistical approach to evaluating the 2009 EEM data with data from previous years has been structured to also consider two levels of scale. The first level of analyses is a coarse screening for differences between Near-field (stations between 250 and 1,000 m from the platform), Mid-field (stations between 1,500 and 3,000 m from the platform), and Far-field (stations 6,000 m from the platform). The division of stations into Near-, Mid- and Far-field groupings for all the data is arbitrary, but has proven effective (HMDC 2003a, 2005, 2008) to reflect the varying spatial extent of contaminant predictions developed in the Hibernia production phase EEM program (HMDC 1996).

The 2009 Hibernia sediment data, as for the 2002, 2004 and 2007 data, have been described as Near-field, Mid-field and Far-field for the sediment chemistry statistical analyses. The "Field" distance descriptors used for the 2009 statistical analyses to describe distances from the Hibernia Platform are provided in Table B.3.

Table B.3 Hibernia 2009 EEM Field Distance Definitions

Field Distance Descriptors	Distance from Hibernia Platform (m)
Near-Field	250, 500, 1,000
Mid-Field	2,000, 3,000
Far-Field	6,000

The second statistical analysis focuses on contaminants within 1,000 m of the platform, by looking explicitly at contaminants along radii at distances of 250, 500 and 1,000 m. The 750-m

distance stations from Hibernia were discontinued in 2004, and therefore are not included in the "Distance" factor for this statistical analysis; however, they are included in the "Field" factor for the years sampled before 2004. In both cases (coarse and Near-field analysis), the analysis explicitly tests for variation in contaminants concentrations attributable to Distance from the platform, either as Field (Near-, Mid- or Far-field) or explicitly as Distance (250, 500 and 1,000 m); the year of the EEM program (Year) and, most importantly, the potential interaction terms (Year x Field or Year x Distance). This same statistical approach to spatial scales was also used for the Microtox toxicity test results on sediment samples.

B.3.1 Values Below the Reportable Detection Limit and Statistical Analysis

The treatment of values below the Reportable Detection Limit (RDL) by substituting one-half the RDL in the statistical analysis of measurable parameters for the Hibernia EEM program has raised general concerns by DFO. DFO suggested that this substitution method may not be the most appropriate approach and indicated that analysis of non-detected or censored data should follow those of Helsel (2005) and methods for censored data sets, such as using the Maximum Likelihood Estimation (MLE) approach to descriptive statistics, ANOVA and regression analysis for example. However, Helsel (2005) notes that analysis of censored data would be important when multiple detection limits are encountered in the non-detected data, comparing descriptive statistics (e.g., mean) that include non-detected data to environmental guideline values for regulatory applications, and when there are >50 data points. In addition, Helsel (1990) cautions that statistical methods such as regression are not reliable when more than 40% of the values are non-detects. Below is a discussion where all these factors for consideration are not inherent in Hibernia EEM data. Regarding the number of samples, on an annual basis, the Hibernia EEM data generally have too few samples (<50) to robustly apply Helsel's MLE method.

Overall, the Hibernia EEM sediment, and for that matter the toxicity testing and fish tissue data sets as well, do not have a problem with non-detects. This would cover most of the sediment trace elements, such as aluminum, chromium, manganese, and strontium for example. Likely contaminants to be detected in sediment and as a result of Hibernia production, which have been retained for statistical analysis in this and previous EEM programs, include barium, weak-acid leachable barium, fuel range hydrocarbon, and lube range hydrocarbon. For these substances, barium and lube range hydrocarbon data were generally measurable parameters and analytically detected above the RDL. Statistical analysis using ANOVA for these substances is by far the most robust and transparent statistical technique to use, and there are no technical reasons why ANOVA should not be used.

It is mainly weak-acid leachable barium and fuel range hydrocarbon data that non-detects are present, and generally less than 40 percent of the data are non-detects. The RDL for these parameters has not changed for all years of the EEM program (5 mg/kg for weak-acid leachable barium), except for fuel range hydrocarbon when the RDL was 10 mg/kg in 1994 and then lowered to 0.25 mg/kg since then (rounded to 0.3 mg/kg by the analytical laboratory as of 2007). However, the fuel range hydrocarbon data for 1994, and that for lube range hydrocarbon data as well, were not retained for statistical analysis because all the data were non-detects for that year. Therefore, estimating means and variances to test for statistical significance with one detection limit and substituting one-half the detection limit to less than 40 % of the data is likely to be statistically robust. In addition, these are unique substances introduced into the

environment primarily at the GBS (and the environmental background for these substances is essentially non-detectable). Therefore, where these substances are detected, it is *de facto* an environmental effect of the GBS (both practically, and as defined under the *Canadian Environmental Assessment Act*). The Hibernia EEM program has been designed to assess both the magnitude (which is measured by the absolute concentrations, where detected) and the spatial extent (the total number and distribution of monitoring stations where the substance was detected). The use of ANOVA for these stations is believed to be protected against statistical errors by the fact that the data are stratified by distance (Field and near-field Distance factors) from the platform, and not simply comparing two groups of data to determine statistically significant differences; the Hibernia EEM program is generally comparing as a function of distance, time, and the distance x time interaction effect – the error variance in these tests is dominated by the higher values (the detected weak-acid leachable barium and fuel range hydrocarbons), not by the substitution values of one-half the RDL for the non-detects. It is believed that the test results from ANOVA are robust and the conclusions valid.

To further illustrate how ANOVA is robust and the subtle contribution non-detected data have to the statistical results for the Hibernia EEM program, ANOVAs were performed to compare non-detected data substituted with one-half the RDL to those substituted with the low values analytically measured by the laboratory but reported as non-detected because of confidence limits. The latter substitution data were provided by Maxxam Analytics, which are assumed to be unbiased estimates and where the uncertainties associated with these data would be randomly distributed. The comparison of ANOVA results was carried out for weak-acid leachable barium and fuel range hydrocarbon in sediment for the 2009 Hibernia EEM data.

Prior to the ANOVA, Pearson correlation coefficients on log-transformed data for the factors Field and Distance between weak-acid leachable barium and grain size index were calculated. These correlation coefficients are provided in Table B.4. The results indicate that sediment grain size does not correlate with weak-acid leachable barium and fuel range hydrocarbon concentrations and therefore is not a covariate for these substances. In addition, the correlation coefficients do not differ substantially between the two types of substitution data for the non-detects, varying mainly at the level of the second significant digit. The overall conclusion, however, remains the same and that weak-acid leachable barium and fuel range hydrocarbon concentrations generally do not vary with sediment grain size.

Table B.4 Comparison of Pearson Correlation Coefficients for Log_{10} Transformed Data Using Values One-half of the RDL and Measured Values Below the RDL for 2009 Hibernia Sediment

Parameters	Factor	Sample Size	Correlation Coefficient Containing Non-detected Data as One-half the RDL	Correlation Coefficient Containing Non-detected Data as Measured Values Below the RDL
Weak-acid Leachable Barium and Grain Size Index	Field [1]	32	-0.182	-0.226
	Distance[2]	20	-0.299	-0.331
Fuel Range Hydrocarbon and Grain Size Index	Field [1]	32	-0.056	-0.060
	Distance[2]	20	-0.292	-0.289

[1] samples in the Near-, Mid-, and Far-fields

[2] samples from only the Near-Field at 250 m, 500 m, 1,000 m from the Hibernia Platform

The comparisons of One-Factor ANOVAs for the 2009 Hibernia EEM data only (i.e., there is no interaction term for Year x Field, or Year x Distance) and between non-detected data substituted with one-half the RDL to those substituted with measured values by the laboratory but reported as non-detected are provided in Tables B.5 to B.8. As for the correlation coefficients, it can be seen in these tables that the ANOVA results generally differ primarily at the second significant digit. However, the outcomes and conclusions for the statistical significance of the ANOVAs are the same. In addition, the probability level for the statistical test is the same when it is significant and relatively high (i.e., $p < 0.001$). The above ANOVA comparisons suggest and support the notion that, in the context of the Hibernia EEM program, there is no difference in the statistical analysis and conclusions reached when non-detected data are substituted with one-half the RDL, and that ANOVA is a statistically robust technique to use for the data analysis.

Table B.5 One-Factor ANOVA for Log_{10} Transformed Weak-acid Leachable Barium Concentration in 2009 Hibernia Sediment Using Values One-half of the RDL

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	*p*
Field (Near-, Mid-, Far-) Factor					
Field	1.350	2	0.675	10.056	< 0.001
Error	1.947	29	0.067		
Distance (in Near-Field – 250, 500, 1,000 m) Factor					
Distance	1.205	2	0.603	24.485	< 0.001
Error	0.418	17	0.025		

Table B.6 One-Factor ANOVA for Log_{10} Transformed Weak-acid Leachable Barium Concentration in 2009 Hibernia Sediment Using Laboratory-measured Values below the RDL

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	*p*
Field (Near-, Mid-, Far-) Factor					
Field	1.545	2	0.773	12.389	< 0.001
Error	1.809	29	0.062		
Distance (in Near-Field – 250, 500, 1,000 m) Factor					
Distance	1.105	2	0.553	27.885	< 0.001
Error	0.337	17	0.020		

Table B.7 One-Factor ANOVA for Log_{10} Transformed Fuel Range Hydrocarbon Concentration in 2009 Hibernia Sediment Using Values One-half of the RDL

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	*p*
Field (Near-, Mid-, Far-) Factor					
Field	0.489	2	0.244	1.481	0.244
Error	4.784	29	0.165		
Distance (in Near-Field – 250, 500, 1,000 m) Factor					
Distance	2.344	2	1.172	13.191	< 0.001
Error	1.511	17	0.089		

Table B.8 One-Factor ANOVA for Log_{10} Transformed Fuel Range Hydrocarbon Concentration in 2009 Hibernia Sediment Using Laboratory-measured Values below the RDL

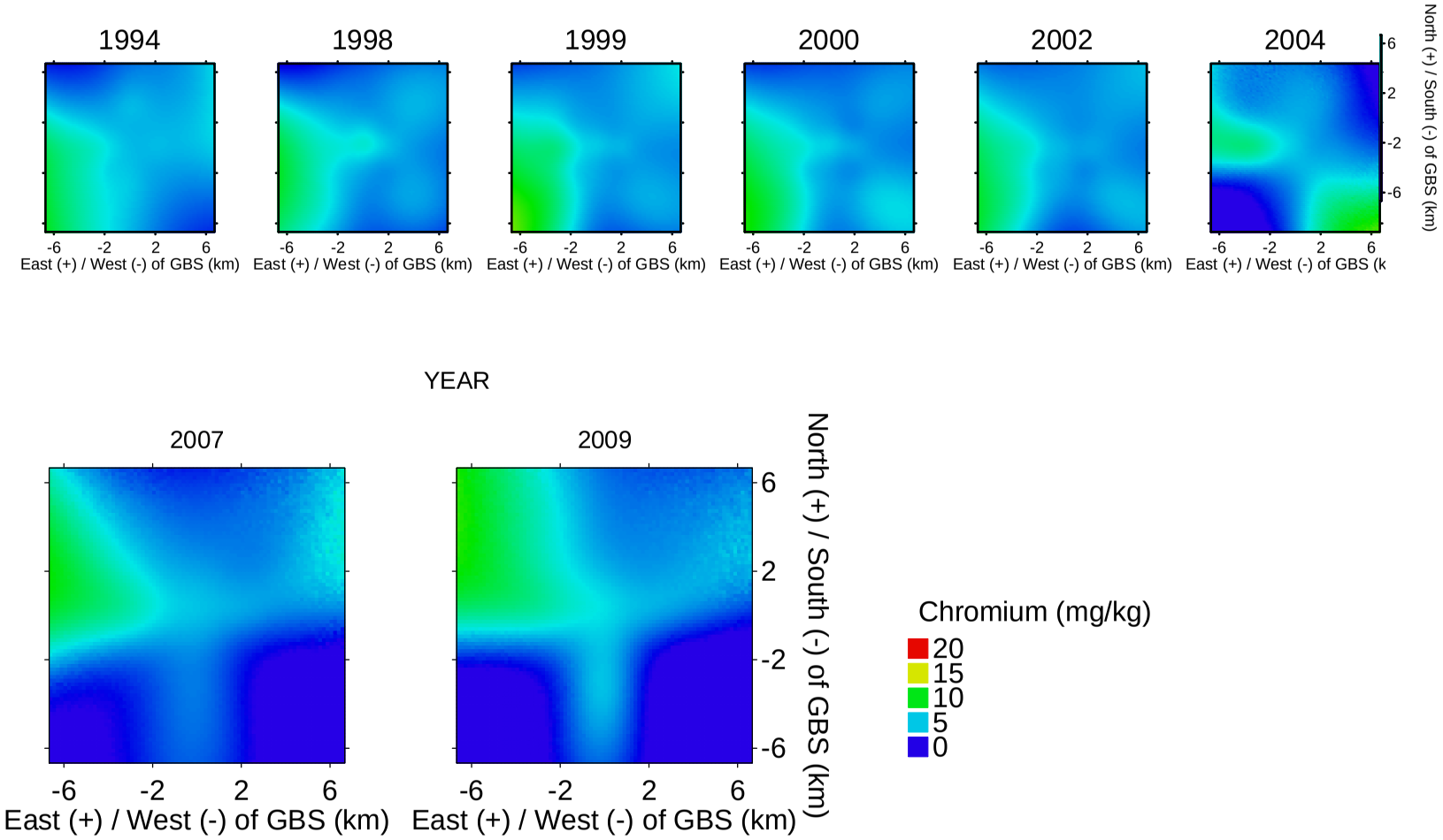
Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	*p*
Field (Near-, Mid-, Far-) Factor					
Field	0.517	2	0.259	1.569	0.225
Error	4.779	29	0.165		
Distance (in Near-Field – 250, 500, 1,000 m) Factor					
Distance	2.334	2	1.167	13.193	< 0.001
Error	1.504	17	0.088		

Another issue to underline where total petroleum hydrocarbons (TPH) are detected or where the analyses of trace elements such as total barium or weak-acid leachable barium have indicated that there is a statistical difference caused by proximity to the GBS, is to determine whether the concentrations of any substance detected might have the potential to cause additional environmental effects (for example, changes to benthic invertebrate community composition or productivity; or direct toxicity to aquatic life). For many substances, this can be achieved by comparison to CCME guidelines. For TPH, there are no CCME guidelines, but benchmarks can

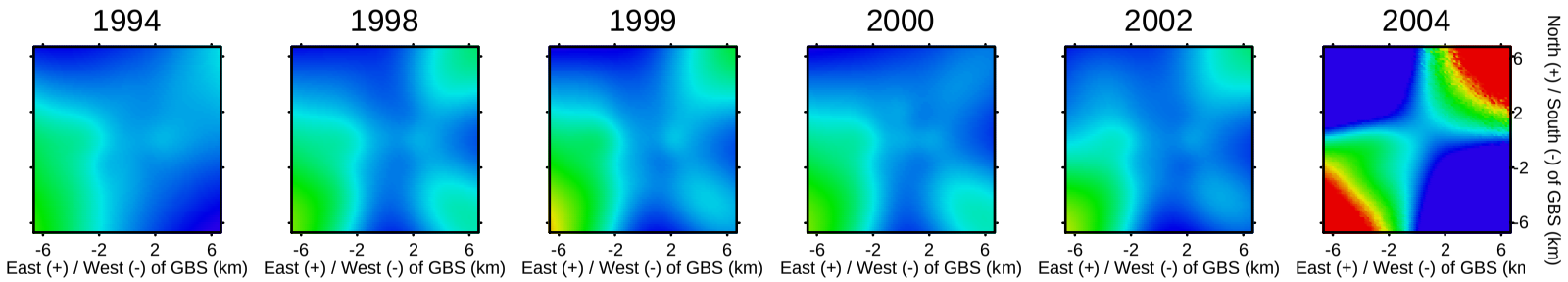
be established that are likely to be protective of the sediment-dwelling community based on theoretical models (e.g., those by Di Toro and others), and by reference to empirical studies relating effects to spilled hydrocarbon concentrations. As long as TPH can be detected at toxicologically relevant concentrations, then we can be comfortable with a relatively higher incidence of “non-detects” in the overall data.

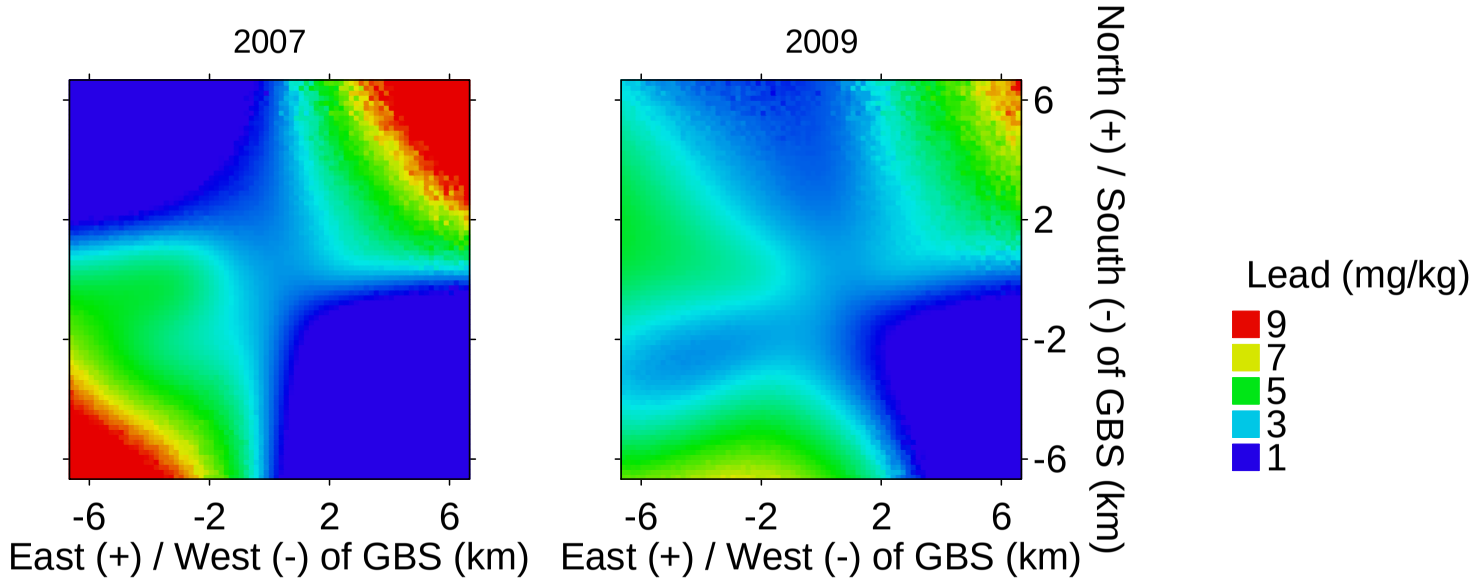
APPENDIX C

Spatial and Temporal Variability of Total Metal Concentrations in Sediment

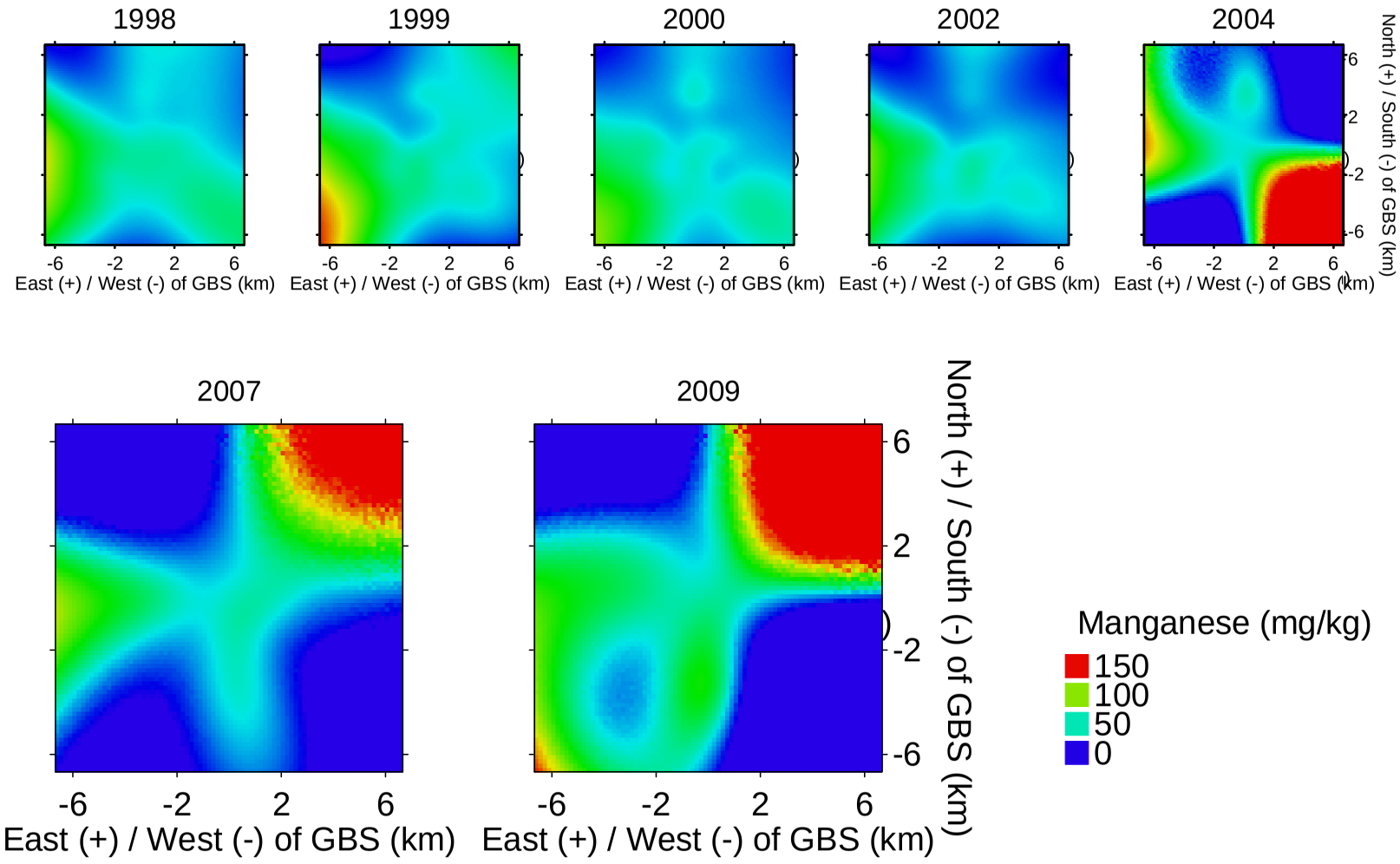

1994
1998
1999
2000
2002
2004
2007
2009
YEAR
-6
-2
2
6
East (+) / West (-) of GBS (km)
North (+) / South (-) of GBS (km)
Chromium (mg/kg)
20
15
10
5
0

Spatial and Temporal Variability of Chromium Total Metal Concentrations in Sediment

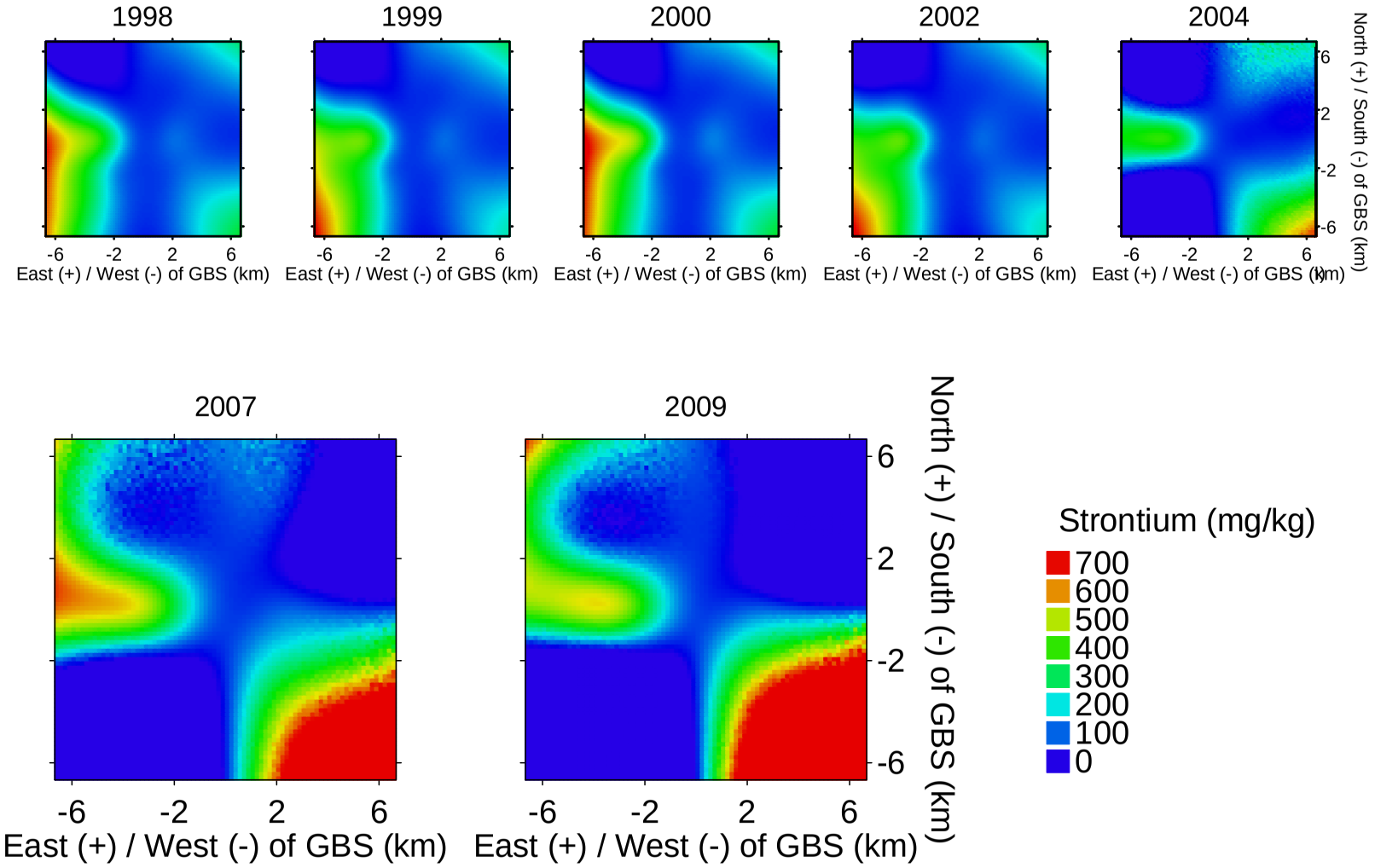




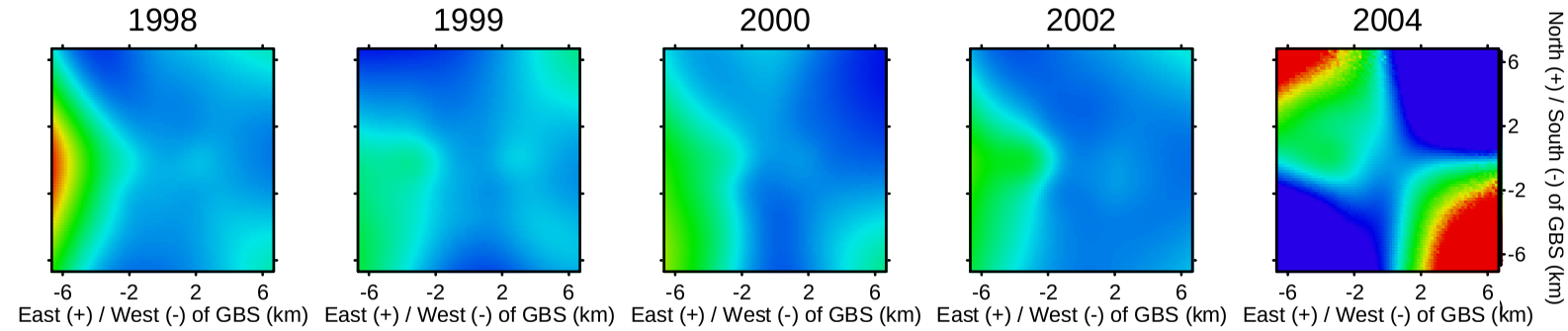
Spatial and Temporal Variability of Lead Total Metal Concentrations in Sediment

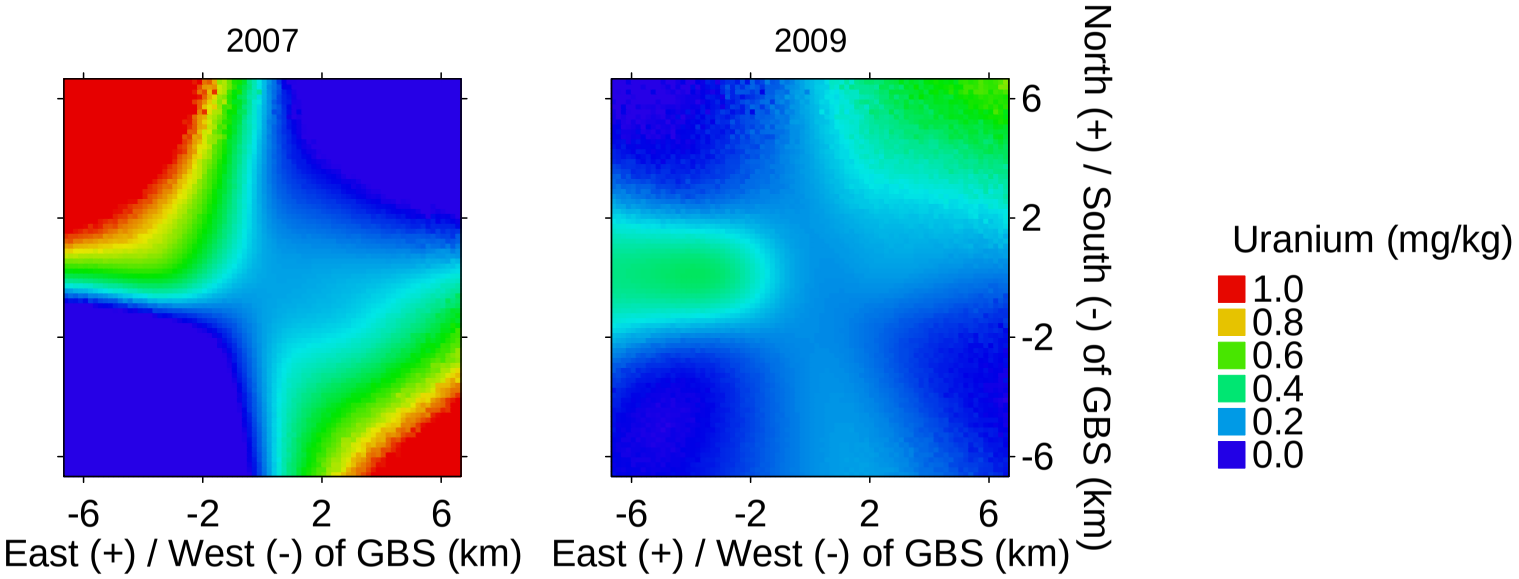


Spatial and Temporal Variability of Manganese Total Metal Concentrations in Sediment

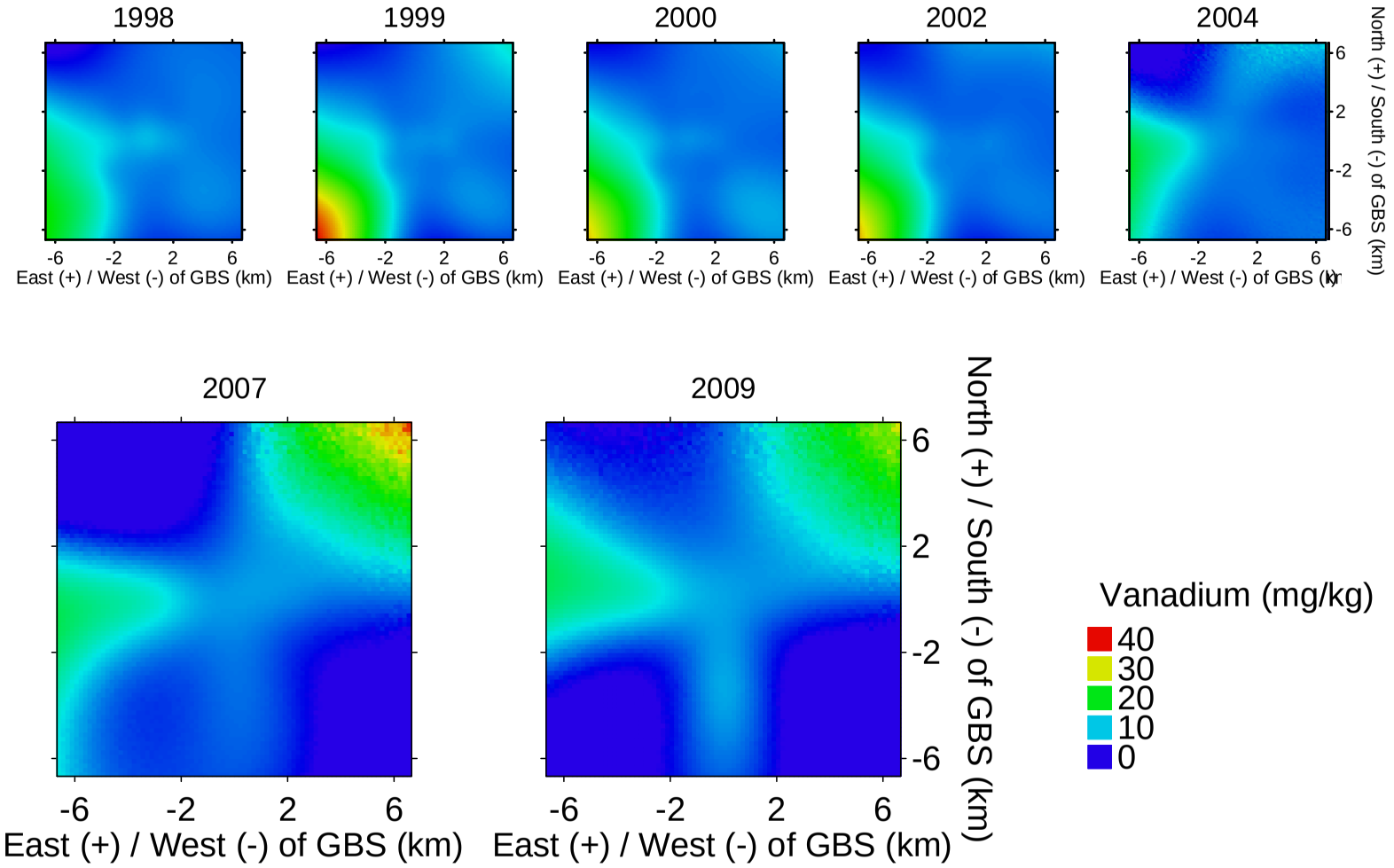


Spatial and Temporal Variability of Strontium Total Metal Concentrations in Sediment

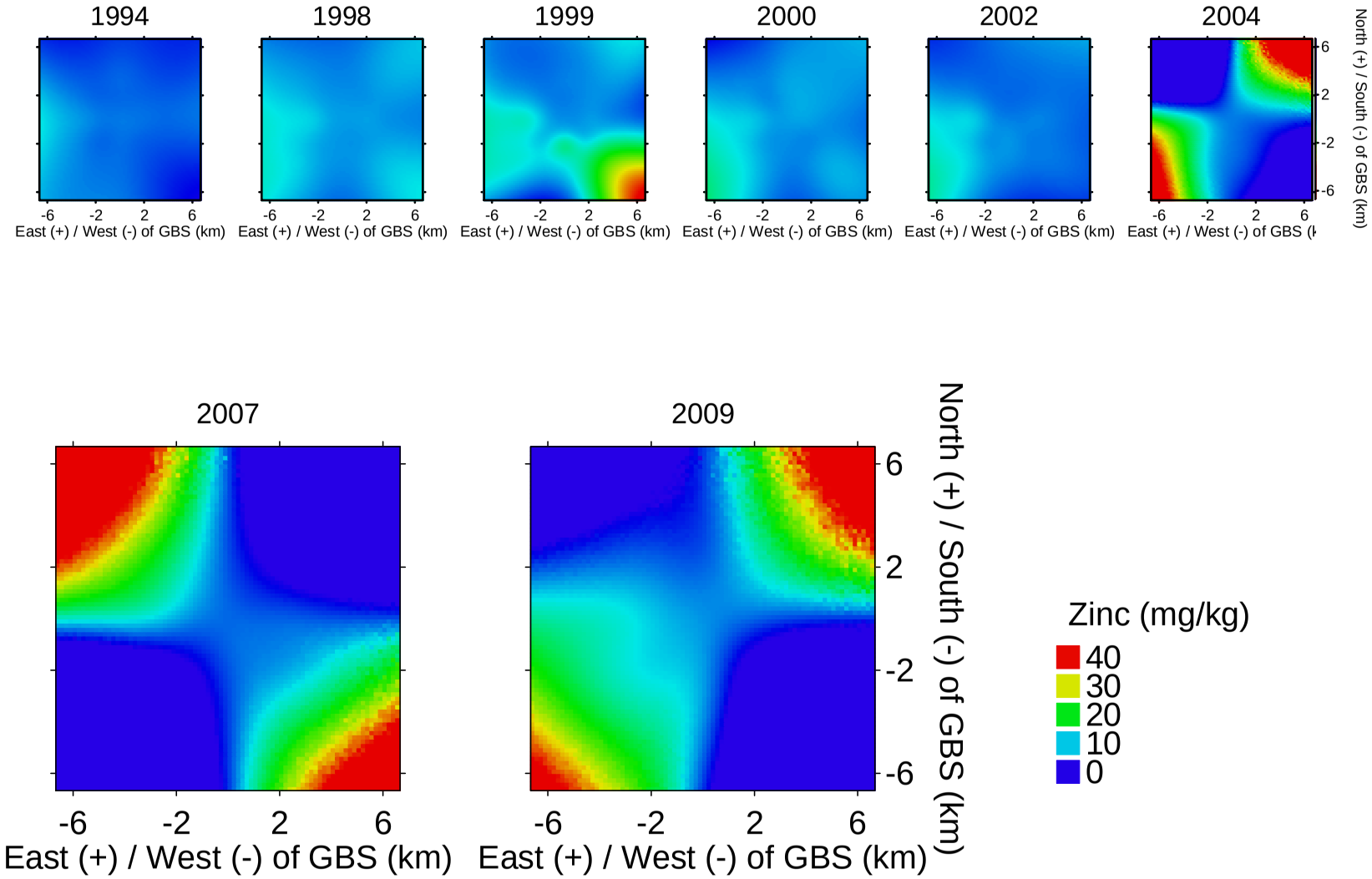




Spatial and Temporal Variability of Uranium Total Metal Concentrations in Sediment



Spatial and Temporal Variability of Vanadium Total Metal Concentrations in Sediment



Spatial and Temporal Variability of Zinc Total Metal Concentrations in Sediment

APPENDIX D

2007 Hydrocarbon Data

Station	Distance from GBS Wall (m)	Depth (from surface) (m)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylenes (mg/L)	C_6-C_{10} (less BTEX) (mg/L)	>C_{10}-C_{21} (Fuel Range) (mg/L)	>C_{21}-C_{32} (Lube Range) (mg/L)	Hydrocarbons BTEX plus TPH (Note [a]) mg/L
1	16.5	-1	0.023	0.016	0.001	0.007	0.02	0.06	<0.1	0.177
		-9	0.012	0.009	< 0.001	0.004	0.01	<0.05	<0.1	0.111
		-11	0.008	0.007	< 0.001	0.003	< 0.01	<0.05	<0.1	0.099
		- 65	0.023	0.015	< 0.001	0.005	0.02	<0.05	<0.1	0.139
2	21.2	-1	0.003	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.087
		-14	0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.083
		-18	< 0.001	0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.083
		-70	0.005	0.004	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.091
3	25.4	-1	0.002	0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.085
		-8	0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.083
		-12	0.002	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.086
		-70	0.007	0.004	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.093
4	21	-1	0.032	0.02	0.001	0.007	0.02	<0.05	<0.1	0.155
		-7	0.033	0.021	0.001	0.009	0.02	<0.05	<0.1	0.159
		-9	0.029	0.02	0.001	0.007	0.02	<0.05	<0.1	0.152
		-70	0.004	0.003	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.089
5	36	-1	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-6	0.04	0.026	0.002	0.009	0.03	0.05	<0.1	0.207
		-14	0.053	0.034	0.002	0.012	0.03	<0.05	<0.1	0.206
		-70	0.038	0.024	0.001	0.009	0.02	<0.05	<0.1	0.167
6	39	-1	0.017	0.011	< 0.001	0.004	0.01	<0.05	<0.1	0.118
		-6	0.012	0.008	< 0.001	0.002	< 0.01	<0.05	<0.1	0.103
		-14	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-70	0.012	0.008	< 0.001	0.002	< 0.01	<0.05	<0.1	0.103
7	24	-1	0.004	0.003	< 0.001	< 0.002	0.01	<0.05	<0.1	0.094
		-10	0.003	0.002	< 0.001	< 0.002	0.01	<0.05	<0.1	0.092
		-15	< 0.001	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.084
		-70	0.008	0.005	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.095
8	20.8	-1	0.09	0.055	0.004	0.021	0.05	0.09	0.1	0.410
		-10	0.044	0.028	0.002	0.011	0.03	<0.05	<0.1	0.190
		-11	0.09	0.006	< 0.001	< 0.002	0.01	<0.05	<0.1	0.183
		-70	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
9	26	-1	0.002	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.086
		-10	0.002	0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.085
		-13	0.001	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.085
		-70	0.003	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.087

Station	Distance from GBS Wall (m)	Depth (from surface) (m)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylenes (mg/L)	C6-C10 (less BTEX) (mg/L)	>C10-C21 (Fuel Range) (mg/L)	>C21-C32 (Lube Range) (mg/L)	Hydrocarbons BTEX plus TPH (Note a) mg/L
10	23.5	-1	0.002	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.086
		-12	0.002	0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.085
		-14	0.002	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.086
		-70	0.003	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.087
11	26.6	-1	0.032	0.02	0.001	0.008	0.02	<0.05	<0.1	0.156
		-10	0.01	0.007	< 0.001	0.002	< 0.01	<0.05	<0.1	0.100
		-16	< 0.001	0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.083
		-70	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
Ref 1	16,000 (Radial 7)	-1	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-25	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-45	< 0.001	0.002	< 0.001	< 0.002	< 0.01	<0.05	<0.1	0.084
		-75	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
Ref 2	16,000 (Radial 1)	-1	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-15	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-25	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
		-80	< 0.001	< 0.001	< 0.001	< 0.002	< 0.01	<0.05	<0.1	< EQL (0.083)
EQL			**0.001**	**0.001**	**0.001**	**0.002**	**0.01**	**0.05**	**0.1**	**0.2**

Note [a]: Hydrocarbons calculation note < EQL for samples that have all values below EQL and provide an estimate in brackets that uses ½ EQL ,. Thus the hydrocarbon calculation provided is a liberal estimate of the hydrocarbon levels for the samples analysed.

APPENDIX E

Triangle Test Statistical Table

TRIANGLE TEST, DIFFERENCE ANALYSIS

Number of Tasters	Number of Correct Answers Necessary to Establish Level of Significance			Number of Tasters	Number of Correct Answers Necessary to Establish Level of Significance		
	5%	1%	0.1%		5%	1%	0.1%
7	5	6	7	57	27	29	31
8	6	7	8	58	27	29	32
9	6	7	8	59	27	30	32
10	7	8	9	60	28	30	33
11	7	8	9	61	28	30	33
12	8	9	10	62	28	31	33
13	8	9	10	63	29	31	34
14	9	10	11	64	29	32	34
15	9	10	12	65	30	32	35
16	10	11	12	66	30	32	35
17	10	11	13	67	30	33	36
18	10	12	13	68	31	33	36
19	11	12	14	69	31	34	36
20	11	13	14	70	32	34	37
21	12	13	15	71	32	34	37
22	12	14	15	72	32	35	38
23	13	14	16	73	33	35	38
24	13	14	16	74	33	36	39
25	13	15	17	75	34	36	39
26	14	15	17	76	34	36	39
27	14	16	18	77	34	37	40
28	15	16	14	78	35	37	40
29	15	17	19	79	35	38	41
30	16	17	19	80	35	38	41
31	16	18	19	81	36	38	41
32	16	18	20	82	36	39	42
33	17	19	20	83	37	39	42
34	17	19	21	84	37	40	43
35	18	19	21	85	37	40	43
36	18	20	22	86	38	40	44
37	18	20	22	87	38	41	44
38	19	21	23	88	39	41	44
39	19	21	23	89	39	42	45
40	20	22	24	90	39	42	45
41	20	22	24	91	40	42	46
42	21	22	25	92	40	43	46
43	21	23	25	93	40	43	46
44	21	23	25	94	41	44	47
45	22	24	26	95	41	44	47
46	22	24	26	96	42	44	48
47	23	25	27	97	42	45	48
48	23	25	27	98	42	45	49
49	23	25	28	99	43	46	49
50	24	26	28	100	43	46	49
51	24	26	29	200	80	84	89
52	25	27	29	300	117	122	127
53	25	27	29	400	152	158	165
54	25	27	30	500	188	194	202
55	26	28	30	1000	363	372	383
56	26	28	31	2000	709	722	737

Source: Larmond 1977.

APPENDIX F

Oceans Ltd. (2005) Report

HEALTH ASSESSMENT OF AMERICAN PLAICE FROM THE HIBERNIA DEVELOPMENT SITE 2009

Prepared for:

Stantec
607 Torbay Road
St. John's, Newfoundland
A1A 4Y6

February 22, 2010

HEALTH ASSESSMENT OF AMERICAN PLAICE FROM THE HIBERNIA DEVELOPMENT SITE 2009

Prepared for:

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February 22, 2010

Doc. Ref. No. 12197.doc

ACKNOWLEDGMENTS

We thank:

- Dr. Mark Myers (Northwest Fisheries Science Center, Seattle, USA) for confirmation of diagnosis of pathological lesions.

- TMC Services Ltd. (St. John's, NL) for ageing otoliths.

TABLE OF CONTENTS

1.0 SUMMARY

OCEANS Ltd. was contracted by Stantec to carry out a health assessment of American plaice (*Hippoglossoides platessoides*) for the Hibernia Environmental Effects Monitoring (EEM) program. Bioindicator studies were carried out on fish sampled in the vicinity of the Hibernia Development Area (Study Area) as well as approximately 50 km Northwest of the rig (Reference Area). Bioindicators have potential to identify adverse health conditions in fish in advance of effects at the population level and thus provide early warning of potential problems. They are of special value for use in EEM programs around development sites on important fishing grounds in the open ocean where population level effects or for instance any site-induced changes in various condition indices could be very difficult to resolve in the absence of major impacts. As such, they can also be a valuable scientific tool for addressing public concerns of a real or perceptual nature about the scope of potential impacts on fish stocks of commercial importance.

The bioindicators studied in this survey included external and internal lesions, fish condition, haematology, mixed-function oxygenase (MFO) enzymes, and a variety of liver and gill histological indices. These bioindicators are in line with those used in many local, national and international monitoring programs. Overall, the results of the fish health survey carried out in 2009 indicated that the present health of American plaice is similar at the Reference Area and the Study Area. Small differences were observed between the two Areas for Fulton's condition factor and hepato-somatic index in females. These differences can reasonably be attributed to natural variability, but a contaminant related link cannot be ruled out indicating the importance of trend data. A slightly higher level of MFO enzyme activity was also observed in females from the Study Area when all maturity stages were pooled. However, when immature females, which were only obtained in the Study Area, were removed from the analysis, enzyme activity was similar in fish from the two Areas indicating that the increase observed was likely linked to differences in maturity stages and not to contamination. Of particular interest was the virtual absence of inter-site variability with respect to health effect indicators more commonly associated with chemical toxicity. This included a wide range of liver and gill lesions as well as visible external and internal abnormalities.

2.0 INTRODUCTION

The effects of environmental contamination can be viewed at different levels of biological organisation, extending from the molecular or biochemical level to effects on organ physiology and histology at the individual animal level and ultimately to the population or community level. Over the past few years, there has been increasing emphasis on the use of individual-level indicators of chemical stress to obtain an appreciation of the degree, extent and severity of potential health effects on populations. These indicators are commonly referred to as bioindicators or health effect indicators**.** Use of such indicators at the sub-organismal or organismal level has the potential to identify adverse conditions in advance of responses at the population level and as such can provide an early warning of problem identification and adverse health effects (e.g. Peakall, 1992; Society of Environmental Toxicology and Chemistry (SETAC) Special Publication Series, 1992; Adams, 2002; Tillit and Papoulias, 2003; Schlenk et al., 2008).

Holdway (2002) has provided a general overview of the acute and chronic effects, including on fishes, of wastes associated with offshore oil and gas production. Payne et al. (2003) and Hylland (2006) have also reviewed the effects of polycyclic aromatic hydrocarbons (PAHs) on fish.

It is important to have background knowledge on selected health effect indicators in fish in order to provide perspective on any future changes which may arise over the life of the Hibernia project. In this regard it is also important to note that in relation to concerns about fisheries, which are of major socio-economic importance in Atlantic Canada in general and in Newfoundland in particular, bioindicators can be a powerful tool for “disproving” as well as “proving” whether effects may be occurring.

All three developers on the Grand Banks, Exxon-Mobil, Petro-Canada and Husky, have included fish health indicators in their EEM programs. Some studies have also been published (Mathieu et al., 2005; Mathieu et al., 2010).

2.1 Selected Health Effect Indicators

Gross pathology, haematology, mixed function oxygenase (MFO) enzymes and tissue histopathology were studied in American plaice. These bioindicators have been extensively used with various fish species in environmental assessments and studies on gross pathology, MFO enzymes and histopathology have been specifically endorsed by agencies such as the Oslo Paris Commission for use in environmental monitoring and assessment programs (Stagg, 1998). A health effect indicator approach has been endorsed more recently by the European Union for use in assessing effects on fish health in such programs as the CITY FISH Program (Mierke et al., 2002) as well as by the US Geological Survey for their Biomonitoring of Environmental Status and Trends (BEST) Program (Schmitt and Dethloff, 2000; Schmitt et al., 2004). The latter program also includes a haematology component. A variety of health effect indicators have also recently been employed in major monitoring programs carried out in the North Sea and the Baltic Sea (Hylland et al., 2006; Lehtonen and Schiedek, 2006).

Presence of visible lesions, alterations in blood parameters and especially tissue histological changes are generally viewed as being pathological or potentially pathological in nature, while induction of MFO is most commonly recognised as an index of chemical exposure. However there is a body of literature associating MFO induction with radical production and mutagenic and carcinogenic processes at the molecular level. Induction in fish has also been specifically linked with effects on reproduction (Spies et al., 1988) as well as effects on the liver at both organ (e.g. Payne et al., 1988) and cellular levels (Au et al., 1999; Au and Wu, 2001; see also additional references under 2.1.3- Mixed-Function Oxygenase (MFO)

Activity). Thus measurement of MFO enzyme status in fish in monitoring programs also has value beyond being an index of chemical exposure.

2.1.1 Gross Pathology

Gross pathology refers to the observation and quantification of the presence of visible diseases, lesions and other abnormalities. These external (ulcers of the fins and trunk, lymphocystis, epidermal papilloma, skeletal disorders) and internal (grossly visible organ lesions) pathologies are often natural but may be exacerbated by various stressors, including contaminants (Bucke et al., 1996). Extensive studies in the USA and Europe have established a causal relationship between gross pathology and levels of contaminants in the environment (reviewed in Lang, 2002 and Au, 2004) and studies on gross pathology are commonly carried out in national and international biological monitoring programs (ICES, 1996; Stagg, 1998; USEPA, 2000; Lang, 2002; Schmitt et al., 2004; Hinck et al., 2006).

2.1.2 Haematology

Haematological parameters such as change in various types of blood cells can provide important insight into potential effects on immune functions and resistance to disease. As such, haematology is being used more extensively in assessing the health of fish (e.g. Blaxhall, 1972; Rice and Arkoosh, 2002). Numerous environmental pollutants have been shown to interact with components of the fish immune system (e.g. Dethloff et al., 1998; Khangarot et al., 1999; Rice and Arkoosh, 2002; Kruzynski et al., 2004; Tierney et al., 2004; Witeska, 2005) which can act as early warning bioindicators of the potential harm of environmental toxicants (Weeks et al., 1992; Tort et al., 2004). Differential white blood cell counts are currently used as bioindicators by the US Geological Survey in their Biomonitoring of Environmental Status and Trends (BEST) Program (Jenkins, 2003 and 2004). Changes in white blood cells have also recently been observed in laboratory studies with fish exposed to wastewaters from oil-sand refining operations (Farrell et al., 2004) and production waters (Payne et al., 2005).

2.1.3 Mixed Function Oxygenase (MFO) Activity

The MFO system refers to a family of enzymes that transforms the structure of organic chemicals and performs a critical role in detoxification and other physiological processes. The system which has iron-containing hemoproteins, cytochromes P-450, as terminal oxidases is also referred to as cytochrome P-450-dependent MFO. The activity of this enzyme system commonly increases in animals in the presence of certain classes of organic pollutants. An increase in activity or induction of this enzyme system can therefore be used to monitor exposure to low levels of such pollutants.

Both field and laboratory studies have demonstrated that MFO induction can be a useful index for assessing exposure to various types of mixed organic pollutants such as petroleum hydrocarbons in fish (e.g. Payne et al., 1987). MFO induction is currently used by eight of the contracting parties to the Oslo Paris Commission for monitoring biological effects of contaminants in the North Sea area (Stagg, 1998). With respect to petroleum contamination, field studies have been carried out around oil rigs in the North Sea (Stagg et al., 1995; Stagg and McIntosh, 1996) as well as in connection with major oil spills such as the Exxon Valdez in Alaska (Woodin et al., 1997), the Braer spill in the Shetland Islands (George et al., 1995) or the Prestige oil spill in Spain (Martinez-Gomez et al., 2006; Martinez-Gomez et al., 2009). Within the past few years, attention has also been given to the MFO induction potential of produced water. This has included some field (Aas and Klungsoyr, 1998; King et al., 2005; Forlin and Hylland, 2006; Abrahamson et al., 2008; Zhu et al., 2008) as well as laboratory

studies (e.g. Payne et al., 2005; Hurst et al., 2005; Casini et al., 2006; Olsvik et al., 2007; Abrahamson et al., 2008).

Variability is common in natural populations for endpoints such as bioindicators in fish or invertebrates and other endpoints such as components of benthic community structure. With respect to MFO, the recognised and adopted practice for many years in monitoring programs is to compare animals of the same sex, within the same size range, taken at similar times of the year (preferably outside the spawning season) at putatively impacted and reference site(s) (Stagg and McIntosh, 1998; Mathieu et al., 2005).

Temporary induction of MFO enzymes for a few days may not be harmful, but prolonged or repeated induction has been associated with a variety of metabolic, cellular, organ and developmental disturbances (Spies et al., 1988; Johnson et al., 1988; Stegeman and Hahn, 1994; Au et al., 1999; Au and Wu, 2001; Carls et al., 2005; Morales-Caselles et al., 2006; Colavecchia et al., 2007).

The term MFO is a generic expression and a functional definition is often applied, depending on the specific catalytic activity being assayed. One of the more convenient and sensitive assays uses 7-ethoxyresorufin as a substrate with the enzyme activity referred to as 7-ethoxyresorufin O-deethylase (EROD) (e.g. Whyte et al., 2000). EROD activity was measured in liver tissues of American plaice in this study.

2.1.4 Histopathology

Histopathology involves the analysis of tissues for the presence of cellular damage (i.e. lesions and tumours) which can indicate chronic, or long-term, exposure to pollutants. The value of histopathology lies in its ability to reflect the integrated effects of various contaminants over time and as such provides an indication of the potential for more serious health impairment in organisms. For the Hibernia EEM surveys, both liver and gill tissues were studied.

The liver plays a major role in metabolism, digestion, excretion, and the storage of various substances and can be a key indicator of chemical toxicity. Various field and laboratory studies have shown the relationship between chemical contamination and pathological lesions, such as neoplasia, hydropic vacuolation, megalocytic hepatosis, nuclear pleomorphism and proliferation of macrophage aggregates in the liver of fish (e.g. Hinton et al., 1992; Myers at al., 1998; Stentiford et al., 2003; Lang et al., 2006). Moreover, decrease in prevalence of hepatic lesions has been reported to occur following reduction in input of chemical contaminants, such as PAHs (Baumann and Harshbarger, 1995; Moore et al., 1996). Capping of contaminated sediment has similarly been shown to reduce the prevalence of various lesions in resident fish (Myers et al., 2008). These relationships clearly indicate the utility of these lesions as bioindicators of contaminant-induced effects in marine organisms. Fish liver histopathology has been adopted as one of techniques to be used in biomonitoring programs designed to assess the environmental quality of waters in Europe (Stagg, 1998; ICES, 2004; Lang et al., 2006), and in the United States (Johnson et al., 1993; Myers et al., 1994; Stehr et al., 1998; Hinck et al., 2006). Khan (2000) has also carried out some preliminary studies on the Grand Banks of Newfoundland.

Gills are important in respiration, acid-base balance, ionic regulation and excretion (e.g. Evans et al., 2005), and gill epithelia are quite sensitive to chemical damage (Haensly et al., 1982; Mallatt, 1985; Stoker et al., 1985; Evans, 1987). Studies carried out in the 1980s demonstrated that gills are damaged upon exposure to relatively low levels of petroleum (Haensly et al., 1982; Solangi and Overstreet, 1982; Kiceniuk and Khan, 1987). Epithelial hyperplasia, fusion of gill lamellae, and separation of respiratory epithelium from underlying tissue were reported in two species of marine fish exposed to crude oil and its water soluble

fractions (Solangi and Overstreet, 1982). A number of studies carried out since the 1980s have further noted the potential of petroleum (Spies et al., 1996; Moles and Norcross, 1998; Morales-Caselles et al., 2006), produced water (Stephens et al., 2000) as well as for instance oil sands wastewater (Van den Heuvel et al., 2000; Farrell et al., 2004; Nero et al., 2006) to damage fish gills. Gill histology has been included in a variety of fish health monitoring studies or programs (e.g. Stentiford et al., 2003; Mathieu et al., 2005; Landman et al., 2006 a and b; Hinck et al., 2007).

Confounding issues for some histopathologic bioindicators include distinguishing changes caused by contaminants from those due to infectious diseases and parasites. Generally, the causes of these lesions can be determined histologically by visualisation of the offending organism or the resultant inflammatory response. Seasonal and hormonal changes may also influence some histopathological biomarkers, however, the basic architectural pattern of the organs is not altered and detection of many important lesions is not compromised (e.g. Hinton et al., 1992; Myers and Fournie, 2002).

In general, the greater the frequency of different lesions known to be associated with chemical toxicity at a study site, the stronger the case can be made for a direct chemical aetiology. However histopathologic studies can also be equally valuable for identifying disease states in tissues which may have arisen from a contaminant mediated increase in disease susceptibility.

2.2 Selected Species

American plaice (*Hippoglossoides platessoides*) is a flatfish of the family Pleuronectidae and inhabits the continental shelves of northeastern North America and northwestern Europe (Scott and Scott, 1988). It is an important commercial species that was abundant before the fisheries moratorium on the northern half of the Grand Bank near Newfoundland (Morgan, 1992). The stock is presently showing signs of recovery. It is generally associated with depths from 80-250 m and cold water temperatures from 1.5 to – 1.8 ºC, prefers sand-mud bottoms and forages for a variety of invertebrates such as brittle stars, sand dollars and sea urchins, and small fish, mainly capelin, sand lance and mailed sculpin. American plaice are batch spawners (Walsh, 1994) and release batches of eggs every few days during the spawning period which extends on the Grand Bank from late April through early July (Maddock and Burton, 1999). Individuals can attain a maximum length of about 75 cm and age in excess of 25 years, with females growing faster than males (Pitt, 1975). Their long life span is of interest with regard to potential for cumulative effects of a chronic nature.

Studies have indicated that American plaice are relatively sedentary in comparison with other groundfish species. Pitt (1969) in a tagging study with 33 % total return for the Northeast Grand Bank reported that most tagged fish were recovered less than 30 miles from the tagging site seven or eight years after tagging. Morgan (1994) reported more movement of fish tagged on the top of the Bank in 3L but the return rates from this experiment were low (4.2%).

It is reasonable to suggest that American plaice are resident for periods of weeks to months in specific areas with any early warning indicators of health such as MFO enzyme induction and selected gill and liver lesions (but probably not carcinomas for instance) being causally linked to those areas. However, fish might move in and out of potential “contaminant” zones very close to study areas, such that exposure is insufficient to result in deleterious effects, yet this is a reflection of ecological relevance. Overall, the presence or absence of health effects in such a commercial species is important from a fisheries and general ecosystem perspective. American plaice was recommended by the Department of Fisheries and Oceans as the species of choice to include in EEM programs on the Grand Banks.

3.0 MATERIALS AND METHODS

3.1 Sampling

Fish Collection

The Hibernia field sampling program was carried out from July 1 and 2, 2009 aboard the Aqviq using an otter trawl. A total of 100 American plaice were collected during the cruise, 50 fish from the Study Area (~1-3 km from the Fisheries Exclusion Zone) and 50 fish from the Reference Area (~ 50 km Northwest of the rig). Table 1 provides information on sampling sets.

Table 1. Information on Fish Sampling Sets

Area	Trawl #	Sampling Date	Depth (m)	Temperature (° C)	Fish Sampled
STUDY AREA	GBS-01	July 1, 2009	77	2.1	7
	GBS-02	July 1, 2009	na	0.7	8
	GBS-03	July 1, 2009	78	0.8	7
	GBS-04	July 1, 2009	79	0.5	7
	GBS-05	July 1, 2009	81	0.3	7
	GBS-06	July 1, 2009	79	0.5	7
	GBS-07	July 1, 2009	79	0.4	7
REFERENCE AREA	REF-01	July 2, 2009	86	0.1	8
	REF-02	July 2, 2009	81	4.1	7
	REF-03	July 2, 2009	82	0.4	7
	REF-04	July 2, 2009	77	1.8	7
	REF-05	July 2, 2009	89	0.2	7
	REF-06	July 2, 2009	na	na	7
	REF-07	July 2, 2009	83	0.5	7

na = data not available

Tissue Sample Collection

Upon drawing blood, fish were killed by severing the spinal cord, measured to the nearest centimetre for total length and weighed to the nearest 2 grams on a sea-going balance. Each fish was assessed visually for any parasites and/or abnormalities observed on the skin, gills and fins or on internal organs (liver, gonads, digestive tract, musculature and spleen) under the general framework of Autopsy-Based Condition Assessment described by Goede and Barton (1990). Fish were dissected and sex and maturity stage were determined by visual examination according to procedures used by the Department of Fisheries and Oceans in the Newfoundland Region (Annex A). Tissues were processed as follows:

- **Blood** – Approximately 0.5 to 1.0 ml of blood was drawn from a dorsal vessel near the tail, dispensed carefully into a labelled tube containing an anticoagulant (EDTA) and gently mixed. Two blood smears were prepared for each fish within 1 hour of blood withdrawal according to standard haematological methods (Platt, 1969). Briefly, a capillary tube was dipped into the blood and a drop was spread across a microscope slide to form a uniformly thin film. Slides were dried in a chamber, fixed in methanol for 2 minutes and stored in slide boxes.

- **Liver** - The entire liver was excised and bisected. A 4-5 mm thick slice was cut from the centre portion of the right half of the liver (along the longitudinal axis) and placed in 10% buffered formalin for histological processing and the remainder of the right half was frozen on Dry Ice until returning to Port when it was placed in a -65^{0}C freezer for MFO analysis.

- **Gill** - The first gill arch on the right side of the fish was removed and placed in 10% buffered formalin for histological processing.

- **Heart, spleen, gonad, and head-kidney** - Tissue samples of these organs were removed and placed in 10% buffered formalin for histological processing, if required.

- **Otoliths** - A pair of otoliths were removed for ageing.

Throughout the dissection process, any internal parasites and/or abnormal tissues were recorded and preserved in 10% buffered formalin for subsequent identification.

It is noted that the left half of the liver which was retained for chemistry, as well as the gonads, were frozen on the boat and weighed upon return to the laboratory.

3.2 Sample Analysis

3.2.1 Haematology

Blood smears were stained with Giemsa stain and examined with a Wild Leitz Aristoplan bright field microscope in order to identify different types of cells based on their general form and affinity for the dye (Ellis, 1976).

Size, shape and degree of haemoglobinization of red blood cells were examined and recorded.

Differential blood cell counts were performed on lymphocytes, neutrophils and thrombocytes and expressed as a percentage of each type of cell on 200 white blood cells counted. Cells were counted under 400 magnification in fields along a row commencing from the front edge of the smear and continuing parallel to the slide edge until the total number of cells were counted.

3.2.2 Mixed Function Oxygenase (MFO) Assay

MFO induction was assessed in liver samples of American plaice as 7-ethoxyresorufin O-deethylase (EROD) activity according to the method of Pohl and Fouts (1980) as modified by Porter et al. (1989).

Sample preparation
Liver samples were thawed on ice within 4 weeks of storage at -65 ^{0}C and homogenised in 4 volumes of 50 mM Tris buffer, pH 7.5, (1 gram liver to 4 ml buffer) using at least ten passes of a glass Ten Broek homogeniser. Homogenates were centrifuged at 9,000 g for 15 minutes at 4 °C and the post-mitochondrial supernatant (S9 fraction) frozen in triplicate at -65 ^{0}C until assayed. All liver samples were held and processed under the same storage and assay conditions. Assays were carried out within 4 weeks of storage of S9 fractions.

EROD assay
The reaction mixture, final volume of 1 ml, contained 50 mM Tris buffer, pH 7.5, 2 µM ethoxyresorufin (Sigma) dissolved in dimethyl sulphoxide, 0.15 mM NADPH and 20 µl of S9

protein (diluted 5 times). After a 15-minute incubation at 27ºC, the reaction was stopped with 2 ml of methanol (HPLC grade) and samples were centrifuged (3600 g for 5 minutes) in order to remove the protein precipitate. The fluorescence of resorufin formed in the supernatant was measured at an excitation wavelength of 550 nm and an emission wavelength of 585 nm using a Perkin-Elmer LS-5 fluorescence spectrophotometer. Blanks were performed as above with methanol added before the incubation. All the samples were run in duplicate. Protein concentration was determined using the Lowry protein method (Lowry et al., 1951) with bovine serum albumin as standard. The rate of enzyme activity in pmol/min/mg protein was obtained from the regression of fluorescence against standard concentrations of resorufin. Two external positive controls (pools of liver homogenates from uninduced cunners and cunners induced with petroleum) were run with each batch of samples to ensure consistency of measurements.

3.2.3 Histopathology

Fixed liver and gill samples were processed by standard histological methods (Lynch et al., 1969) using a Tissue-Tek® VIP Processor. A graded ethyl alcohol series of 70%, 80%, 95%, and two changes of 100%, were used for dehydration of the samples. The tissues were then cleared in four changes of xylene and finally impregnated with three changes of molten embedding media, Tissue Prep 2 ™. The processed tissues were embedded in steel molds using molten embedding media, and topped with labelled embedding rings. After cooling, the hardened blocks of embedded tissues were removed from their base molds. The blocks were then trimmed of excess wax. Sections were cut at 6µm on a Leitz microtome, floated on a 47 ^{0}C water bath, and then picked up on labelled microscope slides. After air drying, slides were fixed at 60 ^{0}C for approximately 2 hours to remove most of the embedding media and allow the tissue to adhere properly to the slide. Sections were stained using Mayers Haematoxylin and Eosin method (Luna, 1968). Coverslips were applied using Entellan ® and the slides were left to air dry and harden overnight.

Histological examination of each tissue was conducted by the same investigator. One slide with 4-6 sections was examined per fish. If an abnormality was found in a section, the other sections were checked for the same abnormality. To minimize interpretive bias, a “blind” system in which the examiner is not aware of the site of capture of specimen was used. This is accomplished by using a “pathology” number on the slide label generated from a random number table matched with the actual specimen number.

Liver

All liver samples were assessed microscopically for the presence of different lesions previously identified as having a putative chemical aetiology in fish (e.g. Myers et al., 1987; Boorman et al., 1997; ICES, 2004; Blazer et al., 2006). Among them were:

1. Nuclear pleomorphism
2. Megalocytic hepatosis
3. Eosinophilic foci
4. Basophilic foci
5. Clear cell foci
6. Hepatocellular carcinoma
7. Cholangioma
8. Cholangiofibrosis
9. Proliferation of Macrophage aggregates
10. Hydropic vacuolation
11. Fibrillar inclusions

Any other observations were also recorded. Among them were hepatocellular vacuolation and parasitic infestation of the biliary system.

Lesions (except macrophage aggregates) were recorded for each fish as not detected (0) or

detected (1).

Macrophage aggregation was recorded on a relative density scale from 0 to 7 and prevalence was calculated for fish showing a proliferation of macrophage aggregates (considered here as 4 or higher on the scale).

The percentage of fish affected by each type of lesions or prevalence of lesion was then calculated.

Gill

Each gill sample was examined microscopically, first under low power (x20) for a general overview of the entire section and to record any abnormalities or parasites present. Four filaments, or primary lamellae, sectioned at a correct angle (with the central venous sinus visible in at least two-thirds of the filament and secondary lamellae of equal length on both sides) were selected and examined under x250 magnification for the presence of gill lesions associated with chemical toxicity (Mallat, 1985). This included observations for epithelial lifting (separation of the epithelial layer from the basement membrane), telangiectasis (dilatation of blood vessel at the tip of the secondary lamellae), lamellar hyperplasia (thickening of the epithelium due to an increase in the number of epithelial cells), fusion (fusion of two or more adjacent secondary lamellae) or oedema (swelling between or within cells).

A semi-quantitative examination was carried out where the total number of secondary lamellae as well as the lamellae presenting the lesions were counted on each selected filament as follows: (1) basal hyperplasia was recorded when an increase in thickness of the epithelium near the base of the lamellae reached at least 1/3 of the total length of the lamellae, (2) distal hyperplasia was recorded when there were more than 2 cell layers all around the two sides of the secondary lamellae and (3) tip hyperplasia was recorded when there were more than 3 cell layers at least 2/3 around the secondary lamellar tip. Results of the lamellar counts for each fish were expressed as the percentage of secondary lamellae presenting the lesion in relation to the total number of lamellae counted. The prevalence of the various types of lesions (presence or absence of each lesion for each fish) was also examined.

Regarding oedema, no count was carried out, but the severity of the condition (here, the swelling within cells) was recorded on a 0-3 relative scale (0-rare, 1-light, 2-moderate and 3-heavy).

3.2.4 Quality Assurance / Quality Control (QA/QC)

QA/QC procedures were used in all facets of the project, including sample collection, preparation, and analysis.

In terms of sample collection, only live fish were sampled and all relevant oceanographic data associated with each sample (i.e. latitude, longitude, depth, water temperature) were recorded.

Observations for gross pathology were carried out under the general framework of Autopsy-Based Condition Assessment described by Goede and Barton (1990).

QA/QC procedures for MFO followed the protocols recommended by Hodson et al. (1991) and Stagg and McIntosh (1998). All liver samples used for MFO analysis were treated and processed in the same manner to provide assurance that any difference in MFO activity was

due to sampling area and not affected by processing. All liver homogenates (S9 fractions) were prepared in triplicate and frozen at -65^{0}C. A stock solution of the enzyme substrate 7-ethoxyresorufin was made up to a consistent peak absorbance (i.e. dissolved in DMSO until the absorbance at 461.5 nm = 1.6 to 1.7). In preparing resorufin standards, the purity of the resorufin was taken into account by measuring it's molar absorbance at 572 nm in 0.1 M phosphate buffer, pH 8.0. Ethoxyresorufin and standard stock solutions were prepared in multiple small aliquots and stored under proper conditions. Blanks (reaction mixture with sample immediately inhibited by methanol) were incorporated in each batch of samples. All assays, including blanks, were run in duplicate. Two external positive controls (pools of liver homogenates from uninduced cunners and cunners induced with petroleum) were run with each batch of samples to ensure consistency of measurements. To insure that the equipment used for MFO assays gives accurate results, duplicate samples are assessed periodically both at Oceans Ltd. and at the Department of Fisheries and Oceans in St. John's to compare enzymatic results.

Protein contents were measured on the individual homogenates used for the MFO assays. A standard stock solution of bovine serum albumin was prepared in multiple aliquots and stored at -20ºC. Two standard concentrations and one blank (reaction mixture with distilled water instead of S9) were incorporated into each batch of samples. All samples were run in duplicate.

QA/QC procedures for haematology followed the practical guidelines described by Blaxhall and Daisley (1973). A blood sample was obtained from the dorsal vein near the tail of each fish. The sample was carefully expelled into a correctly labelled tube containing the anticoagulant EDTA to prevent clot formation. Duplicate blood smears were prepared, fixed and stained with Giemsa by standard haematological protocols (Platt,1969). One slide per fish was examined under bright field microscopy to identify different types of blood cells based on their general form and affinity for the dye (Ellis, 1976). Following this nomenclature, 4 types of blood cells were identified in American plaice: erythrocytes, lymphocytes, neutrophils and thrombocytes. *Erythrocytes* are thin ovoid cells containing a centrally located ovoid nucleus displaying a granular chromatin arrangement. The cytoplasm is eosinophilic with abundant haemoglobin. *Lymphocytes* are small white blood cells with a large nucleus and a small amount of cytoplasm. The nucleus, which occupies a large portion of the cell shows a clear chromatin network and the cytoplasm is basophilic. *Neutrophils* are large white blood cells with a nucleus that is rod or kidney shaped and on occasion is bi-lobed. The cytoplasm stains pale. *Thrombocytes* are oval or spindle cells with projection(s) of cytoplasm in one or both ends of the cell. The cell has a large oval nucleus with granular chromatin. Differential blood cell counts were performed on lymphocytes, neutrophils and thrombocytes and expressed as a percentage of each type of cell on 200 white blood cells counted. Haematological counts were conducted by the same investigator.

QA/QC procedures for histopathology followed the practical guidelines described by Myers and Fournie (2002). With respect to the gills, the first gill arch on the right side of each fish was sampled. Also, in order to ensure consistency, the liver was bisected and a 3-5 mm slice from the center portion was obtained. Tissues were placed in a volume of fixative equal to or greater than 20 times the volume of the tissue collected. For each block of paraffin embedded liver, two microscope slides containing 4-6 sections were prepared. One slide was examined per fish. If an abnormality was found in a section, the other section was checked for the same abnormality. To assure accuracy in histopathological diagnosis, established standardized terminology for liver lesions (e.g. Myers et al., 1987; Boorman et al., 1997; ICES, 2004; Blazer et al., 2006) and gill lesions (Mallat, 1985) were followed. Histological examinations were conducted by the same investigator. To minimize interpretive bias, a "blind" system in which the examiner is not aware of the site of capture of specimen was used. This is accomplished by using a "pathology" number on the slide label generated from a random number table matched with

the actual specimen number. Any questionable lesions are also screened by a world renowned fish pathologist (Dr. Mark Myers) for confirmation of diagnoses.

3.2.5 Statistical Analyses

A Control-Impact design was used and comparisons between the Reference and the Study Areas were conducted using Sigma-Stat 3.5 for the analysis of variance and SAS for the analysis of covariance.

- Length, total and gutted weight, age, condition indices, MFO enzyme activity, degree of oedema and arcsine square root-transformed percentages of blood cells and gill lesions, were analysed by the Unpaired t-test or the Mann-Whitney Rank Sum test, when the groups were not normally distributed.

- Log-log regression of body gutted weight on length as well as liver and ovary weight on gutted body weight were analysed by ANCOVA. When ANCOVA revealed parallel regression slopes between areas, comparisons were made and adjusted means calculated.

- Prevalence of maturity stages, and liver lesions were analysed by the Fisher exact test.

Comparisons between sites having a $p<0.05$ were considered to be statistically significant.

4.0 RESULTS

One hundred American plaice were examined for early warning effects on fish health. Fifty fish were sampled in the vicinity of the Hibernia development area (Study Area) and 50 fish at approximately 50 km Northwest of the rig (Reference Area). Necropsy data are provided in Annex B.

4.1 Biological Characteristics and Condition of Fish

Information on biological characteristics (sex, maturity, size, and age) as well as fish condition is valuable for interpreting the results of early warning effects on fish health. Biological characteristics and fish condition were analysed separately for each sex.

4.1.1 Sex Ratios and Maturity Stages

Thirty three females and 17 males were collected in the Study Area while 31 females and 19 males were collected in the Reference Area. Female:Male (F:M) ratios were not significantly different between the two Areas (p=0.835; Fisher exact Test).

Maturity stages of male and female fish were defined according to procedures used by DFO (Annex A) and results, expressed as frequencies (percentages) of maturity stages, were compared between the Reference and Study Areas with the Fisher exact test.

All males but 1 were mature and no significant differences in frequencies of various maturity stages were observed between the two Areas (Table 2).

Table 2 Frequencies (%) of Maturity Stages of Male American Plaice from the 2009 Hibernia Survey

	N	Immature M-100 [a]	Maturing to spawn this year M-140 [a]	Partly spent M-150 [a]	Spent this year M-160+M-170 [a]	Maturing for next year M-180 [a]
Reference Area	19	5.3	15.8	68.4	10.5	0.0
Study Area	17	0.0	2.4	52.9	23.5	0.0
p Value [b]		1.000	0.684	0.495	0.391	1.000

[a] Maturity stages were defined according to procedures used by DFO (Annex A)
[b] p Value obtained with the Fisher exact test

All females were mature, except for 3 in the Study Area (Table 3), and no significant inter-site differences in frequencies of various maturity stages were observed.

Table 3 Frequencies (%) of Maturity Stages of Female American Plaice from the 2009 Hibernia Survey

	N	Immature F-500 [a]	Maturing to spawn this year F-520 to F-540 [a]	Partly spent F-550 [a]	Spent this year F-560+F-570 [a]	Maturing for next year F-580 [a]
Reference Area	31	0.0	32.3	16.1	48.4	3.2
Study Area	33	9.1	21.2	12.1	57.6	0.0
p Value [b]		0.239	0.400	0.729	0.617	0.484

[a] Maturity stages were defined according to procedures used by DFO (Annex A)
[b] p Value obtained with the Fisher exact test

4.1.2 Size, Age and Condition

Length, total and gutted body weight, liver and gonad weight and age were compared between the Reference and Study Areas using the Unpaired t-test or the Mann-Whitney Rank Sum test, when the groups were not normally distributed.

Fish condition, which can be defined as a state of physical fitness, was assessed by calculating different condition indices (Dutil et al., 1995) such as (a) condition index expressed as Fulton's condition factor and calculated as 100 x body weight/length^3 based on gutted weight (b) hepato-somatic index (HSI) calculated as 100 x liver weight/gutted weight and (c) gonado-somatic index (GSI) calculated as 100 x gonad weight/gutted weight. Since these condition indices are commonly used, they are presented for general interest with comparisons between the two Areas being carried out by the Unpaired t-test. However, since use of these indices assumes that body weight is proportional to the cube of length, and liver and gonad weights are linearly related to gutted weight (which is not always the case), log-log regressions of body gutted weight on length, and liver and gonad weight on body gutted weight were also tested by analysis of covariance (ANCOVA). When ANCOVA revealed equality of regression slopes between areas, comparisons were made and adjusted means calculated.

Males

Information on biological characteristics and condition of male fish (all maturity stages pooled) from the Reference and Study Areas are summarised in Table 4. Data are expressed as means and standard deviations. The complete data set is provided in Annex B.

There were no significant differences in any of the parameters measured in male fish from the two Areas.

Table 4 Biological Characteristics and Condition Indices of Male American Plaice (all Maturity Stages Pooled) from 2009 Hibernia Survey

	Reference Area	Study Area	p Value [d]
Fish number	19	17	
Length (cm)	34.5 ± 2.6	35.0 ± 3.7	0.612
Total body weight (g)	359 ± 90	377 ± 136	0.682
Gutted body weight (g)	314 ± 82	342 ± 121	0.419
Liver weight (g)	2.6 ± 1.8	3.3 ± 2.0	0.288
Gonad weight (g)	3.8 ± 4.8	4.0 ± 4.9	0.911
Age (year)	8.3 ± 0.8	7.8 ± 1.8	0.348
Fulton's condition factor [a]	0.748 ± 0.072	0.763 ± 0.110	0.632
Hepato-somatic index [b]	0.815 ± 0.538	0.941 ± 0.436	0.217
Gonado-somatic index [c]	1.164 ± 1.247	1.134 ± 1.349	0.949

All data are expressed as mean of raw values ± standard deviation
[a] Calculated as 100 x gutted body weight/length ^{3}
[b] Calculated as 100 x liver weight/gutted body weight
[c] Calculated as 100 x gonad weight/gutted body weight
[d] p Value obtained with the Mann-Whitney Rank Sum test

With respect to adjusted means (Table 5), gutted body weight relative to the covariate length as well as liver and gonad weight relative to the covariate gutted weight did not differ significantly between the two Areas after ANCOVA analysis.

Table 5 Adjusted Means of Male American Plaice (all Maturity Stages Pooled) from the 2009 Hibernia Survey

Variable	Covariate	Adjusted Means		p Value [a]
		Reference Area	Study Area	
Gutted weight	**Length**	309	311	0.877
Liver weight	**Gutted weight**	2.1	2.6	0.212
Gonad weight	**Gutted weight**	1.8	1.8	0.943

Adjusted means are predictive mean variable at overall mean covariate
[a] p Value obtained after ANCOVA analysis of log-log regression of variable on covariate

Females

Information on biological characteristics and condition of female fish (all maturity stages pooled) from the Reference and Study Areas are summarised in Table 6. Data are expressed as means and standard deviations and were compared between Areas using the Unpaired t-test or the Mann-Whitney Rank Sum test. The complete data set is provided in Annex B.

Table 6 Biological Characteristics and Condition Indices of Female American Plaice (all Maturity Stages Pooled) from 2009 Hibernia Survey

	Reference Area	Study Area	p Value [d]
Fish number	31	33	
Length (cm)	41.9 ± 4.3	43.4 ± 4.0	0.166
Total body weight (g)	675 ± 230	769 ± 250	0.060
Gutted body weight (g)	568 ± 228	662 ± 231	***0.030****
Liver weight (g)	5.9 ± 3.6	8.9 ± 4.1	***0.002****
Gonad weight (g)	60.3 ± 61.5	60.2 ± 83.2	0.629
Age (year)	10.3 ± 1.7	10.5 ± 1.8	0.508
Fulton's condition factor [a]	0.740 ± 0.100	0.792 ± 0.080	***0.002****
Hepato-somatic index [b]	1.012 ± 0.508	1.351 ± 0.516	***0.004****
Gonado-somatic index [c]	10.317 ± 8.907	8.047 ± 8.556	0.189

All data are expressed as mean of raw values ± standard deviation
[a] Calculated as 100 x gutted body weight/length ^{3}
[b] Calculated as 100 x liver weight/gutted body weight
[c] Calculated as 100 x gonad weight/gutted body weight
[d] p Value obtained with the Mann-Whitney Rank Sum test
* Significantly different ($p<0.05$)

There were no significant differences in length, gonad weight, age or gonado- somatic index in female fish from the two Areas. However, significant differences were observed for gutted body weight ($p = 0.030$) and liver weight ($p = 0.002$) as well as for Fulton's condition factor (0.002) and hepato-somatic index ($p = 0.004$), with fish from the Study Area displaying higher values for all these parameters.

With respect to adjusted means by ANCOVA, there was a significant difference between Reference and Study Areas in gutted weight on length ($p = 0.028$) with a higher value observed in females from the Study Area (Table 7). However, for liver and gonad weight on gutted body weight, since the condition of equality of slopes between the two Areas was not met, comparisons of adjusted means were not suitable for ANCOVA.

Table 7 Adjusted Means of Female American Plaice (all Maturity Stages Pooled) from the 2009 Hibernia Survey

Variable	Covariate	Adjusted Means		p Value [a]
		Reference Area	Study Area	
Gutted weight	**Length**	562	598	***0.028 ** **
Liver weight	**Gutted weight**	ns	ns	ns
Gonad weight	**Gutted weight**	ns	ns	ns

Adjusted means are predictive mean variable at overall mean covariate
ns = not suitable for comparison due to inequality of slopes
[a] p Value obtained after ANCOVA analysis of log-log regression of variable on covariate
* Significantly different ($p<0.05$)

4.2 Gross Pathology

One fish from the Study Area exhibited nematode worms on its liver and another had its intestine protruding from the anus while one fish from the Reference Area had pale gills. No other external or internal lesions/abnormalities were observed on the 100 fish examined.

4.3 Haematology

Blood smears were examined for various types of cells. The red blood cells of all fish appeared to be normal in size and shape. Coloration was also similar indicating a similar degree of haemoglobinisation.

A differential cell count of lymphocytes, neutrophils and thrombocytes was carried out on a total of 95 fish. Blood smears of 1 fish from the Reference Area and 4 fish from the Study area were not suitable for cell counting. For the other blood smears, 200 cells were counted per fish and the results were expressed as mean percentage ± standard deviation of each cell type for each Area (Table 8). The complete data set on the different cells examined is provided in Annex C and a representative photograph of a blood smear (Photo 1) is included in Annex G.

Table 8 Frequencies of Blood Cell Types in American Plaice from the 2009 Hibernia Survey

	Reference Area N = 49	Study Area N = 46	p Value [a]
Lymphocytes	82.9 ± 26.4	82.2 ± 28.4	0.186
Neutrophils	0.5 ± 0.6	0.5 ± 0.8	0.748
Thrombocytes	16.6 ± 2.7	17.3 ± 2.8	0.231

All data are expressed as mean percentage ± standard deviation of each type of cell on 200 white blood cells counted
[a] p Value obtained with Mann-Whitney Rank Sum test after arcsin square root transformation of percentages

Inter-area comparisons were carried out with the Mann-Whitney Rank Sum test analysis after arcsin square root transformation of percentages. There were no significant differences between areas in the percentages of the 3 types of blood cells examined.

4.4 Mixed Function Oxygenase (MFO) Activity

Results of MFO enzyme activity, measured as EROD, in the liver of male and female American plaice (all maturity stages pooled) from the Reference and Study Areas are summarised in Figures 1 and 2. The complete data set is provided in Annex D.

There was no significant difference in EROD activity levels in males between the two Areas (p = 0.924; Unpaired t-test). However, the difference in enzyme activity was marginally significant in females (all maturity stages pooled) with an activity 1.8 fold higher in fish from the Study Area (p = 0.058; Mann-Whitney Rank Sum test).

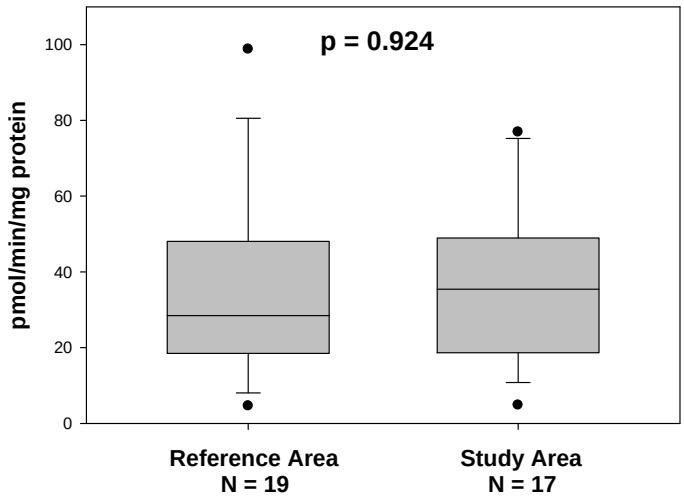

Figure 1 EROD Activity in the Liver of Male American Plaice (All Maturity Stages Combined)

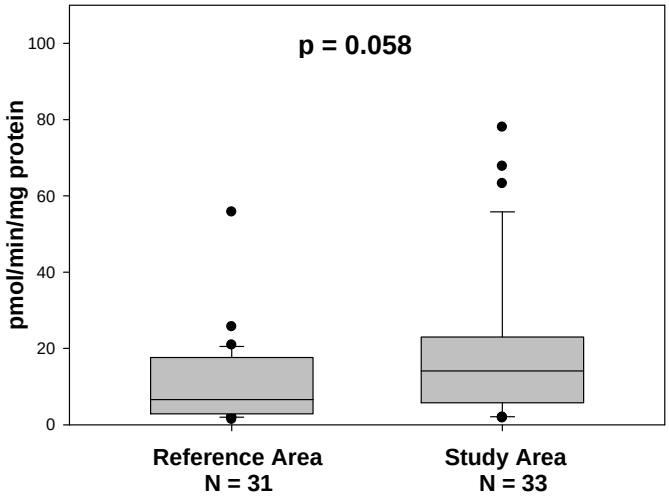

Figure 2 EROD Activity in the Liver of Female American Plaice (All maturity Stages Combined)

Data plotted are median (horizontal line in box), 25th and 75thpercentiles (bottom and top of box) and 10thand 90th percentiles (error bars). Data points beyond the 5th and 95th percentiles are also displayed.

Since EROD activity can vary with the stage of sexual maturity in female fish (e.g. Whyte et al., 2000), comparisons were also carried out separately for various stages including: mature (all

females minus immature females), maturing to spawn this year (including stages F-510 to F-540), partially spent (Stage F-550) and spent of the year (including stages F-560 and F-570), (see Annex A for description of maturity stages as used by DFO). Comparisons are provided in Table 9.

Table 9 Hepatic EROD Activity in Various Maturity Stages of Female American Plaice from the 2009 Hibernia Survey

	Reference Area	Study Area	p-Value [a]
All Females (Stages F-500 to F-580)	(31) 10.4 ± 10.9	(33) 19.0 ± 19.6	0.058
Mature females (all females minus immature females)	(31) 10.4 ± 10.9	(30) 15.3 ± 14.8	0.168
Maturing to spawn (stages F-510 to F-540 pooled)	(10) 5.0 ± 3.8	(7) 4.8 ± 5.8	0.464
Partially spent (stage F-550)	(5) 5.6 ± 7.1	(4) 10.6 ± 10.6	0.190
Spent females (stages F-560 and F-570 pooled)	(15) 21.6 ± 14.8	(19) 31.3 ± 20.8	0.510

All data are expressed as mean of raw values ± standard deviation
Maturity stages were defined according to procedures used by DFO (Annex A)
() = Number of fish analysed
[a] p Value obtained with the Mann-Whitney Rank Sum test

EROD enzyme activities in mature (p = 0.168), maturing to spawn (p = 0.464), partially spent (p = 0.190) and spent (p = 0.510) females were not significantly different between the two Areas. However, it is noted that the number of fish in the partially spent category was low for carrying out comparative analysis.

4.5 Histopathology

4.5.1 Liver Histopathology

Detailed histopathological studies were carried out on liver tissues of American plaice with observations on various lesions that have been commonly associated with chemical toxicity. These included nuclear pleomorphism, megalocytic hepatosis, basophilic, eosinophilic and clear cell foci, fibrillar inclusions, fibrosis, carcinoma, cholangioma, cholangiofibrosis, proliferation of macrophage aggregates and hydropic vacuolation. Any other observations were also recorded and included hepatocellular vacuolation and parasitic infestation of the biliary system. Lesions were recorded for each fish as present or absent (Annex E), except for macrophage aggregation which was rated on a relative scale from 0 to 7.

Results were expressed as percentage of fish affected by each type of lesion/observation (or prevalence of lesion) in the Reference and Study Areas (Table 10). A representative photograph of a normal liver structure is included in Annex G (Photo 2).

Fifty fish from the Study Area and 49 fish from the Reference Area were examined. There were no observation of lesions that have been commonly associated with chemical toxicity.

The frequencies of macrophage aggregates in livers of fish from the various Areas were low (0-2 rating on a relative scale from 0-7) and no cases of moderate to high aggregation (4 or higher on the relative scale) that are considered as a proliferation of macrophage aggregates were observed.

With respect to other observations, a patchy distribution of hepatocellular vacuolation (Photo 3, Annex G), not associated with degenerative changes, was observed in 6.1 % of fish from the Reference Area and in 2% of fish from the Study Area. The difference in incidences between the two Areas was not significant (p=0.362; Fisher's Exact Test). An infestation of the biliary system with a myxosporean parasite, possibly *Myxidium sp*., was also observed in 4.1 % of fish from the Reference Area and in 18.0 % of fish from the Study Area. The infestation did not appear to result in any other pathological changes in hepatic tissues. Inter-site difference in incidence of parasitic infestation was marginally significant (p=0.051; Fisher's Exact Test).

Table 10 Number of American Plaice with Specific Types of Hepatic Lesions and Prevalence of Lesions in the 2009 Hibernia Survey

Lesions	Reference Area (N = 49)		Study Area (N = 50)	
	Fish affected	Prevalence % [a]	Fish affected	Prevalence % [a]
Nuclear pleomorphism	0	0	0	0
Megalocytic hepatosis	0	0	0	0
Fibrillar inclusions	0	0	0	0
Eosinophilic foci	0	0	0	0
Basophilic foci	0	0	0	0
Clear cell foci	0	0	0	0
Carcinoma	0	0	0	0
Cholangioma	0	0	0	0
Cholangiofibrosis	0	0	0	0
Proliferation of macrophage aggregation [b]	0	0	0	0
Hydropic vacuolation	0	0	0	0
Hepatocellular vacuolation	3	6.1	1	2.0
Parasitic infestation of biliary system	2	4.1	9	18.0

[a] Percentage of fish affected
[b] Defined as scores greater than 3 on a 0-7 relative scale.

The observations on hepatocellular vacuolation and parasitism are of general interest but the absence of liver lesions that have been commonly associated with chemical toxicity are more relevant from an EEM perspective.

4.5.2 Gill Histopathology

Gill sections were examined for the presence of various gill lesions commonly associated with chemical toxicity. These included epithelial lifting, basal, distal and tip hyperplasia, fusion, telangiectasis and severe oedema.

For each fish, lamellar counts were performed on four filaments, when possible, and are presented as the percentage of secondary lamellae affected by each type of lesion in relation to the total number of secondary lamellae counted (Annex F). One fish from the Reference Area (which exhibited pale gills during necropsy) displayed extensive proliferation of ovoid and pale staining cells, or X-cells, in the interlamellar spaces of secondary lamellae (Photo 4, Annex G) and tissue structure was altered to such an extent that it was not possible to count the secondary lamellae. Accurate counts were also not possible for another fish from the Reference Area due to inadequate orientation of the primary lamellae. Lamellar counts were thus carried out on gill tissues of 48 fish from the Reference Area and 50 fish from the Study Area. A representative picture of normal gill secondary lamellae is provided in Annex G (Photo 5).

When lesions were observed, they were small and limited to a few secondary lamellae among the large number of lamellae examined (between 352 and 928 lamellae per fish). There were no cases of epithelial lifting in fish from either site and the percentages of lamellae affected by the other lesions were very low, all were less than 4%, except for one fish from the Reference Area with 8.4% of lamellae exhibiting basal hyperplasia as well as 11.3% of lamellae exhibiting fusion (Photo 6). Degree of oedema, which was recorded on a 0-3 relative scale, was quite low in both Areas.

Results, expressed as means ± standard deviations of percentage of secondary lamellae affected by lesions, are summarized in Table 11.

Table 11 Percentages of Secondary Lamellae Affected by Lesions and Rating of Oedema Condition in the Gill Tissues of American Plaice from the 2009 Hibernia Survey

	Reference Area N = 48	Study Area N = 50	p Value [c]
Epithelial lifting [a]	0	0	1.000
Basal hyperplasia 1 [a]	0.22 ± 1.21	0.04 ± 0.13	0.278
Basal hyperplasia 2 [a]	0.02 ± 0.09	0.03 ± 0.16	0.621
Distal hyperplasia [a]	0.16 ± 0.64	0.09 ± 0.22	0.161
Tip hyperplasia [a]	0.09 ± 0.36	0.13 ± 0.33	0.255
Fusion [a]	0.36 ± 1.68	0.20 ± 0.49	0.471
Telangiectasis [a]	0.01 ± 0.03	0.01 ± 0.05	0.992
Oedema condition [b]	0.833 ± 0.907	0.864 ± 0.805	0.669

All data are means ± standard deviations
[a] Mean percentage of lamellae presenting the lesion
[b] Mean of rating on a relative 0-3 scale
[c] p Value obtained with the Mann-Whitney Rank Sum test on arcsine square root-transformed percentages of the lesions or on ranking of oedema

Overall, less than 0.4% of the secondary lamellae were affected by the various lesions and no significant differences between the Study and Reference Areas were observed after the Mann-Whitney Rank Sum test on arcsine square root-transformed percentages of the lesions or on ranking of oedema.

5.0 DISCUSSION

Cellular and sub-cellular bioindicator responses along with observations on visible lesions on skin and internal organs are valuable monitoring tools for identifying adverse health conditions in animals in advance of population level responses. As such, they can provide early warning of potential health effects and aid in identifying their nature, scope and cause (see reviews by Payne et al., 1987; Peakall, 1992; Society of Environmental Toxicology and Chemistry (SETAC) Special Publication Series 1992; Adams, 2002; Tillitt and Papoulias, 2003; Schlenk et al., 2008). They are of special value in this regard for use in EEM programs around development sites on important fishing grounds in the open ocean where population level effects or for instance site-induced changes in various condition indices could be very difficult to resolve in the absence of major impacts (Mathieu et al., 2005). They can also be a valuable scientific tool for addressing public concerns of a real or perceptual nature about the scope of potential impacts on fish stocks of commercial importance. However, it is recognised that as for fish growth, fish organ condition, or for instance benthic community structure, bioindicator endpoints can display some natural variability and the focus should be on the prevalence of observations and recurrences or trends over time.

Various bioindicator studies were carried out on American plaice taken in the vicinity of the Hibernia development area (Study Area) as well as approximately 50 km Northwest of the rig (Reference Area). Blood smears of fish from each Area were examined for changes in red blood cell morphology and staining characteristics as well as changes in different types of white blood cells. EROD activity was determined in male and female liver tissues. Pathological studies were carried out and included observations on gross pathology of fish as well as detailed histopathological studies on liver and gill tissues. Histopathological studies included observations on 13 different indices in liver and 7 in gills. The biochemical and pathological indices studied were generally in line with those used in many local and national monitoring programs and specifically endorsed by the London-Paris Commission (Stagg, 1998). A health effect indicator approach has also more recently been endorsed by the European Union for use in assessing effects on fish health in such programs as the CITY FISH Program (Mierke et al., 2002) as well as by the US Geological Survey for their Biomonitoring of Environmental Status and Trends (BEST) Program (Schmitt and Dethloff, 2000; Schmitt et al., 2004). The latter program also includes a haematology component (Jenkins, 2003 and 2004). A variety of health effect indicators have also recently been employed in major monitoring programs carried out in the North Sea and the Baltic Sea (Hylland et al., 2006; Lehtonen and Schiedek, 2006).

Haematological changes can provide insight into potential contaminant mediated effects on immune function and resistance to disease (e.g. Weeks et al., 1992; Jenkins, 2003; Tort et al., 2004), while induction of MFO enzymes is known to be a sensitive response to selected organic contaminants of environmental concern including petroleum hydrocarbons (e.g. Payne et al., 1987). Pathology can provide information on the potentially injurious and cumulative effects of various contaminants, e.g. petroleum hydrocarbons, metals, etc. (e.g. Evans, 1987; Kiceniuk and Khan, 1987; Hinton et al., 1992; Moore et al., 1996; Myers et al., 1998; Stentiford et al., 2003). Moreover, both general pathology and histopathology have been used extensively in monitoring programs (e.g. Murchelano, 1990; Myers et al., 1994; Baumann and Harshbarger, 1995; Moore et al., 1996; Myers et al., 1998; Stagg, 1998; Hinck et al., 2006).

Biological characteristics and fish condition were also recorded.

5.1 Biological Characteristics and Condition of Fish

Information on fish biological characteristics and condition is valuable for interpreting results of bioindicator studies (Levine et al., 1995; Barton et al., 2002). Although a limited number of fish are being analysed from a population perspective (Dutil et al. 1995), such data could also provide a level of information for assessing major effects on animal condition.

American plaice were sorted by sex and by maturity stages as defined by DFO. The female:male ratio did not differ between the Reference and Study Areas. With respect to fish maturity, there were also no significant inter-site differences in the frequencies of the various maturity stages in male and female fish.

All biological characteristics and condition indices were similar in male fish from the two Areas.

With respect to females, length, gonad weight, age and gonado-somatic were similar in fish from the two Areas. However, gutted body weight, liver weight, Fulton's condition factor and hepato-somatic index were significantly higher in fish from the Study Area.

Condition factor can vary naturally with feeding and reproductive status of fish (Dutil et al., 1995; Maddock and Burton, 1999; Barton et al., 2002) and Morgan (2003) has provided data indicating that condition in American plaice on the Grand Banks can vary in fish collected at sites in close proximity. But, condition factor can also vary in either direction outside the normal range in response to chemical exposure (e.g. Schmitt and Dethloff, 2000).

As for fish condition factor, the relative size of liver can vary naturally with feeding and reproductive status of fish (Dutil et al., 1995; Maddock and Burton, 1999; Barton et al., 2002), but also with various stressors including contaminants. Elevated HSI have been observed in fish at sites contaminated with pulp mill effluents (Hodson et al., 1992; Munkittrick et al., 1992; Servos et al., 1992; Leblanc et al., 1997; Billiard and Khan, 2003; Sepulveda et al., 2004), industrial effluents (Fabacher and Baumann, 1985; Arcand-Hoy and Metcalfe, 1999; Orlando et al., 1999), mixed pollution (Poels et al., 1980; Sloof et al., 1983; Everaarts et al., 1993), sewage sludge and treatment works (Secombes et al., 1995; Lye et al., 1997; Corsi et al., 2003) and metals (Mauk and Brown, 2001; Ozmen et al., 2006).

Overall, the slight differences in condition indices observed in female American plaice between sites have to be interpreted cautiously as the size of samples was small for such population level comparisons (Dutil et al., 1995). Such differences are likely due to natural factors such as nutritional and reproductive status. However, there is a body of literature from field studies indicating that a contaminant-related link cannot be ruled out.

5.2 Gross Pathology

Gross pathology was assessed visually on all fish during the necropsies for any external or internal abnormalities on the skin or fins or on internal organs (gill, liver, gonads, digestive tract, musculature and spleen). Abnormalities were rarely encountered in the two Areas: one fish from the Study area exhibited nematode worms on its liver and another had its intestine protruding from the anus (likely due to trawling pressure) while one fish from the Study Area exhibited pale gills which were confirmed by microscopy to be X-cell lesions. Liver nematodes and anal protrusions are occasionally observed in American plaice on the Grand Banks (personal communication DFO). Likewise, a low frequency of X-cells has been reported in American plaice on the Grand Banks (Mathieu et al., 2005 and 2010).

5.3 Haematology

Haematology, including the analysis of red and white blood cells, has potential to assess the overall health of fish as well as to indicate immunological effects that may be important in disease susceptibility. Payne et al. (2005) have noted changes in white blood cells (in a 50% difference range) in cunner chronically exposed to relatively high levels of produced water under laboratory conditions. Alteration of some immunnlogical parameters has also been observed in codfish chronically exposed for several weeks to produced water from the Grand Banks (Perez Casanova, 2009)

There were no apparent qualitative differences in morphology or staining characteristics of red blood cells and no significant differences in differential white blood cell counts in fish from the two Areas.

5.4 Mixed Function Oxygenase (MFO) Activity

Since basal levels of MFO enzymes can vary seasonally between males and females of the same species (e.g. Walton et al., 1983; Mathieu et al., 1991; Whyte et al., 2000), results were analysed separately for each sex.

There was no significant difference in EROD activity in males between the two Areas. However, the difference in enzyme activity was marginally significant ($p = 0.058$) in females when all maturity stages were pooled with a 1.8-fold higher level in fish from the Study Area. Since reproductive state can influence enzyme activity in female fish (e.g. Whyte et al., 2000), a inter-area comparison of enzyme activity was also carried out on females of different maturity stages. This included mature (all maturity stages except immature), maturing to spawn, partially spent and spent females. Although the number of fish in the partially spent category were quite low for comparisons, there were no significant differences in enzyme levels for the various categories between the Reference and Study Areas. This indicates that the difference observed for all females (immature and mature pooled) was likely due to the presence of immature females that were only found in the Study Area. Immature fish of various species have been reported to have higher EROD activity than mature female fish (e.g. Whyte et al., 2000). A trend towards higher levels of EROD in immature female American plaice has also been observed in other surveys on the Grand Banks.

5.5 Histopathology

With respect to liver histopathology, there were no cases of hepatic lesions that have been associated with chemical toxicity in field and laboratory studies (e.g. Myers and Fournie, 2002; ICES, 2004). These included nuclear pleomorphism, megalocytic hepatosis, basophilic, eosinophilic and clear cell foci, fibrillar inclusions, fibrosis, carcinoma, cholangioma, cholangiofibrosis, proliferation of macrophage aggregates and hydropic vacuolation.

As noted in previous years, a few other hepatic conditions were observed. A “patchy distribution” of hepatocellular vacuolation, not associated with degenerative changes, was observed in similar proportions of fish from each Area and is likely linked to gonadal maturation (Timashova, 1981; Bodammer and Murchelano, 1990; Couillard et al., 1997). Also, an infestation of the biliary system with a myxosporean parasite was found in a number of fish from both Areas, but with a higher proportion (18%) observed in the Study Area ($p = 0.051$). However, similar and higher prevalences of parasitic infestation (up to 58%) have been commonly observed in other surveys on the Grand Banks including at Reference and

Development Areas. It is noted that, as reported in past surveys, the presence of parasites in the biliary system did not appear to result in any other pathological changes in hepatic tissues.

Observations on parasitism and hepatocellular vacuolation are of value in relation to providing general information on their presence in the survey area. However, it is important to note from an EEM perspective that liver lesions more commonly associated with chemical toxicity were absent.

With respect to gills, microstructural changes which have been associated with chemical toxicity (e.g. Mallat, 1985) such as epithelial lifting, hyperplasia, telangiectasis and lamellar fusion were absent or found at very low frequencies (less than 0.5%) in both Areas. The degree of severity of oedema was quite low in the 2 Areas. Also, X-cells, that have recently been shown to be caused by a protozoan parasite (Miwa et al., 2004), were observed in one fish from the Reference Area. A few cases of X-cells have been reported in American plaice in other surveys on the Grand Banks (Mathieu et al., 2005 and 2010).

As for the liver histopathological indices, it is of interest to note from an EEM perspective the absence or extremely low occurrence in the survey area of gill lesions associated with chemical toxicity.

6.0 Conclusions

Overall, the results of the fish health survey carried out in 2009 indicated that the present health of American plaice is similar in fish from the Reference Area and the Study Area. Small differences were observed between the two Areas for Fulton's condition factor and hepato-somatic index in females. These differences can reasonably be attributed to natural variability, but a contaminant related link cannot be ruled out indicating the importance of trend data. A slightly higher level of MFO enzyme activity was also observed in females from the Study Area when all maturity stages were pooled. However, when immature females, which were only obtained in the Study Area, were removed from the analysis, enzyme activity was similar in fish from the two Areas indicating that the increase observed was likely linked to differences in maturity stages and not to contamination. Of particular interest was the virtual absence of inter-site variability with respect to health effect indicators more commonly associated with chemical toxicity. This included a wide range of liver and gill lesions as well as visible external and internal abnormalities.

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ANNEX A

Description of Groundfish Maturity Stages Used by DFO in the Newfoundland Region

Description of Groundfish Maturity Stages Determined by Visual Examination According to DFO in the Newfoundland Region

Male	Female
Immature (100): Testes narrow and translucent, vasa deferentia very narrow and thin walled.	**Immature (500):** Ovary small, grey to pink in colour; membrane thin and translucent; eggs not visible to the naked eye.
Spent L (110): Vasa deferentia wide and opaque, sometimes with residual milt from spawning in previous year; outer edges of testes not pinkish or greyish as in maturing fish; spent in previous (L-last) year.	**Spent L (510):** Ovary thick-walled with no new eggs visible to the naked eye; spent in the previous (L-last) year. **Mat A-P (520):** Eggs visible to naked eye in ovary itself; all eggs opaque; maturing to spawn in present year. **Mat B-P (530):** Opaque and clear eggs present with less than 50% of the volume being clear eggs; maturing to spawn in the present (P) year.
Mat P (140): Testes relatively thick compared with immature, with outer edges pink or grey in early stage and white in later stage; early in the year some testes may show evidence of spawning in a previous year but the edges of the testes indicate recovery; maturing to spawn in present (P) year, i.e. year of capture.	**Mat C-P (540):** 50% or more of the volume are clear eggs; this stage also includes the ripe condition where the ovarian content is almost liquid with clear eggs; to spawn or spawning in the present (P) year.
Partly Spent (150): Some milt extruded in present year, but residual milt in testes and vasa deferentia.	**Partly Spent P (550):** Ovary not full as in Mat C-P; some eggs extruded but many clear eggs remaining.
Spent P (160): Spawning completed in present year; recovery not sufficiently advanced for outer edges of testes to be pinkish or greyish in colour.	**Spent P (560):** Spawning completed in present year but possibly a few clear eggs remaining; no new opaque eggs visible to the naked eye.
Spent P Mat N (170): Spawning completed in present year; outer edges of testes pink or grey or even becoming white in preparation for spawning in the next (N) year; this stage becomes Mat P in January of the next year.	**Spent P Mat N (570):** Spawning completed in present year, and new opaque eggs, for spawning in the next (N) year, visible to the naked eye; this stage becomes Mat A-P in January of the next year.
Mat N (180 or 190): Testes developing from immature stage for spawning in the next (N) year; testes becoming thick, being pink or grey early in this stage and gradually whitening; this stage becomes Mat P in January of the next year.	**Mat A-N (580):** No evidence of previous spawning; but new opaque eggs, for spawning in the next (N) year, visible to the naked eye; this stage becomes Mat A-P in January of the next year.

ANNEX B

Necropsy Data of American Plaice
from the 2009 Hibernia Survey

Necropsy Data of American Plaice from the 2009 Hibernia Survey

Study Area

Fish #	Sex - Maturity [a]	Length (cm)	Total Weight (g)	Gutted Weight (g)	Liver Weight (g)	Gonad Weight (g)	Observations	Age (year)	CF t [b]	CF g [c]	HSI	GSI
1	F-560	42.9	730	620	19.6	0.8		11	0.9246	0.7853	3.1613	0.1290
2	M-160	39.5	542	450	8.6	0.6		11	0.8794	0.7302	1.9111	0.1333
3	F-560	43.5	710	630	7.2	20.4		11	0.8626	0.7654	1.1429	3.2381
4	F-530	40	540	440	4	53.9		9	0.8438	0.6875	0.9091	12.2500
5	F-560	45	950	770	10	34.4		12	1.0425	0.8450	1.2987	4.4675
6	M-150	40.5	570	500	3.6	3.6		9	0.8580	0.7527	0.7200	0.7200
7	F-550	40	570	450	6	15.5		9	0.8906	0.7031	1.3333	3.4444
8	F-560	47.8	964	800	16.4	39		10	0.8827	0.7325	2.0500	4.8750
9	F-560	42.1	640	550	9.8	19.8		9	0.8577	0.7371	1.7818	3.6000
10	F-550	39.5	550	450	7.4	56		9	0.8924	0.7302	1.6444	12.4444
11	F-560	45	870	760	9.2	32.6		11	0.9547	0.8340	1.2105	4.2895
12	F-520	43.3	770	600	12	190.2		13	0.9485	0.7391	2.0000	31.7000
13	F-560	45.6	960	830	7.4	32		14	1.0125	0.8754	0.8916	3.8554
14	M-170	37.4	390	360	3.2	2.4		9	0.7455	0.6882	0.8889	0.6667
15	M-150	29.7	190	170	0.6	1		5	0.7252	0.6489	0.3529	0.5882
16	F-560	43.4	770	660	11.2	41.6		11	0.9419	0.8074	1.6970	6.3030
17	F-560	42.3	640	560	7.4	16.6		7	0.8456	0.7399	1.3214	2.9643
18	M-140	36	390	360	2.6	7.6		8	0.8359	0.7716	0.7222	2.1111
19	M-140	28.5	150	130	0.8	1		6	0.6480	0.5616	0.6154	0.7692
20	M-150	34.1	330	290	3	14.2		7	0.8322	0.7314	1.0345	4.8966
21	F-560	40.1	590	510	7	20.6		9	0.9150	0.7909	1.3725	4.0392
22	F-560	52.5	1280	1090	9.4	60.4	worms on liver	13	0.8846	0.7533	0.8624	5.5413
23	M-150	37.8	490	430	2.8	15.8		9	0.9072	0.7961	0.6512	3.6744
24	F-500	36.5	420	370	3.8	0.2	intestine protruding out near front fins	8	0.8637	0.7609	1.0270	0.0541
25	F-560	47.5	920	800	9.2	37.8		14	0.8584	0.7465	1.1500	4.7250
26	F-500	40.6	640	540	6.2	4.8		10	0.9563	0.8069	1.1481	0.8889
27	M-150	30.1	220	200	1.2	0.4		5	0.8067	0.7334	0.6000	0.2000
28	M-150	39.6	590	530	5.4	10.2		9	0.9501	0.8535	1.0189	1.9245
29	F-520	41.4	650	600	5.8	89		9	0.9160	0.8456	0.9667	14.8333
30	F-530	45.1	890	710	6.2	141		11	0.9702	0.7740	0.8732	19.8592
31	F-530	41.2	710	690	3.4	190.6		9	1.0152	0.9866	0.4928	27.6232
32	F-560	44.6	770	650	6.2	70.2		11	0.8679	0.7327	0.9538	10.8000
33	M-140	33	320	280	1.4	4.2		8	0.8904	0.7791	0.5000	1.5000
34	M-150	38.1	420	410	3.2	2.4		8	0.7594	0.7413	0.7805	0.5854
35	F-560	39.4	520	490	6.6	27.6		9	0.8502	0.8011	1.3469	5.6327
36	F-530	51	1300	1200	19.4	159.4		10	0.9800	0.9046	1.6167	13.2833
37	F-550	45.7	880	800	7	108.4		10	0.9220	0.8382	0.8750	13.5500
38	M-170	37.6	540	500	6	1.4		11	1.0159	0.9406	1.2000	0.2800
39	F-560	44	740	650	8	26.8		11	0.8687	0.7631	1.2308	4.1231
40	M-160	34.7	400	380	3	0.4		7	0.9574	0.9095	0.7895	0.1053

Fish #	Sex - Maturity [a]	Length (cm)	Total Weight (g)	Gutted Weight (g)	Liver Weight (g)	Gonad Weight (g)	Observations	Age (year)	CF t [b]	CF g [c]	HSI	GSI
41	F-560	43.2	750	650	5.4	19.6		10	0.9303	0.8062	0.8308	3.0154
42	M-150	32	300	290	3.2	2.2		6	0.9155	0.8850	1.1034	0.7586
43	M-150	33.4	340	330	4	0.2		7	0.9125	0.8857	1.2121	0.0606
44	F-560	39.6	570	480	9.6	13.2		8	0.9179	0.7730	2.0000	2.7500
45	F-500	39.3	500	460	7.8	0.4		10	0.8237	0.7578	1.6957	0.0870
46	F-560	44.5	690	590	9.8	12.2		11	0.7830	0.6695	1.6610	2.0678
47	F-550	35.4	400	360	6.2	5.4		10	0.9017	0.8115	1.7222	1.5000
48	F-530	51.3	1520	1420	13.8	420		14	1.1259	1.0518	0.9718	29.5775
49	M-140	32.8	230	200	3.8	0.6		8	0.6518	0.5668	1.9000	0.3000
50	F-570	47.5	980	na	16.2	28		12	0.9144	na	na	na

[a] Sex (F = Female and M = Male) and maturity stages expressed as a number (see Annex A for description of the different stages according to DFO).
[b] Fulton's condition factor expressed as 100 x total body weight/length^3.
[c] Fulton's condition factor expressed as 100 x gutted body weight/length^3.
na = data not available

Necropsy Data of American Plaice from the 2009 Hibernia Survey

Reference Area

Fish #	Sex - Maturity [a]	Length (cm)	Total Weight (g)	Gutted Weight (g)	Liver Weight (g)	Gonad Weight (g)	Observations	Age (year)	CF t [b]	CF g [c]	HSI	GSI
51	F-560	43.6	630	500	12.2	24.2		8	0.7601	0.6033	2.4400	4.8400
52	F-560	40.7	610	500	5.6	14.6		na	0.9048	0.7416	1.1200	2.9200
53	M-150	35	370	320	2.4	4.6		8	0.8630	0.7464	0.7500	1.4375
54	F-560	37.7	460	370	4.2	15.4		9	0.8585	0.6905	1.1351	4.1622
55	F-530	50.6	1118	900	8.4	141.4		10	0.8630	0.6947	0.9333	15.7111
56	F-560	43	660	560	8	25.8		10	0.8301	0.7043	1.4286	4.6071
57	F-560	41.5	700	600	8	14		11	0.9794	0.8395	1.3333	2.3333
58	M-150	35	390	330	1	0.4		9	0.9096	0.7697	0.3030	0.1212
59	F-560	46.1	800	660	15.2	22		9	0.8166	0.6737	2.3030	3.3333
60	M-150	38.2	480	410	4.4	1.6		8	0.8611	0.7355	1.0732	0.3902
61	F-560	51.8	1130	1070	10.8	68.4		14	0.8130	0.7698	1.0093	6.3925
62	M-150	33.8	380	330	6.2	10.4		8	0.9841	0.8546	1.8788	3.1515
63	F-550	42.7	750	530	5.2	121.8		10	0.9633	0.6808	0.9811	22.9811
64	F-560	35.5	410	340	6.8	4.2		8	0.9164	0.7600	2.0000	1.2353
65	M-160	34	360	310	5.6	5		7	0.9159	0.7887	1.8065	1.6129
66	F-530	41.8	650	520	3.8	41.4		10	0.8900	0.7120	0.7308	7.9615
67	F-530	38.5	570	400	3.2	96.6		10	0.9988	0.7009	0.8000	24.1500
68	F-550	40.3	500	430	2.2	17		9	0.7639	0.6570	0.5116	3.9535
69	F-560	42.6	620	510	5	23.8		9	0.8020	0.6597	0.9804	4.6667
70	M-150	37.4	460	380	5.4	3		9	0.8793	0.7264	1.4211	0.7895
71	F-530	38.6	530	380	2.2	81		11	0.9215	0.6607	0.5789	21.3158
72	M-150	32.6	260	220	1.8	0.4		9	0.7504	0.6350	0.8182	0.1818
73	F-530	38.1	490	350	3	79.4		10	0.8860	0.6328	0.8571	22.6857
74	F-560	38	460	390	1.4	25.6		10	0.8383	0.7107	0.3590	6.5641
75	M-150	36.3	430	390	1.6	1.8		9	0.8990	0.8154	0.4103	0.4615
76	M-150	36.8	450	390	1.8	0.6		8	0.9030	0.7826	0.4615	0.1538
77	F-580	50.4	1007	910	11.2	41.2		14	0.7866	0.7108	1.2308	4.5275
78	F-560	36.9	440	360	0.4	13.4		10	0.8757	0.7165	0.1111	3.7222
79	M-150	36.5	410	350	1.6	4.4		7	0.8432	0.7198	0.4571	1.2571
80	M-100	31.7	240	190	3	2.8	pale gills	9	0.7534	0.5965	1.5789	1.4737
81	M-150	35.2	400	350	1.8	0.2		10	0.9171	0.8025	0.5143	0.0571
82	F-540	40.1	490	410	3.6	13		11	0.7599	0.6358	0.8780	3.1707
83	M-150	32.1	290	255	0.4	0.6		8	0.8768	0.7709	0.1569	0.2353
84	F-530	36.8	505	350	1.8	94.6		9	1.0133	0.7023	0.5143	27.0286
85	M-150	27.8	170	130	0.4	0.2		7	0.7913	0.6051	0.3077	0.1538
86	F-510	38.8	460	390	2.4	31.2		10	0.7875	0.6677	0.6154	8.0000
87	F-560	42.2	620	550	6.4	20.2		10	0.8250	0.7319	1.1636	3.6727
88	F-550	49	1310	1250	10.2	240		14	1.1135	1.0625	0.8160	19.2000
89	F-550	40.2	610	520	3	100		9	0.9390	0.8004	0.5769	19.2308
90	M-140	34.4	330	320	1.2	2.2		9	0.8107	0.7861	0.3750	0.6875

Fish #	Sex - Maturity [a]	Length (cm)	Total Weight (g)	Gutted Weight (g)	Liver Weight (g)	Gonad Weight (g)	Observations	Age (year)	CF t [b]	CF g [c]	HSI	GSI
91	M-150	32	280	250	1.4	7.6		8	0.8545	0.7629	0.5600	3.0400
92	F-560	40.7	550	490	3.8	18		9	0.8158	0.7268	0.7755	3.6735
93	F-530	41.4	680	600	6	45.6		8	0.9583	0.8456	1.0000	7.6000
94	F-550	41	740	590	6.2	163		9	1.0737	0.8561	1.0508	27.6271
95	M-140	33.8	290	270	1.2	7.2		8	0.7510	0.6992	0.4444	2.6667
96	F-560	47.1	970	840	11	41.8		13	0.9283	0.8039	1.3095	4.9762
97	F-540	44.5	940	880	7	219.4		13	1.0667	0.9986	0.7955	24.9318
98	F-560	39.2	520	470	4.8	12.4		11	0.8633	0.7803	1.0213	2.6383
99	M-140	38.7	510	470	4.2	19.6		8	0.8799	0.8109	0.8936	4.1702
100	M-160	33.3	320	300	3.8	0.2		8	0.8666	0.8124	1.2667	0.0667

[a] Sex (F = Female and M = Male) and maturity stages expressed as a number (see Annex A for description of the different stages according to DFO).
[b] Fulton's condition factor expressed as 100 x total body weight/length^3.
[c] Fulton's condition factor expressed as 100 x gutted body weight/length^3.
na = data not available

ANNEX C

Haematological Results in American Plaice from the 2009 Hibernia Survey

Haematological Results in American Plaice from the 2009 Hibernia Survey

Fish #	Lymphocytes*	Neutrophils*	Thrombocytes*	Red Blood Cells
1	158	3	39	Appeared normal
2	160	0	40	Appeared normal
3	155	3	42	Appeared normal
4	155	5	40	Appeared normal
5	161	5	34	Appeared normal
6	166	2	32	Appeared normal
7	Not suitable for counting cells			
8	159	1	40	Appeared normal
9	165	1	34	Appeared normal
10	163	1	36	Appeared normal
11	165	0	35	Appeared normal
12	168	1	31	Appeared normal
13	170	0	30	Appeared normal
14	167	2	31	Appeared normal
15	155	0	45	Appeared normal
16	165	1	34	Appeared normal
17	164	1	35	Appeared normal
18	169	0	31	Appeared normal
19	172	0	28	Appeared normal
20	Not suitable for counting cells			
21	158	0	42	Appeared normal
22	159	1	40	Appeared normal
23	164	1	35	Appeared normal
24	155	0	45	Appeared normal
25	169	0	31	Appeared normal
26	160	2	38	Appeared normal
27	155	1	44	Appeared normal
28	169	0	31	Appeared normal
29	Not suitable for counting cells			
30	158	0	42	Appeared normal
31	174	0	26	Appeared normal
32	162	2	36	Appeared normal
33	170	3	27	Appeared normal
34	166	0	34	Appeared normal
35	162	1	37	Appeared normal
36	167	1	32	Appeared normal
37	166	1	33	Appeared normal
38	169	5	26	Appeared normal
39	173	0	27	Appeared normal
40	159	3	38	Appeared normal
41	159	0	41	Appeared normal
42	160	1	39	Appeared normal
43	174	0	26	Appeared normal

Fish #	Lymphocytes*	Neutrophils*	Thrombocytes*	Red Blood Cells
44	164	0	36	Appeared normal
45	170	5	25	Appeared normal
46	166	0	34	Appeared normal
47	Not suitable for counting cells			
48	168	0	32	Appeared normal
49	170	1	29	Appeared normal
50	175	0	25	Appeared normal
51	147	0	53	Appeared normal
52	169	0	31	Appeared normal
53	169	1	30	Appeared normal
54	165	3	32	Appeared normal
55	169	5	26	Appeared normal
56	157	2	41	Appeared normal
57	172	0	28	Appeared normal
58	170	0	30	Appeared normal
59	166	1	33	Appeared normal
60	178	0	22	Appeared normal
61	169	3	28	Appeared normal
62	158	0	42	Appeared normal
63	159	0	41	Appeared normal
64	170	1	29	Appeared normal
65	163	1	36	Appeared normal
66	160	2	38	Appeared normal
67	172	0	28	Appeared normal
68	159	1	40	Appeared normal
69	166	0	34	Appeared normal
70	172	0	28	Appeared normal
71	168	0	32	Appeared normal
72	168	1	31	Appeared normal
73	167	0	33	Appeared normal
74	155	0	45	Appeared normal
75	163	3	34	Appeared normal
76	167	0	33	Appeared normal
77	162	1	37	Appeared normal
78	172	0	28	Appeared normal
79	166	3	31	Appeared normal
80	170	0	30	Appeared normal
81	165	2	33	Appeared normal
82	167	1	32	Appeared normal
83	162	2	36	Appeared normal
84	165	2	33	Appeared normal
85	170	0	30	Appeared normal
86	163	0	37	Appeared normal
87	166	3	31	Appeared normal
88	170	0	30	Appeared normal
89	163	0	37	Appeared normal

Fish #	Lymphocytes*	Neutrophils*	Thrombocytes*	Red Blood Cells
90	163	2	35	Appeared normal
91	167	2	31	Appeared normal
92	171	1	28	Appeared normal
93	165	0	35	Appeared normal
94	165	3	32	Appeared normal
95	168	0	32	Appeared normal
96	Not suitable for counting cells			
97	169	3	28	Appeared normal
98	161	0	39	Appeared normal
99	169	1	30	Appeared normal
100	168	0	32	Appeared normal

* Expressed as a percentage of each type of cells identified on two hundred cells counted.

ANNEX D

EROD Activity in the Liver of American Plaice from the 2009 Hibernia Survey

EROD Activity in the liver of Male and Female American Plaice from the 2009 Hibernia Survey

HIBERNIA REFERENCE AREA

Female

Fish #	EROD pmol/min/mg protein	Sex - Maturity [a]
86	6.6	F-510
55	1.9	F-530
66	2.6	F-530
67	13.0	F-530
71	3.5	F-530
73	4.1	F-530
84	2.7	F-530
93	9.9	F-530
82	4.0	F-540
97	1.4	F-540
63	2.8	F-550
68	18.3	F-550
88	2.0	F-550
89	2.0	F-550
94	3.0	F-550
51	3.6	F-560
52	18.5	F-560
54	8.7	F-560
56	2.8	F-560
57	17.6	F-560
59	20.9	F-560
61	12.3	F-560
64	55.8	F-560
69	18.4	F-560
74	6.6	F-560
78	25.7	F-560
87	19.2	F-560
92	12.2	F-560
96	4.7	F-560
98	8.2	F-560
77	8.0	F-580

Male

Fish #	EROD pmol/min/mg protein	Sex - Maturity [a]
80	47.9	M-100
90	21.8	M-140
95	4.6	M-140
99	24.1	M-140
53	8.0	M-150
58	38.7	M-150
60	20.1	M-150
62	14.0	M-150
70	21.5	M-150
72	18.4	M-150
75	16.2	M-150
76	54.5	M-150
79	80.5	M-150
81	48.0	M-150
83	34.6	M-150
85	28.5	M-150
91	62.2	M-150
65	98.8	M-160
100	32.4	M-160

[a] Maturity stage assessed according to procedures used by DFO (Annex A).

HIBERNIA STUDY AREA

Female

Fish #	EROD pmol/min/mg protein	Sex - Maturity [a]
24	78.0	F-500
26	63.2	F-500
45	28.1	F-500
12	2.3	F-520
29	1.8	F-520
4	4.0	F-530
30	1.9	F-530
31	17.8	F-530
36	3.7	F-530
48	1.9	F-530
7	17.4	F-550
10	5.8	F-550
37	3.7	F-550
47	15.3	F-550
1	67.7	F-560
3	44.7	F-560
5	5.9	F-560
8	14.1	F-560
9	17.9	F-560
11	15.1	F-560
13	28.3	F-560
16	15.0	F-560
17	36.6	F-560
21	12.9	F-560
22	10.9	F-560
25	13.8	F-560
32	5.7	F-560
35	7.2	F-560
39	10.4	F-560
41	16.5	F-560
44	38.8	F-560
46	15.2	F-560
50	7.0	F-570

Male

Fish #	EROD pmol/min/mg protein	Sex - Maturity [a]
18	24.5	M-140
19	76.9	M-140
33	32.6	M-140
49	47.0	M-140
6	18.1	M-150
15	35.4	M-150
20	50.9	M-150
23	4.8	M-150
27	12.3	M-150
28	15.7	M-150
34	56.1	M-150
42	39.0	M-150
43	38.6	M-150
2	74.8	M-160
40	19.2	M-160
14	29.2	M-170
38	41.4	M-170

[a] Maturity stage assessed according to procedures used by DFO (Annex A).

ANNEX E

Liver Histopathological Results of American Plaice from the 2009 Hibernia Survey

Liver Histopathological Results of American Plaice from the 2009 Hibernia Survey

HIBERNIA REFERENCE AREA

Fish #	NP [a]	MH [b]	FI [c]	FCA [d]	MA [e]	HV [f]	Par [g]
51	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0
53	0	0	0	0	2	0	0
54	0	0	0	0	0	0	1
55	0	0	0	0	0	0	1
56	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0
69	0	0	0	0	0	1	0
70	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0
72	0	0	0	0	2	0	0
73	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0

Fish #	NP [a]	MH [b]	FI [c]	FCA [d]	MA [e]	HV [f]	Par [g]
82	0	0	0	0	1	0	0
83	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0
88	0	0	0	0	1	1	0
89	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0
96	0	0	0	0	0	1	0
97	0	0	0	0	1	0	0
98	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0

[a] **NP** = Nuclear pleomorphism: 0 (not detected) or 1 (detected).
[b] **MH** = Megalocytic hepatosis: 0 (not detected) or 1 (detected).
[c] **FI** = Fibrillar inclusions: 0 (not detected) or 1 (detected).
[d] **FCA** = Focus of cellular alteration, including eosinophilic, basophilic and clear cell (0=not detected or 1=detected).
[e] **MA** = Macrophage aggregation: Rating on a 1-7 relative scale.
[f] **HV** = Hepatocellular vacuolation: 0 (homogeneous distribution) or 1 (“patchy” distribution).
[g] **Para** = Parasitic infestation of the biliary system: 0 (not detected) or 1 (detected).

HIBERNIA STUDY AREA

Fish #	NP [a]	MH [b]	FI [c]	FCA [d]	MA [e]	HV [f]	Par [g]
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	1	0	0
7	0	0	0	0	0	0	0
8	0	0	0	0	1	0	0
9	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0
11	0	0	0	0	0	0	1
12	0	0	0	0	0	1	1
13	0	0	0	0	1	0	0
14	0	0	0	0	0	0	0
15	0	0	0	0	0	0	1
16	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0
20	0	0	0	0	0	0	1
21	0	0	0	0	0	0	0
22	0	0	0	0	0	0	1
23	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0
25	0	0	0	0	1	0	0
26	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0
30	0	0	0	0	0	0	1
31	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0
33	0	0	0	0	0	0	1
34	0	0	0	0	0	0	0
35	0	0	0	0	1	0	0

Fish #	NP [a]	MH [b]	FI [c]	FCA [d]	MA [e]	HV [f]	Par [g]
36	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0
38	0	0	0	0	1	0	1
39	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0
45	0	0	0	0	0	0	1
46	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0

[a] **NP** = Nuclear pleomorphism: 0 (not detected) or 1 (detected).
[b] **MH** = Megalocytic hepatosis: 0 (not detected) or 1 (detected).
[c] **FI** = Fibrillar inclusions: 0 (not detected) or 1 (detected).
[d] **FCA** = Focus of cellular alteration, including eosinophilic, basophilic and clear cell (0=not detected or 1=detected).
[e] **MA** = Macrophage aggregation: Rating on a 1-7 relative scale.
[f] **HV** = Hepatocellular vacuolation: 0 (homogeneous distribution) or 1 ("patchy" distribution).
[g] **Para** = Parasitic infestation of the biliary system: 0 (not detected) or 1 (detected).

ANNEX F

Gill Histopathological Results of American Plaice from the 2009 Hibernia Survey

Gill Histopathological Results of American Plaice from the 2009 Hibernia Survey

Fish #	Lamellae Counted [a]	% Hyperplasia				% Fusion	% Telangiectasis	Oedema Rating [e]	Parasites
		Distal [b]	Tip [c]	Basal [d] 1/3 to 2/3	Basal [d] > 2/3				
1	584	0.0	0.0	0.0	0.0	0.0	0.0	0	0
2	698	0.0	0.0	0.0	0.0	0.0	0.0	1	0
3	652	0.0	0.0	0.0	0.0	0.0	0.0	1	0
4	528	0.0	0.0	0.0	0.0	0.0	0.0	0	0
5	784	0.0	0.0	0.0	0.0	1.5	0.0	0	0
6	748	0.0	0.0	0.0	0.0	0.0	0.0	0	0
7	680	0.0	0.0	0.0	0.0	0.0	0.0	0	0
8	612	0.2	0.7	0.0	0.0	0.0	0.0	0	0
9	614	0.0	0.0	0.0	0.0	0.0	0.0	0	0
10	536	0.0	0.0	0.0	0.0	0.0	0.0	2	0
11	532	0.0	0.0	0.0	0.0	0.0	0.0	2	0
12	470	0.4	0.0	0.0	0.0	0.0	0.0	2	0
13	450	0.0	0.0	0.0	0.0	0.0	0.0	2	0
14	640	0.0	1.4	0.0	0.0	0.0	0.0	1	0
15	458	0.0	0.0	0.0	0.0	0.0	0.0	2	0
16	648	0.2	0.2	0.5	0.0	0.9	0.0	1	0
17	700	0.0	0.0	0.0	1.1	0.0	0.0	0	0
18	738	0.0	0.0	0.0	0.0	0.0	0.0	1	0
19	520	0.6	0.2	0.0	0.0	0.0	0.0	0	0
20	702	0.0	0.0	0.0	0.0	0.0	0.0	1	0
21	734	0.0	0.0	0.0	0.0	0.0	0.0	0	0
22	928	1.1	0.0	0.0	0.0	0.2	0.0	1	0
23	716	0.0	0.0	0.0	0.0	0.0	0.0	1	0
24	580	0.9	0.3	0.3	0.0	0.5	0.0	1	0
25	890	0.0	0.0	0.0	0.0	0.0	0.0	2	0
26	660	0.0	0.0	0.0	0.0	0.0	0.0	0	0
27	578	0.2	0.0	0.2	0.0	1.0	0.0	0	0
28	472	0.0	0.0	0.0	0.0	0.0	0.0	2	0
29	906	0.1	0.1	0.0	0.0	1.1	0.0	1	0
30	832	0.0	0.0	0.0	0.0	2.4	0.0	1	0
31	764	0.0	0.0	0.0	0.0	0.0	0.0	2	0
32	392	0.0	0.3	0.0	0.0	0.0	0.3	0	0
33	686	0.0	0.0	0.0	0.0	0.0	0.0	1	0
34	464	0.0	0.6	0.0	0.0	0.0	0.0	0	0
35	528	0.0	0.0	0.0	0.0	0.0	0.0	2	0
36	666	0.0	0.0	0.0	0.0	0.0	0.0	1	0
37	520	0.0	0.0	0.0	0.0	0.0	0.2	0	0
38	770	0.0	0.0	0.1	0.0	0.0	0.0	1	0
39	748	0.0	0.1	0.0	0.0	0.0	0.0	2	0
40	602	0.2	0.0	0.0	0.0	0.0	0.0	2	0

Fish #	Lamellae Counted [a]	% Hyperplasia				% Fusion	% Telangiectasis	Oedema Rating [e]	Parasites
		Distal [b]	Tip [c]	Basal [d] 1/3 to 2/3	Basal [d] > 2/3				
41	448	0.2	0.4	0.0	0.0	0.0	0.0	0	0
42	530	0.2	0.0	0.0	0.0	0.0	0.0	2	0
43	620	0.0	0.0	0.0	0.0	0.0	0.0	0	0
44	676	0.0	0.7	0.1	0.0	1.3	0.0	2	0
45	582	0.2	0.0	0.7	0.2	0.7	0.0	0	0
46	696	0.3	1.6	0.1	0.0	0.0	0.0	0	0
47	456	0.0	0.0	0.0	0.0	0.0	0.0	1	0
48	512	0.0	0.0	0.0	0.0	0.0	0.0	1	0
49	602	0.0	0.0	0.0	0.0	0.0	0.0	0	0
50	928	0.1	0.0	0.0	0.0	0.0	0.0	1	0
51	660	0.0	0.0	0.0	0.0	0.0	0.0	3	0
52	844	0.0	0.0	0.0	0.0	0.0	0.0	1	0
53	548	0.0	0.0	8.4	0.0	11.3	0.0	0	0
54	754	0.0	0.0	0.0	0.0	0.0	0.0	0	0
55	584	0.0	0.0	0.0	0.0	0.0	0.0	0	0
56	514	0.0	0.0	0.0	0.0	0.0	0.0	0	0
57	712	0.0	0.1	0.0	0.0	0.0	0.0	3	0
58	742	0.0	0.3	0.0	0.0	0.0	0.0	0	0
59	416	0.0	0.0	0.5	0.0	0.0	0.0	1	0
60	574	0.0	0.0	0.0	0.0	0.0	0.2	0	0
61	768	1.4	0.5	0.0	0.0	2.3	0.0	1	0
62	764	0.0	0.0	0.1	0.0	0.7	0.0	1	0
63	684	0.0	0.0	0.0	0.4	0.0	0.0	1	0
64	646	0.0	0.0	0.0	0.0	0.0	0.0	0	0
65	724	0.0	0.3	0.4	0.0	0.0	0.0	1	0
66	508	0.0	0.0	0.0	0.0	0.0	0.0	1	0
67	828	0.0	0.0	0.0	0.0	0.0	0.0	0	0
68	652	0.0	0.0	0.0	0.0	0.0	0.0	0	0
69	736	0.0	0.0	0.1	0.0	0.1	0.0	0	0
70	704	0.0	0.0	0.0	0.0	0.0	0.0	0	0
71	602	0.0	0.0	0.0	0.0	0.0	0.0	0	0
72	670	1.0	0.6	0.1	0.3	0.0	0.0	0	0
73	622	0.0	0.0	0.0	0.0	0.0	0.0	0	1
74	438	0.0	0.0	0.0	0.0	0.0	0.0	2	0
75	770	0.0	0.4	0.0	0.0	0.0	0.0	1	1
76	750	0.0	0.0	0.0	0.0	0.0	0.0	1	0
77	596	0.0	0.0	0.0	0.0	0.0	0.0	0	0
78	824	0.0	0.0	0.1	0.0	0.0	0.0	0	0
79	654	0.0	0.0	0.0	0.0	0.0	0.2	0	0
80	Proliferation of X-cells preventing the counting of lamellae								
81	590	0.3	0.0	0.2	0.0	0.7	0.0	2	0
82	642	0.0	0.0	0.0	0.0	0.0	0.0	2	0
83	548	0.0	0.0	0.0	0.0	0.0	0.0	1	0

Fish #	Lamellae Counted [a]	% Hyperplasia				% Fusion	% Telangiectasis	Oedema Rating [e]	Parasites
		Distal [b]	Tip [c]	Basal [d] 1/3 to 2/3	Basal [d] > 2/3				
84	682	0.0	0.0	0.0	0.0	0.0	0.0	2	0
85	Could not be read accurately								
86	352	0.0	0.0	0.0	0.0	0.0	0.0	1	0
87	400	0.0	0.0	0.0	0.0	0.0	0.0	2	0
88	532	0.0	0.0	0.0	0.0	0.0	0.0	2	0
89	820	0.0	0.0	0.0	0.0	0.0	0.0	1	0
90	594	0.2	0.0	0.0	0.0	0.0	0.0	0	0
91	508	4.1	0.0	0.0	0.0	0.0	0.0	0	0
92	588	0.0	0.0	0.2	0.0	0.0	0.0	2	0
93	706	0.0	0.0	0.0	0.0	0.0	0.0	2	0
94	722	0.0	0.0	0.0	0.0	0.0	0.0	1	0
95	574	0.0	0.0	0.0	0.0	0.0	0.0	2	0
96	912	0.0	0.0	0.0	0.0	0.0	0.0	2	0
97	852	0.0	0.0	0.2	0.0	0.0	0.0	1	0
98	826	0.0	0.0	0.0	0.0	0.0	0.0	0	0
99	690	0.1	0.0	0.1	0.3	2.0	0.0	0	0
100	602	0.3	2.3	0.0	0.0	0.0	0.0	0	0

[a] Secondary lamellae counted on four filaments per fish.
[b] Distal hyperplasia was recorded when there were more than 2 cell layers all around the two sides of the secondary lamellae.
[c] Tip hyperplasia was recorded when there were more than 3 cell layers at least 2/3 around the secondary lamellae tip.
[d] Basal hyperplasia was recorded when an increase in thickness of the epithelium near the base of the lamellae was reaching 1/3 to 2/3 of the total of the length of the secondary lamellae or more than 2/3.
[e] Rating on a relative 0-3 scale.

ANNEX G

Representative Photographs of American Plaice
from the 2009 Hibernia Survey

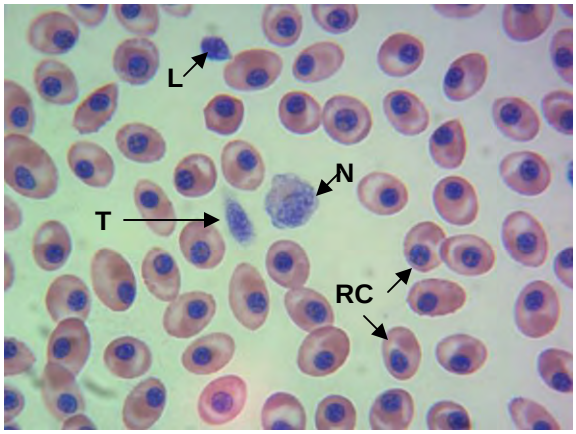

Photo 1: Blood Smear (Giemsa x1000)
RC=red blood cell, L=lymphocytes, N=Neutrophils, T=thrombocyte

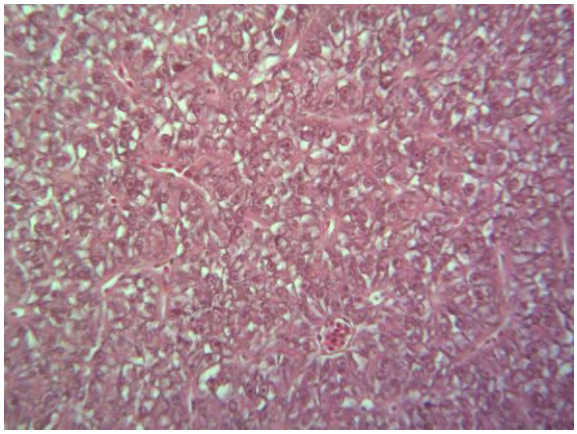

Photo 2: Normal Liver (H&E x250)

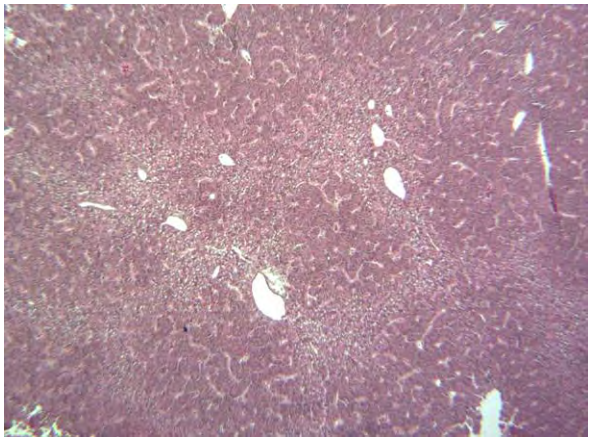

Photo 3: Patchy Hepatocellular Vacuolation (H&E x63)

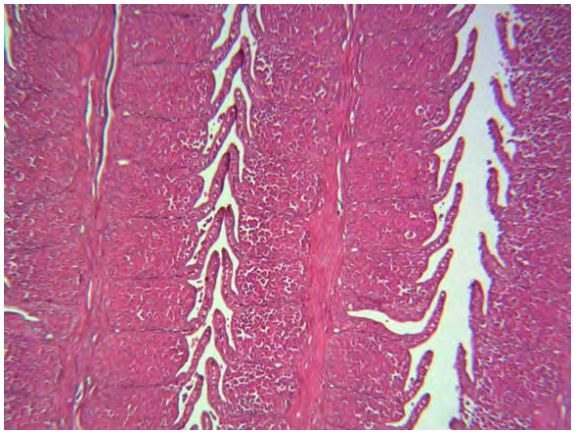

Photo 4: Extensive Proliferation of X-cells between Gill Secondary Lamellae (H&E x63)

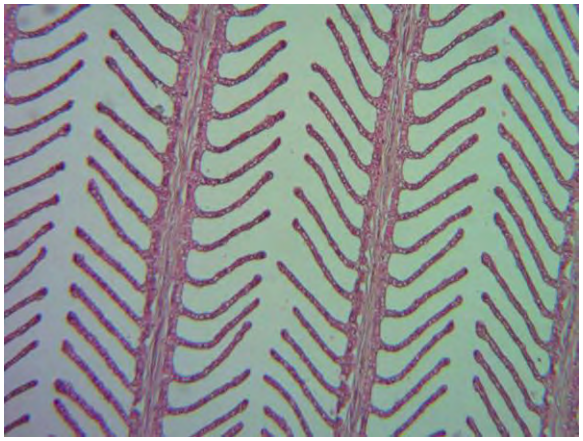

Photo 5: Normal Gill Secondary Lamellae (H&E x63)

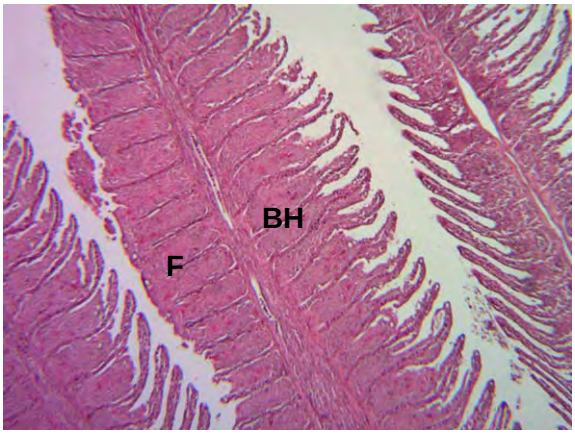

Photo 6: Basal Hyperplasia (BH) and Fusion (F) of Gill Secondary Lamellae (H&E x63)