Appendix J

Cycle 7 EEM Results

Appendix J1 – 2016 EEM with Appendices
Appendix J2 – What is Environmental Effects Monitoring

Appendix J1

2016 EEM with Appendices

Context for 2016 EEM Report

What is an EEM

In May 1992, Environment Canada (now referred to as Environment and Climate Change Canada - ECCC) and the federal Department of Fisheries and Oceans (DFO) amended the Pulp and Paper Effluent Regulations (PPER) under the *Fisheries Act*. These amendments prescribed that all mills were required to conduct an Environmental Effects Monitoring (EEM) program. EEM programs are designed to detect and measure changes in aquatic ecosystems, including assessment of long-term effects. ECCC provides review of study design and monitoring results.

The Current EEM Study Framework is for the Boat Harbour Facility not the Replacement Effluent Treatment Facility

The EEM study results have been requested by the public during 2017 Community Open Houses on the proposed replacement effluent treatment facility. **This EEM study is not directly applicable to the proposed replacement effluent treatment facility and associated treated effluent.** The EEM study evaluates the existing effluent being discharged into the Northumberland Strait from the Boat Harbour Effluent Treatment Facility. Samples for Cycle 7 were collected from the downstream end of the Boat Harbour Estuary and from three reference areas (i.e., Merigomish Harbour, Logan's Point and Little Harbour).

EEM is an on-going monitoring program as required by ECCC. The monitoring will continue for the new discharge location, if approved, subsequent to the Environmental Assessment process; however new EEM methodology would likely be required to address the new area and effluent. A new discharge location would require a new plume delineation study to be conducted to determine the extent of the 1% effluent envelope for the purpose of identifying exposure and reference sampling locations for EEM. The implication of the proposed replacement offshore discharge location is that new benthic and fish survey programs would likely have to be designed and implemented – essentially the EEM field survey program would have to be revamped. The exposure areas that have previously been sampled presumably won't be relevant anymore, and new reference locations may also need to be established to match the new exposure area habitat(s). Also, the overall quality of treated effluent that would be discharged from the replacement effluent treatment system is expected to be better than that of today, thus reaffirming that **this EEM study is not directly applicable to the proposed replacement effluent treatment facility and associated treated effluent.**

EEM Cycle 7 Interpretive Report for the Northern Pulp Nova Scotia Corp. Facility near Pictou, Nova Scotia

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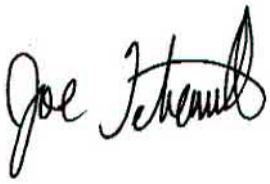
EEM Cycle 7 Interpretive Report for the Northern Pulp Nova Scotia Corp. Facility near Pictou, Nova Scotia



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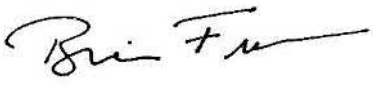

Brian Fraser M.Sc.
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EXECUTIVE SUMMARY

EcoMetrix Incorporated was retained by Northern Pulp Nova Scotia Corp. (NPNS) to implement the seventh-cycle EEM program at its mill near Pictou, Nova Scotia. The program included sublethal effluent toxicity testing, a benthic community survey, and an investigation of cause (IOC) on confirmed effects on fish. This report documents the methods by which the Cycle 7 field program was conducted, the results generated by the field program and the interpretation of these results, as well as the results of the sublethal toxicity testing program. The results of previous EEM studies at the mill, as well as other relevant information, are also included to provide the results of this current study context.

The NPNS mill is located at Abercrombie Point along the shore of Pictou Harbour. A mill has been in operation at this site since 1967. In February 2016, the mill produced on average about 782 ADT/d of bleached Kraft pulp, from mostly softwood chips. Mill effluent is piped several kilometres east from the mill to the Effluent Treatment Centre (ETC). Primary treatment is provided in one of two settling ponds, with retention time of about 12 to 24 hours depending on mill operation. Secondary treatment is provided in an aerated stabilization basin (ASB) that has a five-day retention time. Treated effluent is discharged to the Pictou Road area of the Northumberland Strait and currently satisfies all provincial and federal effluent quality limits.

The spatial extent of the mill's effluent plume in the receiving environment is variable and is affected by the direction and magnitude of the prevailing tide and wind. In some instances (e.g., rising tide, on-shore wind) the 1% effluent concentration envelope does not extend beyond the nearshore area. Alternatively, the 1% effluent envelope sometimes exists (e.g., falling tide, off-shore wind) as a relatively narrow, surface layer that can extend over greater distances towards Logan's Point to the north and beyond Mackenzie Head to the east.

THE CYCLE 7 EEM STUDY

The Cycle 7 EEM program at NPNS included sublethal effluent toxicity testing, a benthic macroinvertebrate survey and an IOC related to effects on fish measured in previous EEM cycles. Water quality was characterized coincident with the fish and benthic field collections. Sediment quality was characterized coincident with the benthic collections.

Sublethal Toxicity

Sublethal effluent toxicity tests using invertebrates (sea urchin; *Lytechinus pictus*) and plants (red alga; *Champia parvula*) were completed semi-annually over the duration of the three-year cycle. Geometric mean IC25 concentrations (i.e., effluent concentrations that result in a 25% decrease in a measured endpoint) for Cycle 7 tests were as follows:

- *Lytechinus pictus* GMIC25 = 2.44%; and
- *Champia parvula* GMIC25 = 0.62%.

The results of seventh-cycle EEM sublethal toxicity testing indicated that the NPNS effluent quality has increased marginally with results for both *Champia* and *Lytechinus* being some of the best reported since Cycle 4. The pattern of toxicity was varied in Cycle 7 with some results showing a positive trend, especially two of the last three for sea urchin and the third last for *C. parvula*. Despite signs of improvement during this current cycle a response to very low concentrations of effluent remains.

In an attempt to address the sublethal toxicity values available effluent treatment system data for the period 2009 until March 2016 was analyzed to determine if there was any relationships between conventional parameters and the sublethal results. However, only dissolved sulphate, COD and volatile suspended solids showed any linear decline over the timeframe and no parameters showed marked increases at times that corresponded with low toxicity results. Given the present data it does not appear likely that a conventional parameter is the potential cause of sublethal toxicity. Therefore, as part of the Cycle 8 EEM consideration will be made to measuring an additional suite of parameters in effluent in conjunction with the toxicity tests.

Benthic Macroinvertebrates

The Cycle 7 benthic invertebrate community survey (ICS) followed a control/impact design with three exposure areas (i.e., near field, far field and far-far-field) and two reference areas (Merigomish Harbour and Logan's Point) sampled. There were no statistical differences between Logan's Point and any exposure area for density or richness whereas the near-field and far-field areas had significantly higher density and all three exposure areas had significantly higher richness compared to the Merigomish Harbour reference area.

The Bray-Curtis comparisons were suggestive of differences in community structure among the sampling areas; however these differences were not limited to exposure vs. reference comparisons, as using Logan's Point as the median also lead to a significant difference from the Merigomish Harbour reference area and vice versa. These community differences seem to be related to the feeding habits of the resident benthos. Generally speaking, the near-field and far-field areas had a larger proportion of deposit feeding taxa than the other areas.

Overall, the nature of differences in benthic invertebrate assemblages seen in the study may be related to or associated with subtle habitat differences. This combined with the fact that the effluent is buoyant and has limited contact with the benthic community likely means that the differences are not mill related. These results are similar to all past EEM benthic surveys at NPNS.

Fish

The Cycle 7 study design was developed to investigate the potential cause of the increased liver size in Boat Harbour Estuary Mummichogs and to confirm the absence of an age effect reported in Cycle 6. Exposure area Mummichogs were collected from the Boat Harbour

Estuary and reference fish were collected from Little Lake near Antigonish. Little Lake has similar lentic habitat to the Boat Harbour Estuary with a seasonal connection to the ocean. Conventional EEM measures related to the investigation of cause were conducted on adult fish from both areas collected in May 2015. Fish from both areas were also collected in August to investigate seasonal differences in the two areas. Mummichogs from both areas were used for liver histological, lipid and glycogen content analysis to investigate the potential cause of the previously confirmed liver effects.

A number of significant differences were noted among the Boat Harbour Estuary and the Little Lake reference area fish for conventional endpoints. Exposure fish were significantly older, larger at a given age and had larger gonads compared to the Little Lake reference area fish. Females from the exposure area also had significantly larger livers whereas there was no difference in the size of male livers between the two areas. There was no significant difference in condition between the two areas and other qualitative measures of condition (i.e., parasites or deformities) were also similar between the reference and exposure fish sampled.

Similar to Cycle 6 a wider variety of sampling gear including minnow traps and trapnets in addition to beach seines were used to confirm the presence of older fish in the Boat Harbour Estuary. Overall fish were older in Boat Harbour compared to previous cycles with the exception of Cycle 6. There was no significant difference in the size of fish captured in shallow or deep gear in Boat Harbour in August 2014. However, a contrary results was evident in May 2015 indicating that the age differences noted in some previous cycles may have been the result of the type of effort employed to capture adult Mummichog. A few marked fish captured during previous studies were recaptured, however they were all captured in the area in which they were clipped. That is, no fish clipped outside of the estuary were captured in the estuary in 2014 or 2015.

Visual inspection of the length frequency histograms from Little Lake and Boat Harbour appeared to indicate that the populations of Mummichog in the two areas were different. However, statistical evaluation of the populations did not detect a statistical difference in between the two areas in either sampling seasons.

The histopathogical analysis identified a number of conditions including minor cell necrosis, metazoans, paripancreatitis, and perihepatitis. Generally, the majority of the observations reported were of minimal significance and the prevalence of the conditions were that expected in wild fish populations. However, Boat Harbour fish appeared to have a higher degree of hepatic lipidosis compared to Little Lake fish. This was confirmed by the lipid analysis. Boat Harbour fish had significantly higher lipid concentrations in liver tissue compared to their Little Lake counterparts. The significant difference for lipids was apparent in sexes indicating it may be area effect and therefore could be the results of effluent exposure. Conversely, there was no detected difference in the glycogen levels in the livers between the two areas.

In terms of the lipids it is established that the Boat Harbour area has more nutrients that could be resulting in a better or more plentiful diet. Higher total lipid in the liver, greater liver mass, and larger size of Boat Harbour Mummichog compared to Little Lake indicate Boat Harbour fish have a different diet than Little Lake. It appears that exposure fish are storing more lipid than other benthivorous fish of similar size indicating that it is not likely that the Little Lake fish have a poor diet. It is also possible that the enlarged livers in the exposure areas and higher lipid content is the result of accumulation of lipophilic organics in Mummichog liver as a result of exposure to mill effluent. Testing for the presence of these organic compounds in the liver tissue should be further investigated to determine if the increased liver size is a direct result of effluent exposure (i.e., organics) or indirect exposure better diet based on increased nutrients in the Boat Harbour Estuary. Preliminary results of the dietary tests indicated that diets did not vary between areas but further study may be needed.

Supporting environmental variables were measured coincident with the fish and benthic collections to assist in the interpretation of the biological data. These included:

Water	**Sediments**
Dissolved oxygen	TOC
Temperature	Carbon to nitrogen ratio (C:N)
Salinity	Total sulphides (TS)
Dissolved organic carbon (DOC)	Sediment particle size
Total organic carbon (TOC)	Sediment Eh
Total Kjeldahl nitrogen (TKN)	
Colour	

The influence of mill effluent on water chemistry within the Boat Harbour was measurable. Nutrient (TKN), colour, TOC and DOC levels were elevated in the exposure area compared to Little Lake. Little Lake salinity was similar to Boat Harbour Estuary. Temperature and dissolved oxygen were higher in the reference than in the exposure area in both the August 2014 and May 2015 sampling seasons.

At the time the ICS was implemented (August), there was little indication of a mill-related water quality influence. Moreover, water quality was similar in top and bottom waters indicating that the water column was well mixed across the ICS study area.

According to the dissolved oxygen and conductivity dataloggers the exposure area had a higher maximum water temperature but overall Little Lake was slightly warmer. Dissolved oxygen showed a high degree of variability in both fish sampling areas. However, on average there was more oxygen in Little Lake than in Boat Harbour. Salinity in both areas was similar from the August deployment until around mid-December. From mid-December until the end of January salinity was higher in the Boat Harbour exposure than in the Little

Lake reference area. From January until the removal of the dataloggers, Little Lake had higher salinity than the exposure area and the salinity was more constant. This is likely attributable to the daily tide cycle experienced in the Boat Harbour area compared to seasonal saltwater intrusions in Little Lake and the large volume of freshwater input associated with the run-off from snowmelt in the Boat Harbour basin. Stress caused by the fluctuating salinity and dissolved oxygen in Boat Harbour may be resulting in some of the differences observed in the Mummichog population.

Overall, the sediment quality data collected as part of the Cycle 7 EEM ICS were within historical ranges for most parameters. Any changes, namely a reduction in sulphide concentrations and C:N ratios compared to previous cycles, were in a positive direction.

Recommendations for Future Monitoring

Decisions regarding nature of future monitoring (Cycle 8) at NPNS will be made within the decision making framework available at the time that the study design is developed and in response to comments provided by Environment Canada regarding the Cycle 7 EEM study. Currently, that study will be comprised of sublethal toxicity testing, a benthic surveillance survey and continued investigation of cause of the effects on liver size in the Boat Harbour Estuary Mummichog population.

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1.0 INTRODUCTION

In May 1992, Environment Canada (EC) and the Department of Fisheries and Oceans (DFO) amended the Pulp and Paper Effluent Regulations (PPER) under the *Fisheries Act*. In addition to limiting the discharge of total suspended solids (TSS), biochemical oxygen demand (BOD), and the acute lethality of effluents, these amendments prescribed that all mills were required to conduct an Environmental Effects Monitoring (EEM) program.

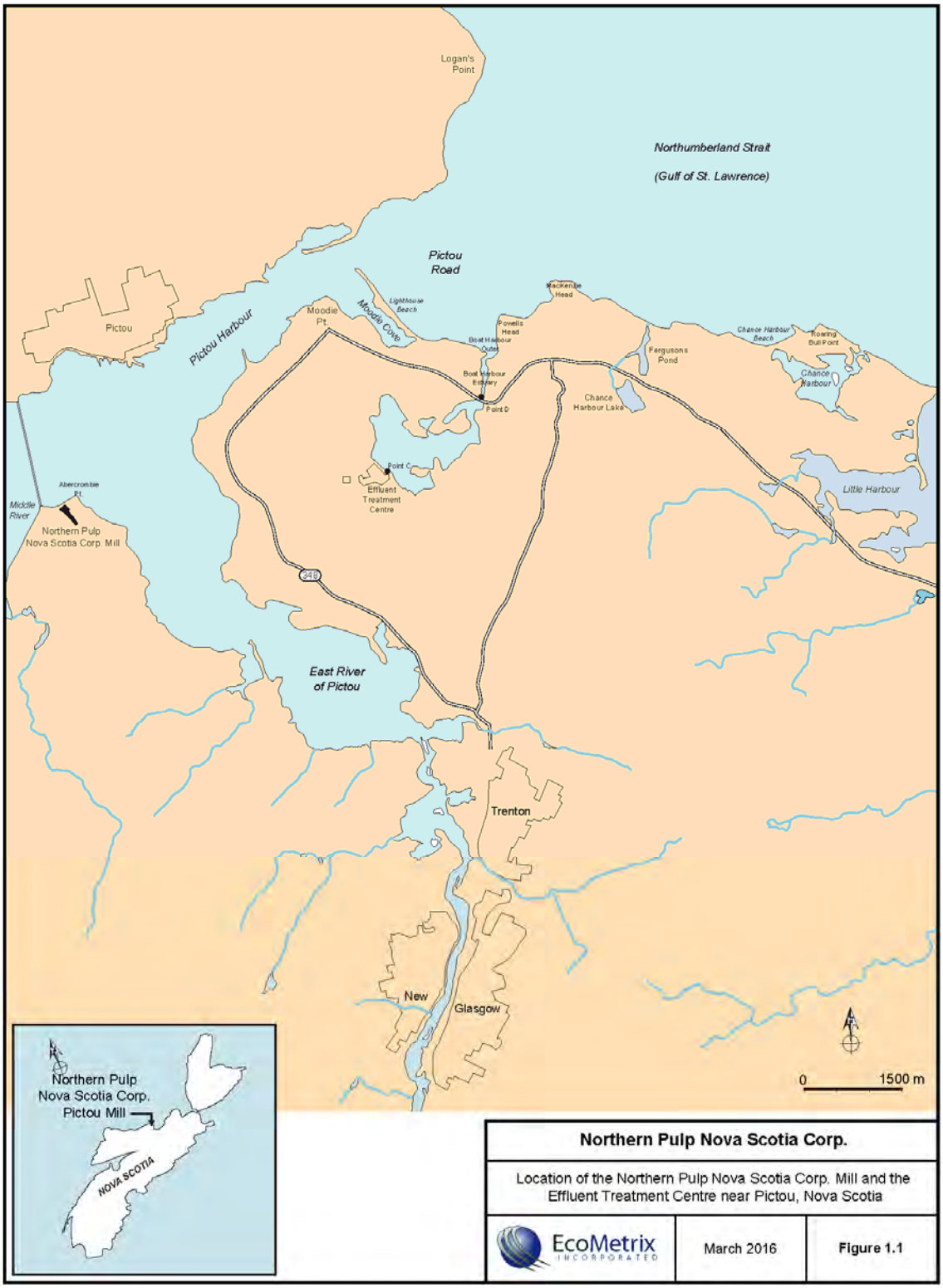
The PPER have been amended on several occasions. The amendments dealt mainly with changes to monitoring and reporting requirements, but also focused on streamlining and improving the original regulation. The objective of the EEM program is to evaluate the effects of effluents on fish, fish habitat and the use of fisheries resources by humans. As such, EEM goes beyond end-of-pipe measurement of chemicals in effluent to examine the effectiveness of environmental protection measures directly in aquatic ecosystems. The program consists of:

- sublethal toxicity testing of mill effluents;
- assessment of benthic invertebrate community structure in the effluent receiving environment;
- assessment of fish population health in the effluent receiving environment; and
- evaluation of effects on fisheries resource use (e.g., dioxins and furans in fish tissue) in the effluent receiving environment.
- Investigation of cause (IOC) and investigation of solutions (IOS)

The first five EEM cycles for all mills in Canada have been completed and study results were reported to EC by 01 April 1996, 01 April 2000, 01 April 2004, 01 April 2007 and 01 April 2010, respectively. The information compiled throughout Canada during Cycles 1 through 6 was reviewed by EC and served, in part, as the basis to identify the monitoring needs for Cycle 7. These needs, as well as monitoring protocols, were defined and revised in the formal program technical guidance (Environment Canada, 2005a), as well as various other related sources. In 2010, EC updated the technical guidance (Environment Canada, 2010) including new decision trees to focus and accelerate action towards identification of the potential cause(s) of mill-related effects and potential solutions to address the effects.

1.1 EEM Cycle 7 at the NPNS Pictou Mill

The Northern Pulp Nova Scotia Corp. mill (NPNS) near Pictou, Nova Scotia (Figure 1.1) retained EcoMetrix Incorporated to implement its seventh-cycle EEM program. The data from the fifth and sixth-cycle EEMs at NPNS were used in conjunction with the new decision trees provided in the revised 2010 technical guidance (Environment Canada, 2010) to determine the required components for the current EEM program. To this end it was determined that an investigation of cause (IOC) fish study initiated in Cycle 6 to determine the cause of the confirmed effects of increased liver size and decreased age of


Logan's Point
Northumberland Strait
(Gulf of St. Lawrence)
Pictou Road
Pictou
Pictou Harbour
Moodie Pt
Moodie Cove
Lighthouse Beach
Mackenzie Head
Powells Head
Boat Harbour Outlet
Boat Harbour Estuary
Point D
Point C
Effluent Treatment Centre
Fergusons Pond
Chance Harbour Beach
Roaring Bull Point
Chance Harbour
Chance Harbour Lake
Little Harbour
Abercrombie Pt
Middle River
Northern Pulp Nova Scotia Corp. Mill
348
East River of Pictou
Trenton
New Glasgow
0
1500 m
Northern Pulp Nova Scotia Corp. Pictou Mill
NOVA SCOTIA
Northern Pulp Nova Scotia Corp.
Location of the Northern Pulp Nova Scotia Corp. Mill and the Effluent Treatment Centre near Pictou, Nova Scotia
EcoMetrix INCORPORATED
March 2016
Figure 1.1

effluent exposed Mummichogs (*Fundulus heteroclitus*) would continue. Additionally, a benthic macroinvertebrate community surveillance survey was implemented, as one had not been conducted since Cycle 5.

The field sampling program component for EEM Cycle 7 consisted of fish collections completed in two stages and a single season of benthic collections. Fish collections were completed from 22 to 27 August 2014 and from 13 to 18 May 2015. Both surveys were completed in a generally consistent manner. The fish survey included collections in one exposure area near the mill outfall and a one reference area. The benthic collections were conducted on 20 and 21 August 2014. Samples were collected from three exposure areas (i.e., near field, far field and far-far field) and two reference areas (i.e., Merigomish Harbour and Logan's Point). In addition, and as required by the PPER, sublethal toxicity testing was completed semi-annually over the duration of the cycle.

1.2 Report Format

Following this introductory section, the remainder of the report is organized as follows:

- Section 2.0 – current mill operations are described and an overview of the ecological aspects of the study area is provided.
- Section 3.0 – the results of sublethal toxicity tests completed with treated mill effluent are provided.
- Section 4.0 – water and sediment quality information collected coincident with the fish and benthic surveys is summarized.
- Section 5.0 – the benthic invertebrate community surveys from past and current EEM studies are detailed.
- Section 6.0 – fish surveys from past and current EEM studies are detailed.
- Section 7.0 – the results of the study components are evaluated together to provide a holistic weight-of-evidence based assessment of the potential cause for the previously observed fish effects within the area surrounding the mill effluent discharge.
- Section 8.0 – recommendations for future EEM programs are provided.
- Section 9.0 - the references consulted in the preparation of this report are given.

Notes on QA/QC measures implemented during this study are provided in Appendix A, and are also embedded in appropriate sections of the report. The remaining appendices contain raw data and other information collected during the completion of the study.

2.0 BACKGROUND INFORMATION

2.1 Mill History and Current Operation

2.1.1 Mill History

Scott Maritimes Ltd. began operating a bleached Kraft sulphate mill at Abercrombie Point, Pictou County, Nova Scotia in 1967. Kimberly-Clark Inc. took possession of the mill from Scott Maritimes Ltd. on January 1, 1996. At this time, operation of the Effluent Treatment Centre (ETC) formerly referred to as the Boat Harbour Treatment Facility, also passed from the provincial government to Kimberly-Clark Inc.. In November 2004, Neenah Paper Inc. (NPI) spun off from Kimberly-Clark Inc. In 2008 NPI sold the Pictou mill to NPNS. NPNS took full control of the Pictou mill and operation of the ETC at that time. In 2011, Paper Excellence purchased NPNS, and the associated forestry company, and is the current owner of the mill.

Mill effluent has been treated at the ETC since the mill was commissioned. Boat Harbour basin was created in 1965 when dams were built across this natural harbour to form a settling and stabilization pond for effluent from the mill. Two 50,000 m^3 earthen settling ponds (the north and south ponds) and a 567,750 m^3 (20 ha) aeration basin were added in 1972. At any given time one of the two earthen ponds is used as the primary settling pond, while the other is used as for the collection of emergency spills.

In 1991, a rock berm was constructed at the mouth of the Boat Harbour Estuary to prevent fish from entering. Prior to the construction of this berm, several fish kills occurred in the estuary because of low dissolved oxygen levels.

Between May 1991 and April 1992, urea and diammonium phosphate were added in bulk to the effluent to improve microbial growth and reduce BOD levels. However, this program ended when toxicity, apparently related to the ammonia addition, was detected in the final effluent.

The aeration system was modified in 1993 to improve aerobic treatment of the effluent and dissolved oxygen levels. Aeration was increased further in 1996 and three curtains were installed in the aeration basin to improve mixing and reduce areas of sludge build-up.

In 1997, an automated nutrient addition system that supplies urea and diammonium phosphate to the effluent before it reaches the aeration basin was installed. As the result of the new delivery system, problems related to final effluent toxicity that had been experienced previously with the bulk nutrient amendment regime have not re-surfaced.

In 2004, further aeration unites were introduced to the aeration basin to improve aerobic treatment of the effluent and dissolved oxygen levels.

2.1.2 Recent Changes to Mill Operations

2.1.3 Current Operations

Mill Furnish

In 2015, seventy-eight percent of the softwood chips used for mill furnish were provided by sawmills. The softwood chip furnish comprises primarily fir and spruce, as well as small quantities of hemlock, pine and larch. Harwood chips have been used to supplement the furnish in a couple of instances recently, and in 2015 hardwood chips comprised 18% of the mill's furnish. In 2015, the mill processed 1.117 million tonnes of softwood chips.

Water Supply

Process water for the mill is drawn from the Middle River at an average rate of about 95 m^3 per air-dried tonne (ADt) of production, down from about 120 m^3 per ADt in the late 1990s. The mill continues to look for additional ways by which to reduce its process water needs, with the goal of eventually having intake water rates in the range of 80 m^3 per ADt.

Products

NPNS produces about 782 ADt per day of bleached Kraft pulp. Over the period 1996 to 2016, total production rates have increased by about 21% (Table 2.1).

Table 2.1: NPNS Production Rates from 1996 to 2015.

Year	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Mean Adt/day[1]	644	631	693	679	707	736	748	723	748	727	734
Year	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016[2]**	
Mean Adt/day[1]	758	700	687	795	734	766	773	730	741	782	

[1] Data are annual average daily production rates.

[2] Only include February 2016, January was an abnormal production month.

Pulping Process

Chips are fed into a pressurized digester and cooked for about four hours in the presence of steam caustic soda and sodium sulphide, known as white liquor, to separate the lignin from the cellulose fiber. Cooked pulp (brown stock) is washed in a series of wash stages to remove the spent liquor (weak black liquor) and lignin from the cellulose. This brown stock pulp is screened to remove knots and uncooked chips if there are any. The remainder is washed, bleachable fiber.

Bleaching Process

A 5-stage bleaching sequence is utilized by the mill and since 1998 the mill has employed 100% chlorine dioxide substitution, thereby effectively reducing dioxin and furan levels in mill effluent to non-detectable levels. In addition to eliminating elemental chlorine usage in the bleaching process, the substitution has also led to a noticeable improvement in the colour of the mill effluent entering Pictou Road.

A summary of the types and amounts of chemicals used in the bleaching process are provided in Table 2.2.

Table 2.2: Chemical Used in the Bleaching Process at the NPNS Pictou Mill

Bleaching Chemical	1991 (kg/day)	1997 (kg/day)	2001 (kg/day)	2004 (kg/day)	2008 (kg/day)	2009 (kg/day)	2010 (kg/day)	2011 (kg/day)	2012 (kg/day)	2013 (kg/day)	2014 (kg/day)	2015 (kg/day)	2016[1] (kg/day)
Chlorine	24,578	9,174	0	0	0	0	0	0	0	0	0	0	0
Caustic	17,747	15,972	13,622	17,003	17,958	19,156	21,624	18,311	24,852	17,867	18,427	17,928	17,908
Sodium chlorate	19,756	36,366	39,451	42,664	43,686	43,939	47,700	41,731	40,411	43,044	39,521	39,088	36,200
Sulphuric acid	13,628	24,222	28,553	32,510	28,367	28,635	30,766	27,158	26,271	28,875	26,094	25,381	23,338
Salt	379	0	0	0	0	0	0	0	0	0	0	0	0
Oxygen	3,549	3,366	3,469	4,008	2,418	3,282	4,134	3,606	3,566	3,796	3,125	3,548	3,906
Methanol	1,808	3,366	3,587	3,933	4,031	4,144	4,452	4,048	3,922	4,134	3,825	3,646	3,418
Hydrogen peroxide	117	1,222	1,839	2,879	2,418	2,307	3,180	3,459	3,369	4,442	3,603	3,467	7,823

[1] Only includes February 2016, January was an abnormal production month.

Chemical Recovery

Spent cooking chemicals from the digester, known as "weak black liquor", are sent to the evaporators to remove excess water which was used to wash the liquor out of the brown stock. The liquor leaving the evaporators, known as “heavy black liquor”, is used as fuel in the Recovery Boiler which burns off all the lignin and other organic material leaving behind the chemical which is recovered for reuse. The liquor as it leaves the recovery boiler is known as “green liquor”. The green liquor is clarified to remove impurities and is then sent to the Recausticizing plant to regenerate the green liquor back into white liquor which is returned to the digester for reuse.

Effluent Treatment

The treatment of mill effluent is achieved via primary and secondary treatment at the ETC. Mill effluent is sent to the ETC via a 0.86 m (i.e., 34”) diameter pipeline that passes under the East River of Pictou.

Primary treatment is provided in one of two settling ponds. Total retention time in the settling ponds is about 12 to 24 hours, depending on mill operation. The settling pond not in use is used to collect emergency spills as is necessary.

Secondary treatment is provided in an aerated stabilization basin (ASB). As effluent flows from the settling basin to the ASB, a nutrient addition system delivers urea and diammonium phosphate to the effluent to increase microbial activity and thereby decrease

BOD. Aeration in the basin is provided by eight 100 Hp mechanical surface aerators and nineteen 50 Hp and twelve 75 Hp floating aerators. The ASB has a five day retention period, after which mill effluent flows via gravity via Point C into the Boat Harbour Basin (200 ha) then to the outfall structure know as Point D, where it is released into the Pictou Road area of the Northumberland Strait. The Boat Harbour Basin has a 25 to 30 day retention time.

The regulated discharge point was at Point D until 30 June 2010 when it was changed to Point C. The outfall structure located at Point D remains in place with no changes. All usual measurements and data continue to be collected at Point D, though as indicated Point C is the PPER compliance point.

Effluent Flow Measurement

The total amount of untreated mill effluent that is discharged at Point C is measured with a Parshall flume. The effluent flow to Pictou Road through Point D is measured using a TeleSafe micro 16 SCADA Controller connected to two level transmitters.

Sludge Disposal

Sludge and solids dredged from the settling ponds are landfilled. Dredged materials from the ASB are pumped into the Sludge Disposal Cell on-site.

Spills

On 10 June 2014 the line that transfers effluent from the mill to the effluent treatment center experienced a leak. This leak was located on the east side of the East River. The leak was reported and once repaired the mill restarted on 22 June 2014.

2.2 Effluent Quality

2.2.1 Applicable Effluent Quality Regulations

All Canadian pulp and paper mills must meet effluent quality limits set out in the *Pulp and Paper Effluent Regulations* (PPER). The PPER prescribes limits for the discharge of BOD_5, TSS, chlorinated dioxins and furans, as well as, Rainbow Trout and *Daphnia magna* toxicity in all effluent streams. Nova Scotia has adopted the federal limits for its own provincial effluent quality regulations. Calendar year 2015 PPER limits for NPNS are provided in Table 2.3.

Table 2.3: PPER Limits Relevant to NPNS Pictou Mill

Parameter	Units	Daily limit	Monthly limit
BOD	kg/R.P. rate[2]	12.5	7.5
	t/day	11.7	7.0
TSS	kg/R.P. rate	18.8	11.3
	t/day	17.6	10.6
Dioxins (2,3,7,8-TCDD)		ND[3]	ND
Furans (2,3,7,8-TCDF)		ND	ND
Trout toxicity	Pass/Fail	- -	Pass
Daphnia toxicity[4]	Pass/Fail	- -	Pass

[1] Federal *Pulp and Paper Effluent Regulations* under the *Fisheries Act*

[2] R.P. rate = reference production rate (938 tonnes)

[3] Non-detectable (as defined by the Pulp and Paper Effluent Chlorinated Dioxins and Furans Regulations)

[4] *Daphnia* tests are performed weekly

2.2.2 Effluent Chemistry

Effluent chemistry data for the period 1995 to 2015 are summarized in Table 2.4. NPNS currently meets the strictest applicable effluent guideline for each of the effluent chemistry parameters for which limits exist.

BOD_5

On average, BOD_5 loadings from NPNS are currently in the range of about 2,849 kg/day. Current BOD_5 loadings are higher than they have been since 1995 (Table 2.4).

TSS

On average, TSS loadings from NPNS are currently in the range of 1,874 kg/day, similar to values reported during the last Cycle, although higher than most values from Cycles 2 and 3 (Table 2.4).

Conductivity

On average, conductivity in final effluent has varied considerably over the period of record shown in Table 2.4. Over this time, conductivity has ranged from close to 1,000 μmhos/cm in 1995 to just over 2,000 μmhos/cm in 1998, 2007 and 2014. The current conductivity of final mill effluent is about 1,395 μmhos/cm.

Previously, conductivity varied highly on a seasonal basis, typically highest late in the fall during particularly stormy weather. Rough seas often resulted in seawater intrusions into the downstream end of the Boat Harbour basin. Conductivity was typically lowest at the height of the spring freshet when non-mill related freshwater inputs (e.g., snowmelt) to the ETC were greatest. The change of the discharge point from Point D to Point C has decreased the within-

year variation in conductivity by eliminating both the freshwater and seawater inputs at the location of parameter measurement. However, it may also result in increased conductivity.

pH

Over the period 1995 to 2015, final mill effluent has largely been circumneutral to slightly basic (7.0 to 8). pH in 2012 was around 8, whereas in 2013 through 2016 the pH of mill effluent has been around 7.8.

Dioxins and Furans and AOX

From 1994 to 1997, between 70% and 100% of chlorine used in the bleaching process was substituted with chlorine dioxide to reduce dioxins and furans in the mill effluent. This was increased to 100% substitution in 1998.

Because the mill no longer uses elemental chlorine in its bleaching process, it is not required to routinely measure average adsorbable organic halide (AOX) levels.

Table 2.4: Mean Monthly Effluent Chemistry Data (Mean Annual Values) for NPNS - 1995 to 2016

	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010[3]	2011	2012	2013	2014	2015	2016[4]
pH	7.2	7.2	7.5	7.3	7.1	7.2	7.2	7.2	7.2	7.2	7.0	7.3	7.6	7.8	7.6	7.8	7.7	8.0	7.8	7.7	7.8	7.70
Conductivity (µmhos/cm)	1,013	1,087	1,200	2,009	1,904	1,888	1,742	1,823	1,789	1,852	1,745	1,798	2,077	1,599	1,600	1,920	1,400	1,950	1,869	2,148	1,708	1,395
TSS (kg/d)	3,342	3,082	1,237	712	907	1,163	1,174	1,448	1,187	2,017	2,191	1,012	1,030	1,620	1,639	1,655	1,831	1,887	1,463	1,649	1,293	1,874
BOD_5 (kg/d)	3,283	2,039	610	706	648	767	902	875	949	1,661	1,554	1,087	1,354	1,699	1,528	1,607	1,566	1,664	1,407	1,466	2,028	2,849
96-Hr Rainbow Trout Acute Toxicity[1]	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none
48-Hr *Daphnia Acute* Toxicity[2]	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none	none

[1] Rainbow trout acute toxicity has been evaluated via monthly testing on undiluted effluent since 1993. No acute toxicity to undiluted effluent has ever been recorded.
[2] *Daphnia* acute toxicity has been evaluated via weekly testing on undiluted effluent since 1993. No acute toxicity to undiluted effluent has ever been recorded.
[3] Data are from 6 months of Point D and 6 months of Point C.
[4] Data is from February 1st to 25th, 2016 only, January was an abnormal production month.

2.2.3 Effluent Toxicity

Acute Toxicity

NPNS effluent has not been acutely lethal to either Rainbow Trout or *Daphnia magna* since regular testing of their treated effluent began in 1993 (Table 2.4).

Sublethal Toxicity

In 1993, prior to EEM Cycle 1, sublethal toxicity testing was completed on effluent from the stabilization basin. These tests indicated that sea urchin fertilization was affected at stabilization effluent concentrations of greater than 12%. Sublethal effects were not observed among larval inland silversides, even in 100% effluent from the stabilization basin.

Results of sublethal toxicity tests completed over the period 1995 to 2013 (EEM Cycles 1 to 6) generally indicated that the magnitude of response of both sea urchin and algae has increased. The GM IC25 (i.e., the geometric mean [GM] effluent concentration that cause a 25% reduction in a measured endpoint [IC25]) for sea urchin (*Lytechinus pictus*) fertilization was 3.3%, 5.1%, 3.2%, 2.1%, 0.76% and 1.7% in Cycles 1, 2, 3, 4, 5 and 6, respectively. The GM IC25 for algae (*Champia parvula)* sexual reproduction was 8.4%, 5.5%, 6.4%, 2.0%, 0.30% and 0.21% in Cycles 1, 2, 3,4, 5 and 6, respectively.

Five of the six sets of tests have been completed and reported for EEM Cycle 7. Results for Cycle 7 tests are similar to previous results. A more detailed discussion of the effluent sublethal toxicity test results and their potential environmental implications are provided in Section 3.1 of this report.

2.2.4 Volume of Effluent Discharge

The rates of effluent discharge (monthly means) at the NPNS from 1994 to 2016 are provided below (Table 2.5). Discharges reported for Point D include effluent generated via mill processes, as well as natural runoff entering the ETC from the surrounding landscape. This can be considerable at times where the runoff contributes as much as 50% of the final effluent flow (JWEL, 1993a, b).

Point C is not influenced by the surrounding landscape runoff and is, by in large, just mill effluent. Due to the change in the location of the regulated discharge point from Point D to Point C, the reported 2010 flow rates are an average of 6 months discharge at point D and 6 months discharge at Point C.

Treated effluent discharged from NPNS at Point C currently averages about 69,400 m^3/day. Discharge rates during Cycle 7 represent an approximate 32% decrease since Cycle 5.

Table 2.5: NPNS Discharge Rates from 1994 to 2016

Year	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**
1,000 m^3/day	101.4	105.5	108.5	82.5	80.8	72.7	83.5	78.5
Year	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**
1,000 m^3/day	80.7	79.1	84.9	93.1	86.6	85.6	98.6	93.7
Year	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016[1]**	
1,000 m^3/day	77.9	67.9	71.8	67.1	68.9	63.6	69.4	

[1] Only includes February 2016, January was an abnormal production month.

2.2.5 Zone of Effluent Mixing

Attempts to delineate the spatial extent of the effluent plume at Pictou Road have been undertaken several times. The first study was conducted in 1965 (Krauel, 1969), the second in 1993 (ASA Consulting Ltd., 1994) and the third over the period 1997 through 1999 (Peter Cranford, DFO, pers. comm., 1998, 2001). The results of these studies are represented in Figures 2.1 and 2.2 and are described in the text below.

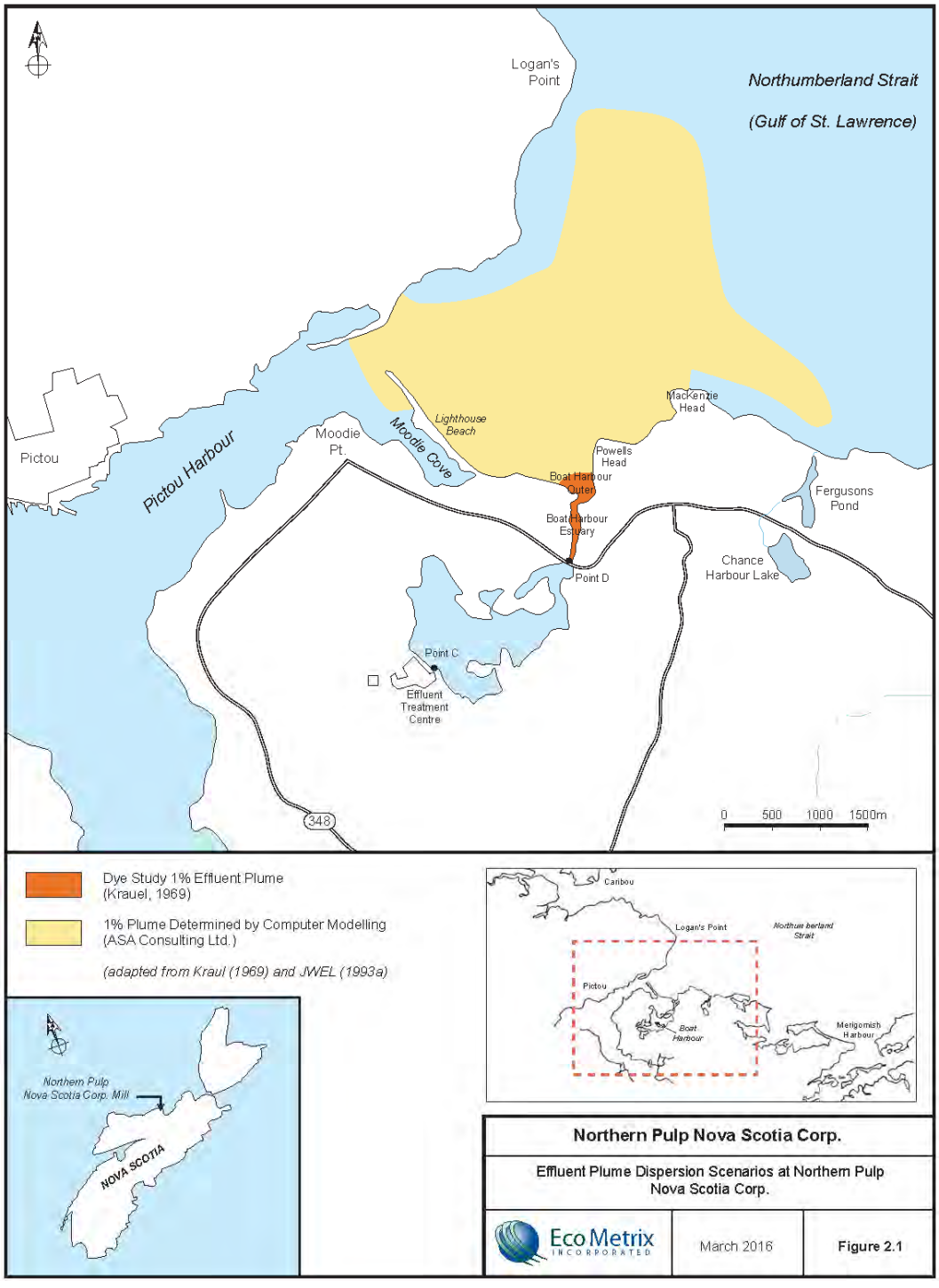
Krauel (1969) injected rhodamine dye at a constant rate over a 26.8-hour period into the mill's outfall in order to track the effluent plume flowing from the ETC. The dye injection indicated that the 1% effluent concentration zone existed near the Boat Harbour basin outlet, typically in the adjacent intertidal zone and within the Boat Harbour Estuary itself (Figure 2.1). The effluent plume usually existed as a thin surface film floating on colder, denser seawater. As a result, it did not come in contact with benthos in the subtidal zone, although it may influence the extreme nearshore (intertidal) community.

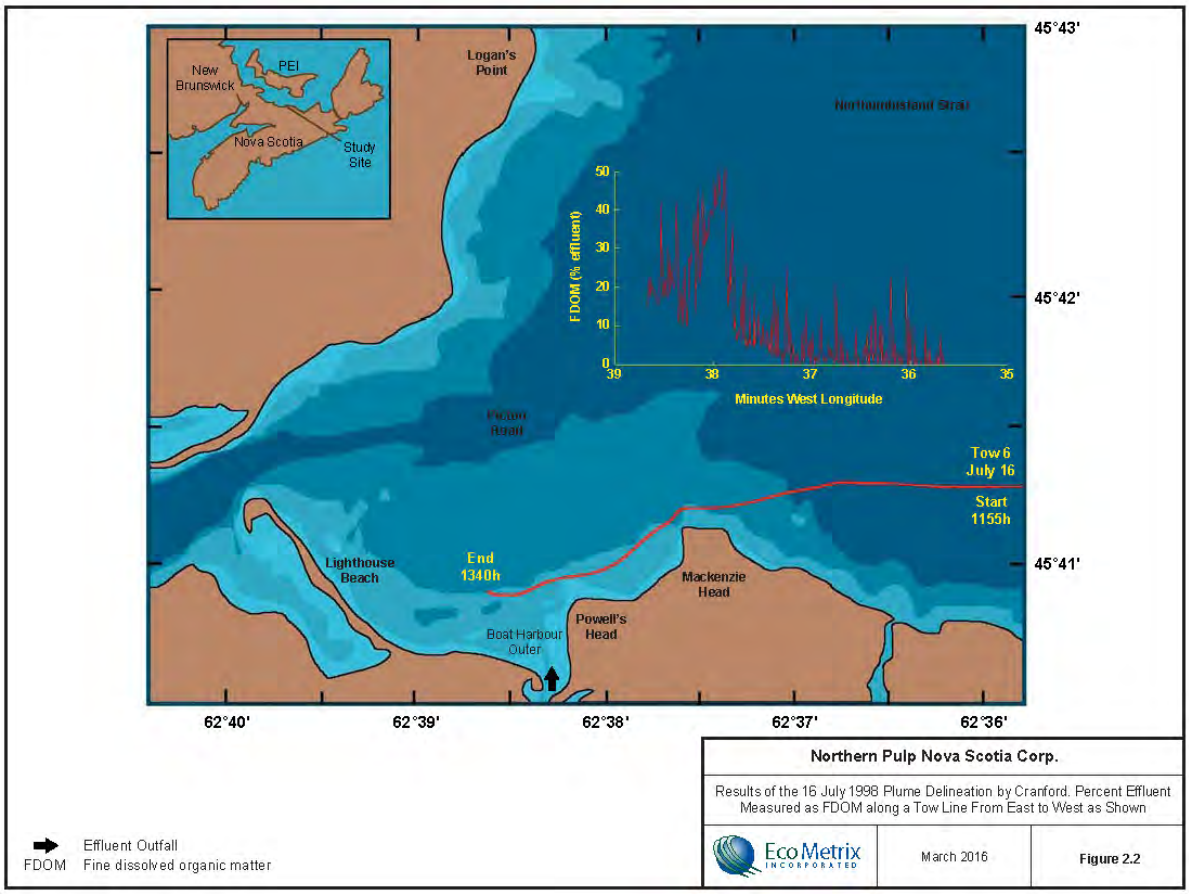
In October 1993, ASA Consulting Ltd. carried out an oceanographic field survey of features and processes in the Pictou Road area (ASA Consulting Ltd., 1994). Their work revealed that the water column is usually stratified in terms of salinity - a shallow surface brackish layer (~2 m thick) originating from river discharge usually floats over the denser waters of the Strait. This new information on salinity and currents was incorporated into computer simulations used to predict the dispersion of mill effluent in Pictou Road. This simulation of the discharge configuration predicted a much larger 1% effluent plume than the one demonstrated in 1965 by Krauel's dye experiment. The 1% effluent envelope extended to the north to the vicinity of Logan's Point, to the east beyond MacKenzie Head and to the west into the mouth of Pictou Harbour (Figure 2.1). The simulated plume from the outflow existed primarily as a thin layer floating on the ocean's surface. This prediction was supported by measurements of salinity, colour and TSS undertaken in the Cycle 1 EEM.

The differences between the modeled (ASA Consulting Ltd., 1994) and dye-tested plume (Krauel, 1969) may be partially explained by the highly variable nature of the plume due to local weather conditions (especially winds and storm surges), tides and ambient water temperatures. The buoyant nature of the plume makes it extremely susceptible to even slight weather changes and hence, the spatial extent of the plume can alter dramatically from day-to-day and certainly from season to season. Although its range is variable throughout the year, the 1% effluent plume in the fall exists as a surface layer that may extend to MacKenzie Head, and occasionally as far as Logan's Point. The effluent is dispersed more effectively in the summer because the ocean water is warmer.

In fall 1997, a research team from the Bedford Institute of Oceanography began characterizing the effluent plume by measuring dissolved organic carbon (DOC) *in situ* with a fluorimeter (Peter Cranford, pers. comm., 1998, 2001). Plume contouring was completed in 1998 and the results generally agreed with the information provided in the Cycle 2 Pre-design and Study Design Report (BEAK, 1998). This work indicates that the effluent tends to move northeast

towards and beyond MacKenzie Head and is confined to a relatively narrow pathway (Figure 2.2).





Most recently, effluent concentrations were determined in Pictou Road via spectrophotometric measurements over a sixteen-week period (June through September) in 2003, coincident with continuing Environment Canada caged mussel research in the area (St. Jean, unpublished data). Water samples were collected weekly on the same day and time over the study period and analyzed for colour. The contrast of the tea-coloured effluent and the receiving water makes it possible to measure effluent concentrations in Pictou Road via colour to a level of about 1% with reasonable accuracy (St.-Jean, pers. comm., 2004). The samples were collected at the same time every week to ensure that the full ranges of tidal and weather conditions were adequately represented in the data. At each sampling event water samples were taken along a gradient extending from the discharge into Pictou Road from the top of the water column, mid-depth and the bottom of the water column. Samples of 100% effluent and non-effluent-exposed water (i.e., reference water) from Northumberland Strait were also collected at each sampling event to derive a standard colour curve specific for that day. Colour was measured spectrophotometrically in the receiving water samples and the relative effluent

concentration (% v/v) for that sample derived from the standard curve. The results of the sampling indicated that on average:

- mill effluent was confined to the top of the water column;
- mill effluent was diluted to less than 1% v/v within 300 to 400 metres of the shoreline; and
- mill effluent travels along the surface of the water following a reasonably narrow pathway in a north by northwest direction.

Overall, the current characterization of the plume satisfies EEM requirements, as it provides sufficient information to appropriately locate exposure and reference sampling stations.

2.3 Ecological Aspect of the Study Area

2.3.1 Habitat Inventory and Classification

The physical habitat in the vicinity of the NPNS mill was characterized during the Pre-Design phase of EEM Cycle 1 (JWEL, 1993a, and b) according to a U.S. Fish and Wildlife Service classification scheme (Cowardin *et al*., 1979). A detailed description of the receiving environment and reference area habitats is available in the Cycle 1 Pre-Design Report (JWEL, 1993a, b) and is summarized in the Cycle 2, Cycle 3, Cycle 4, Cycle 5, Cycle 6 and Cycle 7 Pre-Design/Study Design Reports (BEAK, 1998, 2002 and EcoMetrix 2005, 2008, 2011, 2013). A summary of this information is provided in the following paragraphs along with updates reflecting any changes since Cycle 6 (also see Table 2.6).

In Cycle 1, specific habitat information was obtained through a variety of sources, including discussions with DFO fisheries officers in Pictou, a reconnaissance field survey and review of scientific literature and data reports. All habitat information was compiled to develop maps of the physical habitat characteristics of the receiving environment and the reference area. For the purpose of the EEM Cycle 2 program, the habitat maps created by JWEL during Cycle 1 were reviewed and updated based on information obtained during the Cycle 1 field study, as well as information provided by topographical maps published by the Survey and Mapping Branch of the Department of Energy, Mines and Resources and hydrographic charts produced by the Department of Fisheries and Oceans. Subsequent updates in Cycles 3, 4, 5 and 6 were made based on observations made during the preceding cycle field work as well as through discussions with relevant sources in the area.

For the purposes of Cycle 7, the habitat information has been updated again via observations made during the Cycle 7 field work, as well as, discussions with relevant sources (e.g., DFO fisheries officers) in the area. The habitat conditions in the receiving environment and the benthic reference area are represented in Figures 2.3 and 2.4 and are summarized in the text of the following subsections.

Table 2.6: Habitat Classification of the Study Area for the NPNS EEM Program (based on Cowardin *et al*., 1979)

Hierarchy Category	Study Area Location	
	Boat Harbour Estuary (and mouth)	Pictou Road and environs (nearshore Northumberland Strait)
System	estuarine	marine
Subsystem	intertidal	subtidal
Substrate class	unconsolidated bottom	unconsolidated bottom
Substrate subclass	sand / mud	sand / silt
Salinity	mixohaline	polyhaline
Water regime	tidal - regularly flooded	tidal - subtidal

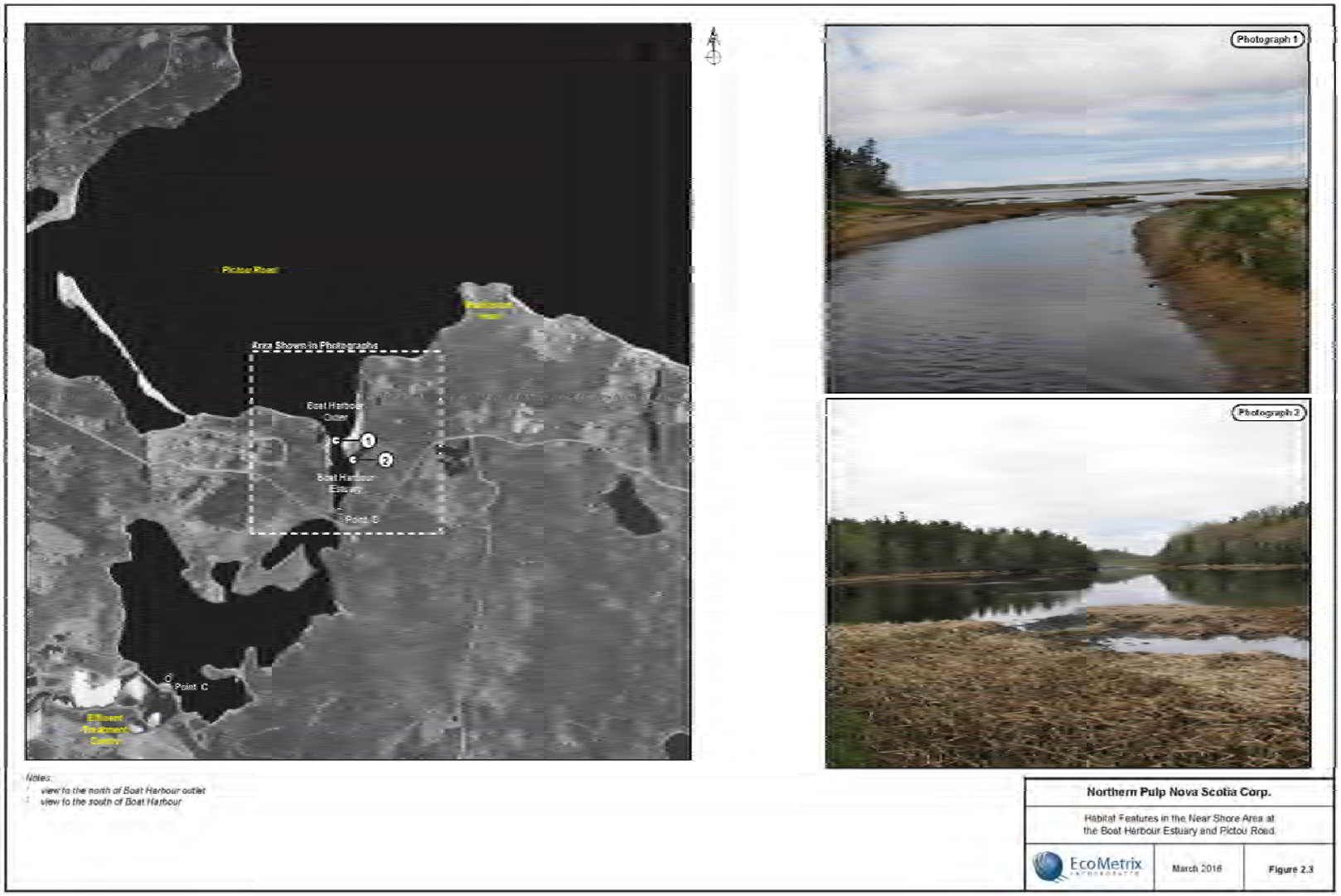
(Modified from JWEL 1993a)

The potential zone of influence of NPNS effluent includes both the intertidal zone at the Boat Harbour Estuary, as well as the nearshore, subtidal zone in Pictou Road.

Intertidal Zone

Effluent from the NPNS treatment facility passes through the Boat Harbour Estuary and then into Pictou Road through a channel across the sandy beach. The intertidal area near the effluent outfall provides a diversity of habitat types including cobble/gravel, exposed boulders, and a sand beach with clumps of grasses (Figure 2.3). Other than the nearby First Nations Fishers Grant Reserve and a United Church Youth Camp at Powells Head, there is little human activity in the area surrounding the ETC and Boat Harbour basin, and much of the adjacent land is covered with mixed forest.

Nearest to the outlet channel of the estuary, the intertidal zone is largely comprised of unconsolidated sand, cobble and gravel. These sediments tend to remain saturated during low tide, largely because of the flow of diluted effluent. Fragmented macroalgae (beach wrack) can be abundant in the upper intertidal area where it is deposited by waves and tides. The most abundant flora washed ashore is rockweed (the brown alga *Fucus serratus*), although other species including kelp (the brown alga *Laminaria agardhii*), a red


Pictou Road
Area Shown in Photographs
Boat Harbour Outlet
Boat Harbour Estuary
Point B
Point C
Effluent Treatment Facility
Photograph 1
Photograph 2
Notes:
1 view to the north of Boat Harbour outlet
2 view to the south of Boat Harbour
Northern Pulp Nova Scotia Corp.
Habitat Features in the Near Shore Area at the Boat Harbour Estuary and Pictou Road
March 2016
Figure 2.3

alga (*Furcellaria* sp.), sea lettuce (the green alga *Ulva lactuca*), Irish moss (a red alga, *Chondrus crispus*) and eel grass (a marine angiosperm, *Zostera marina*) are also found. The invertebrate community living within the beach wrack is relatively diverse and abundant.

To the east of the estuary outlet, the beach and tidal flat consist of sand with some cobble and gravel. The relief of this area is steep relative to the broad flat area near the estuary mouth. Bedforms occurring in areas of unconsolidated coarse sand are likely caused by wind and wave action, rather than tidal currents. Macrofauna is not abundant along this area, and consists of limited wetted patches where soft shelled clams (*Mya arenaria*) can be found. During low tide, the upper intertidal zone drains quickly and tends to be too dry to support most marine organisms. Decaying macroalgae fragments are common at the high water mark, and plant species generally associated with salt marshes (e.g., smooth cord grass [*Spartina alterniflora*]). Closer to Powells Head, gravel, cobble and boulders replace the sandy beach, especially in the lower intertidal zone. Fragmented seaweed is common around the rocks, as is the alga *Enteromorpha*.

Immediately to the west of the estuary mouth the intertidal substrate is comprised of cobble and gravel interspersed with silty sand. Beyond the outlet, the intertidal habitat is somewhat variable, consisting of patches of coarse sand, cobble/gravel and cord grass intermixed with a number of tidal pools. Further west, the shoreline is rocky and more steeply sloped.

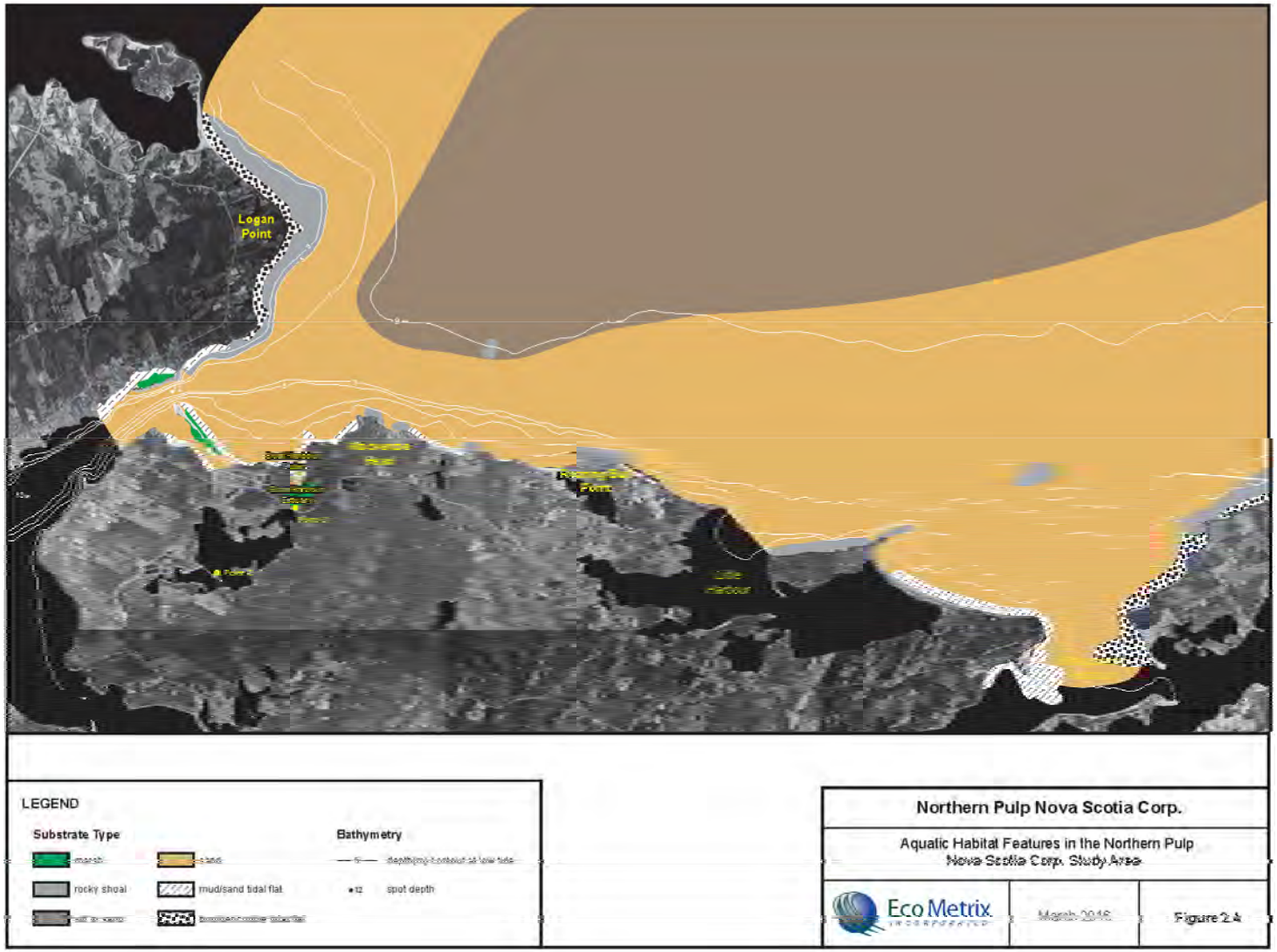
Subtidal Zone

Bottom substrates in the nearshore area off the Boat Harbour Estuary are dominated by unconsolidated sand. Bottom substrates further offshore are comprised of silty sand (Figure 2.4). To the north of the Boat Harbour Estuary, south of Logan's Point, the nearshore subtidal area comprises a rocky shoal (Figure 2.5). Water depths in the Pictou Road area range between 1 m and 5 m at low tide (Figure 2.5).

Perennial seaweeds in this area are usually found only in sheltered crevices and offshore reefs. *Fucus serratus* is the most common macroalgae, along with *Furcellaria fastigiata*, *Chondrus crispus, Corda filum* and *Laminaria* sp.

2.3.1.1 Reference Area

To the north of Logan's Point, the nearshore subtidal area is a rocky shoal (Figure 2.5). To the east of the Boat Harbour Estuary towards Roaring Bull Point and beyond, bottom substrates are sand in nature with isolated rocky shoal areas near shore. The habitat along the shoreline in the area is similar all of the way to the mouth of Merigomish Harbour. In nearshore areas, bottom substrates are sand in nature with isolated pockets of rock and cobble (Figure 2.5). Further offshore the substrates are finer, and are comprised of fine sand and silt fractions.


Logan Point
LEGEND
Substrate Type
rocky shoal
mud/sand tidal flat
Bathymetry
spot depth
Northern Pulp Nova Scotia Corp.
Aquatic Habitat Features in the Northern Pulp
Nova Scotia Corp. Study Area
Eco Metrix
Figure 2.4

For the fish survey in Cycles 4 and 5 three reference areas were chosen as appropriate locations to collect fish (Mummichogs) for comparison to the Boat Harbour Estuary. These included: Caribou Island (a tidal channel area), West River (mouth of Sawmill Brook) and Merigomish Harbour (mouth of Balmoral Brook). These three locales spanned an array of habitats utilized by Mummichogs in the coastal areas surrounding the exposure area. The Caribou Island location was completely marine in nature without any direct freshwater inputs. The sampling site was a small (1 m to 1.5 m wide) tidal channel that was inundated with the tidal cycle. The Merigomish Harbour location was at the mouth of a small brook that becomes inundated with seawater on the rising tide but subsequently becomes largely freshwater on the falling tide. The West River area sampling location was similar to the Merigomish Harbour one in that it was alternately influenced by the rising and falling tides. The brook at the West River area is larger however, and therefore the influence of the freshwater inputs was greater there.

For the purposes of Cycle 6 Mummichogs were collected in all three of the aforementioned areas. In Cycle 6, Mummichogs were also captured at an additional two areas not previously sampled. Little Lake was selected as a reference for comparison of the conventional EEM effect endpoints. Little Lake is located approximately 17 km north of Antigonish, Nova Scotia, has similar maximum depth (~3 m) to the Boat Harbour Estuary and is seasonally connected to the ocean. Little Lake was again sampled during Cycle 7. The final reference area was at Rear Brook on the East River of Pictou. This location is heavily influenced by the tidal cycle and contained emergent vegetation during low tide. Generally the East River location is similar to Boat Harbour Outer.

2.3.2 Resource Inventory

Information pertaining to the use of the receiving waters for recreational, municipal and industrial uses, was originally compiled during EEM Cycle 1 (JWEL, 1993a,b) and updated subsequently in Cycles 2, 3, 4, 5 and 6 (BEAK, 1998; 2002 and EcoMetrix, 2005, 2008, 2011). This information is summarized below. A discussion of fisheries resources is presented separately (Section 2.6).

Industrial and municipal pollutants from the towns of Stellarton, New Glasgow, Trenton, Westville and Pictou enter the East River of Pictou and Pictou Harbour. These include non-point runoff from streets, parking lots, lawns and construction sites (including road construction). Municipal sewage from the towns of New Glasgow, Westville, Stellarton and Trenton is treated at the East River Pollution Abatement System in New Glasgow. There is also a treated municipal waste outfall for the local First Nations community located to the west of the effluent outfall at Lighthouse Beach.

A limited amount of ship traffic passes through Pictou Harbour. The number of ships docking at Pictou has increased somewhat in recent years, and now averages five or six ships a month. There have been no dredging activities in the area.

2.3.3 Fisheries Resources

The identification of aquatic resources, and uses of those resources, which may potentially be affected by mill effluent, is critical to the design of the EEM study and the selection of appropriate sentinel species for the fish survey. As part of the pre-design requirements for EEM, a description of fisheries resources within the study area is required. This information should include the identification of fish and/or shellfish that are presently exploited in commercial and non-commercial fishing, that may potentially be exploited in the future, and any species recognized by federal or provincial authorities as rare, threatened or endangered, as well as any species which may be present in sufficient numbers to be considered as a monitoring species.

As part of the pre-design phase of EEM Cycle 1 at the NPNS mill, fisheries resources were identified and reported in detail in the Cycle 1 Pre-Design Report (JWEL, 1993a, b). An updated summary of this information was provided in the Cycle 2, 3, 4, 5, 6 and 7 pre-design and study design reports (BEAK, 1998; 2002 and EcoMetrix, 2005, 2008, 2011, 2013). The following subsection provides a further update to this information. A list of common fish and shellfish species found within the greater Pictou Road area is provided in Table 2.7.

2.3.3.1 Species

Estuary

A number of small fish species are found within the salt/estuarine marsh environment of the Boat Harbour Estuary. Large numbers of Atlantic Silverside (*Menidia menidia*), Mummichogs and sticklebacks ((F) Gasterosteidae) can be found on the downstream side of the effluent outfall.

Intertidal Zone

Diversity within the intertidal zone is limited to a number of shellfish species, and these are generally found in areas that remain wetted, though not inundated, under most tidal conditions. Common shellfish in the area include the Soft Shelled Clam *(Mya arenaria)*, Blue Mussels (*Mytilus edulis*), Horseshoe Mussels (*Modiouls modiolus*), Oysters (*Crassostrea virginica*), Razor Clams (*Ensis directus*), Surf Clams (*Spisula solidissima*) and Moon Snails (*Polinices heros*). Hermit crabs can also be found.

Subtidal Zone

The subtidal fish community includes shellfish, groundfish, and pelagics and finfish. Shellfish commonly found in soft-bottom subtidal habitats are the same as those found in the intertidal area, although they tend to be less abundant than in the intertidal area. Some of the shellfish species found in the rocky reefs that predominate along the north-west shoreline of Pictou Road near Logan's Point include American Lobsters (*Homarus americanus*) and Rock Crabs (*Cancer irroratus*).

Table 2.7: List of Common Fish and Invertebrate Species Reported from the Pictou Road Area of the Northumberland Strait, Nova Scotia

Scientific Name	Common Name
FISH	
Alosa pseudoharengus	Gaspareau or Alewife
Anguilla rostrata	American Eel
Clupea harengus	Atlantic Herring
Family Gasterosteidae	stickleback
Fundulus heteroclitus	Mummichog
Hemitriterus americanus	Atlantic Sea Raven
Lycodes sp.	eelpout
Macrozoarces americanus	Ocean Pout
Menidia menidia	Atlantic Silverside
Microgadus tomcod	Atlantic Tomcod
Morone saxatilus	Striped Bass
Myoxocephalus octodecemspinosus	Longhorn Sculpin
Osmerus mordax	Rainbow Smelt
Pseudopleuronectes americanus	Winter Flounder
Raja sp.	skate
Salmo salar	Atlantic Salmon
Salvelinus fontinalis	Brook Trout
Scomber scombrus	Atlantic Mackerel
Scophthalmus aquosus	Windowpane
Tautogolabrus adspersus	Cunner
Urophycis sp.	hake
INVERTEBRATES	
Cnidaria	
Metridium dianthus	sea anenome
Mollusca	
Crassostrea virginica	oyster
Ensis directus	razor clam
Littorina littorea	periwinkle
Modiolus modiolus	horse mussel
Mya arenaria	soft shelled clam
Mytilus edulus	blue mussel
Placopecten magellanicus	sea scallop
Polinices heros	moon snail
Spisula solidissima	surf clam
Crustacea	
Pagurus bernhardus	hermit crab
Balanus crenatus	barnacle
Carcinus maenas	green crab
Cancer irroratus	rock crab
Homarus americanus	American lobster
Echinodermata	
Asterias sp.	sea star
Echinarachnius parma	sand dollar
Henricia sanguinolenta	sea star
Strongylocentrotus drobachiensis	sea urchin

Common groundfish in the area include flounders (e.g., Winter Flounder, *Pseudopleuronectes americanus*), hake (*Urophycis* sp.) Tomcod *(Microcadus tomcod)* and skates (*Raja* sp.). Groundfish, common specifically in hard-bottom areas, include Cunners (*Tautogolabrus adspersus*) and Longhorned Sculpins *(Myoxocephalus octodecemspinosus*)

Fish that utilize the area on a seasonal basis include mackerel (*Scomber scombrus*), Herring (*Clupea harengus*), Alewife (*Alosa pseudoharengus*), Rainbow Smelt (*Osmerus mordax*), American Eel (*Anguilla rostrata*), sea-run Brook Trout (*Salvelinus fontinalis*) and Atlantic Salmon (*Salmo salar*).

2.3.3.2 Fisheries Resource Use

Sport Fishery

Recreational fishing in the Pictou Road area primarily involves sea-run Brook Trout, Mackerel, Atlantic Salmon and Rainbow Smelt. The sport fishery for Striped Bass (*Morone saxatilus*) was reopened in 2013 with two short retention periods in May and August. This followed the fishery being closed for a number of years. In 2015 and 2016 the season was increase to four short retention periods (i.e., May, August, September and October).

Commercial Fishery

Commercial fishing has been an important contributor to the local economy for many years. Commercial fishery resources in the area are shown on Figure 2.5. The two most important commercial species in the area are lobster and Herring. Lobsters are trapped from 30 April to 30 June, typically close to shore around rocky shoals. Herring are harvested at the approaches to Pictou Harbour, from September to October. Other fish collected in the area include migratory anadromous species, such as, Alewife, smelt, eels and Rock Crabs. The clam and mussel shellfish fishery in the area is currently closed due to bacterial contamination as is common in most harbours.

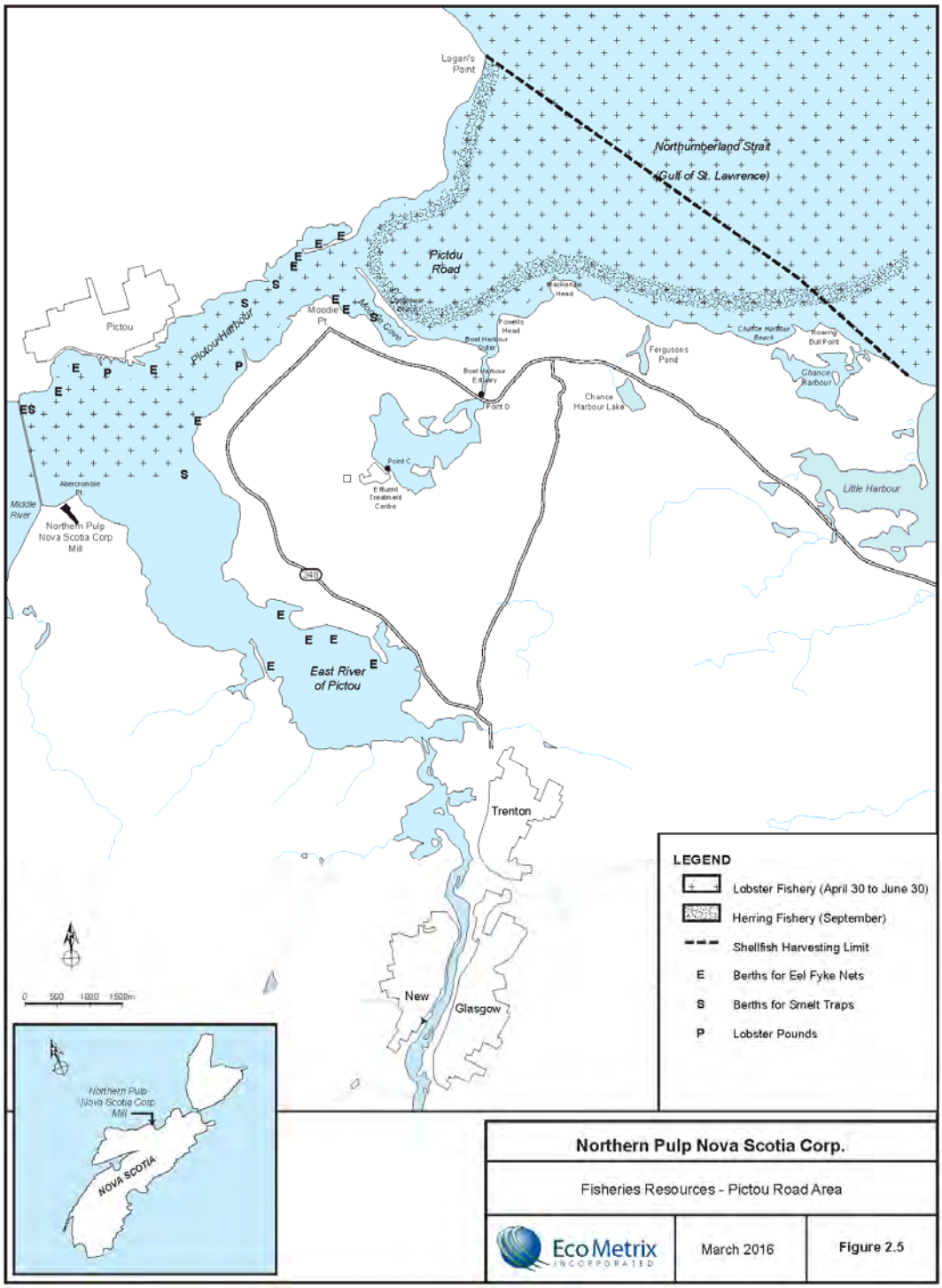
Subsistence Fishery

Residents of the Pictou Landing Band residential reserve (Fisher's Grant Reserve 24) harvest a variety of species in the Pictou Road area, including lobster, Rock Crab, Herring and eels. The First Nation fishery also included the collection of shellfish and eels from Boat Harbour area prior to its conversion into a wastewater treatment facility.

2.3.3.3 Contaminants in Fish

Documentation of contaminant levels in fish and shellfish tissue at Pictou dates back to the late 1980s. At this time, DFO looked at levels of dioxins and furans in fish and shellfish near pulp and paper mills at various sites in the Maritimes. Although low levels of dioxins and furans were detected in all composite samples of crabs, lobsters, clams and Blue Mussels

collected 100 m to 3 km from the effluent outfall, consumption of these species was not considered to be a human health hazard (JWEL, 1993a, b).



Similar results were found for tissue samples collected as part of the Cycle 1 EEM study. Dioxin and furans were measured in mussels collected near both the Boat Harbour Estuary outlet and a reference area at Caribou Island (JWEL, 1996). Although furans were not detected in either composite sample, the dioxin congeners H7CDD (0.8 ppt) and O8CDD (2.6 ppt) were detected in the Pictou Road sample. H7CDD was non-detectable at the Caribou Island reference site, but O8CDD concentrations were actually higher at 4.3 ppt, possibly because of higher lipid levels in the Caribou Island samples. These concentrations were well within published health-related guideline criteria. Resin and fatty acids were not detected in the hepatopancreas tissues of rock crabs or liver tissues of Winter Flounder collected during the Cycle 1 EEM (JWEL, 1996).

As part of NPNS's second-cycle EEM program, mussel, tissue and shell were collected from cages deployed in areas exposed to and not exposed to NPNS effluent for chemical characterization (Andrews and Parker, 1999; Table 2.8). Laboratory analysis of pre-deployment and post-deployment tissue samples targeted parameters typically associated with bleached Kraft mill effluents (BKME), including chlorophenolic compounds, resin acids, plant sterols and metals. The study did not detect any contamination in mussel tissues or shells exposed to NPNS effluent.

Shellfish collection is currently prohibited in Pictou Harbour and Pictou Road in an area that extends from south-east of Roaring Bull Point to Logan's Point (English, Personal Communication, 2013). Shellfish collection in the inner portion of Pictou Harbour was also banned in 1991 because of heavy metal contamination (JWEL, 1993a, b).

2.3.3.4 Fish Tainting

Since Cycle 1, there have been no complaints of tainted fish or shellfish in the Boat Harbour area. Historical concerns over the potential for lobster tainting prompted a lobster tainting evaluation to be included in the Cycle 1 EEM. Although lobster exposed to the 20% effluent were generally described as different, most panelists did not consider them unacceptable. Some panelists detected an unpleasant odour in the exposed tissue (JWEL, 1996). The evaluation panel also detected a difference in sweetness between the 10% effluent treatment and the control samples. Under no circumstances would it be expected that lobster collected in the greater Pictou area would be exposed to effluent concentrations at these levels, given known effluent dispersion characteristics.

2.3.4 Historical Receiving Environment Information

The review of historical (ten-year) and more recent monitoring data, which have been collected for other purposes, is an important component of the pre-design information requirements. This information may assist in identifying known impacts, if any, and will aid in determining selection of exposed and reference areas. Such review should also identify other site-specific monitoring programs (e.g., intensive dissolved oxygen monitoring programs) which should be undertaken or are being conducted on a site-specific basis and

Table 2.8: Tissue Chemistry Results from Pre- and Post-Exposure Samples of Mussels Caged at Five Site in and Around Pictou Road for 90 Days (source: Andrews and Parker, 1999)

Analytical parameter	Mussel Cage Location					
	Baseline[1]	Pictou Road (effluent exposed)	Northumberland Strait (sometimes effluent-exposed)	Northumberland Strait (potentially effluent-exposed)	Pictou Road (reference)	Pictou Harbour (reference)
Chlorophenols (mg/g)	ND	ND	ND	ND	ND	ND
Total resin acids (mg/g)	0.24	ND	ND	ND	ND	ND
Total plant sterols (mg/g)	1,580	1,590	1,610	1,050	1,670	1,580
Metals[2] (mg/g)	Fe = 5; Mn = 0.5	Elevated levels of Fe (range 29-68) and Mn (range 3-9.6) above baseline in all samples				
% lipids	1.3	2.3	2.5	2.1	2.3	2.0
% moisture	82	77	77	81	79	79

[1] Includes results for tissues from mussels that were obtained from the same source as the others but not deployed for the study.

[2] full ICP-MS metals scan completed - only selected parameters shown

identify past problems or concerns, such as tainting, shellfish or fisheries closures, bacterial contamination and sediment fibre mats.

In EEM Cycles 1 through 6 a review of available historical data for the receiving environment at the NPNS mill was completed as part of the Pre-Design Phase (JWEL, 1993a, b; BEAK, 1998, 2002; EcoMetrix, 2005, 2008, 2011). The findings of these reviews are summarized in the following paragraphs.

Prior to EEM Cycle 1, several studies identified water and sediment quality concerns and impacts to the biological community of the ETC, formerly the Boat Harbour Water Treatment Facility, (e.g., Rades, 1981, 1985).

2.3.4.1 Water Quality

Prior to the commissioning of the secondary treatment facility at the ETC, there had been a number of observations and/or complaints by local residents and federal government agencies regarding water quality issues attributable to the mill. Examples included excessive effluent foam, beach closures and fish kills associated with low oxygen levels. However, following the advent of secondary effluent treatment at the ETC, incidents of this sort ceased as the direct result of the dramatic improvement in effluent quality.

Examples of how these improvements in effluent quality have resulted in improvements in receiving water quality include:

- the effluent plume being less visible in Pictou Road (J. Van Buskirk, NPI, pers. comm., 1998; 2002) ;
- dissolved oxygen levels in the study site's surface and bottom waters being at or near saturation (JWEL, 1996; BEAK, 2000; Stantec, 2004a, EcoMetrix 2007a, 2010); and
- chlorinated phenolic compounds being undetectable in both surface and bottom water samples from the study site (JWEL, 1996).

2.3.4.2 Sediment Quality

Sediment quality at Pictou Road in the vicinity of the mill discharge has been investigated a number of times outside of the EEM program (e.g., Rades, 1981, 1985, 1991; JWEL, 1994). These studies provided no evidence of changes neither in composition nor in organic matter content of sediments near the Boat Harbour Estuary outflow.

Sediment granulometry and total organic carbon (TOC) levels were measured at reference and exposure benthic stations as part of EEM Cycles 1 through 5 (JWEL, 1996; Beak, 2000; Stantec, 2004a; EcoMetrix, 2007a, 2010) (Table 2.9). In each of the studies, bottom substrates in the study area were comprised of sand-sized fractions almost exclusively (> 85%). Sediment analysis during Cycle 1 revealed TOC levels were about 3.1 to 3.4% in Pictou Road, and about 2.6% in Merigomish Harbour. In Cycles 2 through 5, TOC levels were almost an order of magnitude lower than found in Cycle 1 at all sampling areas (reference and exposure). Although there was a cycle–to-cycle difference in TOC levels, both studies indicated that there was no measurable accretion of organic matter from the treatment facility.

Additional sediment quality parameters measured in Cycles 2 through 5 included total nitrogen (TN), carbon to nitrogen ratio (C:N), sediment total sulphides (TS) and sediment redox potential (Eh) (BEAK, 2000; Stantec, 2004a; EcoMetrix, 2007a, 2010) (Table 2.10). For all parameters, either sediment quality was similar among the sampling areas or where differences were noted all values were still within what would be considered indicative of normal, non-impacted conditions (BEAK, 2000; Stantec, 2004a; EcoMetrix, 2007a, 2010).

2.3.4.3 Fisheries Research

There has been extensive fisheries research conducted by DFO, along with various partners, related to the development of new biomarkers to be used in the assessment of marine environmental health (e.g., St.-Jean, 2002; St.-Jean *et al.*, 2003). The research particularly relevant to NPNS broadly fits into three categories of response variables, including:

- physiological (feeding rate),
- immunological, and
- developmental (incidence of abnormalities).

Table 2.9: Grain Size and TOC in Sediments from the NPNS EEM Study Area

	GRAIN SIZE (%)				TOC (%)
	Gravel	Sand	Silt	Clay	
CYCLE 1					
NF	< 0.1	97.8	0.4	1.8	3.4
FF	1.0	88.8	5.8	4.3	3.1
FFF	-	-	-	-	-
REF[1]	< 0.1	98.5	0.1	1.5	2.6
REF[2]	-	-	-	-	-
CYCLE 2					
NF	1.6	96.6	1.8	0.0	0.1
FF	3.1	93.7	1.9	0.9	0.2
FFF	-	-	-	-	-
REF[1]	0.3	98.7	0.9	0.0	0.1
REF[2]	-	-	-	-	-
CYCLE 3					
NF	3.5	91.6	1.8	3.1	0.3
FF	2.1	91.3	3.4	3.2	0.2
FFF	0.5	85.0	8.2	6.3	0.3
REF[1]	0.5	97.6	0.2	1.7	0.1
REF[2]	0.0	87.2	7.4	5.4	0.6
CYCLE 4					
NF	0.6	97.3	2.7	-	0.2
FF	0.1	95.6	4.3	-	0.3
FFF	0.1	97.0	2.9	-	0.2
REF[1]	2.8	95.8	1.4	-	0.1
REF[2]	3.6	92.5	1.9	-	0.2
REF[3]	1.9	83.0	15	-	0.1
CYCLE 5					
NF	-	96.0	3	<2	0.1
FF	-	97.0	3	1.2	0.1
FFF	-	97.0	3	<2	0.1
REF[1]	-	98.0	<2	<2	0.1
REF[2]	-	97.0	<2	2.0	0.1
REF[3]	-	93.0	5	2.0	0.1

[1] Merigomish Harbour
[2] Logan's Point
[3] Chance Harbour

Table 2.10: Chemical Measures in Sediments from the NPNS EEM Study Area

	TS (mg/kg)[4]	TN (%)	C:N	Eh (mV)
CYCLE 2				
NF	5.4	0.17	1.5	205
FF	27.2	0.21	1.3	228
FFF	-	-	-	-
REF[1]	1.1	0.14	1.1	223
REF[2]	-	-	-	-
CYCLE 3				
NF	1.8	0.03	8.6	122
FF	1.6	0.04	6.5	129
FFF	1.6	0.04	8.9	136
REF[1]	2.0	0.01	9.5	134
REF[2]	2.0	0.05	11.0	131
CYCLE 4				
NF	1.9	0.19	11.0	72
FF	1.1	0.25	11.0	78
FFF	1.1	0.21	12.0	79
REF[1]	1.1	0.15	8.0	76
REF[2]	0.8	0.18	9.0	75
REF[3]	2.6	0.15	9.0	75
CYCLE 5				
NF	1363	0.02	16.9	23
FF	2954	0.02	6.7	6
FFF	234	0.02	6.6	97
REF[1]	~ 0	0.01	8.0	198
REF[2]	1510	0.02	5.6	62
REF[3]	3156	0.02	6.2	57

[1] Merigomish Harbour

[2] Logan's Point

[3] Chance Harbour

[4] TS was measured using a different method in Cycle 5 compared to previous cycles as per Environment Canada recommendations (units = µM).

Physiological Biomarkers

DFO deployed a new automated biological effects monitoring system called HABITRAP at Pictou (BIO, 2000; P. Cranford, pers. comm., 2001). The HABITRAP provides a continuous record of bivalve feeding rates, a response that is sensitive to the presence of contaminants

and relevant to population health as it is closely related to growth and reproduction (BIO, 2000). In 2000, the HABITRAP was deployed at Pictou Road within the effluent plume of NPNS for a period of 60 days. Preliminary results indicated that, although the feeding rate of mussels at Pictou Road was reduced relative to reference, the reduced feeding rate was not the result of effluent toxicity. Rather, the reduced feeding rate measured in the effluent-exposed mussels was the result of the influence of the freshwater input from effluent outfall (P. Cranford, pers. comm., 2001).

Immunological Biomarkers

In the early 2000s the DFO was conducting a research project with the overall objective of developing a suite of immunological biomarkers in fish and bivalve molluscs for assessing potential impacts of industrial and sewage effluents (S. St-Jean, per. comm., 2002). The objective of the project was to quantify spatial and temporal variation in immune function in order that anthropogenically induced changes in the immune system, or immunomodulation, can be discerned. Immunological endpoints of interest included haemocyte counts and viability; phagocytic activity; lysosome retention; nitro blue tetrazolium reduction (a measure of the oxygen superanion production, peroxide production); *p*-nitrophenyl acid glucosaminide assay (which measures the production of bactericide); total protein; and bacterial clearance (or bacterial challenge).

The first phase of the study was completed in conjunction with the caged bivalve study completed as part of the NPNS Cycle 2 EEM program (see Section 6.0). Briefly, the study included the deployment of five mussel cages at locations in Pictou Harbour and Pictou Road, including within the area potentially influenced by NPNS effluent. At the end of the 90-day exposure period, haemocyte function and bacterial challenge assays were performed. The results of the study suggested that mussels exposed to NPNS effluent may have been immuno-suppressed (e.g., lower phagocytic activity, lower lysosomal retention, higher mortality rate during immune challenge) (S. St-Jean, per. comm., 2002). It was also suggested that there may have been other factors contributing to the patterns observed in overall levels of immune response and that to resolve the apparent contradiction more endpoints and reference sites were needed. Interestingly, the immuno-suppression apparently observed in the mussels was not manifested at the population level, as mussel morphometric endpoint data collected during the same exposure period indicated that mussel survival and growth were not adversely affected by NPNS effluent (Andrews and Parker, 1999).

The follow-up project was designed as a three-year immunomodulation monitoring study to diagnose sublethal stress in the Blue Mussel and Mummichogs (S. St-Jean, pers. comm., 2002). In order to characterize the influence of different anthropogenic inputs, mussels were caged at thirteen potentially impacted sites, including at NPNS, and one reference site. Wild Mummichog populations were sampled from the same areas in which mussels were caged, where possible (6 out of 14 of the sites). The monitoring covered the ice-free seasons of 1999 to 2001, inclusively.

The results of the mussel assays suggested that the site most similar to the reference site (i.e., potentially the least stressed) was Logan's Point, whereas, the most different site (i.e., potentially the most stressed) was the outfall of untreated municipal wastewater from the town of Pictou (S. St-Jean, pers. comm., 2002). The cages situated near the NPNS outfall appeared to be different from the reference site in terms of immune response, but ranked among the upper half of the 14 sites for overall health (i.e., least stressed). There also appeared to be somewhat of a gradient response to effluent-exposure, as the mussels deployed at MacKenzie Head were less stressed than those deployed in closer proximity to the Boat Harbour Estuary. Similar patterns to those observed in mussels were observed in the fish portion of the project (S. St-Jean, pers. comm., 2002). That is, fish collected within Pictou Harbour tended to display the most overt signs of stress. Fish from Pictou Road appeared to be different from reference, although the differences appeared to be relatively minor.

Developmental Biomarkers

Juvenile Mummichogs were collected from several sites receiving industrial and sewage effluents in New Brunswick and Nova Scotia in order to quantify the incidence of developmental abnormalities, such as spinal curvatures, relative to Mummichogs from unimpacted sites (BIO, 2000; S. St-Jean, pers. comm., 2002). This included fish from the lower end of Boat Harbour Estuary where Mummichogs are plentiful. Field-collected fish from Boat Harbour Estuary did not show development abnormalities at rates above what was found at unimpacted sites (e.g., <1%) (S. St-Jean, pers. comm., 2002). Laboratory exposures of juvenile Mummichogs using NPNS effluent were also undertaken. There was some indication that the rate of abnormalities was greater in the laboratory than in the natural population (2 to 3%) but it was still relatively low (S. St-Jean, pers. comm., 2002).

3.0 SUBLETHAL TOXICITY OF MILL EFFLUENT

3.1 Summary of EEM Cycles 1 through 6

Results of sublethal toxicity tests completed over the period 1995 to 2013 generally indicated that the magnitude of response of each of the test species to mill effluent had increased (Table 3.1). The GM IC25 (i.e., the geometric mean [GM] of effluent concentrations that caused a 25% reduction in a measured endpoint [IC25]) for sea urchin (*Lytechinus pictus*) fertilization was 3.3%, 5.1%, 3.2%, 2.1%, 0.76% and 1.68% in Cycles 1, 2, 3, 4, 5 and 6 respectively. The GM IC25 for algae (*Champia parvula)* sexual reproduction was 8.4%, 5.5%, 6.4%, 2.0%, 0.30% and 0.21% in Cycles 1, 2, 3, 4, 5 and 6, respectively.

3.2 EEM Cycle 7 Program

3.2.1 Requirements and Objectives

Under the PPER, mills are required to complete sublethal toxicity testing of their final treated effluent semi-annually using representative invertebrate and plant species.[1]

3.2.2 Testing Methods

At the time of report preparation results from five of the required six undiluted effluent samples from the NPNS mill submitted for sublethal testing were available

Testing Period	Summer 2013	Winter 2014	Summer 2014	Winter 2015	Summer 2015	Winter 2016
Date (dd/mm/yy)	22-Jul-13	27-Jan-14	21-Jul-14	17-Mar-15	6-Jul-15	RNA

RNA – Results not available

Each effluent grab sample was collected by mill personnel in 10-litre plastic containers and stored at between 1 and 7°C in a cooler. Samples were shipped by air transport to AQUATOX Testing and Consulting Incorporated (Aquatox) Laboratory in Guelph, Ontario. The samples were received at Aquatox laboratory and all tests were initiated within three days of sample collection. The discharge compliance point “Point C” is located at the outlet of the ASB.

[1] In 2008, amendments to the PPER removed the requirement for mills to conduct sublethal toxicity testing with a fish species (Inland Silverside). Testing prior to 2008 included fish, invertebrate and plant test species.

The toxicity tests were conducted according to the following protocols:

Test	Endpoint(s) (duration)	Protocol
Sea Urchin	fertilization	EPS 1/RM/27 (Environment Canada, 2011)
Champia parvula	growth inhibition (3-day)	EPA/821/R-02/014, (USEPA, 2002)

3.2.3 Test Results

The results of sublethal toxicity tests completed for Cycle 7 are summarized together with the previous six EEM cycles worth of data in Table 3.1. Full laboratory toxicity reports are provided in Appendix B.

The GM IC25 for the five Cycle 7 sublethal tests using sea urchin was 2.44% v/v effluent, and IC25s ranged between 0.43% to 15.40% v/v effluent (Table 3.1).

Champia parvula has generally been the most sensitive test species to NPNS effluent. The IC25 values for *Champia* ranged between 0.09% and 4.64% v/v effluent, resulting in a GM IC25 0.62% v/v effluent.

3.2.4 Potential Spatial Extent of Effluent Effects

In the receiving environment, the 1% effluent concentration zone is often confined to the nearshore area, within a few of hundred metres of the Boat Harbour outlet, especially within the intertidal zone (Krauel, 1969; ASA, 1994). However, its range is variable and the 1% effluent envelope sometimes exists as a surface layer that can extend as far as Logan's Point to the north and beyond MacKenzie Head to the east, a distance of 4.5 km or greater, as the result of ambient buoyant spreading (P. Cranford, pers. comm., 1998; 2001). Under these conditions the plume is a thin film floating on colder, denser seawater and effluent concentrations beneath this surface layer are predicted to be less than 1%.

Based on the two effluent plume dispersion scenarios it is possible to estimate the spatial extent to which sublethal effects might be anticipated in Pictou Road using GMIC25 values corresponding to each EEM Cycle (Table 3.2). For algae, under the nearshore plume scenario, the potential effect zone is 323 metres. Under the offshore plume scenario potential, effects on algal reproduction may occur as far away 7.3 km from the discharge. For sea urchin, under the nearshore plume scenario, the potential effect zone is 82 metres. Under the offshore plume scenario the potential effect zone for sea urchin fertilization could extend to 1.8 km from the discharge.

It should be emphasized that these effect predictions are only meant to provide an overall indication of the spatial extent to which effects could occur based on laboratory testing. Effluent mixing particularly in marine environments is affected by a complex set of factors (including density and temperature differences between effluent and the receiving water,

waves, the tidal cycle and weather conditions) and therefore potential exposure of biota in the receiving environment to effluent must be considered within this context.

Table 3.1:Sublethal Effluent Toxicity (IC25[1] Values) to Inland Silverside, Sea Urchin and *Champia parvula* for EEM Testing

	Inland Silverside Survival and Growth Test	Sea Urchin Fertilization Assay	Red Algae Sexual Reproduction Test
EEM Cycle 1			
18-Apr-95	88	2.8	6.7
04-Jul-95	>100	4.2	19
16-Nov-95	>100	1.2	4.6
12-May-95	>100	8.2	8.6
EEM Cycle 1 GM IC25	***> 97***	***3.3***	***8.4***
EEM Cycle 2			
21-Oct-97 (7-Nov-97)	>72	3.8	2.9
14-Apr-98	>71	7.9	13
04-Aug-98	>67	4.3	4.0
12-Apr-99	>67	6.3	5.0
14-Sep-99	>67	6.1	6.0
25-Jan-01	> 71	3.63	6.0
EEM Cycle 2 GM IC25	***>67***	***5.1***	***5.5***
EEM Cycle 3			
22-Aug-00 (25-Sep-00)	> 71	1.2	2.2
19-Mar-01	> 71	12.2	10.1
25-Jul-01	> 71	6.9	8.2
14-Jan-02	>70	11.2	11.5
10-Sep-02	>100	0.63	4.1
18-Feb-03	71	1.3	11.7
09-Sep-03	>100	6.4	9.4
16-Feb-04	>100	1.9	3.0
EEM Cycle 3 GM IC25	***>81***	***3.2***	***6.4***
EEM Cycle 4			
10-Aug-04	74	1.2	2.4
22-Feb-05	> 100	2.3	2.4
25-Jul-05	>100	3.4	2.2
21-Feb-06	>100	5.2	5.5
24-Jul-06	>100	2.5	1.2
26-Mar-07	>100	0.8	0.9
EEM Cycle 4 GM IC25	***> 96***	***2.1***	***2.0***
EEM Cycle 5			
23-Jul-07	>100	0.35	0.17
21-Jan-08	>100	0.33	0.34
21-Jul-08	>100	2.74	2.28
26-Jan-09	N/A	0.37	0.20
6-Jul-09	N/A	0.13	0.05
25-Jan-10	N/A	12.3	0.50
EEM Cycle 5 GM IC25	***>100***	***0.76***	***0.30***
EEM Cycle 6			
05-Jul-10	*N/A*	*2.19*	*0.52*
31-Jan-11	*N/A*	*2.57*	*0.16*
11-Jul-11	*N/A*	*0.30*	*0.20*
30-Jan-12	*N/A*	*12.50*	*0.48*
06-Jul-12	*N/A*	*0.07*	*0.07*
14-Jan-13	*N/A*	*15.30*	*0.14*
EEM Cycle 6 GM IC25	***N/A***	***1.68***	***0.21***
EEM Cycle 7			
22-Jul-13	*N/A*	*0.43*	*0.69*
27-Jan-14	*N/A*	*0.55*	*0.09*
21-Jul-14	*N/A*	*13.40*	*4.64*
17-Mar-15	*N/A*	*1.77*	*0.37*
06-Jul-15	*N/A*	*15.40*	*0.88*
14-Jan-16	*N/A*	*RNA*	*RNA*
EEM Cycle 7 GM IC25	***N/A***	***2.44***	***0.62***

[1] IC25 - effluent concentration that causes a 25% reduction in a measured endpoint.

N/A - Sublethal toxicity sampling using a fish species is no longer required by the PPER.

RNA - Results not available

Table 3.2: Estimated Spatial Extent of Potential Sublethal Effects Zone in Pictou Road Based on EEM Sublethal Effluent Toxicity Test Results

Test Species	GM IC25							Potential Effect Zone (distance from discharge in metres)														
								Nearshore Plume							Offshore Plume							
	Cycle 1	*Cycle 2*	*Cycle 3*	*Cycle 4*	*Cycle 5*	*Cycle 6*	*Cycle 7*	*Cycle 1*	*Cycle 2*	*Cycle 3*	*Cycle 4*	*Cycle 5*	*Cycle 6*	*Cycle 7*	*Cycle 1*	*Cycle 2*	*Cycle 3*	*Cycle 4*	*Cycle 5*	*Cycle 6*	*Cycle 7*
Champia parvula (algae)	8.4	5.5	6.4	2.0	0.30	0.21	0.62	24	36	31	100	667	952	323	536	818	703	2,250	15,000	21,429	7,258
Lytechinus pictus (sea urchin)	3.3	5.1	3.2	2.1	0.8	1.7	2.4	61	39	63	95	263	119	82	1,364	882	1,406	2,143	5,921	2,679	1,844
Menidia beryllina (inland silverside)	97	> 67	> 81	>96	>100[1]	N/A	N/A	2	< 3	< 2	< 2	< 2[1]	N/A	N/A	46	< 67	< 56	< 47	< 45[1]	N/A	N/A

[1] - Values based on three sets of data. Sublethal toxicity testing using a fish species was removed from the regulation and is no longer mandatory.
N/A = Not applicable because Inland Silverside were removed from the regulation.

3.2.5 Temporal Trends in Effluent Toxicity

Any comparisons of temporal trends in the effluent toxicity is complicated by the change in the compliance point in June 2010 from Point D at the control structure in the lower Boat Harbour Estuary at the outlet of the Boat Harbour basin to Point C at the outlet of the ASB. The change in the point of effluent collection from Point D to Point C effectively removes between 25 to 30 days of retention time in the system. Therefore the Cycle 6 and 7 data are not directly comparable to previous cycles.

Cycle 6 and 7 data can be directly compared because all samples submitted for sublethal toxicity testing were from the Point C location. The results of seventh-cycle sublethal toxicity testing indicate that there has been a slight decrease in the effect of the effluent on both sea urchin fertilization and red algae reproduction. In Cycle 6, the effluent concentration that cause an IC25 in sea urchin ranged from 0.07% to 15.30% v/v with a GM IC25 of 1.68% v/v whereas in Cycle 7 the GM IC25 was 2.44% v/v with a range of 0.43% to 15.40% v/v. The values of 13.4% effluent for July 2014 and 15.4% effluent for the July 2015 samples represent the highest concentrations since the EEM program began with the exception of the January 2013 sample.

Cycle 7 red algae tests also resulted a higher GM IC25 (i.e., 0.62% v/v) compared to the Cycle 6 value of 0.21% v/v. Generally, the range of results in Cycle 6 and 7 were similar except for the July 2014 Cycle 7 test, which produced a result of 4.64% effluent. Although there has been an increase in the GM IC25 since Cycle 6 the GM IC25 remains much lower than observed during cycles 1 through 4. Overall, the last sample for which results are available show a positive trend in that the 15.4% for sea urchin and 0.88% for red algae reported from the July 2015 sample submission were the highest ever reported and highest since 2008 for these species, respectively, excluding the 4.64% for algae in July 2014.

3.3 Investigation of Effluent Chemistry and Sublethal Toxicity Test Results

Sublethal toxicity results have been low since the inception of the EEM program. Discussions with AquaTox, the laboratory that conducts the tests, indicate that some of the low results may be associated with the dark colour of the NPNS effluent. The colour negatively effects the algae and the sea urchin test species and according to the lab is an issue for a number of mills. Additionally, mill personnel indicated that some of the low results in recent time may be the result of the shifting contributions of differing furnish and the fact that the proportion of high quality chips compared to other chips can cause the mill to experience some upset resulting in lower sublethal values.

Available effluent treatment centre (ETC) data were used to ascertain if there were any relationships between the effluent chemistry parameters that are routinely measured and the sublethal results that were not captured in the regular monitoring.

The ETC monitors for a number of parameters on a weekly or more frequent basis. These parameters include: nitrate, nitrite, Total Kjeldhal nitrogen, ammonia, BOD, COD, TOC, colour, orthophosphate, total phosphorus, dissolved sulphate, sulphide, volatile suspended solids and more recently total sodium. Data for these parameters from 2009 until March 2016 were plotted to investigate any readily available patterns that relate to the sublethal toxicity results. These graphs are provided in Appendix B. Investigation of the plots shows that of the 20 plots created only dissolved sulphate, volatile suspended solids and COD showed any linear decline.

Given the present data it does not appear likely that a conventional parameter is the potential cause of sublethal toxicity. Therefore, as part of the Cycle 8 EEM consideration will be made to measuring an additional suite of parameters in effluent in conjunction with the toxicity tests.

4.0 SUPPORTING ENVIRONMENTAL INFORMATION

Following the guidance provided in Environment Canada technical guidance (Environment Canada, 2010), as well as the results of past EEM studies at the mill, supporting environmental variables were measured coincident with the fish and benthic collections to assist in the interpretation of the biological data. These included:

Water	Sediments
Dissolved oxygen[1]	TOC[1]
Temperature[1]	Carbon to nitrogen ratio (C:N)[1]
Salinity[1, 2]	Total sulphides (TS)[1]
Dissolved organic carbon (DOC)[3, 4]	Sediment particle size[1]
Total organic carbon (TOC)[3, 4]	Sediment Eh[1]
Total Kjeldahl nitrogen (TKN)[3, 4]	
Colour[3]	

[1] Required measure (see Environment Canada, 2010).
[2] Salinity is potentially an important measure at Boat Harbour because of the relatively high freshwater inputs to Pictou Road.
[3] Site-specific measure.
[4] Previous studies indicate that the spatial patterns in DOC, TOC and TKN in water in Pictou Road may be consistent with effluent dilution in the area and are therefore useful measures, if only spot measures, for EEM purposes.

In addition to laboratory measures and field spots measurements an HOBO optical Dissolved oxygen logger and a HOBO conductivity logger were both placed in Boat Harbour and Little Lake to aid in the interpretation of potential differences from the fish survey.

Latitude and longitude coordinates for each sampling location (station) were obtained using a Garmin Map 60CX Handheld GPS (Appendix C). Water depths were measured to the nearest 0.1 m with a portable depth sounder or by metre stick (Appendix C). Files containing all supporting environmental information collected during the field sampling program are provided in Appendix C.

Given the variable nature of the effluent plume (due to the effects of tides, wind and the ambient nature of the effluent in the marine environment), it was not practical to confirm that the exposure areas, especially the far-field and far far-field areas, were exposed to effluent at the time of sampling. The locations of the sampling areas are based on *in situ* plume measurements, as well as model scenarios, which indicated that under given tidal and wind conditions the plume occupies given areas. The sampling areas represent the potential spatial extent of the effluent plume over an extended period of time, rather than at any single point in time. Nevertheless, in-field measurements of salinity TOC and TKN were taken to help to provide a snapshot of the effluent plume location at the time of sampling.

4.1 Water Quality

4.1.1 Methods

Water quality data (conventional parameters and laboratory measures) were collected coincident with the fish and benthic invertebrate surveys.

Samples for water quality were collected at the top of the water column (i.e., where the buoyant effluent is limited to) and at the bottom of the water column (i.e., where the benthic community resides) at each ICS sampling area (see Figure 5.1and Figure 5.2). Measures of DO, temperature, pH and salinity (top and bottom) were taken at each benthic station. These conventional measures were collected using an YSI 600 QS Sonde and YSI 650 MDS display-datalogger. The unit was calibrated to manufacturers specifications prior to commencement of the sampling and the calibrations checked periodically throughout the project.

Samples (top and bottom) were also collected within each ICS sampling area for the analysis of DOC, TOC and TKN. These samples were collected with the aid of a 2.2-L Van Dorn bottle. Samples were collected from three stations within each of the sampling areas, as this number is adequate to characterize water chemistry in a given area.

Measures of conventional water quality were made at each fish survey area as well (see Figure 6.1). These measurements were made using an YSI 600 QS Sonde and YSI 650 MDS display-datalogger. The unit was calibrated to manufacturers specifications prior to commencement of the sampling and the calibrations checked periodically throughout the project. Samples were also collected from each fish sampling area for the analysis of DOC, TOC, TKN and colour.

Laboratory analysis of all water samples was conducted by Maxxam Analytics (Mississauga, Ontario).

The HOBO data loggers for DO and conductivity were deployed and removed from Boat Harbour and Little Lake on 24 August 2014 and 13 May 2015 and 25 August 2014 and 15 May 2015, respectively. The logger were moored using an anchor and buoy system to suspend them just off the bottom. Temperature and conductivity were logged every 30 minutes, and daily mean, maximum and minimum dissolved oxygen were calculated by each logger. HOBOware Pro software was used to download and produce graphs of the results for analysis.

4.1.2 Results

Data Quality

The water quality meter calibration records indicated that there were no problems with the meters while in use. It is expected therefore that the instruments provided true and accurate measure of the desired parameters.

For those analytes that required laboratory analyses the quality assurance/quality control (QA/QC) program included checks on the integrity of field collection methods and laboratory analyses. Field checks included the analysis of field duplicates, field blanks and trip blanks. Laboratory QA/QC comprised the analysis of method blanks, concentration standards and run duplicates.

Comparisons of the duplicates indicated that precision between samples was acceptable and met the data quality objectives (Table 4.1). The analysis of the May 2015 field and trip blank samples resulted in values above detection for at least one parameter. However, only one of these three instances did not meet the data quality objective. Additionally, the result that did not meet the objective was for DOC and the results from the reference and exposure areas for this analyte are an order of magnitude different and therefore this result of the field blank does not affect the interpretation of the supporting water quality.

Table 4.1: Data Quality Assessment for the Duplicate Water Samples

Parameter	Units	RDL	Sample ID	Duplicate ID	RPD (%) or AD
			FF3-T	Duplicate	
			20-Aug-14	20-Aug-14	
Total Organic Carbon (C)	mg/L	5	<5	<5	BD
Total Kjeldahl Nitrogen	mg/L	0.5	<0.5	<0.5	BD
Parameter	**Units**	**RDL**	**Sample ID**	**Duplicate ID**	**RPD (%) or AD**
			Exposure	**Duplicate**	
			18-May-15	**18-May-15**	
Colour	mg/L	10	430	350	14
Total Kjeldahl Nitrogen (TKN)	mg/L	0.50	2.1	1.7	0.4
Dissolved Organic Carbon	mg/L	0.20	43	42	2
Total Organic Carbon (TOC)	mg/L	2.0	56	60	5

Notes:
RDL - Reported detection limit
RPD - relative percent difference; is calculated for analytes with concentrations greater than or equal to five times the RDL and should be less than or equal to 20%
AD - absolute difference; for samples having concentrations less than five times the RDL, the difference between the sample and duplicate should not be greater then two times the RDL
BD - The sample and/or duplicate had analyte concentrations below the RDL
Bold Font - indicates that the data quality objective was not achieved

Water Chemistry

Conventional water quality data and water chemistry measures collected during the benthic and fish surveys are summarized in Table 4.2 and Table 4.3, respectively.

Table 4.2: Conventional Water Quality Parameters and Water Chemistry in the Invertebrate Community Survey Sampling Areas – August 2014

Sampling area	Depth (m)	Salinity (ppt)		pH		DO (mg/L)		Temp (°C)		TKN (mg/L)		TOC (mg/L)	
		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
NF	3.6	24	24	7.9	7.9	7.8	7.7	18.8	18.3	<0.50	2.43	<5	<5
FF	3.6	23	23	7.9	7.9	8.0	8.1	19.4	18.9	<0.50	<0.50	<5	<5
FFF	5.6	23	23	8.0	7.9	8.1	7.9	19.2	18.6	0.51	<0.50	<5	<5
REF[1]	5.4	24	24	8.0	8.0	7.7	7.6	18.8	18.5	0.83	0.8	<5	<5
REF[2]	6.0	24	24	8.1	8.0	8.5	8.4	18.3	18.2	<0.50	<0.50	<5	<5

[1] Merigomish Harbour
[2] Logan's Point

Table 4.3: Conventional Water Quality Parameters and Water Chemistry in the Fish Survey Sampling Areas – August 2014 and May 2015

Sampling area	Sample Season	Temperature (°C)	Salinity (ppt)	DO (%sat)	Colour (TCU)	TKN (mg/L)	DOC (mg/L)	TOC (mg/L)
Boat Harbour Estuary	Aug-14	15.0 - 23.2	1.2 - 19.4	3.7 - 43.4	-	4.0	-	76
	May-15	8.3 - 13.1	1.9 - 19.8	1.7 - 23.4	430	2.1	43	56
Little Lake	Aug-14	22.6 - 25.7	9.1 - 14.7	33.4 - 138.8	-	<0.50	-	6.6
	May-15	10.0 - 16.9	3.6 - 31.5	99.8 - 131.3	20	1.2	4.9	5.4

At the time the ICS was implemented (August), there was little indication of a mill-related water quality influence. Moreover, water quality was similar in top and bottom waters indicating that the water column was well mixed across the ICS study area. Salinity was about 24 ppt and the pH of the water was around 8.0. Dissolved oxygen (DO) levels were between 7.5 and 8.5 mg/L and tended to be around 0.1 mg/L higher in surface waters versus bottom waters. Surface water temperatures were 18.3 to 19.4°C. Bottom waters tended to be about 0.4°C cooler. TKN ranged from below the detection limit of 0.5 mg/L to 2.4 mg/L throughout the study area. TKN in the bottom waters of the near-field area were elevated compared to the surface, whereas within all other areas the TKN concentrations were similar top and bottom. TOC levels were below the detection limit of 5 mg/L in all samples collected during the ICS survey (Table 4.2).

There was considerable variability in conventional water quality parameters within the fish collection areas in May and August, and the influence of mill effluent on some parameters was measurable, as were tidal influences.

At the Boat Harbour Estuary the salinity was between 1 and 19.4 ppt depending on the tide cycle with lower salinity during the falling tide. This results from the influence of the relatively large and constant inputs of freshwater in the form of mill effluent, as well as runoff that is generated within the ETC drainage area.

The Little Lake reference area also had relatively low salinity on the lower end (i.e., ~ 4 ppt) but increased to around 31.5 ppt at depth. Little Lake is seasonally inundated with saltwater through a channel depending on tide cycles and wind and weather conditions and may also experience saltwater intrusions through the beachhead.

Water temperatures overall were higher in Little Lake than in the Boat Harbour Estuary despite the input of treated effluent. In August 2014 the reference temperature ranged from around 23°C to 26°C and 15°C to 23°C in the reference and exposure areas, respectively. In May 2015 temperature were more comparable between the two areas with ranges of around 8°C to 13°C in Boat Harbour and 10°C to 17°C in Little Lake.

Dissolved oxygen saturation also varied widely at all sites (2 to 139%). DO in Little Lake was super saturated at the surface but did decrease at depth and potential anoxic conditions may occur in the deepest parts of the lake based on the catches of dead fish in a trapnet in August 2014. In the Boat Harbour Estuary the DO was relatively low throughout with a maximum of around 43%.

Colour and carbon measures were an order of magnitude higher in the Boat Harbour Estuary than in Little Lake. TKN measured in August 2014 was also an order of magnitude higher in the exposure. However, contrary to most previous studies in May 2015 the concentrations of TKN in the two areas were comparable (Table 4.3).

Similar spatial patterns (or lack thereof) have been seen at the fish sampling locations in past EEM surveys. The influence of mill effluent on water quality within the exposure fish sampling area is readily apparent. Parameter levels and concentrations measured in Cycle 7 were within historical ranges based on previous EEM studies.

Data Logger Results

Measurements from both the conductivity and the dissolved oxygen loggers were used to create time series graphs of the daily minimum, maximum and average values from August 2014 until the logger membrane expired (March 2015) or were removed (May 2015). Descriptive statistics of all logger values are presented in Table 4.4. The time series plots are provided in Figure 4.1.

Daily temperature in the Little Lake reference and the Boat Harbour exposure areas were generally similar from August until January. Starting in January and continuing until mid-February Little Lake had higher minimum, maximum and average daily temperatures than Boat Harbour. From mid-February until early May the opposite was true with Boat Harbour having higher temperatures. This may be the result of the input of effluent that prevents the exposure area from freezing whereas, Little Lake becomes completely ice covered. Boat Harbour had a maximum recorded temperature of 35.8°C compared to a maximum of 27.7°C in Little Lake. However, the 95th percentile of measures was higher in Little Lake (24.4°C) compared to Boat Harbour (19.7°C). All other summary statistics were similar

between the two areas. For example the average and median temperatures were 7.7°C and 5.4°C and 8.0°C and 6.5°C in Boat Harbour and Little Lake.

Dissolved oxygen showed a high degree of variability in both fish sampling areas. However, on average there was more oxygen in water in Little Lake than in Boat Harbour. Rarely, did the oxygen levels in Little Lake decrease to levels approaching zero, whereas in Boat Harbour this occurred frequently throughout the logging period. Mean oxygen saturation was also lower in Boat Harbour compared to Little Lake with values of 6% and 56%, respectively. Although Mummichog are known to be able to tolerate oxygen concentrations down to 1 mg/L and will resort to surface respiration if required, prolonged periods could still be potentially causing stress to fish and may have manifested in other measures as an effect from mill effluent exposure. This potential is discussed in further detail in Section 6.0.

Salinity in both areas was similar from the August deployment until around mid-December. From mid-December until the end of January salinity was higher in the Boat Harbour exposure than in the Little Lake reference area. Unlike some reference areas used in past cycles the salinity in the Little Lake reference never approached that of pure seawater at the location of the logger deployment. From January until the removal of the loggers, Little Lake had higher salinity than the Boat Harbour exposure area and the salinity was more constant. This is likely attributable to the daily tide cycle experienced in the Boat Harbour area compared to seasonal saltwater intrusions in Little Lake and the large volume of freshwater input associated with the run-off from snowmelt in the Boat Harbour Basin. The range of salinity in Boat Harbour was 0 to 19.4 ppt whereas in Little Lake it was 8.6 to 19.5 ppt. The mean salinity from these two areas was 13.5 and 14.8 ppt, respectively. All other summary statistics were relatively similar with Little Lake having salinity measures that were between 1 and 2 ppt higher. Mummichog are an estuarine species and can reside in areas that have varying salinity conditions. However, similar to oxygen, the fluctuations in salinity from the tidal cycle, combined with the large amount of freshwater effluent input may be causing physiological stress on the Boat Harbour population. Fluctuations would not normally hinder Mummichog but perhaps exposure to effluent enhances the effects of the shifting water quality conditions.

Table 4.4: Summary of Logger Data in Boat Harbour and Little Lake

	Boat Harbour					Little Lake				
	Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (mg/L)	Dissolved Oxygen (%)	Salinity Adjusted Dissolved Oxygen (mg/L)	Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (mg/L)	Dissolved Oxygen (%)	Salinity Adjusted Dissolved Oxygen (mg/L)
N	12,662	12,662	10,128	10,128	10,128	12,624	12,624	10,226	10,226	10,226
Minimum	-0.3	0.0	0.00	0	0.00	-1.1	8.6	-0.08	-0.6	-0.07
Maximum	35.8	19.4	11.69	100	10	27.7	19.5	18.87	169	17
Average	7.7	13.5	0.78	6	0.69	8.0	14.8	6.73	56	6
Median	5.4	13.1	0.03	0.2	0.03	6.5	15.0	6.74	57	6
5th Percentile	0.5	8.6	0.01	0.1	0.01	-0.1	9.3	1.26	11	1
25th Percentile	2.1	11.3	0.02	0.1	0.02	1.3	12.3	3.55	33	3
50th Percentile	5.4	13.1	0.03	0.2	0.03	6.5	15.0	6.74	57	6
75th Percentile	13.1	16.1	0.04	0.4	0.04	13.0	17.9	9.30	83	9
95th Percentile	19.7	18.9	5.46	39	5	24.4	18.8	12.72	94	11

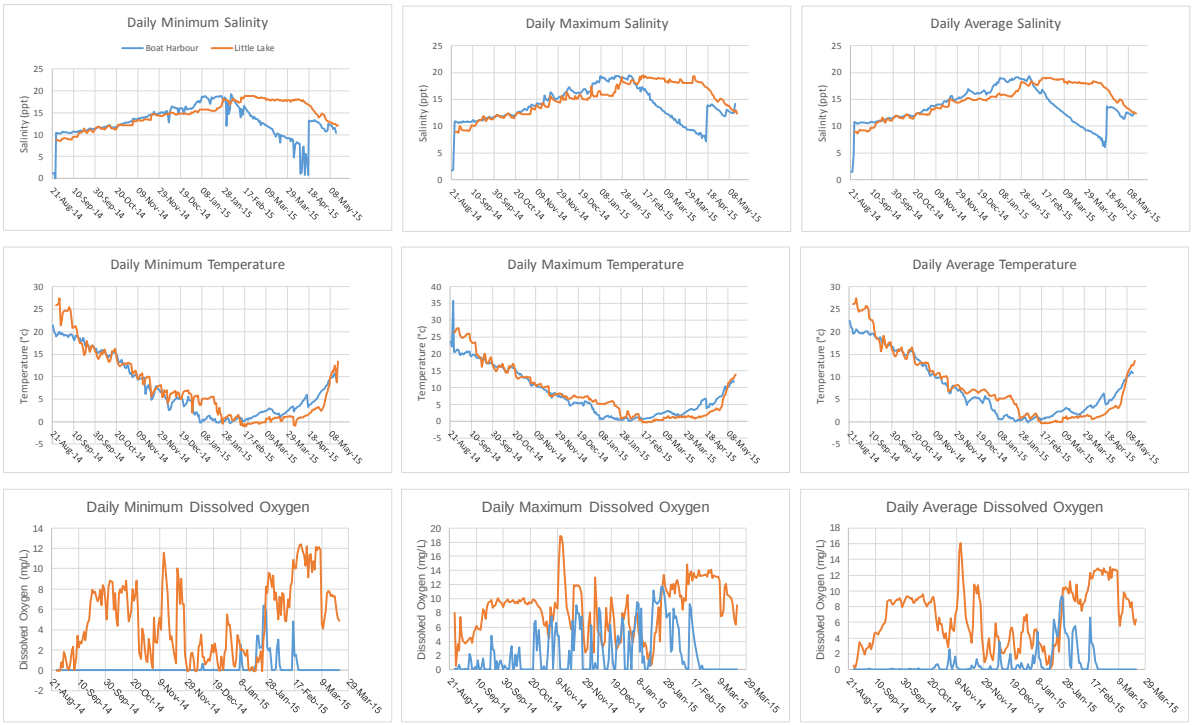


Figure 4.1: Daily Summary of Salinity, Dissolved Oxygen and Temperature from Data Loggers in Little Lake and Boat Harbour in 2014 and 2015

4.2 Sediment Quality

4.2.1 Methods

Surficial (top 5 cm) sediment samples were collected at each of the invertebrate community survey (ICS) sampling stations (see Figure 5.1 and Figure 5.2) for the chemical analysis of TOC, the characterization of grain-size distribution and the determination of the carbon to

nitrogen ration (C:N). Cellulose derived from trees typically has a much higher organic carbon content than other sources and as a result high C:N ratios are typically indicative of fibre-related sources (Environment Canada, 1998).

Redox potential (Eh) was measured in the top 5 cm of sediments at each ICS station using a Hanna Handheld mV meter. This was done immediately after collection, and care was taken to ensure minimal disturbance of the samples. Total sulphides were measured using a Accumet AP125 meter with a sulphide ion probe. All samples were kept in a cool, dark anoxic container until all samples were collected so measurements could be conducted under the same conditions. The procedures used were those recommended in the "Additional Technical Guidance for Conducting Redox and Sulphide Measurements in Marine Sediments" (Environment Canada, 2003).

Laboratory analysis of all sediment samples was conducted by Maxxam Analytics (Mississauga, Ontario).

4.2.2 Data Quality

The redox potential meter calibration records indicated that there were no problems with the meter while in use. It is expected therefore that the instrument provided true and accurate measures. Calibration of the meter used to measure sulphides was conducted by Ferris Chemicals (Saint John, New Brunswick). No calibration issues were identified during use of the meter in the field and therefore it is assumed that the measurements collected were accurate.

For those analytes that required laboratory analyses, quality assurance/quality control (QA/QC) measures included the analysis of standard reference materials, laboratory spikes and laboratory duplicates. Analysis of QA samples was reported to be within internal laboratory standards.

Overall, there were no readily apparent QA/QC issues noted with the laboratory sediment chemistry data and therefore the data were deemed to be of acceptable quality.

4.2.3 Results

Sediment Chemistry and Granulometry

Sediment chemistry measures are summarized in Table 4.5.

Table 4.5: Sediment Quality Measures in the NPNS EEM Study Area – EEM Cycle 7

Sampling area	Grain Size				TOC (%)	TS (µM)	C:N	eH (mV)
	% Gravel	% Sand	% Silt	% Clay				
NF	0.5	97	0.4	1.6	0.12	25	6.3	150
FF	0.1	98	0.5	1.8	0.11	53	6.4	165
FFF	0.5	97	0.8	2.1	0.10	1	5.9	158
REF[1]	0.3	98	0.3	1.7	0.09	9	6.9	221
REF[2]	1.0	96	0.6	2.1	0.08	43	4.5	220

[1] Merigomish Harbour

[2] Logan's Point

TOC levels were low across the study area (0.08 to 0.12%) and within the range of the levels seen in past surveys (e.g., Stantec, 2004a; EcoMetrix, 2007a, 2010).

The ration of carbon to nitrogen (C:N ratio) in study area sediments was in the range of about 5 to 7, with the highest area average in the Merigomish Harbour reference. Low C:N ratios suggest that the carbon in sediments is not likely from mill-related sources. Cellulose derived from trees typically has a much higher organic carbon content than other sources and as a result C:N ratios in areas with fibre deposition are high (Environment Canada, 1998). In a study completed by EcoMetrix in an area where there are significant pulp and paper mill-related fibre deposits C:N ratios were in the range of 50:1 in the fibre mat and about 10:1 in unimpacted areas (EcoMetrix, 2007b).

Overall, sediments from all areas were relatively well oxygenated with all eH values greater than 0.

Sulphide levels varied throughout the study area, with exposure and reference area values in the same range. Area-wide means varied from around 0 to 53 µM in the reference areas and from 1 to 53 µM in the exposure areas. According to Wildish *et al.* (1999) based on sulphide levels the sediment would be considered normal.

Bottom substrates were dominated by the sand-sized fractions (generally greater than > 96%), with smaller amounts of gravel, silt and clay. The laboratory results confirmed observations made during the field work as the sediments within all of the sampling areas were characterized qualitatively as “fine sand”.

Overall, the sediment quality data collected as part of the Cycle 7 EEM program were within historical ranges for most parameters. Any changes, namely a reduction in sulphide concentrations and C:N ratios compared to previous cycles were a positive.

5.0 BENTHIC INVERTEBRATE COMMUNITY

5.1 Objective

Generally, an assessment of the benthic invertebrate community serves as a basis to delineate the extent and magnitude of fish habitat degradation, if any, due to organic or nutrient enrichment, or other forms of physical and chemical contamination potentially associated with mill operations. It also provides a basis for an evaluation of the aquatic food resources available for fish communities in the receiving environment. "Effects" are assessed on the basis of a series of "EEM effect endpoints", which describe key attributes of benthic invertebrate community structure.

5.2 Results of Previous EEM Studies

In both Cycles 1 and 2 at NPNS benthic invertebrate samples were collected in the subtidal and intertidal zones. In Cycles 3, 4 and 5 only subtidal surveys were completed.

5.2.1 Subtidal Benthic Invertebrate Community

EEM Cycle 1

The Cycle 1 subtidal ICS was intensive and sampling encompassed one reference area in Merigomish Harbour and two exposure areas (near-field and far-field) in Pictou Road (JWEL, 1996) (Figure 5.1 and Figure 5.2). Sand was the dominant substrate type at all three sampling areas. Benthic samples were collected using a 0.1 m^2 Van Veen grab sampler. Four stations were sampled in each area, each consisting of three replicate grabs. Cycle 1 benthic data are summarized in Table 5.1.

Total invertebrate abundance tended to be highest at the reference area (44.1 organisms/ 0.3 m^2), intermediate at the near-field area (22.2 organisms/0.3 m^2), and lowest at the far-field area (13.0 organisms/0.3 m^2). The trend for species richness was the same, with corresponding values of 11.3, 7.2 and 5.0 taxa/0.3 m^2. However, variability within each study area also tended to be high, especially for the near-field and reference areas.

A total of 35 individual taxa were collected at the three sampling areas. The most common benthic taxa included the polychaete worms *Nephtys bucera* and *Streptosyllis varians*, the clam *Tellina aegilis* and nematodes. Correspondence analyses of the data indicated that the near-field and reference areas supported similar benthic communities, whereas, the far-field area was different. This may have reflected a slight difference in grain size between the areas (the far-field sediment contained more silt and clay), rather than mill-related effects. EEM Cycle 1 results also indicated that there was no substantial deposition or accumulation of organic matter in Pictou Road from the ETC.

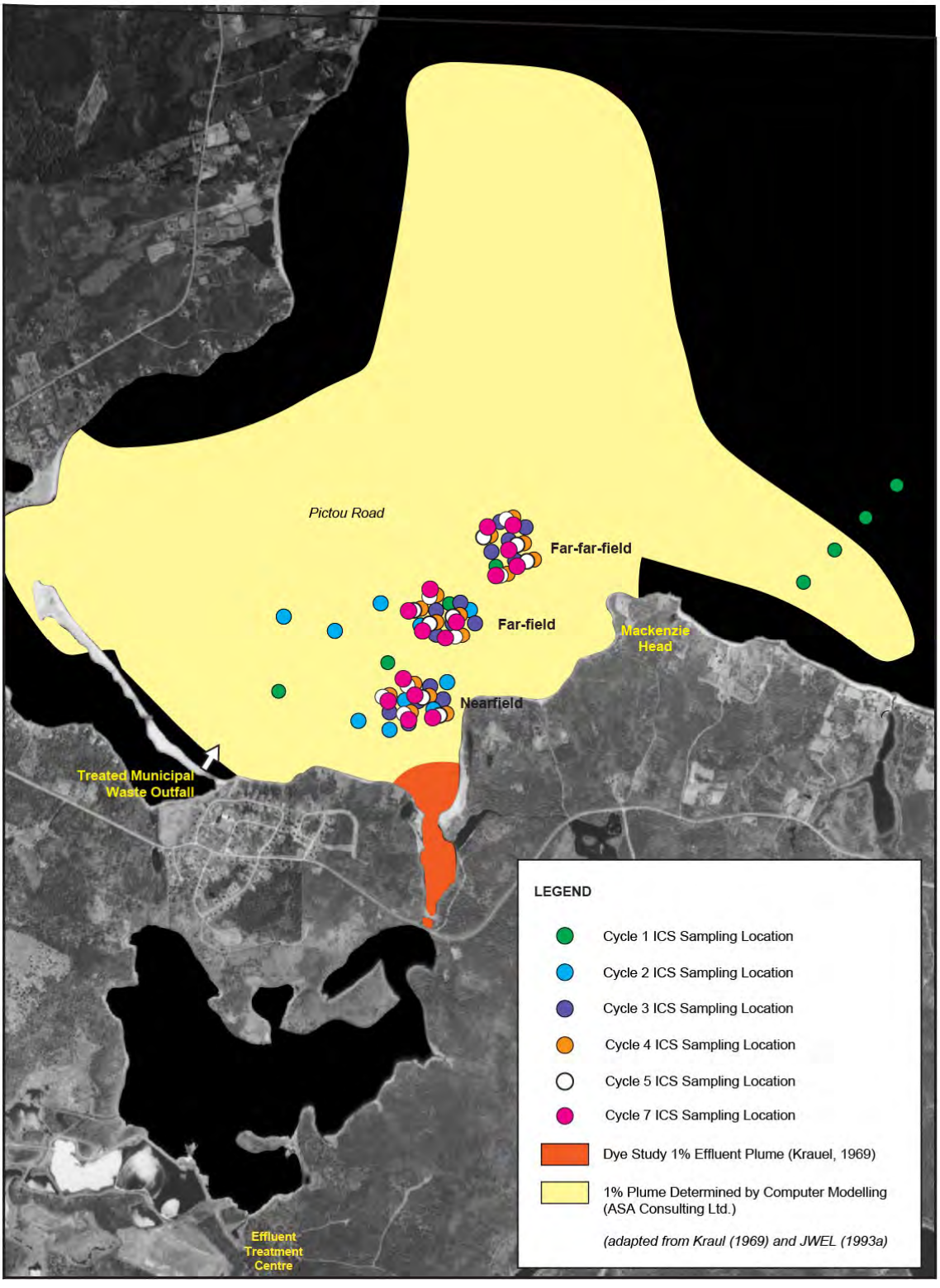


Figure 5.1: EEM ICS Exposure Area Sampling Station Locations

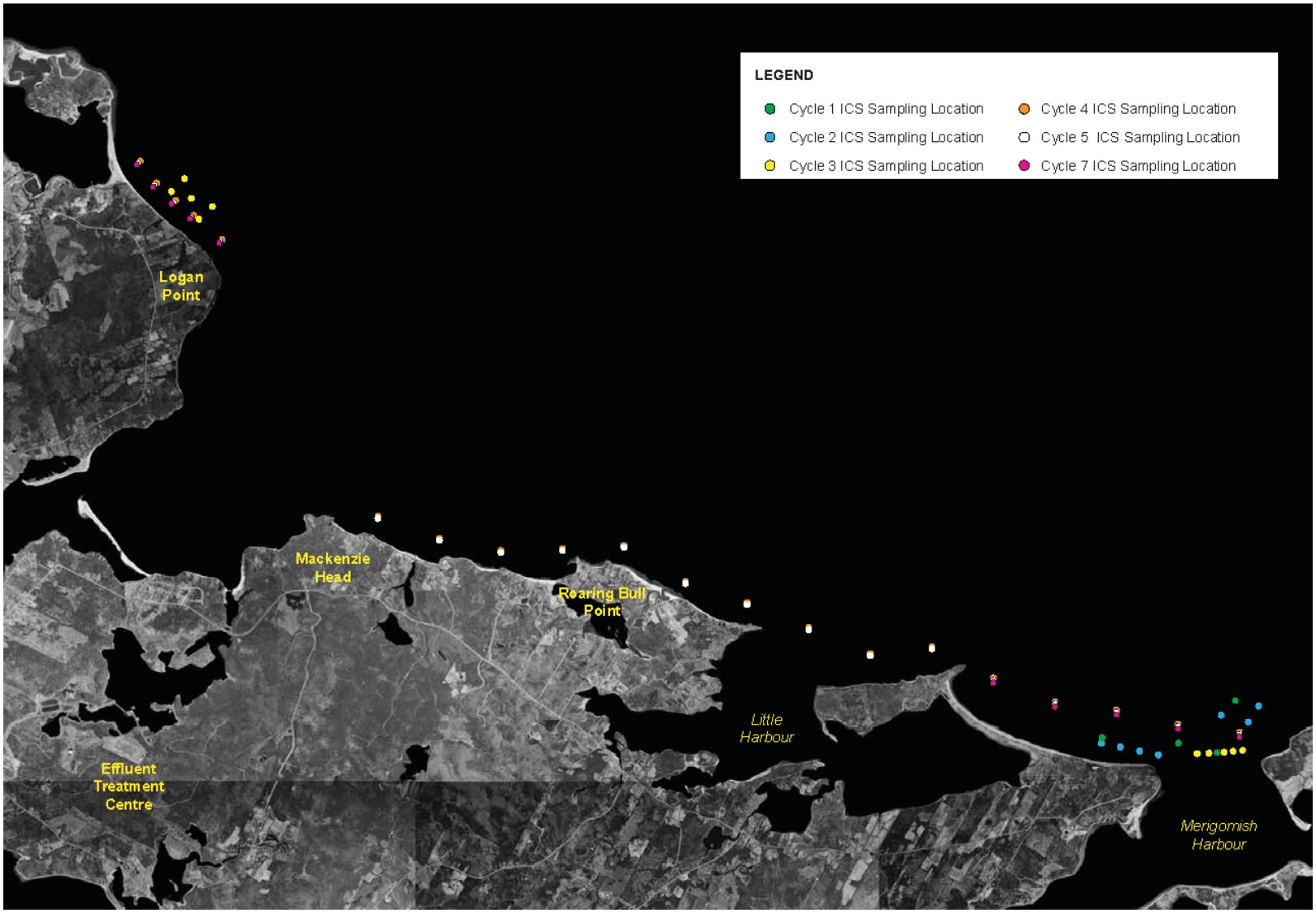


Figure 5.2: EEM ICS Reference Area Sampling Station Locations

Table 5.1: Benthic Invertebrate Density and Relative Abundance of Major Taxonomical Groups for EEM Cycle 1 and 2 Data[1]

	Reference				Near-field		Far-field		Far-far-field	
	Merigomish		Logan's Point		Pictou Road		Pictou Road		Pictou Road	
	No.	%	No.	%	No.	%	No.	%	No.	%
Cycle 1										
P. Nemertea	8	4	na	na	6	2	-	-	na	na
P. Nematoda	71	12	na	na	-	-	-	-	na	na
P. Annelida										
Cl. Polychaeta	252	57	na	na	167	51	38	26	na	na
Cl. Polychaeta (Errantia)	133	36	na	na	42	13	7	5	na	na
Cl. Polychaeta (Sedentaria)	119	21	na	na	125	38	31	21	na	na
P. Arthropoda										
Cl. Ostracoda	-	-	na	na	-	-	-	-	na	na
Cl. Crustacea										
O. Cumacea	34	6	na	na	8	2	-	-	na	na
O. Tanaidacea	-	-	na	na	-	-	-	-	na	na
O. Isopoda	2	<1	na	na	-	-	5	3	na	na
O. Amphipoda	18	3	na	na	5	2	7	4	na	na
O. Mysidacea	-	-	na	na	-	-	-	-	na	na
O. Decapoda	8	1	na	na	-	-	3	0	na	na
P. Mollusca										
Cl. Gastropoda	5	1	na	na	14	4	3	2	na	na
Cl. Pelecypoda	40	12	na	na	92	28	43	29	na	na
P. Echinodermata										
Cl. Stelleroidea	-	-	na	na	-	-	-	-	na	na
Cl. Echinoidea	17	3	na	na	5	2	40	27	na	na
Total	456	100	na	na	333	100	149	100	na	na
Cycle 2										
P. Nemertea	10	1	na	na	-	0	-	0	na	na
P. Nematoda	87	7	na	na	21	1	-	0	na	na
P. Annelida										
Cl. Polychaeta	642	39	na	na	683	24	1,899	47	na	na
Cl. Polychaeta (Errantia)	598	35	na	na	605	21	1,579	39	na	na
Cl. Polychaeta (Sedentaria)	45	4	na	na	78	3	320	8	na	na
P. Arthropoda										
Cl. Ostracoda	-	0	na	na	-	0	10	0	na	na
Cl. Crustacea										
O. Cumacea	106	11	na	na	69	3	68	2	na	na
O. Tanaidacea	66	6	na	na	114	5	4	0	na	na
O. Isopoda	20	1	na	na	7	0	16	1	na	na
O. Amphipoda	45	2	na	na	2	0	42	1	na	na
O. Mysidacea	-	0	na	na	-	0	2	0	na	na
O. Decapoda	2	0	na	na	-	0	12	0	na	na
P. Mollusca										
Cl. Gastropoda	193	11	na	na	197	6	628	16	na	na
Cl. Pelecypoda	284	20	na	na	1,843	61	1,238	33	na	na
P. Echinodermata										
Cl. Stelleroidea	1	0	na	na	-	0	-	0	na	na
Cl. Echinoidea	12	1	na	na	-	0	28	1	na	na
Total	1,457	100	na	na	2,929	100	3,948	100	na	na

[1] See Figures 5.1 and 5.2 for sampling area locations.

EEM Cycle 2

The Cycle 2 subtidal ICS followed a Control/Impact design. Samples were collected at one reference area (Merigomish Harbour) and two exposure areas in Pictou Road (near-field [NF] and far-field [FF]) (BEAK, 2000) (Figure 5.1 and Figure 5.2). Sand was the dominant substrate type at all three sampling areas. Benthic samples were collected using a 0.023 m^2 petite Ponar grab. Five stations were sampled in each exposure area, whereas seven stations were sampled in the reference area. Each station sample consisted of five replicate grabs pooled. Cycle 2 benthic data are summarized in Table 5.1.

Water quality measurements indicated that there was very little difference in bottom water chemistry between the near-field, far-field and reference area, although salinity was lower in the near-field area. Differences in salinity at the top and bottom of the water column illustrated the floating nature of NPNS's effluent plume. The sediment at all the ICS stations largely consisted of medium or fine-grained sand. Supporting sediment chemistry (e.g., total organic carbon and total carbon) suggested that there was no substantial deposition of carbon-based materials, such as wood fibre, in any of the sampling areas.

Mean invertebrate densities were highest in the far-field area, intermediate in the near-field area and lowest in the reference area. The only statistically significant difference, in terms of density, was between the far-field and the reference areas. Mean number of taxa was also significantly greater in the far-field area compared to the reference area. The near-field and reference areas were not significantly different in terms of taxa numbers. Although not compared statistically, both mean invertebrate abundance and taxa richness were greater in EEM Cycle 2 than Cycle 1 in all three areas. This was likely a result of several factors, including the relocation of the far-field area, changes in methods and improvements to effluent quality.

With only a few exceptions, benthic communities at most stations were represented by a broad variety of organisms, with few clearly dominant taxa. Overall, polychaete worms and molluscs were the most dominant taxonomic groups. The two most abundant taxa were the polychaete *Protodriloides* and the clam *Tellina aegilis*. Other common species included the clam *Spisula solidissima,* the tanaidacean *Leptognatha caeca*, and the snail *Retusa canaliculata.*

Principal Component Analysis (PCA) of the benthic macroinvertebrate data suggested that there were subtle differences between areas based on taxa composition, but it did not generally appear that these groupings were influenced by differences in pollution tolerance.

Although the Pictou Road exposure areas appeared to be slightly organically enriched (i.e., more productive) and somewhat different in terms of benthic community structure compared to the Merigomish Harbour reference area, there was little evidence to suggest that the benthic invertebrate community in Pictou Road was impaired. The benthic community in the far-field area was diverse and contained few taxa that are typically

associated with degraded marine environments. Although the small reduction in invertebrate abundance and numbers of taxa in the near-field area relative to the far-field may be partially caused by exposure to effluent, wave activity and ice scour in the shallow, unstable near-field area may also limit the distribution of benthic invertebrates. In summary, there was very little support that the mill effluent was having an adverse effect on benthic communities and fish habitat.

EEM Cycle 3

The Cycle 3 ICS followed a Control/Impact Design (Stantec, 2004a). Benthic invertebrates were collected via a grab sampler (petite Ponar) from three effluent-exposed areas (near-, far- and far far-field [FFF]) and two reference areas (Merigomish Harbour, Logan's Point) (Figure 5.1and Figure 5.2). Supporting environmental information (water and sediment chemistry) was collected coincident with the sampling program within each of the sampling areas. Cycle 3 benthic data are summarized in Table 5.2. A summary of statistical comparisons of the Cycle 3 data is provided in Table 5.3.

Table 5.2: Summary of EEM Cycle 3 ICS Data (Absolute and Relative Abundance) For NPNS

TAXANOMIC GROUP	REFERENCE AREA 1 (MERIGOMISH)		REFERENCE AREA 2 (LOGAN'S POINT)		NEAR-FIELD		FAR-FIELD		FAR-FAR-FIELD	
	no./m^2	%	no./m^2	%	no./m^2	%	no./m^2	%	no./m^2	%
FORAMINIFERA	0	0.0	7	0.0	0	0.0	0	0.0	0	0.0
ROUNDWORMS	2	0.1	2	0.0	0	0.0	0	0.0	0	0.0
UNSEGMENTED WORMS	5	0.2	17	0.1	0	0.0	0	0.0	2	0.0
BRISTLE WORMS	824	27.0	3,743	16.1	1,950	27.6	6,023	49.8	1,162	24.5
Cl. Polychaeta (Errantia)	605	19.7	1,412	6.4	1,080	15.2	2,833	23.2	562	11.9
Cl. Polychaeta (Sedentaria)	219	7.3	2,330	9.7	870	12.5	3,190	26.6	600	12.5
MITES	0	0.0	5	0.0	0	0.0	0	0.0	0	0.0
SEED SHRIMPS	5	0.1	400	1.6	47	0.5	12	0.1	73	1.6
WATER SCUDS	5	0.2	118	0.7	45	0.7	64	0.5	68	1.6
CUMACEANS	71	2.5	82	0.3	9	0.1	17	0.1	12	0.3
AQUATIC SOW BUGS	7	0.3	37	0.1	5	0.1	24	0.2	26	0.6
OPOSSUM SHRIMPS	16	0.6	3	0.0	0	0.0	2	0.0	2	0.0
CRABS and SHRIMP	7	0.2	31	0.2	2	0.0	14	0.1	10	0.2
SNAILS	958	31.3	11,757	49.1	4,151	47.5	4,322	35.9	1,569	36.4
CLAMS	1,063	35.1	5,859	31.4	1,986	23.3	1,511	12.6	1,473	33.5
SAND DOLLARS	68	2.3	19	0.1	2	0.0	24	0.2	52	1.2
SEA CUCUMBERS	2	0.1	17	0.1	5	0.1	49	0.4	9	0.2
SEA STARS	2	0.1	0	0.0	0	0.0	0	0.0	0	0.0
INVERTEBRATE ABUNDANCE	3,035	100.0	22,097	100.0	8,202	100.0	12,063	100.0	4,457	100.0

Chemical analysis of water collected from the surface, mid-depth and bottom of the water column in Pictou Road indicated nitrogen (as TKN) and carbon (as TOC and DOC) can be found at concentrations exceeding background near the water's surface in the vicinity of the Boat Harbour Effluent Treatment Facility discharge. The spatial pattern measured in these

parameters was consistent with the most recently completed effluent dispersion studies, which indicated that on average mill effluent travels at the surface of the water column in a northeast direction from Boat Harbour towards Mackenzie Head.

Bottom sediments in the study area were dominated by sand-sized fractions. Chemical characterization of bottom sediments (TOC, TKN, total sulphides, redox potential) indicated that the presence of mill effluent near the surface of the water column does not affect sediment chemistry in the exposure area. No accumulation of organic matter, nor any indication of excessive processing of organic material, was measured in sampling areas proximate to the Boat Harbour discharge.

The benthos at the exposure areas were largely different from the reference area benthos for both statistical (i.e., $p < 0.05$) and "ecological" (i.e., differences exceeding 2 SDs) criteria. There were also large differences between the two reference areas in terms of the calculated community indices. Examination of the benthic data at a finer taxonomic level helped to explain differences in community structure among sampling areas, but it did not indicate how the patterns in community structure might have or might not have been mill-related.

Table 5.3: Summary of Statistical Comparison of EEM Cycle 3 Effect Parameters

Trophic Level	Effect Endpoint	Comparison	Effect? (p < 0.05)	Direction	Magnitude (%)	Magnitude (Ref SD)[1]
Benthos	**Abundance**	REF1 vs REF2	✓	REF1 < REF2	593	7.3
		REF1 vs NF	✓	REF1 < NF	163	3.7
		REF1 vs FF	✓	REF1 < FF	312	5.4
		REF1 vs FFF	✓	REF1 < FFF	50	1.5
		REF2 vs NF	✓	REF2 > NF	62	2.2
		REF2 vs FF	×	-	-	-
		REF2 vs FFF	✓	REF2 > FFF	78	3.5
	Richness	REF1 vs REF2	✓	REF1 < REF2	59	6.0
		REF1 vs NF	✓	REF1 < NF	42	4.3
		REF1 vs FF	✓	REF1 < FF	37	3.8
		REF1 vs FFF	✓	REF1 < FFF	64	6.5
		REF2 vs NF	×	-	-	-
		REF2 vs FF	×	-	-	-
		REF2 vs FFF	×	-	-	-
	Simpson's Evenness	REF1 vs REF2	✓	REF1 < REF2	48	6.7
		REF1 vs NF	×	-	-	-
		REF1 vs FF	×	-	-	-
		REF1 vs FFF	✓	REF1 < FFF	25	13.6
		REF2 vs NF	×	-	-	-
		REF2 vs FF	✓	REF2 < FF	104	5.6
		REF2 vs FFF	✓	REF2 < FFF	141	5.5
	Bray-Curtis Dissimilarity	REF1 vs REF2	✓	REF1 < REF2	319	6.0
	REF 1 MEDIAN	REF1 vs NF	✓	REF1 < NF	184	3.5
		REF1 vs FF	✓	REF1 < FF	256	4.8
		REF1 vs FFF	✓	REF1 < FFF	127	2.4
	Bray-Curtis Dissimilarity	REF1 vs REF2	✓	REF2 < REF1	70	11.7
	REF 2 MEDIAN	REF2 vs NF	✓	REF2 < NF	139	2.8
		REF2 vs FF	✓	REF2 < FF	135	2.8
		REF2 vs FFF	✓	REF2 < FFF	205	4.2
	Bray-Curtis Dissimilarity	REF_ALL vs REF1	×	-	-	-
	REF_ALL MEDIAN	REF_ALL vs REF2	×	-	-	-
		REF_ALL vs NF	×	-	-	-
		REF_ALL vs FF	×	-	-	-
		REF_ALL vs FFF	×	-	-	-

✓ = yes

× = no

[1] Magnitude of difference in Ref SD (reference standard deviations), as determined by: (area mean - reference mean)/reference standard deviations

EEM Cycle 4

The Cycle 4 ICS consisted of a control/impact survey, in which benthos were collected from three exposure (NF, FF, FFF) and two reference (Merigomish Harbour, Logan's Point) areas. In addition, supplementary reference stations were sampled within the vicinity of Pictou Road to define a regional reference condition so as to be able to analyze the exposure area data in a Reference Condition Approach (RCA) manner (see Figure 5.2).

Univariate comparisons of total invertebrate density, taxa richness and evenness indicated that exposure values for these metrics were within those seen in the reference areas (Logan's Point, Merigomish Harbour) (Table 5.4). In the few instances where statistical differences were seen (6 out of 18 reference vs. exposure area tests), the exposure area was different from one reference area but not the other (e.g., near-field density < Logan's Point but near-field density = Merigomish Harbour) (Table 5.5). With this in mind, it was suggested that these differences should not be interpreted as a mill-related effect (EcoMetrix, 2007a). The Bray-Curtis comparisons were suggestive of differences in community structure among the sampling areas; however these differences were not limited to exposure vs. reference comparisons but were also between the two reference areas. Moreover, from a magnitude perspective the differences were generally below the level at which ecologically significant differences are implied (< 2 reference standard deviations; Environment Canada, 2005a) (Table 5.5).

Table 5.4: Summary of EEM Cycle 4 ICS Data (Absolute and Relative Abundance) for NPNS

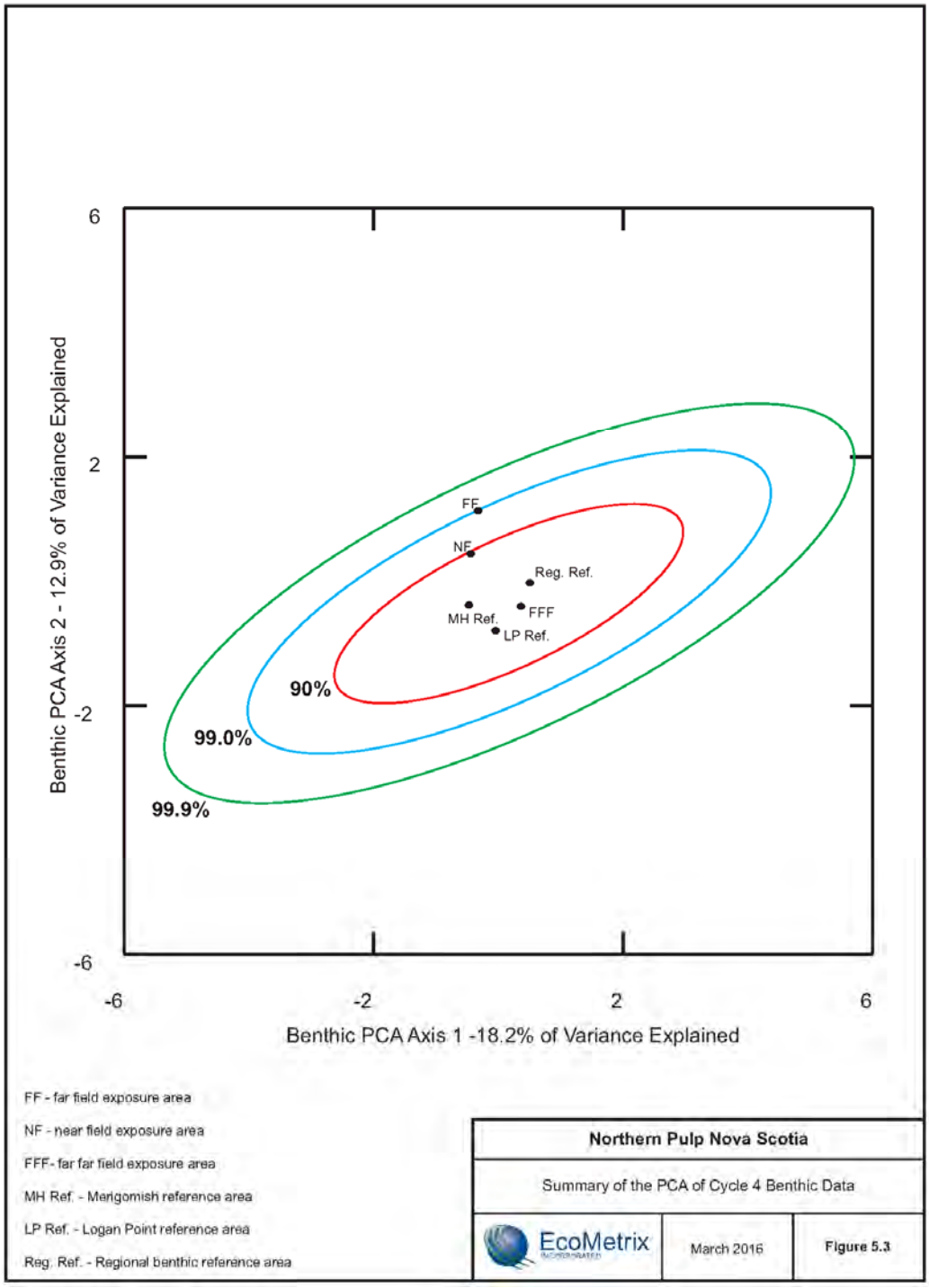
	NEAR-FIELD		FAR-FIELD		FAR-FAR-FIELD		REFERENCE AREA 1 (MERIGOMISH)		REFERENCE AREA 2 (LOGAN'S POINT)		REGIONAL REFERENCE AREA	
TAXANOMIC GROUP	no./m^2	%	no./m^2	%	no./m^2	%	no./m^2	%	no./m^2	%	no./m^2	%
FORAMINIFERA	9	0.39	14	0.38	17	0.14	3	0.07	0	0.00	30	0.47
ROUNDWORMS	0	0.00	0	0.00	41	0.28	3	0.15	35	0.67	3	0.08
UNSEGMENTED WORMS	3	0.1	0	0.0	3	0.1	9	0.3	0	0.0	10	0.1
BRISTLE WORMS	843	42.6	2,414	54.3	7,255	71.7	1,519	48.2	4,110	66.3	3,506	59.6
Cl. Polychaeta (Errantia)	*241*	*12.9*	*522*	*11.1*	*6,307*	*56.3*	*1,125*	*35.1*	*3,783*	*57.7*	*2,533*	*43.2*
Cl. Polychaeta (Sedentaria)	*603*	*29.7*	*1,893*	*43.2*	*948*	*15.3*	*394*	*13.1*	*328*	*8.6*	*972*	*16.3*
MITES	0	0.0	0	0.0	3	0.0	0	0.0	0	0.0	0	0.0
SEED SHRIMPS	0	0.0	0	0.0	6	0.1	0	0.0	3	0.1	41	0.6
WATER SCUDS	6	0.4	6	0.1	177	1.6	17	0.5	20	0.6	62	1.0
CUMACEANS	32	1.8	35	0.7	41	0.6	14	0.4	20	0.3	54	0.8
AQUATIC SOW BUGS	0	0.0	9	0.2	49	0.5	17	0.5	14	0.3	42	0.6
OPOSSUM SHRIMPS	0	0.0	3	0.1	0	0.0	3	0.1	0	0.0	1	0.0
CRABS and SHRIMP	0	0.0	6	0.1	6	0.0	3	0.1	9	0.1	4	0.1
SNAILS	84	4.1	357	7.5	1,151	11.9	800	23.2	351	9.6	939	16.3
CLAMS	968	50.2	1,684	36.6	951	13.0	722	24.9	1,165	20.6	1,119	19.1
SAND DOLLARS	6	0.3	3	0.1	20	0.2	46	1.5	72	1.5	35	1.1
SEA CUCUMBERS	3	0.1	0	0.0	0	0.0	0	0.0	3	0.0	3	0.0
INVERTEBRATE ABUNDANCE	1,954	100	4,530	100	9,719	100	3,157	100	5,803	100	5,849	100
TAXA RICHNESS	16.8	-	21.8	-	26.2	-	19.4	-	24.2	-	29.0	-
EVENNESS	0.294	-	0.221	-	0.121	-	0.229	-	0.150	-	0.172	-

Table 5.5: Summary of Comparisons of Cycle 4 ICS Effect Endpoints

	Comparison	p < 0.1?	Direction	Magnitude (%)	Magnitude (reference standard deviation)
Logan Point Reference					
Abundance (log)	REF vs NF	Yes	EXP < REF	66	1.2
	REF vs FF	No	-	-	-
	REF vs FFF	No	-	-	-
	REF vs MH	No	-	-	-
Richness	REF vs NF	No	-	-	-
	REF vs FF	No	-	-	-
	REF vs FFF	No	-	-	-
	REF vs MH	No	-	-	-
Evenness	REF vs NF	Yes	EXP > REF	96	1.1
	REF vs FF	No	-	-	-
	REF vs FFF	No	-	-	-
	REF vs MH	No	-	-	-
B-C Dissimilarity	REF vs NF	Yes	EXP > REF	139	2.2
	REF vs FF	Yes	EXP > REF	125	2.0
	REF vs FFF	No	-	-	-
	REF vs MH	Yes	MH > REF	77	1.4
Merigomish Harbour Reference					
Abundance (log)	REF vs NF	No	-	-	-
	REF vs FF	Yes	EXP > REF	49	1.2
	REF vs FFF	Yes	EXP > REF	170	2.9
	REF vs LP	No	-	-	-
Richness	REF vs NF	No	-	-	-
	REF vs FF	No	-	-	-
	REF vs FFF	No	-	-	-
	REF vs LP	No	-	-	-
Evenness	REF vs NF	Yes	EXP < REF	46	1.2
	REF vs FF	No	-	-	-
	REF vs FFF	Yes	EXP < REF	47	1.3
	REF vs LP	No	-	-	-
B-C Dissimilarity	REF vs NF	Yes	EXP > REF	83	1.9
	REF vs FF	Yes	EXP > REF	84	1.9
	REF vs FFF	Yes	EXP > REF	75	1.7
	REF vs LP	Yes	LP > REF	42	1.0

Few statistical differences were detected between the reference and exposure areas in Cycle 4 (Table 5.5). This was true when the benthic community data were analyzed within both the control/impact and the RCA frameworks (see Figure 5.3). Taken on balance, the Cycle 4 results, as well as the measured cycle-to-cycle changes seen in benthic community structure, were not interpreted as being mill-related, but rather part of a natural variability in benthic community structure in the study area. Benthic invertebrate community differences

seemed to be related to the feeding habits of the resident benthos. Generally speaking, the near-field and far-field areas had a larger proportion of deposit feeding taxa than the other areas. This same pattern was seen in the Cycle 3 study (Stantec, 2004a).



EEM Cycle 5

The Cycle 5 invertebrate community survey comprised two parts and was similar in scope to the previous surveys. First, samples were collected from exposure (NF, FF, and FFF) and multiple reference areas with data analyzed in a control/impact framework. Secondly, additional "reference" stations were sampled in the region so that the benthic data could the evaluated in a fashion consistent with the reference condition approach (RCA) (Figure 5.2). Benthic data from Cycle 5 is summarized in Table 5.6.

Similar to Cycle 4, a limited number of statistical differences were detected between the reference and exposure areas in Cycle 5, all of which were limited to comparisons to the Logan's Point reference area (Table 5.7). When the benthic community data were analyzed within both the control/impact and the RCA frameworks results indicated that generally exposure and reference areas were similar or within the range of reference as defined herein.

Table 5.6: Summary of EEM Cycle 5 ICS Data (Absolute and Relative Abundance) for NPNS

	NEAR-FIELD		FAR-FIELD		FAR-FAR-FIELD		REFERENCE AREA 1 (MERIGOMISH)		REFERENCE AREA 2 (LOGAN'S POINT)		REGIONAL REFERENCE AREA	
TAXANOMIC GROUP	no./m^2	%	no./m^2	%	no./m^2	%	no./m^2	%	no./m^2	%	no./m^2	%
FORAMINIFERA	19	0.3%	64	0.9%	48	0.8%	3	0.0%	3	0.0%	103	1.4%
ROUNDWORMS	21	0.2%	16	0.2%	1,300	4.4%	34	1.4%	66	0.4%	39	0.3%
UNSEGMENTED WORMS	9	0.1%	3	0.1%	83	0.3%	21	0.5%	19	0.1%	28	0.3%
BRISTLE WORMS	2,520	31.4%	2,377	32.8%	12,315	72.6%	4,900	50.7%	14,076	74.6%	6,175	64.7%
Cl. Polychaeta (Errantia)	*1,145*	*14.3%*	*1,064*	*15.7%*	*10,963*	*64.3%*	*4,388*	*40.6%*	*13,599*	*71.5%*	*4,600*	*50.0%*
Cl. Polychaeta (Sedentaria)	*1,376*	*17.1%*	*1,314*	*17.1%*	*1,352*	*8.4%*	*512*	*10.0%*	*478*	*3.2%*	*1,575*	*14.7%*
SEED SHRIMPS	22	0.3%	38	0.5%	5	0.1%	12	0.2%	24	0.1%	35	0.3%
WATER SCUDS	38	0.5%	31	0.4%	19	0.3%	22	0.2%	67	0.5%	96	1.1%
CUMACEANS	19	0.3%	29	0.4%	22	0.3%	148	2.1%	84	0.5%	181	2.2%
AQUATIC SOW BUGS	24	0.3%	47	0.6%	45	0.6%	50	1.0%	22	0.2%	93	1.1%
OPOSSUM SHRIMPS	48	0.7%	7	0.1%	17	0.3%	16	0.2%	3	0.0%	22	0.3%
TANAID SHRIMPS	3	0.1%	0	0.0%	10	0.0%	10	0.1%	0	0.0%	3	0.0%
CRABS and SHRIMP	19	0.2%	3	0.0%	3	0.1%	19	0.3%	22	0.2%	11	0.1%
SNAILS	1,109	13.3%	1,196	16.4%	388	5.9%	867	14.3%	638	4.3%	802	8.9%
CLAMS	3,979	52.2%	3,386	47.4%	1,005	14.1%	1,477	26.4%	2,958	17.1%	1,131	15.7%
SAND DOLLARS	9	0.1%	10	0.2%	10	0.2%	167	2.6%	271	2.0%	180	3.6%
SEA CUCUMBERS	0	0.0%	0	0.0%	5	0.1%	0	0.0%	3	0.0%	3	0.1%
TUNICATES	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	2	0.0%
TOTAL NUMBER OF ORGANISMS	7,839	100	7,208	100	15,276	100	7,748	100	18,259	100	8,903	100
TAXA RICHNESS	33	-	35	-	26	-	30	-	38	-	36	-
Simpson's D	0.84	-	0.87	-	0.56	-	0.73	-	0.50	-	0.73	-
Simpson's Evenness	0.22	-	0.23	-	0.10	-	0.19	-	0.05	-	0.14	-
Bray-Curtis (MHR)	0.53	-	0.53	-	0.44	-	0.38	-	0.50	-	0.41	-
Bray-Curtis (LPR)	0.66	-	0.68	-	0.49	-	0.54	-	0.25	-	0.48	-

Univariate comparisons of total invertebrate density, taxa richness and evenness indicated that exposure area values for these metrics were within those seen in the reference areas (Logan's Point, Merigomish Harbour). In all instances where statistical differences were seen, the exposure area was different from the Logan's Point reference area but not Merigomish Harbour, as mentioned. With this in mind, it is suggested that these differences should not be interpreted as a mill-related effect (Figure 5.4).

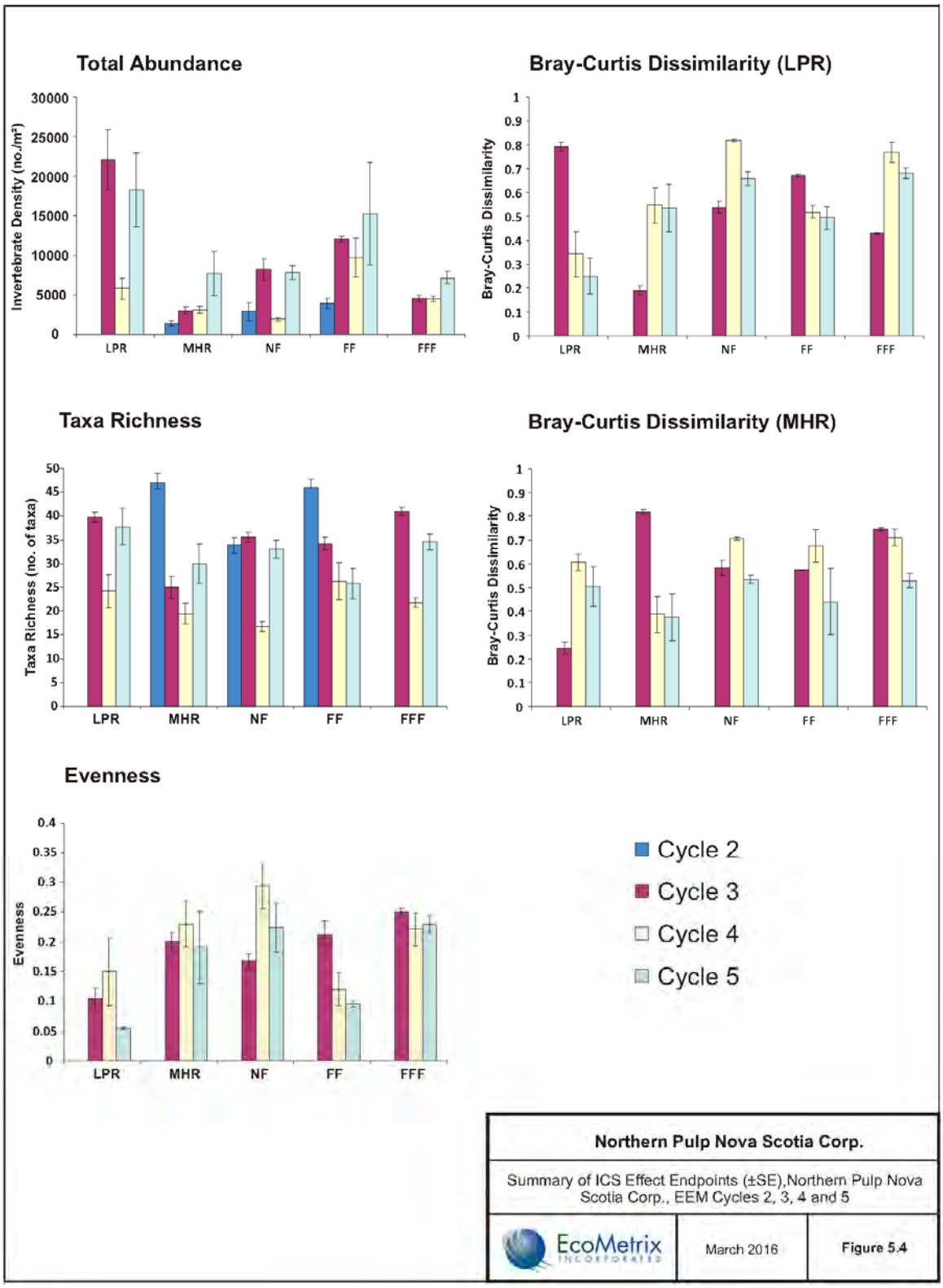
The Bray-Curtis comparisons were suggestive of differences in community structure among the sampling areas; however these differences were not limited to exposure vs. reference

comparisons, as using Logan's Point as the median also lead to a significant difference from the Merigomish Harbour reference area. From a magnitude perspective the differences were around the level at which ecologically significant differences are implied (> 2 reference standard deviations is ecologically significant; Environment Canada, 2005a) therefore the differences could possibly be significant.

Table 5.7: Summary of Comparisons of Cycle 5 ICS Effect Endpoints

	Comparison	p < 0.1?	Direction	Magnitude (%)	Magnitude (reference standard deviation)
Logan's Point Reference					
Abundance (log)	REF vs NF	Yes	REF > NF	52	1.3
	REF vs FF	Yes	REF > FF	56	1.4
	REF vs FFF	No	-	-	-
	REF vs MH	Yes	REF > MH	63	1.8
Richness	REF vs NF	No	-	-	-
	REF vs FF	No	-	-	-
	REF vs FFF	Yes	REF > FFF	32	1.4
	REF vs MH	No	-	-	-
Evenness	REF vs NF	Yes	EXP > REF	307	34.7
	REF vs FF	Yes	EXP > REF	317	35.8
	REF vs FFF	Yes	EXP > REF	74	8.3
	REF vs MH	Yes	MH > REF	247	27.8
B-C Dissimilarity	REF vs NF	Yes	EXP > REF	163	2.4
	REF vs FF	Yes	EXP > REF	172	2.6
	REF vs FFF	Yes	EXP > REF	98	1.5
	REF vs MH	Yes	MH > REF	114	1.7
Merigomish Harbour Reference					
Abundance (log)	REF vs NF	No	-	-	-
	REF vs FF	No	-	-	-
	REF vs FFF	No	-	-	-
	REF vs LP	Yes	REF > LP	172	1.2
Richness	REF vs NF	No	-	-	-
	REF vs FF	No	-	-	-
	REF vs FFF	No	-	-	-
	REF vs LP	No	-	-	-
Evenness	REF vs NF	No	-	-	-
	REF vs FF	No	-	-	-
	REF vs FFF	No	-	-	-
	REF vs LP1	Yes	REF > LP	71	0.99
B-C Dissimilarity	REF vs NF	No	-	-	-
	REF vs FF	No	-	-	-
	REF vs FFF	No	-	-	-
	REF vs LP	No	-	-	-

1 Analyzed using T-test assuming unequal variance
2 Analyzed using Non-parametric Mann-Whitney U-test due to non-normality


Total Abundance
Invertebrate Density (no./m²)
30000
25000
20000
15000
10000
5000
0
LPR
MHR
NF
FF
FFF
Bray-Curtis Dissimilarity (LPR)
Bray-Curtis Dissimilarity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Taxa Richness
Taxa Richness (no. of taxa)
50
45
40
35
30
25
20
15
10
5
Bray-Curtis Dissimilarity (MHR)
Evenness
Evenness
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
Cycle 2
Cycle 3
Cycle 4
Cycle 5
Northern Pulp Nova Scotia Corp.
Summary of ICS Effect Endpoints (±SE),Northern Pulp Nova Scotia Corp., EEM Cycles 2, 3, 4 and 5
EcoMetrix INCORPORATED
March 2016
Figure 5.4

Although some of the major taxa were ubiquitous across the study area and found in similar densities among all sampling locations (e.g., the clam *Tellina agilis*, the snail *Nassarius trivittatus*), others were not. For the most part, however, the differences in community structure among sampling areas were associated with, albeit not exclusively, differences in abundance of major taxa as opposed to the presence and absence of taxa. For example, the polychaete *Protodriloides* was present in all sampling areas but differed in abundance among the sampling areas. *Protodriloides* was the least abundant in the near-field and far-field areas, moderately abundant in the regional reference and Merigomish Harbour and highly abundant in the Logan's Point and far far-field areas. The snail *Acteocina canaliculata* was less abundant in the far far-field compared to all other areas (Table 5.8). The sand dollar *Echinarachnius parma* was also present at relatively low abundance in all sampling areas but was less abundant in all three of the exposure areas compared to the reference areas.

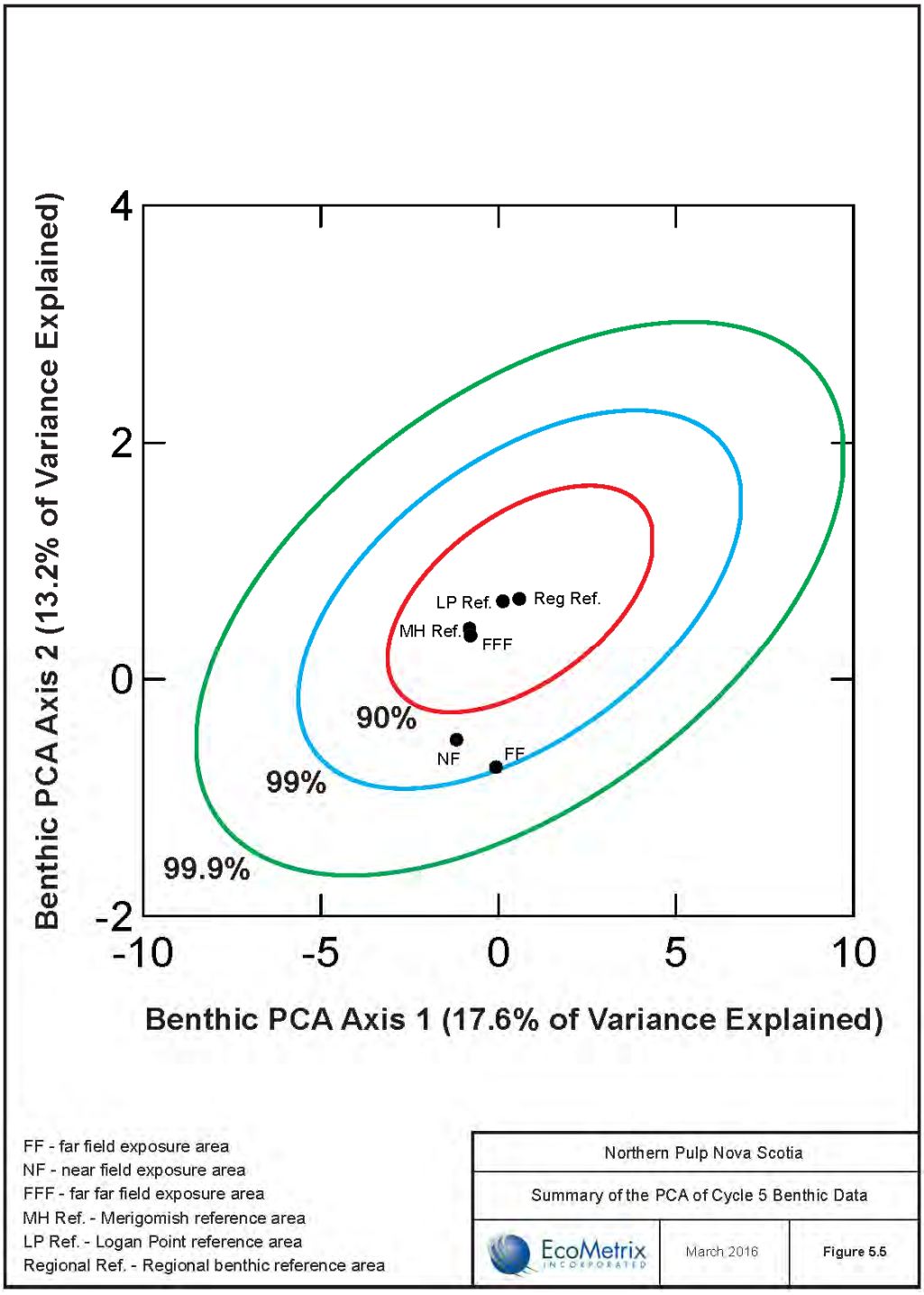
These community differences seem to be related to the feeding habits of the resident benthos. Generally speaking, the near-field and far-field areas had a larger proportion of deposit feeding taxa than the other areas. This same pattern was seen in the Cycle 3 and 4 studies (Stantec, 2004a, EcoMetrix, 2007a).

Table 5.8: Summary (% Abundance) of Major Taxonomic Groups collected in EEM Cycle 2 through 5

Area	Polychaete				Snails				Clams			
	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Merigomish Harbour	39	27	48	51	11	31	23	14	20	35	25	26
Logan's Point	-	16	66	65	-	49	10	4	-	31	21	17
Near-field	24	28	33	31	6	48	4	13	61	23	50	52
Far-field	47	50	54	33	16	36	8	16	33	13	37	47
Far far-field	-	25	72	73	-	36	12	6	-	34	13	14

The outlet of Boat Harbour Estuary is somewhat sheltered, especially the near-field and far-field areas, and is therefore the natural depositional zone for the estuary. Sediment chemistry data indicate, however, that organic material does not accumulate within these areas to a greater extent than other nearby locations (e.g., Logan's Point). Overall, the nature of differences in benthic invertebrate assemblages seen in the study may be related to or associated with subtle habitat differences among the sampling areas and do not seem to be mill related. The RCA analysis for the Cycle 5 data placed the near-field and far-field areas within the 99% probability ellipse and the far far-field fell within the 90% ellipse. This indicates that the near-field and far-field are "possibly different" from the reference condition, whereas the far far-field is "equivalent" to reference. This essentially mimics the results of this same analysis in EEM Cycle 4 (Figure 5.3 and Figure 5.5).

Taken on balance, the Cycle 5 results, as well as the measured cycle-to-cycle changes seen in benthic community structure, were not interpreted as being mill-related, but rather part of a natural variability in benthic community structure in the study area.



EEM Cycle 6

There was no benthic invertebrate study conducted as part of the Cycle 6 EEM program.

Other Relevant Research

In 2002, Kimberly-Clark Nova Scotia Inc. (now NPNS) commissioned Stantec Consulting Limited (Stantec) to repeat a 1965 study of the benthic environment in Pictou Harbour (NS) and surrounding waters (see Stantec, 2004b). The objectives of this study were threefold:

- to review and report on the 1965 study with respect to how sediment quality and invertebrate community structure was characterized;
- to spatially characterize the current (i.e., 2002) invertebrate community structure within Pictou Harbour and environs; and
- to compare the results of the 1965 and 2002 studies to identify changes in sediment quality and the invertebrate community within the context of changes in the local landscape, land/water use and development within the greater Pictou area.

In both the 1965 and 2002 surveys, benthic invertebrates were obtained from sediment samples collected at a total of 59 stations in Pictou Harbour and the Northumberland Strait from Logan's Point to Roaring Bull Point. Detailed taxonomical analyses, which were consistent with the standard methodologies of the eras in which they were undertaken, were completed on the invertebrate samples. These data were utilized to assess the structure of the invertebrate community in the study area with the goal of identifying the overlying factors affecting observed patterns in invertebrate abundance and distribution.

Based on a qualitative assessment of the benthic data, the 1965 study concluded that overall Pictou Harbour and the surrounding waters contained "*a rich and varied bottom fauna*" and that "*an excellent water quality was apparent*". The only exception to this characterization was the most upstream reaches of the East River sampled. This area received industrial and municipal waste discharges from the communities of Trenton and New Glasgow and was characterized as impacted.

Overall, with the exception of the areas near the town of Pictou's municipal sewage outfall and the upper reach of the East River, the spatial pattern in invertebrate community structure was one that was reflective of both gross (e.g., inside vs. outside of the harbour) and subtle (e.g., nearshore exposed vs. nearshore protected) habitat differences within the study area. Invertebrates that typically burrow into soft sediments were found within the depositional habitat of Pictou Harbour, whereas those which tend to exist more in the surface layer of the sediments were found outside the harbour in the Northumberland Strait, where the bottom habitat comprises relatively hard-packed sand. Again, with the exception of two localized areas there was no indication that current land use or industrial/municipal practices are negatively influencing benthic invertebrate communities in the study area.

The spatial pattern identified in the 2002 benthic invertebrate community data was very similar to the pattern identified in the original 1965 study. The most conspicuous difference was the change (negative) in the structure of the invertebrate community resident in the bottom substrates near what is now proximate to the town of Pictou's municipal sewage discharge. In each of the surveys the numerically dominant taxa within the harbour lead a burrowing existence and are most often deposit feeders. Conversely, a relatively greater proportion of the taxa found at stations in the Northumberland Strait were typically surface dwellers and suspension feeders.

Based on the results of the study it was concluded that:

- in 1965 Pictou Harbour and the surrounding waters contained "*a rich a varied bottom fauna*" and that "*an excellent water quality was apparent*". The only exception to this characterization was the upstream reach of the East River, which received industrial and municipal waste discharges from the communities of Trenton and New Glasgow;
- in 2002 the benthic community within the study area was generally divisible into a series a station clusters that were based on either their spatial distribution relative to their proximity to Pictou Harbour (e.g., in the harbour or not) or their proximity to local point source discharges (e.g., Town of Pictou sewage discharge); and
- there has been little change in the structure of the benthic community in the area, with the exception of the negative, localized impact of the town of Pictou's municipal waste discharge. The pulp and paper mill effluent discharge at Boat Harbour has not adversely affected the benthos or sediment quality in Pictou Road.

5.2.2 Intertidal Benthic Invertebrate Community

EEM Cycle 1

A semi-quantitative intertidal survey was conducted at the mouth of the Boat Harbour Estuary in July 1995. The intertidal study was conducted by surveying 30 m by 30 m adjacent grids, beginning at the point of effluent discharge to a distance of 150 m along the shoreline, both to the east and west of the outfall. The intertidal zone was assessed from the mean high tide mark to the water's edge at low tide. The presence or absence of flora and fauna was noted, along with estimates of their densities using a 0.25 m^2 quadrat (JWEL, 1996).

Faunal diversity associated with the sandy beach to the east of the outfall was limited by the lack of coarse substrate. A few clams were found within 5 m of the outfall, and they became abundant at a distance of 75 m. The invertebrate community was more diverse in the rocky areas to the west of the outfall. The only organisms found in the area directly in front of the outfall were marine algae (e.g., *Enteromorpha intestinalis*) and amphipods. The

intertidal survey indicated that the effluent plume influenced the distribution of intertidal organisms within 90 m of the Boat Harbour outfall, but these effects were rapidly dissipated further away. These patterns may be related to the freshwater nature of the plume.

EEM Cycle 2

A semi-quantitative survey of the intertidal area adjacent to the Boat Harbour discharge was conducted in EEM Cycle 2 in an attempt to determine the influence of mill effluent on the intertidal community. The results of this survey suggested that habitat, rather than effluent exposure, was the most limiting factor controlling invertebrate distribution in the intertidal area around Boat Harbour. For example, the harsh, unstable conditions presented by the sand beach limited the occurrence of many invertebrates. One exception to this was the soft-shelled clam (*Mya arenaria*), which was common in the wetter intertidal areas. Bivalve clams (e.g., *M. arenaria* and *Macoma balthica*) were numerous in the area immediately adjacent to the discharge, despite exposure to high concentrations of mill effluent. Mummichogs were also abundant in and around the effluent stream. Where rocky habitat presented itself and salinity was adequate, barnacles, mussels and snails were very abundant. Osmotic stress imposed by the freshwater effluent is likely the largest limiting factor to marine invertebrate populations in the immediate vicinity of the discharge. Nutrient enrichment in the intertidal area was indicated by the lush growth of *Enteromorpha*, a green encrusting algae tolerant of fluctuating salinities. It is quite possible that the nutrient addition system implemented at the Boat Harbour facility in 1997 contributed to this algal growth. The small degree of nutrient enrichment did not appear to be problematic, and low sediment TOC levels in the intertidal area demonstrated that there had been no substantial deposition of organic material from the mill or other sources.

EEM Cycle 3 through 6

Due to the transient nature of the intertidal zone, the lack of the suitable reference area and previous observations that the intertidal area near the Boat Harbour discharge was not impacted by mill effluent no intertidal benthic community survey was undertaken in Cycles 3, 4, 5 or 6.

5.3 EEM Cycle 7 Study Design and Methods

5.3.1 Overview and Rationale

The Cycle 7 ICS at NPNS followed a similar approach used in Cycles 4 and 5 although no regional reference approach collections were conducted. The survey was comprised of a control/impact design. The original rationale for the combined approach for Cycle 4 and 5 that included the regional reference was driven largely by the results of the earlier EEM surveys (in particular the results of the EEM Cycle 3 benthic community survey [Stantec, 2004a]) and the additional study in which benthic samples were collected from a relatively large area centered around Pictou Harbour (Stantec, 2004b). However, after discussions

with Environment Canada the regional reference portion of the study was dropped and a more conventional focus was undertaken.

5.3.2 Sampling Areas

The exposure areas sampled in Cycles 4 and 5 were utilized again for Cycle 7. Generally these areas comprise the geographical extent of the area of Pictou Road that is potentially exposed (albeit in surface waters only due to the buoyant effluent) to mill effluent at concentrations of 1% or more (Figure 2.2 and Figure 2.3). Three previously established exposure sampling areas at increasing distance along a north-by-northeast gradient from the Boat Harbour outlet were used, including: the near field (about 300 m from the outlet), the far field (about 600 m from the outlet) and the far-far field (about 1,250 m from the outlet).

Reference data were collected within nearshore locations at Logan's Point in the northwest and Merigomish Harbour in the southeast (Figure 5.2). These areas have similar bottom substrates that are comprised of similar-sized fractions to those found in the exposure area outside Boat Harbour at the same depths from which exposure samples were collected. Recent work also suggests that the benthos within this area is similar at least by gross community measures (e.g., abundance, diversity).

Each station comprised a revisitable location with dimensions of 10 m x 10 m.

5.3.3 Sample Collection and Effort

Benthic community sampling methods in Cycle 7 were the same as those employed in Cycles 2 through 5. Samples were collected using a petite Ponar grab (area of 0.023 m^2). Each sample consisted of a composite of five individual grabs (i.e., subsamples) representing a total sample area of 0.115 m^2. Analysis of historical benthic data from this site indicates that this level of replication exceeds the number of replicates required to achieve a precision of 20%, based on organism abundance, as required by the pulp and paper technical guidance (Environment Canada, 2010), (see BEAK, 1998). Five stations were sampled in each of the three exposure areas, as well as the Logan's Point and Merigomish Harbour reference areas. All samples were sieved in the field through a 500 µm mesh and preserved to a level of 10% buffered formalin in receiving water within hours of collection.

Specific field sampling and laboratory processing procedures (including QA/QC) followed Standard Operating Procedures (SOPs).

Sampling location coordinates were obtained with a Garmin Map 60CX Handheld GPS set for the North American Datum (NAD) 1983 geodetic datum. Coordinates were recorded at each sampling station and expressed as degrees, minutes and seconds (dd mm'ss"). Water depths were measured to the nearest 0.1 m with a boat mounted depth sounder (Appendix 2).

5.3.4 Timing

The Cycle 7 ICS was conducted in August 2014 representing a similar time of the year as the Cycle 4 and 5 surveys.

5.3.5 Sample Processing (Laboratory)

Detailed taxonomic identifications were completed by Zaranko Environmental Assessment Services (ZEAS) in Nobleton (ON). When the samples arrived at ZEAS office, each was logged and crosschecked with the field and chain of custody forms to ensure that all of the samples were received and that all of the samples were labeled correctly. Samples were also checked to ensure that they had been properly preserved in the field.

The samples were stained to facilitate the removal of the invertebrates from the associated debris. Prior to further processing, the samples were washed free of formalin. Samples were sorted with the aid of a dissecting microscope at ten times magnification. All benthic fauna were identified to the lowest practical level, as in past surveys. Subsampling error was estimated to determine the precision of the density estimates derived from the subsampling procedure. Recovery checks (i.e., re-sorting of samples to determine initial sorting efficacy) were undertaken on 10% of the samples collected, as required. A voucher collection, including representatives of identified taxa, was compiled and is catalogued at ZEAS.

Data Management, Data Summary and Statistical Analyses

Field data was tabulated on custom data entry sheets as the field program was completed. Upon return from the field these sheets were scanned electronically and held on disc (CD) as a permanent record of the data.

Field data, as well as data derived from sample analyses (water, sediments, benthos), were transcribed into the Environment Canada's EEM data entry system.

Benthic data were analyzed using two different approaches. First, the data were analyzed using the standard EEM approach to identify potential differences between effluent-exposed and reference areas in terms of key effect endpoints. Secondly, the data were analyzed using PCA to assess whether effluent-exposed sampling stations differed from reference areas. These approaches are explained in more detail in the following paragraphs.

5.3.5.1 Effect Endpoint Comparisons

Raw benthic data were summarized and several benthic invertebrate community metrics were calculated using embedded Microsoft® Excel™ spreadsheet functions. These metrics included those defined as "EEM effect parameters" (Environment Canada, 2010), as well as several others that helped to better reveal patterns of invertebrate community structure:

- total invertebrate density[2];
- invertebrate richness[2];
- Evenness (E) [2];
- Bray-Curtis Dissimilarity Index (B-C) [2];
- Taxon density; and
- Taxon proportion (relative density).

Total invertebrate density was determined as the total number of individuals of all taxonomic categories collected at a station expressed per unit area (numbers/m^2).

Invertebrate richness was expressed as the total number of unique taxonomic categories collected at a sampling station.

Evenness (E) is an expression of how equitably taxa are represented within a given sample. In some cases, disturbance or stress can create conditions whereby relatively few taxa are favored and those taxa become disproportionately abundant in that location. In this instance the effect is manifested as relatively low evenness. Evenness ranges from zero to approaching one (with higher scores representing samples in which taxa have the more similar abundance) and is calculated as in Smith and Wilson (1996):

$$E = 1/\sum_{i=1}^{s}(p_i)^2 / S$$

where: E = Evenness
p*i* = the proportion of the *i*th taxon (family) at the station
S = the total number of taxa (families) at the station

The B-C Index is a distance co-efficient that reaches a maximum value of 1 for two sites that are entirely different and a minimum value of 0 for two sites that possess identical descriptors. These distance coefficients measure the amount of association between sites and the B-C Index is a member of the class of distance coefficients known as a semimetric that some prefer to call dissimilarity coefficients. The B-C Index measures the percentage of difference between sites (Legendre and Legendre, 1983) where the distance statistic is calculated as below:

[2] Total invertebrate abundance, richness, E and B-C are “EEM effect parameters”.

$$B - C = \frac{\sum_{i=1}^{n} \left| y_{i1} - y_{i2} \right|}{\sum_{i=1}^{n} \left(y_{i1} + y_{i2} \right)}$$

where: B-C = Bray-Curtis distance between sites 1 and 2;
Y_{i1} = count for species (family) *i* at site 1;
Y_{i2} = count for species (family) *i* at site 2;
n = total number of species (families) present at the two sites.

In the present application, the B-C distances for each of the sampling stations, as well as mean B-C distances for each sampling area, are expressed as distances relative to a calculated reference median condition based on the Merigomish Harbour results.

Descriptive statistics (n, mean, median, standard error, standard deviation) were determined for each of the key endpoints in Microsoft Excel or ACCESS spreadsheets.

Univariate among-area (near field, far field, far far-field, Logan's Point reference, Merigomish reference as defined in EEM Cycles 3 through 5) statistical comparisons of Cycle 7 effect parameter data were made in one of three ways (in order of preference, as well as statistical power):

- Analysis of Variance (ANOVA), for data that were both normal and homoscedastic (i.e., equal variance);
- t-test, for data that were normal but not homoscedastic; and
- Mann-Whitney U Test for data that were neither normal nor homoscedastic.

In order to assess changes that may have occurred in the study area over time, cycle-by-cycle comparisons of effect endpoint data were made via two-way ANOVA (cycle * area). Differences among groups of data are considered statistically significant at α <0.10 (i.e., a 10% probability of committing a Type I error or rejecting a true null hypothesis). Consistent with Environment Canada guidance, no correction of α (e.g., Bonferroni correction) was made, as might typically be done when multiple statistical tests are completed on the same data set, to compensate for the multiplicative nature of probability. In this application, fixing α at 0.10 rather than correcting for multiple tests errs on the side of being protective of the environment.

It should be noted that Environment Canada requested that for the purpose of determining an "effect" only comparisons using Merigomish Harbour reference be assessed. However, to facilitate the determination of potential temporal trends statistics were also conducted on

the Logan's Point reference area data although these results were not used to determine the path forward for subsequent EEM Cycles.

Principal Component Analysis (PCA)

Multivariate ordination (Principal Components Analysis) was used to evaluate spatial and temporal patters in the benthic data[3]. PCA will define the regional variability (i.e., all reference data) in XY space and also distinguish between those exposure area sampling stations that could be characterized as "equivalent to reference", "possibly different", "different" or "very different". This analysis was completed on the 2014 benthic data set (to assess current spatial trends).

5.4 Results

Tables containing the raw benthic invertebrate community data are provided in Appendix D. Benthic invertebrate community data are summarized by sampling area in Table 5.9. A temporal comparison of Cycle 2 through 7 data is provided in Figure 5.6. A summary of the results of the statistical comparisons of the Cycle 7 effect endpoint measures is provided in Table 5.10.

Note that there were a high number of forminiferans in a few of the samples. This taxon is difficult to distinguish from the sand remaining in the sample. Consequently, forminiferans were removed them statistical analysis to not unduly influence the results. The raw density including this taxon are provided in Appendix D.

5.4.1 Data Quality

A number of the benthic samples were subsampled prior to sorting. A list of the sample fractions that were sorted is provided in Appendix D.

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[3] In multivariate ordination the reference invertebrate assemblage is described by its distribution in an ordination space, and the assemblage at any given test site (i.e., exposure station) is characterized by its position in that XY space. The greater the similarity between sites the closer together they are in the XY space. The likelihood of the test site being the same as the reference sites is quantified by constructing probability ellipses that are defined by the reference data only. The 90% probability ellipse is the threshold for a site being considered "equivalent to reference". Sites (i.e., exposure sampling locations) located in ordination space inside the 90% probability ellipse are considered as equivalent to reference and therefore unstressed. Two other probability ellipses (99%, 99.9%) are also used to describe further divergence from the reference state. Exposure stations between the 90% and 99% probability ellipses are considered "possibly different" (there is a 1 in 10 chance that sites will fall in this band through normal variability). Exposure sampling stations between the 99% and the 99.9% probability ellipse are considered "different", as there is a 1 in 100 chance that these sites would incorrectly be described as different. Exposure stations located outside the 99.9% ellipse are designated as "very different".

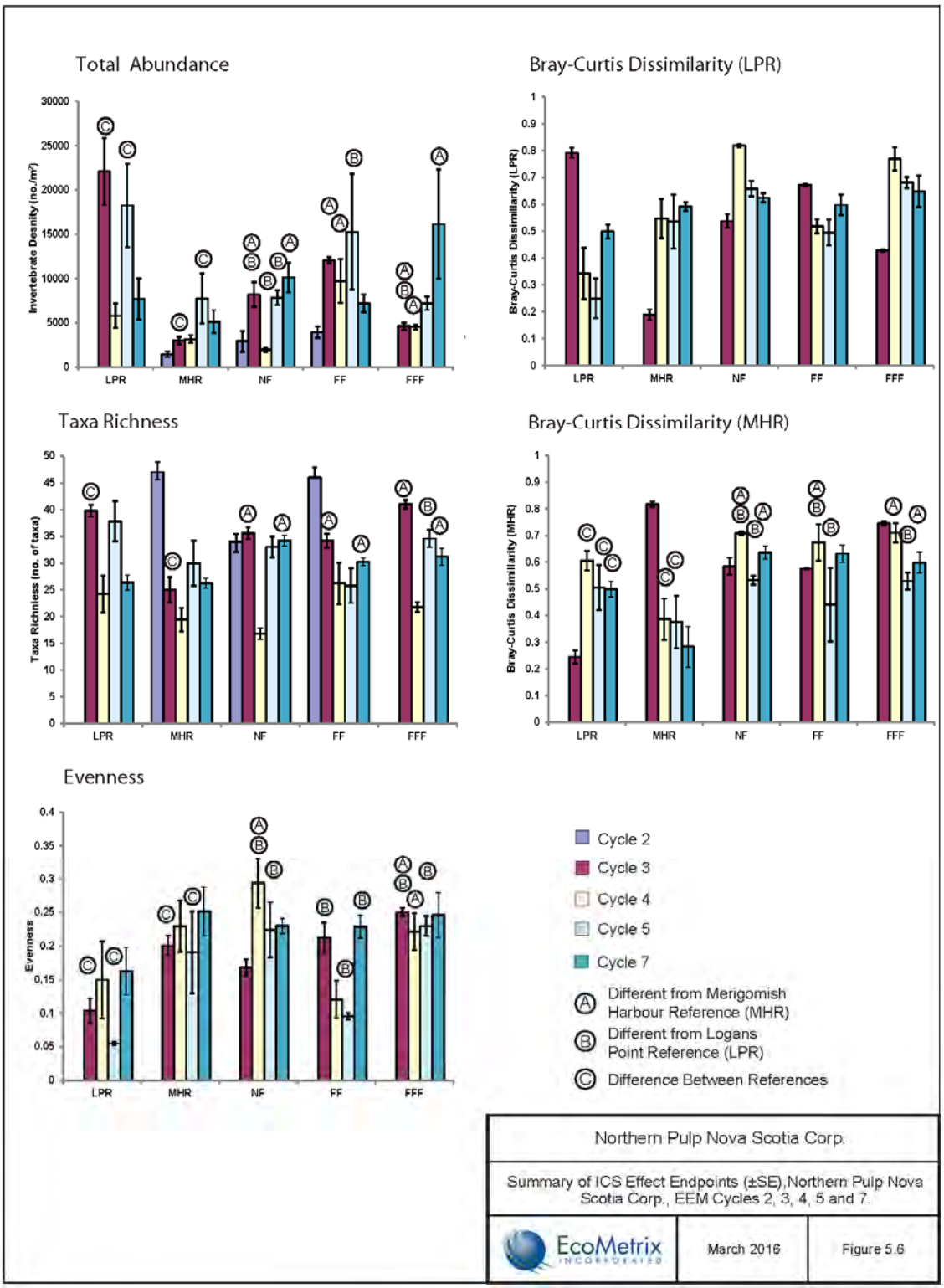
Invertebrate recovery checks (i.e., the number of animals remaining in previously sorted sample fractions) were completed for four benthic samples. Recovery in these samples ranged from 99.0% to 99.6%. Recovery standards as set for the EEM program by Environment Canada indicate that 90% recovery is acceptable (Environment Canada, 2010).

Subsampling precision estimates were calculated for two of the benthic samples. Subsampling precision met the requirements of 20% outlined by Environment Canada (Environment Canada, 2010) (Appendix D).

Subsampling accuracy estimates were also calculated on these same samples. The reported accuracy also met the Environment Canada suggested subsampling accuracy guideline of 20% (Environment Canada, 2010).

Table 5.9: Summary of EEM Cycle 7 ICS Data (Absolute and Relative Abundance) for NPNS

	NEAR-FIELD		FAR-FIELD		FAR-FAR-FIELD		REFERENCE AREA 1 (MERIGOMISH)		REFERENCE AREA 2 (LOGAN'S POINT)	
TAXANOMIC GROUP	no./m^2	%	no./m^2	%	no./m^2	%	no./m^2	%	no./m^2	%
FORAMINIFERA	531	4.8%	717	11.9%	352	2.8%	0	0.0%	9	0.2%
ROUNDWORMS	3	0.0%	0	0.0%	2,014	5.2%	26	0.6%	541	5.7%
FLATWORMS	7	0.1%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
UNSEGMENTED WORMS	14	0.2%	3	0.1%	16	0.1%	12	0.2%	4	0.2%
SEGMENTED WORMS	0	0.0%	0	0.0%	0	0.0%	0	0.0%	38	0.4%
BRISTLE WORMS										
Cl. Polychaeta (Errantia)	*996*	*9.2%*	*395*	*6.2%*	*4,482*	*18.9%*	*703*	*13.0%*	*2,636*	*29.1%*
Cl. Polychaeta (Sedentaria)	*1,174*	*11.1%*	*928*	*12.8%*	*2,876*	*19.5%*	*1,171*	*22.2%*	*1,257*	*12.8%*
MITES	*0*	*0.0%*	*0*	*0.0%*	*28*	*0.1%*	*0*	*0.0%*	*0*	*0.0%*
SEED SHRIMPS	17	0.2%	14	0.2%	7	0.1%	17	0.4%	7	0.1%
WATER SCUDS	45	0.7%	17	0.3%	228	0.9%	16	0.3%	45	0.5%
CUMACEANS	28	0.3%	24	0.3%	62	0.5%	167	2.8%	74	1.4%
AQUATIC SOW BUGS	21	0.2%	17	0.2%	55	0.3%	12	0.2%	14	0.3%
OPOSSUM SHRIMPS	7	0.1%	0	0.0%	0	0.0%	3	0.1%	3	0.0%
TANAID SHRIMPS	14	0.1%	0	0.0%	0	0.0%	10	0.2%	41	0.4%
CRABS and SHRIMP	21	0.2%	16	0.3%	62	0.5%	14	0.4%	10	0.5%
SNAILS	3,379	33.4%	2,627	34.2%	2,579	19.6%	1,738	31.4%	536	9.1%
SEA SLUG	0	0.0%	0	0.0%	14	0.0%	0	0.0%	0	0.0%
CLAMS	3,858	39.3%	2,450	33.4%	3,307	31.0%	1,209	25.8%	2,346	36.1%
SAND DOLLARS	0	0.0%	7	0.1%	33	0.3%	53	2.1%	117	2.9%
SEA CUCUMBERS	0	0.0%	3	0.1%	0	0.0%	0	0.0%	0	0.0%
TOTAL NUMBER OF ORGANISMS	10,118	100	7,218	100	16,154	100	5,162	100	7,703	100
TAXA RICHNESS	34	-	30	-	31	-	26	-	31	-
Simpson's D	0.87	-	0.85	-	0.85	-	0.84	-	0.77	-
Simpson's Evenness	0.23	-	0.23	-	0.25	-	0.25	-	0.16	-
Bray-Curtis (MHR)	0.52	-	0.45	-	0.59	-	0.28	-	0.64	-
Bray-Curtis (LPR)	0.64	-	0.63	-	0.65	-	0.59	-	0.50	-


Total Abundance
Invertebrate Desnity (no./m²)
LPR
MHR
NF
FF
FFF
Bray-Curtis Dissimilarity (LPR)
Bray-Curtis Dissimilarity (LPR)
Taxa Richness
Taxa Richness (no. of taxa)
Bray-Curtis Dissimilarity (MHR)
Bray-Curtis Dissimilarity (MHR)
Evenness
Evenness
Cycle 2
Cycle 3
Cycle 4
Cycle 5
Cycle 7
A Different from Merigomish Harbour Reference (MHR)
B Different from Logans Point Reference (LPR)
C Difference Between References
Northern Pulp Nova Scotia Corp.
Summary of ICS Effect Endpoints (±SE), Northern Pulp Nova Scotia Corp., EEM Cycles 2, 3, 4, 5 and 7.
EcoMetrix INCORPORATED
March 2016
Figure 5.6

5.4.2 Benthic Invertebrate Community Characteristics

5.4.2.1 Near-Field

Total invertebrate density in the near-field area was in the range of about 3,600 to 13,000 animals per square metre of bottom surface area (m^2), with a mean density of around 10,800 animals/m^2. A total of 55 distinct invertebrate taxa were identified in the near-field. Taxa richness ranged from 32 to 37 animals per sampling station with a mean richness of 34. Numerically, clams, snails and polychaete worms were dominant and comprised on average greater than 92% of total benthic invertebrate density.

Clams comprised about 39% of mean invertebrate abundance in the near-field. A total of 10 clam taxa were identified. Of these, *Tellina agilis* was the most numerically important accounting for about 20% of total invertebrate density. *Spisula solidissima* was also relatively abundant and accounted for about 15% of total invertebrate density.

Snails comprised approximately 33% of the mean total invertebrate density. A total of seven snail taxa were identified. The most abundant snail was *Acteocina canaliculata* comprising approximately 18% of the total invertebrate density. Other abundant snail taxa included *Haminoea solitaria*, *Boonea bisuturalis* and *Nassarius trivittatus* comprising a mean of 9.7%, 2.6% and 2.3% of the total invertebrate density, respectively.

Polychaete worms comprised about 20% of mean total invertebrate density. Free-moving polychaetes (the so-called “errantia”) were represented by 12 taxa and were on average slightly less abundant (% of mean total density) than sedentary polychaetes (the so-called “sedentaria”), which comprised 11 taxa and accounted for about 11% of mean invertebrate abundance. Of the errantia, *Protodriloides* was the most numerically important taxon, accounting for 7% of the mean total abundance in the near-field area. This taxon is a tiny worm found in the interstitial spaces between sediment grains (Read, 1996). *Spiophanes bombyx* was the most numerous of the sedentary polychaetes and accounted for about 3% of total density. The second most abundant sedentary polychaete was *Pygospio elegans* accounting for around 2% of the mean invertebrate density. Relatively low abundance of *Mediomastus* (1.4%) a deposit feeder that can be associated with organic enrichment may indicate that little enrichment occurs from mill effluent in the near-field area.

The only other taxonomic group that together comprised greater than 1% of mean total density in the near-field area was the forminiferans (amoebid protist).

5.4.2.2 Far-Field

Total invertebrate density in the far-field area was in the range 5,200 to 10,500 animals/m^2, with a mean density of 7,218 animals/m^2. A total of 45 distinct invertebrate taxa were identified in the area. Taxa richness ranged from 28 to 32 animals per sampling station with a mean richness of nearly 30. Numerically, snails, clams, polychaetes and

forminiferans were dominant and comprised on average greater than 98% of the benthic invertebrate density.

Snails were the most dominant taxa and comprised about 34% of mean total invertebrate density in the far-field area. A total of six snail taxa were identified. The most abundant snail was *Acteocina canaliculata*, which comprised 21% of mean total invertebrate abundance in the far-field area. *Nassarius trivittatus* and *Boonea bisuturalis* were also well represented accounting for mean total invertebrate abundance of 4.9% and 4.2%, respectively.

Clams comprised about 33% of mean invertebrate abundance in the far-field. A total of six clam taxa were identified. Of these, two were particularly abundant including *Tellina agilis* and *Spisula solidissima* which accounted for 17.6% and 14.4% of mean total abundance in the area, respectively.

Polychaete worms comprised about 19% of mean total invertebrate density. Free-moving polychaetes were represented by five taxa and accounted for about 6% of mean invertebrate abundance. Sedentary polychaetes, which comprised 13 taxa, accounted for about 13% of mean invertebrate abundance. Of the errantia, *Protodriloides* was the most numerically important taxon, accounting for about 5.5% of total invertebrate abundance. No other free-moving polychaete was found in relative abundance (>1% of total invertebrate abundance). *Mediomastus ambiseta* and *Spiophanes bombyx* were the numerically dominant sedentary polychaetes and accounted for about 3.3% of mean total invertebrate abundance each. Three other taxa of sedentary polychaete were relatively abundant (>1% of total abundance) and included: *Clymenella torquata*, *Scoloplos armiger* and *Pygospio elegans*.

Forminiferans, on average, comprised 12% of the total abundance. As mentioned above the density of this taxon may not be representative of the true community in each area. Some samples had large numbers of forminiferans.

5.4.2.3 Far Far-Field

Total invertebrate density in the far far-field area was more variable than the other sampling areas, and in the range of about 7,300 to over 40,000 animals/ m^2. Mean invertebrate density in the far far-field was 16,154 animals/m^2. One far far-field stations had an abundance that was more than double any of the other stations (i.e., 40,436 animals/m^2), two stations near the mean (i.e., 13,137 and 12,301 animals/m^2) and two stations were lower (i.e., 7,293 and 7,603 animals/m^2). A total of 65 distinct invertebrate taxa were identified in the area. Taxa richness ranged from 25 to 34 animals per sampling station with a mean richness of nearly 31. Numerically, polychaetes, clams and snails were dominant and comprised on average around 90% of benthic invertebrate density.

Polychaete worms comprised about 38% of mean total invertebrate density. Free-moving and sedentary polychaetes were each represented by 14 taxa and each accounted for

about 19% of mean invertebrate abundance. Of the errantia, *Protodriloides* was the most numerically important taxon, accounting for 15.4% of total invertebrate abundance. No other free-moving polychaetes were found with relative abundance of greater than 1% of total invertebrate abundance. Spiophanes bombyx was the numerically dominant sedentary polychaete and accounted for about 11.7% of mean total invertebrate abundance. *Paraonis fulgens* a worm that inhabits inorganic sands in shallow subtidal areas (Pearson and Rosenberg, 1978) and *Clymenella torquata,* a predominantly intertidal species, accounted for 3.2% and 2.1%, respectively. No other sedentary polychaete accounted for greater the 1% of total invertebrate abundance in the far far-field area.

Clams on average comprised about 31% of mean invertebrate abundance in the far far-field. A total of seven clam taxa were identified. Of these *Tellina agilis* and *Spisula solidissima* were the most numerically important. *T. agilis* and *S*. *solidissima* accounted for about 12.9% and 12.2% of the mean total abundance in the area. *Ensis directus* was another clam taxa that accounted for at least one percent of mean total invertebrate abundance in the far far-field (i.e., 2.1%).

Snails comprised about 20% of mean total invertebrate density. A total of eight snail taxa were identified in the far far-field area. *Acteocina* canaliculata was by far the most dominant snail taxa comprising 8.9% of the total invertebrate abundance. However, four other species *Crepidula fornicata* (3.3%), *Boonea bisuturalis* (2.9%), *Nassarius trivitattus* (2.7%) and Mitrella lunata (1.3%) were relatively abundant (i.e., comprised greater than 1% of total invertebrate abundance). The other three snail taxa were found in relatively low abundances (i.e. < 1% of total invertebrate abundance).

Roundworms and forminiferans comprised about 5.2% and 2.8% of the mean total invertebrate density, respectively.

No other taxonomic group comprised greater than 1% of mean total density in the far far-field area.

5.4.2.4 Merigomish Harbour (Reference)

Total invertebrate density at Merigomish Harbour was in the range of about 1,600 to 9,500 animals/ m^2, with a mean density of 5,162 animals/m^2. A total of 47 distinct invertebrate taxa were identified in the area. Taxa richness ranged from 24 to 29 animals per sampling station with a mean richness of 27. Numerically, polychaetes, clams and snails were dominant and comprised on average greater than 92% of benthic invertebrate density.

Polychaete worms comprised about 35% of mean total invertebrate density. Free-moving polychaetes were represented by 13 taxa and accounted for about 13% of mean invertebrate abundance. Sedentary polychaetes, which comprised 10 taxa, accounted for about 22% of mean invertebrate abundance. Of the errantia, *Protodriloides* was the most numerically important taxon, accounting for about 8.6% of total invertebrate abundance.

The only other free-moving polychaetes that were found with relative abundance of greater than 1% of total invertebrate abundance were *Nephtys bucera and Glycera*. Two sedentary polychaete taxa, *Clymenella torquata* and *Spiophanes bombyx* were relatively abundant whereas all other taxa comprised less than 1% of the total invertebrate abundance.

Snails comprised about 31% of mean total invertebrate density. A total of seven snail taxa were identified in samples from Merigomish Harbour. The most abundant taxon was *Acteocina canaliculata*, which accounted for about 27.6% of mean total density. *Nassarius trivitattus* (2.3%) also accounted for greater than 1% of total invertebrate density.

Clams on average comprised about 26% of mean invertebrate abundance at Merigomish. A total of four clam taxa were identified. Of these *Tellina agilis* was the most numerically important. *T. agilis* accounted for about 14.8% of mean total abundance in the area. The only other clam taxa that accounted for at least one percent of mean total invertebrate abundance at Merigomish Harbour was *Spisula solidissima*, which accounted for 9.6% of mean total invertebrate abundance.

Other taxonomic groups that together comprised greater than 1% of mean total density at Merigomish Harbour were the sand dollars and cumaceans. The sand dollar *Echinarachnius parma*, a burrowing deposit feeder associated with sand substrate (Pearson and Rosenberg, 1978) comprised about 2.1% of mean total density. Cumaceans, also called hooded shrimp, were represented by three taxa and accounted for 2.8% of the mean total invertebrate density. The most dominant of the four taxa of hooded shrimp was *Oxyurostylis smithi* (2.0%).

5.4.2.5 Logan's Point (Reference)

Total invertebrate density at Logan's Point was in the range of about 1,543 to 14,094 animals/ m^2, with a mean density of 7,703 animals/m^2. Three stations had densities of around 10,000 animals/m^2 with the other two stations having densities of less than 3,400 animals/m^2. A total of 60 distinct invertebrate taxa were identified in the area. Taxa richness ranged from 24 to 40 animals per sampling station with a mean richness of around 31. Numerically, polychaetes, clams and snails were dominant and comprised on average 87% of benthic invertebrate density.

Polychaete worms comprised about 42% of mean total invertebrate density. Free-moving polychaetes were represented by 14 taxa and accounted for about 29% of mean invertebrate abundance. Sedentary polychaetes, which comprised 13 taxa, accounted for about 13% of mean invertebrate abundance. Of the errantia, *Protodriloides* was the most numerically important taxon, accounting for about 24.7% of total invertebrate abundance. The only other taxon that comprised 1.0% or more was *Glycera*. No other free-moving polychaete was found with relative abundance of greater than 1% of total invertebrate abundance. *Para fulgens* (7.8%) and *Spiophanes bombyx* (1.7%) were the only two taxa of sedentary polychaetes that comprised > 1% of the relative mean total abundance.

Clams on average comprised about 36% of mean invertebrate abundance at Logan's Point. A total of eight clam taxa were identified. Of these *Tellina agilis* was the most numerically important. *T. agilis* accounted for about 19% of mean total abundance in the area. *Spisula solidissima* was only slightly less abundant (i.e., ~15%). *Enis* directus was another clam taxa that accounted for at least 1% of mean total invertebrate abundance at Logan's Point.

Snails comprised about 9.1% of mean total invertebrate density. A total of six snail taxa were identified in samples from Logan's Point. The most abundant taxon was *Acteocina canaliculata*, which accounted for 6.2% of mean total density. *Nassarius trivitattus* also accounted for around 1% of total invertebrate density. All other snail taxa comprised less than 1% of the mean total invertebrate abundance.

Other taxonomic group that comprised greater than 1% of mean total density at Logan's Point were sand dollars, roundworms and cumaceans. The sand dollar *Echinarachnius parma* comprised about 2.0%, roundworms comprise 5.7% and cumaceans comprised 1.4% of mean total density.

5.4.3 Statistical Comparisons of EEM Effect Endpoints

5.4.3.1 Area by Area Comparisons of Cycle 7 Data

Density

Overall, total invertebrate densities in the exposure areas (NF, FF, FFF) were within densities seen in the reference areas (Logan's Point, Merigomish Harbour). Total density was significantly higher in the near-field and far far-field than at Merigomish Harbour. The magnitudes of these differences were 86% or 1.5 reference standard deviations and 183% or 1.6 reference standard deviations, respectively (Table 5.10). There was no significant difference in invertebrate density between the Logan's Point reference area and any of the exposure areas, nor was there a difference between reference areas with respect to density.

Taxa Richness

Taxa richness in the exposure areas was generally higher than in the Merigomish Harbour reference area and within the range of richness seen in the Logan's Point area. All three exposure areas had significantly higher richness than the Merigomish Harbour reference area, whereas there was no statistical differences detected among the exposure areas and the Logan's Point reference area. The magnitude of difference between the near field, far field and far far-field and the Merigomish Harbour reference area were 27% and 3.3 reference area standard deviations, 11% and 1.4 reference area standard deviations and 17% and 2.0 reference area standard deviations, respectively (Table 5.10). No difference was detected between the two reference areas in terms of taxa richness.

Table 5.10: Summary of Comparisons of Cycle 7 5 ICS Effect Endpoints

Parameter	Comparison	p < 0.1?	Direction	Magnitude (%)	Magnitude (reference standard deviation)	Comments
Merigomish Harbour Reference						
Total Density	**Ref vs NF**	Yes	NF > Ref	86	1.5	
	Ref vs FF	No	-	-	-	
	Ref vs FFF[1]	Yes	FFF > Ref	183	1.6	
	Ref vs LPR	No	-	-	-	
Richness	**Ref vs NF**	Yes	NF > Ref	27	3.3	
	Ref vs FF	Yes	FF > Ref	11	1.4	
	Ref vs FFF	Yes	FFF > Ref	17	2.0	
	Ref vs LPR[2]	No	-	-	-	
Diversity	**Ref vs NF**	No	-	-	-	
	Ref vs FF	No	-	-	-	
	Ref vs FFF	No	-	-	-	
	Ref vs LPR	Yes	LPR < Ref	8	1.6	
Evenness	**Ref vs NF**	No	-	-	-	
	Ref vs NF[2]	No	-	-	-	Extreme outliers MHR-2 and MHR-4 removed
	Ref vs FF	No	-	-	-	
	Ref vs FF[2]	No	-	-	-	Extreme outliers MHR-2 and MHR-4 removed
	Ref vs FFF	No	-	-	-	
	Ref vs FFF	No	-	-	-	Extreme outliers MHR-2 and MHR-4 removed
	Ref vs LPR	No	-	-	-	
	Ref vs LPR	No	-	-	-	Extreme outliers MHR-2 and MHR-4 removed
Bray-Curtis	**Ref vs NF**[2]	Yes	NF > Ref	82	1.4	
	Ref vs FF	No	-	-	-	
	Ref vs FFF[3]	Yes	FFF > Ref	89	-	
	Ref vs FFF[2]	Yes	FFF > Ref	72	1.2	Extreme outlier FFF-1 removed
	Ref vs LPR	Yes	LPR > Ref	126	2.1	
Logan Point Reference						
Total Density	**Ref vs NF**	No	-	-	-	
	Ref vs FF	No	-	-	-	
	Ref vs FFF[1]	No	-	-	-	
	Ref vs MHR	No	-	-	-	
Richness	**Ref vs NF**[2]	No	-	-	-	
	Ref vs FF[2]	No	-	-	-	
	Ref vs FFF[2]	No	-	-	-	
	Ref vs MHR	No	-	-	-	
Diversity	**Ref vs NF**	Yes	NF > Ref	12	1.5	
	Ref vs FF	Yes	FF > Ref	11	1.4	
	Ref vs FFF	Yes	FFF > Ref	10	1.3	
	Ref vs MHR	Yes	MHR > Ref	8	1.1	
Evenness	**Ref vs NF**[2]	No	-	-	-	
	Ref vs FF	Yes	FF > Ref	49	1	
	Ref vs FFF	No	-	-	-	
	Ref vs MHR	No	-	-	-	
	Ref vs MHR	No	-	-	-	Extreme outliers MHR-2 and MHR-4 removed
Bray-Curtis	**Ref vs NF**	Yes	NF > Ref	25	1.9	
	Ref vs FF	Yes	FF > Ref	20	1.5	
	Ref vs FFF	Yes	FFF > Ref	30	2.2	
	Ref vs MHR	Yes	MHR > Ref	19	1.4	

1 - Abundance (log) used for statistical tests
2 - Analyzed using T-test assuming unequal variance
3 - Analyzed using Non-parametric Mann-Whitney U-test due to non-normality

Evenness

Generally, evenness was low (~ 0.1 to 0.3) across the study area reflecting the numerical dominance of relatively few taxa in all samples.

Evenness was not significantly different between any of the exposure areas and the Merigomish Harbour reference area or between the reference areas, whereas the far-field area had greater evenness than the Logan's Point reference area. The magnitude of the significant difference between the far-field area and Logan's Point was 49% or 1.0 reference standard deviations.

Bray-Curtis

The Bray-Curtis Dissimilarity Index analysis yielded a number of significant differences between the exposure areas and reference area median condition for both reference areas. B-C scores for near-field and far far-field areas were significantly higher than the Merigomish Harbour and Logan's Point reference areas. The magnitude of difference was 82% and 1.4 reference area standard deviations and 89% (reference area standard deviations are not calculable) for the Merigomish Harbour comparisons, respectively, and 25% and 1.9 reference areas standard deviations and 30% and 2.2 reference standard area deviations for the Logan's Point reference condition, respectively. B-C scores in the far-field area were significantly higher than the Logan's Point reference condition by 20% and 1.5 reference area standard deviations but not different than the Merigomish Harbour reference condition. The reference areas were significantly different than the other reference area median condition. The Merigomish Harbour reference area was different than the Logan's Point reference condition by 126% and 2.1 reference area standard deviations, whereas the magnitude of difference was 19% and 1.4 reference area standard deviations based on the opposite comparison.

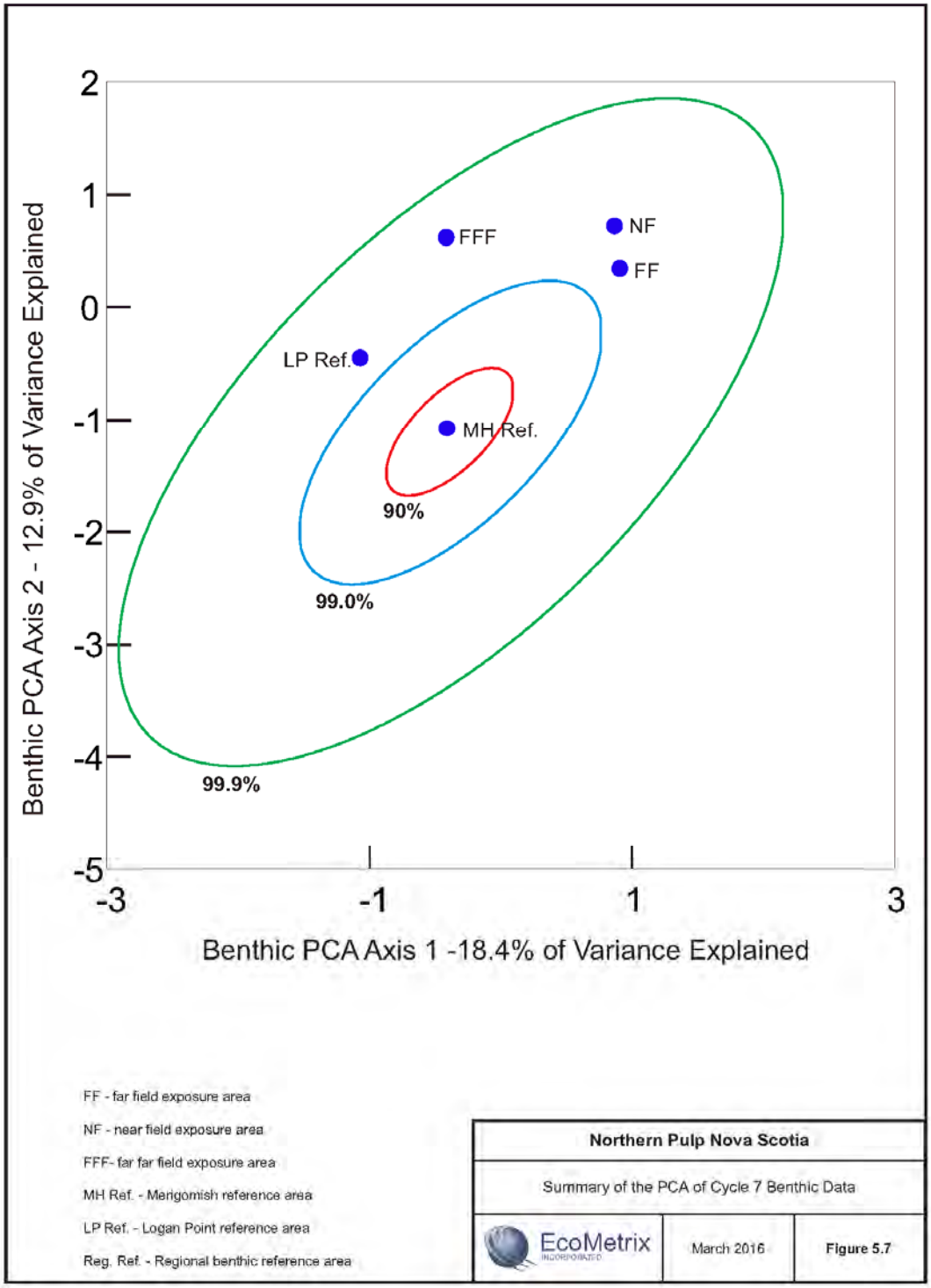
Principal Components Analysis

Overall, the PCA solution was not particularly strong. Together, PCA axes 1 and 2 explained only 31.3% of the variance in the invertebrate community data.

Figure 5.7 shows the average PCA axis 1 and axis 2 scores for each of the ICS sampling areas, as well as the 90%, 99% and 99.9% probability ellipses as defined by the reference station data. It is important to note that the reference was comprised of only five stations from Merigomish Harbour in Cycle 7 rather than 10 as used in Cycles 4 and 5. This explains why the ellipses are smaller this cycle.

On average, all three exposure areas and Logan's Point were inside the 99.9% ellipse but outside the 99.0% ellipse and by definition are considered "different" from the reference. As mentioned the two axes of the PCA only explained 30% of the variance and therefore should not be heavily relied upon for interpretation of a mill effect. Logan's Point is another

reference area used historically and it is as different from the Merigomish Harbour reference as the three mill effluent expose areas.



5.4.3.2 Cycle-to-Cycle Comparisons of Endpoint Data

No significant interaction term was noted in the two-way ANOVA for invertebrate density, taxa richness and B-C using the Merigomish Harbour reference area indicating any changes in these endpoints over time were similar (magnitude and direction) across the study area (Table 5.11).

Evenness, the supporting endpoint Diversity and B-C using the Logan's Point reference area resulted in significant interaction terms (monitoring cycles*sampling areas) in the two-way ANOVA (Table 5.11). This means that the shift in the endpoint in all areas was not in the same direction and magnitude over time.

Evenness increased between Cycles 5 and 7 for both reference areas and at the far-far field area, whereas it was unchanged in the near-field and far-field areas.

The B-C values compared to the Logan's Point reference condition were quite varied between cycles. The value increased by around 0.15 from Cycle 5 to Cycle 7 for the Logan's Point reference, and the far-far-field areas, whereas it only slightly increased in the Merigomish Harbour reference. The near field and far-field areas B-C values decreased between the two cycles. The lack of a consistent pattern is the cause of the significant interaction term in the two-way ANOVA (Table 5.11).

For Simpson's Diversity there was an increase in all areas except in the far field, which remained relatively unchanged between cycles.

Table 5.11: Two-way ANOVA Summary of Cycle-to-Cycle ICS Effect Endpoint Comparisons

Dependent Variable:Total Invertebrate Abundance (Log)					
Source of Variation	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*P-value*
Area	0.778	4	0.195	2.540	**0.055**
Period	0.080	1	0.080	1.039	0.314
Area * Period	0.465	4	0.116	1.519	0.215
Error	3.063	40	0.077		
Dependent Variable:Taxa Richness					
Source of Variation	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*P-value*
Area	353.720	4	88.430	2.757	**0.041**
Period	15.680	1	15.680	0.489	0.489
Area * Period	264.120	4	66.030	2.058	0.104
Error	1283.200	40	32.080		
Dependent Variable:Simpson's Evenness					
Source of Variation	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*P-value*
Area	0.110	4	0.027	5.644	**1.07E-03**
Period	0.050	1	0.050	10.298	**2.62E-03**
Area * Period	0.043	4	0.011	2.232	**8.27E-02**
Error	0.195	40	0.005		
Dependent Variable:Simpson's Diversity					
Source of Variation	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*P-value*
Area	0.381	4	0.095	11.901	**1.84E-06**
Period	0.248	1	0.248	30.921	**1.96E-06**
Area * Period	0.204	4	0.051	6.355	**4.68E-04**
Error	0.321	40	0.008		
Dependent Variable:Bray-Curtis Dissimilarity Index - Logan's Point Reference Median Condition					
Source of Variation	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*P-value*
Area	0.514	4	0.128	9.642	**1.48E-05**
Period	0.078	1	0.078	5.867	**0.020**
Area * Period	0.156	4	0.039	2.934	**0.032**
Error	0.533	40	0.013		
Dependent Variable:Bray-Curtis Dissimilarity Index - Merigomish Harbour Reference Median Condition					
Source of Variation	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*P-value*
Area	0.342	4	0.086	3.108	**0.026**
Period	0.005	1	0.005	0.173	0.680
Area * Period	0.135	4	0.034	1.229	0.314
Error	1.102	40	0.028		

5.5 Discussion

5.5.1 Cycle 7 ICS Data

In Cycle 7 there were a number of statistical differences detected between the reference and exposure areas. The detected differences were apparent for comparisons to both the Logan's Point and Merigomish Harbour reference areas. However, there were more

differences detected between the exposure areas and the Merigomish Harbour reference area than between the exposure areas and the Logan's Point reference area. In all instances of statistical difference the exposure areas had higher values than the reference areas.

There were no statistical difference between Logan's Point and any exposure area for density or richness whereas the near-field and far-field areas had significantly higher density and all three exposure areas had significantly higher richness compared to the Merigomish Harbour reference area. Diversity, a supporting endpoint was significantly lower at Logan's Point than the all three exposure areas and the Merigomish Harbour reference area whereas there was no difference between the exposure areas and Merigomish Harbour.

The Bray-Curtis comparisons were suggestive of differences in community structure among the sampling areas; however these differences were not limited to exposure vs. reference comparisons, as using Logan's Point as the median also lead to a significant difference from the Merigomish Harbour reference area and vice versa.

From a magnitude perspective three of the differences detected were above the level at which ecologically significant differences are implied (> 2 reference standard deviations is ecologically significant; Environment Canada, 2010) therefore the differences could possibly be ecologically meaningful. Richness in the near-field area was greater than the Merigomish Reference areas by 3.3 reference area standard deviations. The other two comparisons exceeded 2 reference areas standard deviations were the Bray-Curtis between the two reference areas using Merigomish Harbour as the reference condition and between the Bray-Curtis between the far-far-field area and the Logan's Point reference condition.

Some of the major taxa were ubiquitous across the study area and found in similar densities among all sampling locations (e.g., the clams *Tellina agilis* and *Spisula solidissima*, the snail *Acteocina canaliculata*), others were not. For the most part, however, the differences in community structure among sampling areas were associated with, albeit not exclusively, differences in abundance of major taxa as opposed to the presence and absence of taxa. For example, the polychaete *Protodriloides* was present in all sampling areas but differed in abundance among the sampling areas. *Protodriloides* was the least abundant in the Merigomish Harbour and far-field areas, moderately abundant in the near-field area and highly abundant in the Logan's Point and far far-field areas. The sedentary polychaete *Spiophanes bombyx* was equally abundant in the near-field, far-field and both reference areas but more than twice as abundance in the far far-field area. The snail *Nassarius trivittatus* was less abundant in the Logan's Point area compared to all other areas.

These community differences seem to be related to the feeding habits of the resident benthos. Generally speaking, the near-field and far-field areas had a larger proportion of

deposit feeding taxa than the other areas. This same pattern was seen in the Cycle 3 and 5 studies (Stantec, 2004a, EcoMetrix, 2007a, 2010).

The outlet of Boat Harbour is somewhat sheltered, especially the near-field and far-field areas, and is therefore the natural depositional zone for the estuary. Sediment chemistry data indicate, however, that organic material does not accumulate within these areas to a greater extent than other nearby locations (e.g., Logan's Point). Overall, the nature of differences in benthic invertebrate assemblages seen in the study may be related to or associated with subtle habitat differences among the sampling areas and do not seem to be mill related. Habitat differences, a buoyant effluent that has limited contact with benthic organisms and statistical results that indicate the two reference areas are as different from each other as the exposure areas are from the Merigomish Harbour reference indicate the mill is not likely the cause of the differences observed during the Cycle 7 EEM ICS.

5.5.2 Temporal Comparisons of Benthic Community Structure

Cycle-to-cycle statistical comparisons of the benthic data indicated that there has not been a substantial change in total invertebrate abundance, taxa richness or the Bray-Curtis across the study area over time. There was a significant change in evenness, reflected in increases in the reference areas and in the far-far-field, but constant numbers in the near field and far field. A significant difference was also detected for diversity which reflected an increase in the all areas except the far-field that remained relatively unchanged since Cycle 5. An increase in the Bray-Curtis values when using the Logan's Point reference condition in the Merigomish reference, far-far-field and Logan's Point comparisons and slight decreases in the near field and far-field areas were the cause of the temporal change.

On an individual taxon level, the temporal patterns in the relative abundance of the major taxa in the far far-field area and the Logan's Point reference area seem to mimic each other (Figure 5.8). In both locations, the relative abundance of polychaetes increased between Cycles 3 and 4 then remained similar for Cycle 5 followed by a decrease in Cycle 7. The opposite trend was generally observed for the relative abundance of clams and snails. In Cycle 7 polychaetes decreased in all areas by similar amounts, a pattern not before reported and potentially an indication of a region wide change.

Snail increased in abundance in all areas between Cycle 5 and Cycle 7 and with the exception of Logan's Point this increase was of similar magnitude. Clams did not show a consistent pattern in the reference or exposure areas. The near-field and far-field have followed a similar pattern since Cycle 2 with a decrease between Cycle 2 and 3, and increase between Cycle 3 and Cycle 4 and smaller increase between Cycle 4 and 5 and a decrease between Cycle 5 a Cycle 7. With the exception of a slight increase between Cycle 4 and Cycle 5 clams in the far-far-field have showed the opposite trend to the other exposure areas. Similar to snails and polycheates Logan's Point trend for clams are similar to the far-far-field whereas in Merigomish Harbour clams have remained relatively stable since Cycle 2 (Figure 5.8).

The fact that general temporal patterns seen in the two sampling area groupings (Merigomish, near-field, far-field and Logan's Point, far far-field) were similar with the habitat types in the areas. The Merigomish, near-field and far-field areas are likely more depositional in nature, and the relative prominence of deposit feeding benthic taxa in each of these areas is supportive of this notion. In contrast, the far far-field area and Logan's Point are more exposed. Taken on balance, the measured cycle-to-cycle changes seen at Pictou are not interpreted as being a mill-related phenomena, but rather part of a natural variability in benthic community structure in the study area.

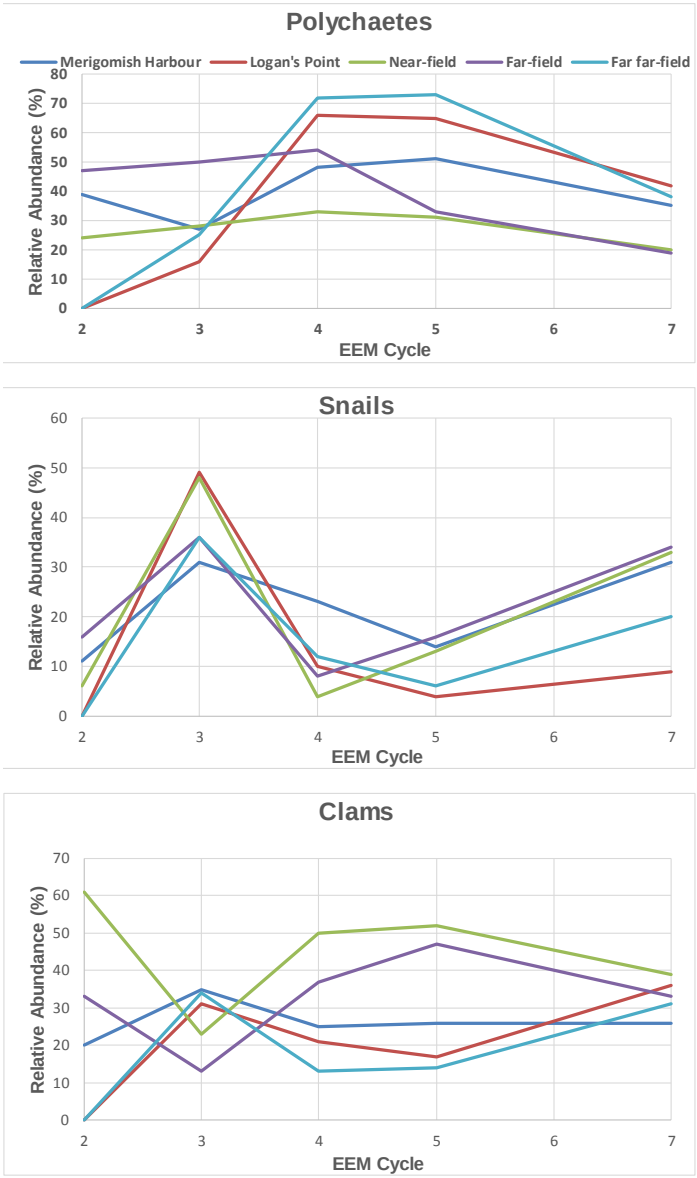


Figure 5.8: Summary (% Abundance) of Major Taxonomic Groups Collected in EEM Cycle 2 through 7

6.0 FISH SURVEY AND FISH USE

6.1 Objectives

For the purposes of EEM, the assessment of potential effluent-related effects on fish includes an evaluation of fish health and an assessment of fisheries resource usability. The objectives of each component are described below.

6.1.1 Fish Survey

The objective of fish monitoring in the EEM program is to determine whether or not mill effluent has an "effect" on the health of fish populations in the mill's effluent receiving environment. This assessment is accomplished by evaluating the growth, reproduction, survival and condition of representative fish populations in reference and effluent exposed areas.

6.1.2 Fisheries Resource Use

For pulp and paper EEM, fish resource usability is evaluated through the measurement of chlorinated dioxin and furan concentrations in the edible portion of a given fish species. Mills that use or have used chlorine bleaching may be required to conduct these analyses if dioxins and furans are an issue for the receiving environment.

Over the first three cycles of EEM, fish usability was also evaluated on the basis on the potential for mill effluents to taint fish. As tainting was not identified as an issue of national concern the requirement for tainting evaluations as part of EEM has been discontinued.

6.2 Results of Previous EEM Studies

6.2.1 Fish Survey

6.2.1.1 EEM Cycle 1

The Cycle 1 Fish Survey included the collection of both male and female rock crab and winter flounder (JWEL, 1993a, b, 1996). The exposure area was located in Pictou Road between Powell's Head and Mackenzie Head and the reference area was situated in the vicinity of Merigomish Harbour (Figure 6.1).

Rock crabs were collected using crab traps on June 6, 1995. Adequate numbers of male and female rock crabs were collected to satisfy EEM requirements (i.e., 20 in each area). Collection of winter flounder was considerably more difficult. Three field trips were necessary to collect eighty winter flounder (June 6, July 11-13 and September 26). Almost all flounder were captured on the latter date using otter trawls. The near absence of flounder in June and July raised doubts as to whether fish captured in the exposure area were exposed to effluent for any length of time. Adequate numbers of female flounder were

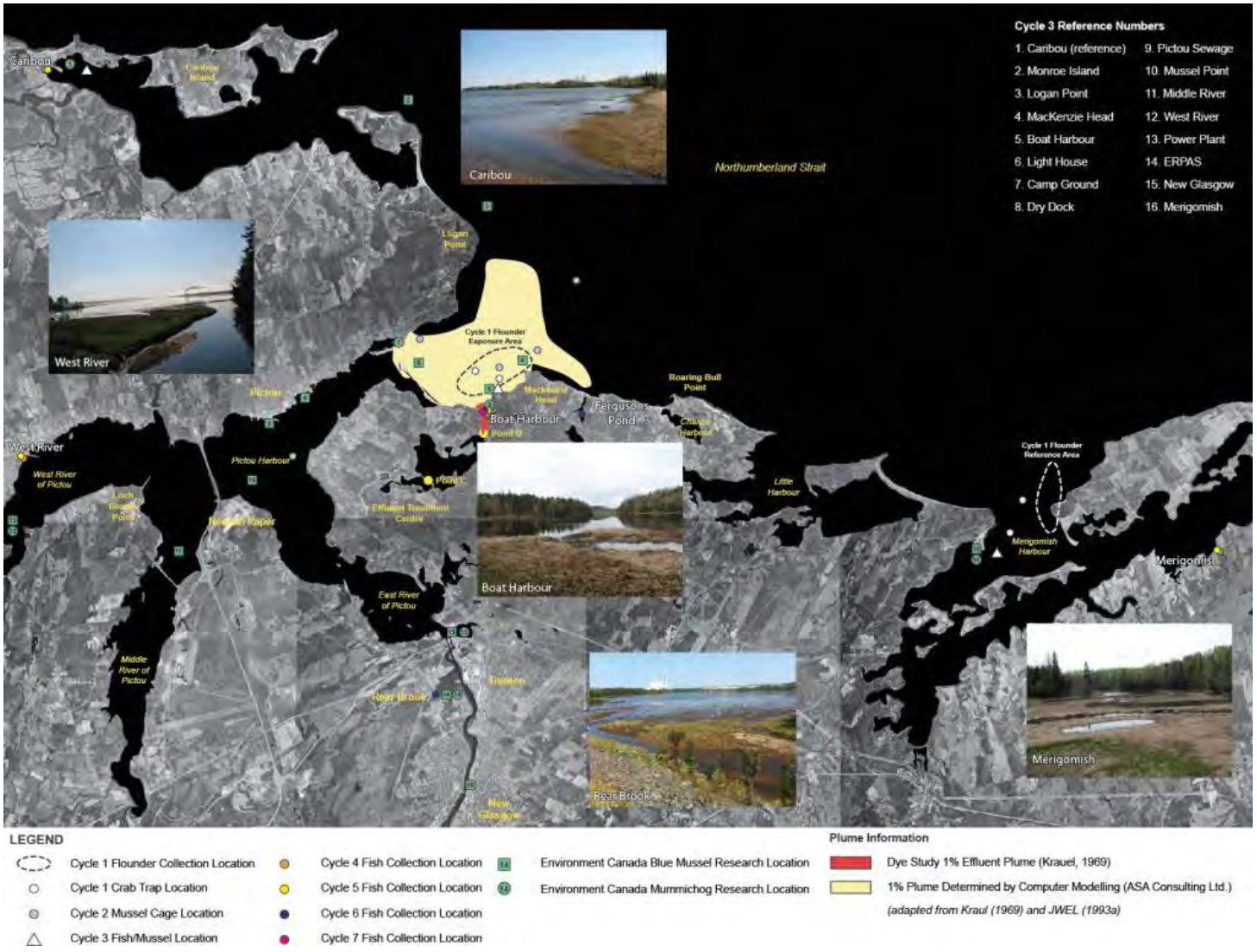


Figure 6.1: Sampling Locations for EEM Fish and Mussel Collections

collected to satisfy EEM requirements (i.e., 20 in each area). Insufficient numbers of males were captured.

Tracer measurements (resin and fatty acids (RFAs) in fish livers and crab hepatopancreas and chlorinated phenols in water) did not confirm exposure of exposure area fish nor did they confirm non-exposure of reference fish.

Comparative statistical analyses indicated that there was virtually no difference in the measured parameters between rock crabs in the reference and exposure areas. One exception to this was that male crabs in the reference area had slightly larger carapace widths than their exposure area counterparts. No age related comparisons in rock crabs were possible, as crabs do not have reliable ageing structures.

Winter flounder from the exposure area (Pictou Road) were slightly smaller and an average of one year younger than those captured in the reference area. Female flounder were slightly larger in all parameters relative to males captured in the same location; however, these comparisons were based upon extremely low numbers of males.

6.2.1.2 EEM Cycle 2

In conjunction with Environment Canada, NPNS fulfilled their Cycle 2 regulatory obligation through involvement in a caged bivalve pilot project (Andrews and Parker, 1999). Five mussel (*Mytilus edulis*) cages, each containing 400 mussels, were deployed at locations in Pictou Harbour and Pictou Road (Figure 6.1). The study utilized a 90-day exposure period (July to October 1998). Measured physiological/morphological endpoints included survival, whole animal wet weight (WAWW), shell length and tissue chemistry.

Based on the survival, growth and bioaccumulation measurements made on caged blue mussels during the study, NPNS effluent did not adversely affect fish health in Pictou Road. The study did suggest that the area in the vicinity of the Boat Harbour discharge might be subjected to increased nutrients and increased productivity from the treatment plant effluent.

6.2.1.3 EEM Cycle 3

In 2002 NPNS participated in an Environment Canada study that specifically compared the response of caged bivalves (blue mussels) and fish (Mummichog) to exposure to the mill's effluent to fulfill its Cycle 3 obligations. The objective of the study was to evaluate whether the response(s) of fish and caged bivalves to effluent exposure was similar, as the caged bivalve study that had recently been approved by the EEM Science Committee for use in EEM as a fish survey alternative.

The fish and caged bivalve components of the study followed similar designs. Samples (i.e., mussels or fish) were collected from three sampling areas including Boat Harbour Estuary (effluent-exposed), Merigomish Harbour (reference) and Caribou Island (reference)

(Figure 6.1). For each species, key morphometric endpoints (survival, growth, energy storage, reproduction) were used to compare the potential effects of exposure to NPNS effluent. The results associated with the study components are provided below.

Results

Supporting Measures

Generally, colour was an effective means by which to measure relative effluent concentrations. A consistent pattern emerged from the data that indicated that nearshore areas at Pictou Road were exposed to effluent concentrations of about 5%, and up to 15%. Effluent concentrations decreased with distance from shore such that effluent concentrations were often measurable above background, but less than 1%, at a distance of about 1 km out from shore. These colour data confirmed the results of previous studies that indicated that NPNS effluent travels out from the discharge along the surface of the water within a thin ribbon extending to the northeast (Cranford, pers. comm., 1998, 2001).

The influence of the discharge from the Boat Harbour Effluent Treatment Facility (including inputs from the facility's watershed) on conventional water chemistry measures in the receiving environment was also apparent, especially early in the spring during the freshet. Salinity of the water within the nearshore area of Pictou Road was as low as 3 to 5 ppt vs. 17 ppt at Merigomish and 33 ppt at Caribou. Dissolved oxygen saturation was in the order of 80% at Pictou Road vs. 100% at Merigomish and Caribou. Water temperatures tended to be a degree or two warmer at Pictou Road than at Merigomish, although both locations were typically 5ºC warmer than Caribou, likely the result of the freshwater inputs at both Pictou Road and Merigomish.

Mussels

Differences between effluent-exposed and reference area mussels were detected for measures of both condition (CF) and the gonadal somatic index GSI (Appendix E, Table E.1). CF was greater in exposure area mussels, whereas GSI was lower. These results seemed to be consistent with a mild/moderate enrichment effect within the vicinity of Boat Harbour in Pictou Road. Differences measured in GSI were not differences in absolute gonad tissue growth (which was similar among the sampling areas). Rather, the differences detected in GSI were the result of differences in gonad tissue growth relative to somatic tissue growth. Overall, there was more absolute somatic growth in effluent-exposed mussels than in mussels in the reference areas. The greater amount of somatic growth in mussels deployed in the vicinity of Boat Harbour was likely the result of nutrient inputs from Boat Harbour, and was consistent with past observations. For example, Andrews and Parker (1999) suggested that there was some evidence of higher growth rates in caged mussels in the vicinity of the Boat Harbour discharge, as the area might be subjected to increased nutrients and increased productivity from the treatment plant effluent. Further, dissolved organic carbon (DOC) in water has been used at this site as a means to track

effluent dispersion in Pictou Road as DOC levels are high enough in effluent relative to background to spatially characterize dispersion to levels of about 1% v/v (Peter Cranford, pers. comm., 1998, 2001).

Mummichog

Fish numbers were relative low at Boat Harbour Outer (Appendix E, Table E.2). A total of eighteen seine hauls over a seven day period were required to collect the requisite number of fish. Conversely, more than ten times this number of fish was collected at both Caribou and at Merigomish in a single sampling effort.

A summary of the results of the statistical tests of fish endpoint comparisons is provided in Appendix E, Table E.3. The most conspicuous difference among the effluent-exposed and reference area Mummichog was the age distribution of the populations. Fish collected at Boat Harbour were almost exclusively 1+, whereas the fish at both Caribou and Merigomish were largely 2+ and, to a lesser extent 3+. It is reasonable to assume that the uneven age distribution of the study populations biased the results of the statistical comparisons to some degree. The tests used to compare the effect endpoint data assume that growth patterns are linear (or log–linear) in nature and therefore relatively constant throughout the life cycle of the average individual. However, with a short-lived fish such as the Mummichog there is no reason to believe that this is necessarily the case (see Abraham, 1985).

Because of the limited age distribution of the exposure Mummichog population, the statistical tests used in the study had essentially compared fish at different stages of development. It is conceivable that growth patterns differ within the life cycle of the Mummichog to the extent that at least some of the differences seen among exposure and reference populations were the result of an age dependent sampling bias. For instance, LSI was higher among effluent-exposed fish than those collected at either of the reference areas. Increased relative liver size is typically explained as being related to the liver's role in energy storage (i.e., as a sink for glycogen – a biological energy source) or the liver's role in the detoxification. Given that mill effluent had no sublethal effects on fish (*Menidia beryllina*) in laboratory testing, it seemed perhaps unlikely that the increased LSI in exposure area fish was in some way related to effluent toxicity. The most simple, although untested, explanation for the difference detected in LSI was that the reference and exposure fish populations have differing liver function largely because they were at different life history stages and therefore had different metabolic requirements (i.e., the 1+ fish have greater energy stores than the 2+ and 3+ fish).

In a similar fashion, increased female GSI (relative to reference) at Boat Harbour Estuary could have been an artifact of the age bias among the sampling areas. An alternative explanation of the difference in GSI among female study area fish may be related to the fact that the fish at the reference area were collected over such a narrow time frame (hours). The underlying assumption of the comparison of the reproductive status of fish from

different sampling areas as done in EEM is that the fish from all sampling areas are, from a timing point of view, synchronized with respect to the development of reproductive tissue. When fish are collected over a relatively long period (e.g., week or weeks), differences in the rate of development that can occur within a local geographical area might be blurred or lessened somewhat. Conversely, when fish are collected over a relatively short timeframe, any local variability in the rate of tissue development will be highlighted and captured within sample collections. Data collected by Environment Canada as part of the research in the Pictou area strongly suggested that this in fact may be a plausible explanation as to why differences in GSI were seen among Mummichog in the sampling area in the 2002 study (St.-Jean, pers. comm., 2004). Fish collected from these sampling areas over several consecutive weeks indicated that there was considerable variability in the rate at which reproductive tissues developed at different locations in the study area (St.-Jean, pers. comm., 2004).

Discussion of the age-distribution bias confounding the interpretation of the nature and magnitude of differences between exposure and reference fish would be incomplete without some recognition that the age differences observed could itself have been an effluent related effect. This is especially true when it is considered that this same age distribution had been measured in the study area in previous studies of the same species (St.-Jean, pers. comm., 2004), in the study area in previous studies of different species (winter flounder; JWEL, 1996) and even at other maritime sites at which EEM surveys have been completed (e.g., Stantec, 2004c). Changes in the age structure of a fish population are commonly reported as the result of some stress related event or set of circumstances in receiving environments (Gibbons and Munkittrick, 1994). Age in the EEM context is used as a potential indicator of survival, or alternatively recruitment success/failure. Of these two scenarios, a lower mean age is likely to result from adult mortality, whereas recruitment failure leads to an older population. These types of generalized responses were developed by observations of relatively long-lived fish and may not hold true for fish like the Mummichog that have short life spans. Nevertheless, the age difference seen among the Mummichog populations in the study area had to be the result of some causative agent. Mummichog in the exposure area were either not surviving beyond their first spawning season or they were leaving the area after their first spawning season, with this niche being filled by the young-of-the-year from the previous season. The former explanation seems very unlikely though, as no sublethal effects had been measured in laboratory test fish (silversides) over the previous ten years. The degree to which the latter explanation (dispersal and recolonization) may have been true was untested, prior to Cycle 6, with no empirical data to support or refute it.

Comparison of Blue Mussel and Mummichog Endpoint Responses

Although data collected during the 2002 study indicated that both Mummichog and mussels “responded” to effluent exposure (or at least there were measurable differences between exposure and reference fish/mussels), the nature of the response was somewhat different between the species (Appendix E, Table E.4). Measures of growth were not different for either

mussels or fish among the sampling areas. CF was higher in exposure mussels than in reference mussels, whereas no differences in CF were measured in fish within the entire study area. Exposure area fish had higher LSI than reference area fish. An equivalent measure of LSI for mussels did not exist at that time, but was in development (St.-Jean, pers. comm., 2004). GSI was lower in effluent-exposed mussels than in reference mussels. There was no difference in GSI for Mummichog males across the study area, whereas females collected in the exposure area had higher GSI than females collected in the reference areas.

The response signal measured in mussels following the 90-day exposure period appeared to be clearer than what was seen in Mummichog. A mild/moderate enrichment effect as the result of the mill effluent was consistent with the results of previous studies at this site (e.g., Andrews and Parker, 1999; Cranford, pers. com., 1998, 2001). While not discounting the potential that the difference seen in age distribution in the Mummichog among the sampling areas was a discharge related phenomena, the fact that there was little overlap in the age distribution between reference and exposure fish greatly reduced the interpretive power of the data.

6.2.1.4 EEM Cycle 4

The Cycle 4 survey focused on the collection of small fish (Mummichog) from Boat Harbour Estuary (exposure area) and three reference areas in the greater Pictou area (Merigomish, Caribou Island, and West River of Pictou) during May 2006 (Figure 6.1). Collections made at the study areas are summarized in Table 6.1. A summary of statistical comparisons of the key effort endpoints are summarized in Table 6.2 (males) and Table 6.3 (females).

Overall exposure area Mummichog showed no signs of growth or reproductive impairment, but as a group both male and female exposure area fish were younger than their cohorts in the reference areas. In addition, female Mummichog from Boat Harbour Estuary had increased liver size relative to the three reference areas.

Table 6.1: Summary of Seine Hauls for NPNS EEM Cycle 4 Fish Survey – May 2006

Common name	Scientific name	West River	Caribou	Merigomish	Boat Harbour
Threespine Stickleback	*Gasterosteus aculeatus aculeatus*	98	593	43	0
Ninespine Stickleback	*Pungitius pungitius*	120	219	2	0
Fourspine Stickleback	*Apeltes quadracus*	0	1	5	0
Blackspotted Stickleback	*Gasterosteus wheatlandi*	1	192	20	0
Creek Chub	*Semotilus atromaculatus*	1	0	0	0
White Sucker	*Catostomus commersonii*	8	0	0	0
Banded Killifish	*Fundulus diaphanus diaphanus*	11	0	0	0
Northern Redbelly Dace	*Phoxinus eos*	3	0	0	0
Atlantic Silverside	*Menidia menidia*	0	0	32	0
Mummichog	*Fundulus heteroclitus heteroclitus*	2,885	1,811	1,210	266
	Total	**3,127**	**2,816**	**1,312**	**266**
	Area (m^2)	**100**	**68**	**110**	**379**
	CPUA[1, 2] (fish/m^2)	**28.9**	**26.6**	**11.0**	**0.7**

[1] CPUA is catch-per-unit-effort
[2] CPUA calculated using only mummichogs

Table 6.2: Summary of Statistical Comparisons of EEM Effect Endpoints for Male Mummichog Collected at Pictou in EEM Cycle 4 (where statistical difference were detected the magnitude of the difference (%) is indicate in parentheses)

Effect Endpoint	Car vs. BH	Mer vs. BH	WR vs. BH	Car vs. Mer	Car vs. WR	Mer vs. WR
Age	BH < Car (29.9)	BH < Mer (31.6)	BH < WR (16.9)	NS	WR < Car (15.6)	WR < Mer (17.7)
Condition	SI[1]	BH < Mer (4.3)	BH < WR (13.0)	NS	Car < WR (12.8)	Mer < WR (11.3)
Relative Liver Size	NS	Mer < BH (42.6)	WR < BH (26.0)	Mer < Car (35.7)	WR < Car (23.2)	Mer < WR (33.2)
Size-at-age	NS	SI[2]	BH < WR (12.5)	SI[3]	Car < WR (23.8)	SI[4]
Relative Gonad Size	Car < BH (7.2)	Mer < BH (29.8)	WR < BH (25.8)	Mer < Car (11.7)	WR < Car (8.9)	Mer < WR (12.7)

NS = not significant

WR = Caribou; Mer = Merigomish Harbour; WR = West River; BH = Boat Harbour

[1] Based on the covariate plots it appears as though Caribou fish increase in weight at a faster rate than Boat Harbor fish, however this relationship may be skewed as there were no larger, older fish collected at Boat Harbour.

[2] There seemed to be little growth (weight gain) in Merigomish fish between 3 and 4 years, and as a result the covariate relationship (size-at-age) between Merigomish and Boat Harbour was not equivalent.

[3] There seemed to be little growth (weight gain) in Merigomish fish between 3 and 4 years, and as a result the covariate relationship (size-at-age) between Merigomish and Caribou was not equivalent.

[4] There seemed to be little growth (weight gain) in Merigomish fish between 3 and 4 years, and as a result the covariate relationship (size-at-age) between Merigomish and West River was not equivalent.

Table 6.3: Summary of Statistical Comparisons of EEM Effect Endpoints for Female Mummichog Collected at Pictou in EEM Cycle 4 (where statistical difference were detected the magnitude of the difference (%) is indicate in parentheses)

Effect Endpoint	Car vs. BH	Mer vs. BH	WR vs. BH	Car vs. Mer	Car vs. WR	Mer vs. WR
Age	BH < Car (35.8)	BH < Mer (34.9)	BH < WR (30.0)	NS	WR < Car (8.2)	NS
Condition	NS	NS	BH < WR (14.2)	Mer < Car (6.2)	Car < WR (10.4)	Mer < WR (17.6)
Relative Liver Size	Car < BH (19.5)	Mer < BH (68.2)	WR < BH (27.3)	Mer < Car (25.2)	WR < Car (10.4)	Mer < WR (12.6)
Size-at-age	SI[1]	SI[2]	SI[3]	Mer < Car (19.5)	Car < WR (20.9)	Mer < WR (47.6)
Relative Gonad Size	NS	BH < Mer (27.5)	WR < BH (62.3)	NS	WR < Car (55.0)	WR < Mer (56.3)
Fecundity	Car < BH (73)	Mer < BH (70)	WR < BH (56)	NS	Car < WR (15)	NS

NS = not significant

Car = Caribou; Mer = Merigomish Harbour; WR = West River; BH = Boat Harbour

[1] BH fish were lighter at a given age than Car fish, however BH fish were larger at age 2 than Caribou fish. The lighter weight at older ages is likely an artifact of a younger age distribution and limited measures of older fish at BH.

[2] BH fish gain weight more slowly as they age than Merigomish fish, although the lack of older fish at BH likely exaggerated this trend.

[3] BH fish gain weight more slowly as they age than WR fish, although the lack of older fish BH likely exaggerated this trend.

6.2.1.5 EEM Cycle 5

The Cycle 5 EEM study at NPNS followed the same design as Cycle 4 in order to confirm (or refute) the response patterns apparent in Cycle 4 (i.e., increased liver size in exposure females and decreased age in both sexes of exposure fish). Table 6.4 summarizes the fish

catches during the Cycle 5 EEM. A summary of statistical comparisons of the key effort endpoints are summarized in Table 6.5 (males) and Table 6.6 (females).

The results of the Cycle 5 survey were increased liver size in both sexes of Mummichog from Boat Harbour Estuary, increased reproductive investment (i.e., relative gonad size) in female Mummichog at Boat Harbour Estuary and possibly a reduced population age structure especially in males. Similar response patterns were seen in Cycles 3 and 4, which would seem to provide further evidence as to their validity. The sex in which the endpoint was significantly different may have been variable between studies but a pattern indicating a mill related effect appeared to be present in Boat Harbour Estuary Mummichog.

Table 6.4: Summary of Seine Hauls for NPNS EEM Cycle 5 Fish Survey – May 2009

Common name	Scientific name	West River[1](2)	Caribou (2)	Merigomish (1)	Boat Harbour (11)
Threespine Stickleback	*Gasterosteus aculeatus*	1017	388	136	0
Ninespine Stickleback	*Pungitius pungitius*	237	128	10	4
Fourspine Stickleback	*Apeltes quadracus*	0	2	2	1
Blackspotted Stickleback	*Gasterosteus wheatlandi*	605	229	94	0
Rainbow Smelt	*Osmerus mordax*	25	0	0	0
White Sucker	*Catostomus commersonii*	25	0	0	0
Banded Killifish	*Fundulus diaphanus*	3	0	0	0
Grubby	*Myoxocephalus aenaeus*	0	0	0	1
Atlantic Silverside	*Menidia menidia*	13	0	3	0
White Perch	*Morone americana*	0	0	0	1
Atlantic Salmon	*Salmo salar*	1	0	0	0
Mummichog	*Fundulus heteroclitus*	3,013	1,549	1,481	2,426
	Total	**4,939**	**2,296**	**1,726**	**2,433**
	Area (m^2)	**80**	**31**	**30**	**960**
	CPUA[2, 3] (fish/m^2)	**37.7**	**50.0**	**49.4**	**2.5**

1 Total number of seine hauls in parenthesis
2 CPUA is catch-per-unit-area
3 CPUA calculated using only Mummichogs

Table 6.5: Summary of Statistical Comparisons (p values) of EEM Effect Endpoints for Male Mummichog Collected at Pictou in EEM Cycle 5 (where statistical difference were detected the magnitude of the difference (%) is indicate in parentheses)

Effect Endpoint	Car vs. BH	Mer vs. BH	WR vs. BH	Car vs. Mer	Car vs. WR	Mer vs. WR
Age	BH < Car (19.2)	BH < Mer (25.3)	BH < WR (13.2)	NS	NS	WR < Mer (13.9)
Condition	Car < BH (7.3)	Mer < BH (11.2)	NS	NS	Car < WR (8.7)	Mer < WR (10.4)
Relative Liver Size	Car < BH (41.9)	Mer < BH (96.3)	WR < BH (36.7)	Mer < Car (28.9)	NS	Mer < WR (45.5)
Size-at-age	BH < Car (31.4)	SI	BH < WR (28.9)	Mer < Car (13.7)	NS	Mer < WR (17.8)
Relative Gonad Size	NS	NS	NS	SI	NS	Mer < WR (20.8)

NS = not significant
SI = Significant Interaction
Car = Caribou; Mer = Merigomish Harbour; WR = West River; BH = Boat Harbour

Table 6.6: Summary of Statistical Comparisons (p values) of EEM Effect Endpoints for Female Mummichog Collected at Pictou in EEM Cycle 5 (where statistical difference were detected the magnitude of the difference (%) is indicate in parentheses)

Effect Endpoint	Car vs. BH	Mer vs. BH	WR vs. BH	Car vs. Mer	Car vs. WR	Mer vs. WR
Age	BH < Car (21.3)	BH < Mer (15.7)	NS	NS	WR < Car (18.7)	WR < Mer (12.9)
Condition	Car < BH (10)	Mer < BH (12.3)	NS	NS	Car < WR (9.8)	Mer < WR (11.8)
Relative Liver Size	Car < BH (67.9)	Mer < BH (127)	WR < BH (47.5)	Mer < Car (26.1)	Car < WR (10)	Mer < WR (53.5)
Size-at-age	BH < Car (24.6)	BH < Mer (15.9)	BH < WR (25.5)	Mer < Car (11.2)	NS	Mer < WR (15)
Relative Gonad Size	Car < BH (51.1)	Mer < BH (93.6)	WR < BH (38.2)	Mer < Car (23.5)	NS	SI
Fecundity	NS	Mer < BH (15.5)	NS	Mer < Car (14.3)	NS	Mer < WR (24.5)

NS = not significant
SI = Significant Interaction

Car = Caribou; Mer = Merigomish Harbour; WR = West River; BH = Boat Harbour

6.2.1.6 EEM Cycle 6

The Cycle 6 study design was developed to investigate the potential causes of the confirmed effects of decreased age and increased liver size in Boat Harbour estuary Mummichog. Exposure area Mummichog were collected from the Boat Harbour estuary and from Pictou Road just outside of the estuary. Reference fish were collected from five reference areas including a new Little Lake reference area near Antigonish that has similar lentic habitat to the Boat Harbour estuary (Figure 6.2). Conventional EEM measures were conducted on fish from the two exposure areas and Little Lake. Fish from all seven areas were used for histopathological and stomach content analyses to investigate the potential cause of the previously confirmed age and liver confirmed effects.

Table 6.7 and Table 6.8 summarize the fish catches during the Cycle 6 EEM May and August sampling events. A summary of statistical comparisons of the key effort endpoints from the May collections are summarized in Table 6.9 (Boat Harbour estuary versus Little Lake), Table 6.10 (Boat Harbour Outer versus Little Lake) and Table 6.11**.** (Boat Harbour estuary versus Boat Harbour outer).

A number of significant differences were noted among the three areas compared for conventional endpoints. Results indicated that the habitat differences between Boat Harbour estuary and the reference areas used in Cycles 4 and 5 may have been the cause of the age effect results. There was no significant difference in ages of males or females in the Little Lake and Boat Harbour estuary.

A wider variety of sampling gear including minnow traps and trapnets was used to test the hypotheses of sampling bias based on gear type. Two sampling seasons were used to test the hypothesis of emigration of fish after one year. Fish captured in offshore habitats within the Boat Harbour estuary using these new gear types did not indicate older fish are more

prevalent in these areas. There was no evidence that the younger fish in the estuary move into deeper water of the estuary after year one. Only a single marked fish was captured outside the estuary that was previously captured inside. Only a limited number of fish were marked and therefore solid conclusions regarding mass emigration of younger fish out into Pictou Road were not possible from the Cycle 6 study.

Stomach content analysis did not indicate that there were substantial differences in the diets or availability of food that would account for the liver size increase. A number of foodstuffs were identified in the fish analyzed however, a large proportion of gut contents from fish from all areas was indistinguishable digest and detritus, impeding interpretations. The histopathological analysis identified a number of different conditions in a variety of tissues in Mummichog from all seven areas (Table 6.12). The most significant result for the histopathology assessment was the increase incidence of thyroid hyperplasia in fish from Boat Harbour. This condition was only noted in Boat Harbour estuary and fish collected in the outer harbour.

Table 6.7: Summary of Fishing Effort for NPNS EEM Cycle 6 Fish Survey – May 2012

Common name	Scientific name	Little Lake	Ferguson's Pond	Boat Harbour Outer	Boat Harbour Estuary
Threespine Stickleback	*Gasterosteus aculeatus aculeatus*	10	0	0	0
Ninespine Stickleback	*Pungitius pungitius*	5	0	0	0
Fourspine Stickleback	*Apeltes quadracus*	177	0	1	0
White Perch	*Morone americana*	1,229	924	0	0
Striped Bass	*Morone saxatilis*	5	0	0	0
Alewife	*Alosa pseudoharengus*	1	0	0	0
White sucker	*Catostomus commersonii*	0	156	0	0
Golden Shiner	*notemigonus crysoleucas*	0	81	0	0
Yellow Perch	*Perca flavescens*	0	33	0	0
American Eel	*Anguilla rostrata*	0	8	0	0
Banded Killifish	*Fundulus diaphanus diaphanus*	1,086	0	0	0
Northern Pipefish	*Syngnathis fuscus*	1	0	0	0
Atlantic Silverside	*Menidia menidia*	5	0	0	0
Mummichog	*Fundulus heteroclitus heteroclitus*	419	0	827	670
	Total	**2,938**	**1,202**	**828**	**670**
	Area (m^2)	**675**	**0**	**450**	**375**
	CPUA[1, 2] (fish/m^2)	**0.5**	**N/A**	**1.8**	**0.7**
	Gear Soak Time	**411.2[3]**	**98.8**	**29.2**	**280.2[3]**
	CPUE[3]	**0.2**	**N/A**	**0.0**	**1.4**

[1] CPUA is catch-per-unit-effort
[2] CPUA calculated using only Mummichogs
[3] Only includes minnow traps
[4] CPUE calculated using only Mummichogs and minnow traps

Table 6.8: Summary of Fishing Effort for NPNS EEM Cycle 6 Fish Survey – August 2012

Common name	Scientific name	Little Lake	Boat Harbour Estuary	Boat Harbour Outer	Merigomish	Caribou	West River	East River
Fourspine Stickleback	*Apeltes quadracus*	8	0	0	0	0	6	3
White Perch	*Morone americana*	1	0	8	0	0	0	0
Winter Flounder	*notemigonus crysoleucas*	0	0	1	0	0	0	0
Banded Killifish	*Fundulus diaphanus diaphanus*	117	3	0	0	0	0	0
Northern Pipefish	*Syngnathis fuscus*	2	0	0	0	0	0	0
Atlantic Silverside	*Menidia menidia*	0	0	2	0	22	5	1
Mummichog	*Fundulus heteroclitus heteroclitus*	595	341	108	497	400	453	1,111
	Total	**723**	**344**	**119**	**497**	**422**	**464**	**1,115**
	Area (m^2)	**450**	**675**	**1,125**	**2,250**	**225**	**450**	**225**
	CPUA[1, 2] (fish/m^2)	**0.12**	**0.42**	**0.08**	**0.19**	**0.79**	**0.70**	**4.19**
	Gear Soak Time	**7.66**	**12.00**	**3.0**	**2**	**11.25**	**3.33**	**5.92**
	CPUE[3]	**70.89**	**4.58**	**7.67**	**29.50**	**19.82**	**41.74**	**28.38**

[1] CPUA is catch-per-unit-effort
[2] CPUA calculated using only Mummichogs
[3] Only includes minnow traps
[4] CPUE calculated using only Mummichogs

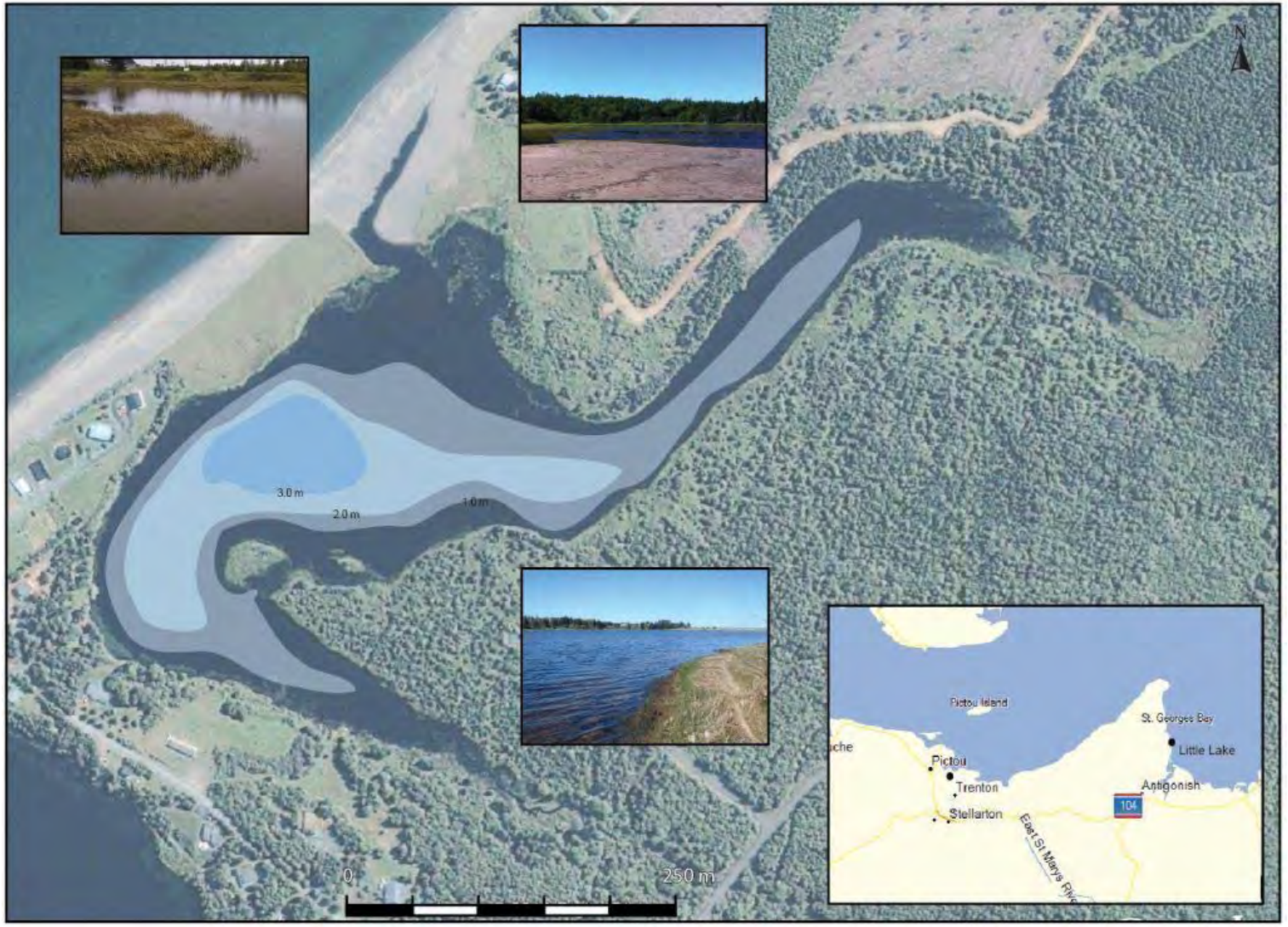


Figure 6.2: Location and Bathymetry of Little Lake Reference

Table 6.9: Summary of Statistical Comparisons (p values) of EEM Effect Endpoints for Mummichog Collected at Boat Harbour Estuary, in EEM Cycle 6 – May 2012

Sex	Type of Response	Endpoint	Endpoint Effect or Support Analysis	Primary Analysis					Reference Adjusted Means			Exposure Adjusted Means			Power Analysis[6] Effect Size =10% for CF and 25% for GW	Interaction Resolution Method[9]
				Interaction Statistic[2] (P)	Intercept Statistic[3] (P)	SSD[4]	Direction	Magnitude[5] (%)	Mean	SE	N	Mean	SE	N		
Reference Area - Little Lake vs. Boat Harbour Estuary																
Female	Survival	Mean Age[7]	Effect	na	0.644	No	-	-	2.810	0.200	26	2.58	0.113	26	nc	-
	Condition	Condition (body weight @ total length)[1]	Effect	**0.005**	0.367	No	-	-	0.967	0.009	26	0.955	0.009	27	20	2
		Adjusted body weight[7]	Support	na	**0.004**	Yes	Ref > Exp	47	12.427	2.683	26	6.627	0.306	27	-	-
		Total length[7]	Support	na	**0.010**	Yes	Ref > Exp	12	9.662	0.425	26	8.463	0.140	27	-	-
		Liver Weight @ Adjusted Body Weight[1]	Effect	0.105	**2.46E-11**	Yes	Exp > Ref	53	-0.558	0.015	26	-0.373	0.014	27	11	-
		Liver Weight @ Total Length[1]	Support	0.759	**9.87E-10**	Yes	Exp > Ref	48	-0.550	0.015	26	-0.381	0.015	27	-	-
	Energy Use	Body Weight @ Age[1] - Small fish (2 years old)	Effect	**2.84E-06**	na	No	-	-7							-	3
		Body Weight @ Age[1] - Large fish (4 years old)	Effect	**2.84E-06**	na	Yes	Ref > Exp	-51.0							-	3
		Total Length @ Age[1] - Small fish (2 years old)	Support	**7.57E-05**	na	No	-	-4							-	3
		Total Length @ Age[1] - Large fish (4 years old)	Support	**7.57E-05**	na	No	-	-17							-	3
	Reproduction	Gonad size (gonad weight @ adjusted body weight)[1]	Effect	0.858	0.462	No	-	-	-0.058	0.036	26	-0.097	0.035	27	56	-
		Gonad size (gonad weight @ total length)[1]	Support	0.407	0.376	No	-	-	-0.054	0.036	26	-0.101	0.036	27	-	-
Male	Survival	Mean Age[7]	Effect	na	0.272	No	-	-	2.25	0.111	28	2.33	0.092	27	nc	-
	Condition	Condition (body weight @ total length)[1]	Effect	0.877	0.142	No	-	-	0.891	0.005	28	0.880	0.005	27	8	-
		Adjusted body weight[7]	Support	na	0.775	No	-	-	8.366	0.885	28	7.591	0.902	27	-	-
		Adjusted body weight[7,8]	Support	na	0.775	No	-	-	0.849	0.023	27	0.855	0.027	27	-	-
		Total length[7]	Support	na	0.755	No	-	-	8.761	0.265	28	8.730	0.191	27	-	-
		Total length[7,8]	Support	na	0.579	No	-	-	8.530	0.141	27	8.730	0.191	27	-	-
		Liver Weight @ Adjusted Body Weight[1]	Effect	0.180	**2.8392E-13**	Yes	Exp > Ref	68	-0.886	0.016	28	-0.660	0.017	27	15	-
		Liver Weight @ Total Length[1]	Support	0.214	**1.5946E-11**	Yes	Exp > Ref	62	-0.878	0.017	28	-0.668	0.017	27	-	-
	Energy Use	Body Weight @ Age[1]	Effect	0.160	0.399	No	-	-	0.900	0.024	28	0.871	0.025	27	31	-
		Total Length @ Age[1]	Support	0.180	0.580	No	-	-	0.942	0.008	28	0.935	0.008	27	-	-
	Reproduction	Gonad size (gonad weight @ adjusted body weight)[1] - 0.119 vs. 0.148 g	Effect	**0.022**	na	No	-	24							-	3
		Gonad size (gonad weight @ adjusted body weight)[1] - 0.209 vs. 0.456 g	Effect	**0.022**	na	Yes	Exp > Ref	118							-	3
		Gonad size (gonad weight @ total length)[1] - 0.124 vs. 0.154 g	Support	**0.022**	na	No	-	24							-	3
		Gonad size (gonad weight @ total length)[1] - 0.217 vs. 0.482 g	Support	**0.022**	na	Yes	Exp > Ref	123							-	3

[1] calculated using log10-transformed data

[2] test for equal slopes

[3] test for equality of test groups

[4] statistically significant difference

[5] difference between reference and exposure relative to reference mean

[6] Statistical analysis conducted at α = 0.1 and power assuming β=0.1

[7] non-parametric Mann-Whitney U-test used due to non-normality

[8] Analysis rerun after removal of statistical extreme outlier LL-17

[9] Interaction resolution approach applied according to Environment Canada TGD (2010) - Section 7

n/a - not applicable

nc - not calculable due to non-normal distribution

Table 6.10: Summary of Statistical Comparisons (p values) of EEM Effect Endpoints for Mummichog Collected at Boat Harbour Outer, in EEM Cycle 6 – May 2012

Sex	Type of Response	Endpoint	Endpoint Effect or Support Analysis	Primary Analysis					Reference Adjusted Means			Exposure Adjusted Means			Power Analysis[6] Effect Size =10% for CF and 25% for GW	Interaction Resolution Method[11]
				Interaction Statistic[2] (P)	Intercept Statistic[3] (P)	SSD[4]	Direction	Magnitude[5] (%)	Mean	SE	N	Mean	SE	N		
Reference Area - Little Lake vs. Boat Harbour Outer																
Female	Survival	Mean Age[8]	Effect	na	**0.068**	Yes	Exp > Ref	9	2.81	0.200	26	3.07	0.106	27	nc	-
	Condition	Condition (body weight @ total length)[1]	Effect	**0.005**	**1.17E-04**	Yes	Exp > Ref	13	1.142	0.009	26	1.195	0.008	27	18	2
		Adjusted body weight[1]	Support	na	**5.83E-04**	Yes	Exp > Ref	57	0.986	0.052	26	1.183	0.017	27	-	-
		Adjusted body weight[1,9]	Support	na	**1.0913E-06**	Yes	Exp > Ref	71	0.950	0.039	25	1.183	0.017	27	-	-
		Total length[8]	Support	na	**3.7585E-05**	Yes	Exp > Ref	14	9.662	0.425	26	11.033	0.152	27	-	-
		Total length[9]	Support	na	**2.6127E-06**	Yes	Exp > Ref	18	9.34	0.289	25	11.033	0.152	27	-	-
		Liver Weight @ Adjusted Body Weight[1]	Effect	**0.052**	**4.37E-15**	Yes	Exp > Ref	77	-0.395	0.015	26	-0.148	0.015	27	11	2
		Liver Weight @ Total Length[1]	Support	0.456	**6.83E-11**	Yes	Exp > Ref	75	-0.393	0.016	26	-0.15	0.016	27	-	-
	Energy Use	Body Weight @ Age[1] - Small fish (2 years old)	Effect	**6.21E-05**	na	Yes	Exp > Ref	147							-	3
		Body Weight @ Age[1] - Large fish (4 years old)	Effect	**6.21E-05**	na	No	-	-0.1							-	3
		Total Length @ Age[1] - Small fish (2 years old)	Support	**7.44E-03**	na	Yes	Exp > Ref	26							-	3
		Total Length @ Age[1] - Large fish (4 years old)	Support	**7.44E-03**	na	No	-	-2							-	3
	Reproduction	Gonad size (gonad weight @ adjusted body weight)[1]	Effect	0.850	**1.54E-10**	Yes	Exp > Ref	158	0.043	0.034	26	0.454	0.034	27	50	-
		Gonad size (gonad weight @ total length)[1]	Support	0.779	**1.40E-10**	Yes	Exp > Ref	159	0.042	0.035	26	0.455	0.034	27	-	-
Male	Survival	Mean Age[8]	Effect	na	**4.60E-08**	Yes	Exp > Ref	42	2.25	0.111	28	3.19	0.076	27	nc	
	Condition	Condition (body weight @ total length)	Effect	0.240	**2.27E-06**	Yes	Ref > Exp	15	13.482	0.225	28	11.476	0.231	27	10	-
		Adjusted body weight[8]	Support	na	**1.04E-08**	Yes	Exp > Ref	86	8.366	1.101	28	15.6	0.539	27	-	-
		Adjusted body weight[10]	Support	na	**1.54E-16**	Yes	Exp > Ref	112	7.346	0.431	27	15.6	0.539	27	-	-
		Total length[8]	Support	na	**6.15E-09**	Yes	Exp > Ref	27	8.761	0.265	28	11.167	0.140	27	-	-
		Total length[9]	Support	na	**1.15E-18**	Yes	Exp > Ref	31	8.530	0.135	27	11.167	0.140	27	-	-
		Liver Weight @ Adjusted Body Weight[1]	Effect	0.677	**2.31E-12**	Yes	Exp > Ref	117	-0.761	0.022	28	-0.425	0.022	27	16	-
		Liver Weight @ Total Length[1]	Support	0.917	**4.42E-11**	Yes	Exp > Ref	115	-0.759	0.023	28	-0.427	0.024	27	-	-
	Energy Use	Body Weight @ Age[1]	Effect	0.263	**3.84E-03**	Yes	Exp > Ref	34	0.985	0.025	28	1.112	0.026	27	21	-
		Total Length @ Age[1]	Support	0.358	**2.44E-03**	Yes	Exp > Ref	11	13.482	0.225	28	11.476	0.231	27	-	-
	Reproduction	Gonad size (gonad weight @ adjusted body weight)[1]	Effect	0.664	**4.21E-04**	Yes	Exp > Ref	84	-0.786	0.028	28	-0.536	0.049	27	23	-
		Gonad size (gonad weight @ adjusted body weight)[1,7]	Effect	0.415	**1.05E-09**	Yes	Exp > Ref	90	-0.783	0.022	28	-0.504	0.023	26	16	-
		Gonad size (gonad weight @ total length)[1]	Support	0.868	**7.29E-07**	Yes	Exp > Ref	84	-0.784	0.029	28	-0.525	0.048	27	-	-
		Gonad size (gonad weight @ total length)[1,7]	Support	0.603	**2.56E-09**	Yes	Exp > Ref	89	-0.782	0.022	28	-0.505	0.023	26	-	-

[1] calculated using log10-transformed data

[2] test for equal slopes

[3] test for equality of test groups

[4] statistically significant difference

[5] difference between reference and exposure relative to reference mean

[6] Statistical analysis conducted at α = 0.1 and power assuming β=0.1

[7] Analysis rerun after removal of statistical extreme outlier BO-11

[8] non-parametric Mann-Whitney U-test used due to non-normality

[9] Analysis rerun after removal of statistical extreme outlier LL-16

[10] Analysis rerun after removal of statistical extreme outlier LL-17

[11] Interaction resolution approach applied according to Environment Canada TGD (2010) - Section 7

n/a - not applicable

nc - not calculable due to non-normal distribution

Table 6.11: Summary of Statistical Comparisons (p values) of EEM Effect Endpoints for Mummichog Collected at Boat Harbour Estuary and Boat Harbour Outer in EEM Cycle 6 – May 2012

Sex	Type of Response	Endpoint	Endpoint Effect or Support Analysis	Primary Analysis					Reference Adjusted Means			Exposure Adjusted Means			Power Analysis[6] Effect Size =10% for CF and 25% for GW	Interaction Resolution Method[9]
				Interaction Statistic[2] (P)	Intercept Statistic[3] (P)	SSD[4]	Direction	Magnitude[5] (%)	Mean	SE	N	Mean	SE	N		
Boat Harbour Estuary vs. Boat Harbour Outer																
Female	Survival	Mean Age[7]	Effect	na	**0.003**	Yes	BO > BI	19	2.58	0.113	26	3.07	0.106	27	nc	-
	Condition	Condition (body weight @ total length)[1]	Effect	0.838	**4.17E-07**	Yes	BO > BI	34	1.022	0.012	27	1.150	0.012	27	18	-
		Adjusted body weight[1]	Support	na	**1.13E-19**	Yes	BO > BI	136	0.810	0.020	27	1.183	0.017	27	-	-
		Total length	Support	na	**3.23E-17**	Yes	BO > BI	30	8.463	0.140	27	11.033	0.152	27	-	-
		Liver Weight @ Adjusted Body Weight[1]	Effect	0.661	0.470	No	-	-	-0.242	0.022	27	-0.270	0.022	27	9	-
		Liver Weight @ total length[1]	Support	0.692	0.210	No	-	-	-0.281	0.022	27	-0.231	0.022	27	-	-
	Energy Use	Body Weight @ Age[1]	Effect	0.705	**1.22E-19**	Yes	BO > BI	138	0.898	0.018	27	1.274	0.018	27	15	-
		Total Length @ Age[1]	Support	0.795	**4.62E-15**	Yes	BO > BI	26	0.934	0.006	27	1.034	0.006	27	-	-
	Reproduction	Gonad size (gonad weight @ adjusted body weight)[1]	Effect	0.801	**1.81E-04**	Yes	BO > BI	182	-0.041	0.061	27	0.410	0.061	27	64	-
		Gonad size (gonad weight @ total length)[1]	Support	0.722	**2.70E-17**	Yes	BO > BI	345	-0.140	0.038	27	0.509	0.035	27	-	-
Male	Survival	Mean Age[7]	Effect	na	**1.76E-07**	Yes	BO > BI	37	3.19	0.076	27	2.33	0.092	27	nc	-
	Condition	Condition (body weight @ total length)[1]	Effect	**0.074**	0.306	No	-	-	1.051	0.007	27	1.039	0.007	27	7	2
		Adjusted body weight[7]	Support	na	**7.92E-09**	Yes	BO > BI	106	15.60	0.539	27	7.591	0.592	27	-	-
		Adjusted body weight[1,8]	Support	na	**6.36E-18**	Yes	BO > BI	41	1.18605	0.016	27	0.838	0.022	26	-	-
		Total length[7]	Support	na	**7.33E-09**	Yes	BO > BI	28	11.167	0.140	27	8.730	0.191	27	-	-
		Total length[1,8]	Support	na	**3.12E-18**	Yes	BO > BI	12	1.04702	0.005	27	0.933	0.007	26	-	-
		Liver Weight @ Adjusted Body Weight[1]	Effect	0.645	0.139	No	-	-	-0.456	0.022	27	-0.515	0.022	27	13	-
		Liver Weight @ total length[1]	Support	0.323	**0.078**	Yes	BO > BI	19	-0.448	0.024	27	-0.523	0.024	27	-	-
	Energy Use	Body Weight @ Age[1]	Effect	0.938	**9.28E-07**	Yes	BO > BI	63	1.156	0.024	27	0.935	0.024	27	20	-
		Total Length @ Age[1]	Support	0.846	**1.64E-06**	Yes	BO > BI	18	1.028	0.008	27	0.957	0.008	27	-	-
	Reproduction	Gonad size (gonad weight @ adjusted body weight)	Effect	0.385	0.818	No	-	-	0.289	0.021	27	0.298	0.021	27	12	-
		Gonad size (gonad weight @ total length)	Support	0.647	0.895	No	-	-	0.291	0.022	27	0.296	0.022	27	-	-

[1] calculated using log10-transformed data

[2] test for equal slopes

[3] test for equality of test groups

[4] statistically significant difference

[5] difference between reference and exposure relative to reference mean

[6] Statistical analysis conducted at α = 0.1 and power assuming β=0.1

[7] non-parametric Mann-Whitney U-test used due to non-normality

[8] Analysis rerun after removal of statistical extreme outlier BH-16

[9] Interaction resolution approach applied according to Environment Canada TGD (2010) - Section 7

n/a - not applicable

nc - not calculable due to non-normal distribution

Boat Harbour Inner used as Reference location

Table 6.12: Mummichog Histopathology Analysis for the NPNS Cycle 6 EEM –August 2012

Condition	Thyroid hyperplasia	Myocarditis	Minor inflammation with no agents not gills	Branchitis with any agent but trematode eggs/larvae*	Branchitis with trematode eggs/larvae	Bulbous with trematode eggs/larvae**	Myxosporean cysts anywhere but gill*	Metazoans not mentioned	Significant inflammatory process - likely bacterial
AREA									
Boat Harbour estuary	2	2	2	5	0	0	4	0	0
Boat Harbour outer	2	2	3	1	0	0	3	2	0
Little Lake	0	0	1	1	4	4	0	1	1
Caribou Island	0	1	2	1	5	4	3	2	0
East River	0	0	1	3	5	0	4	0	0
Merigomish Harbour	0	0	0	3	4	0	4	0	0
Sawmill Creek	0	0	1	4	3	1	0	1	0

* complete accuracy would require going back to original notes - at home
**not all tissues were present for every fish

6.2.1.7 Other Relevant Research

Background

The mill was involved in a multi-year study beginning in 1999 that attempted to: (1) develop and evaluate a new bioindicator tool to be used in assessments of Marine Environmental Health (MEH); and (2) better understand the dynamics between anthropogenic stresses and the Marine Environmental Health (MEH) of Pictou Harbour (St.-Jean, 2002; St.-Jean, pers. comm., 2004). To achieve these objectives, a top-down monitoring approach was proposed to diagnose sublethal stress in the blue mussel and Mummichog. The choice of those species allowed the examination of representatives of both major groups of coastal marine fauna: vertebrates represented by the Mummichog and invertebrates represented by the blue mussel. Both species are sedentary (or at least relatively so), native to Pictou Harbour, abundant, and easily sampled within Pictou Harbour and adjacent waters. The bioindicators explored incorporated a suite of immunological biomarkers in fish and bivalve mollusks that would produce a comprehensive understanding of the MEH of the Pictou Harbour region. Further, in an effort to evaluate potential damage to the health of wild resident fish, histological and scanning electron microscopy were also performed on the gills and livers of fish during the second year of the project. The intent of this project was to quantify normal variation in immune function over space and time in order that anthropogenically induced changes in the immune system, or immunomodulation, could be discerned. The methods and results of this study are summarized below.

Study Design and Results

The Pictou Harbour/Pictou Road area receives liquid effluents from a variety of industrial and municipal sources (Figure 6.1). In order to characterize the influence of these different anthropogenic inputs, mussels were caged at thirteen potentially impacted sites and one reference site (Figure 6.1). Mummichog were collected where possible from the same

areas in which mussels were caged and in which wild populations of Mummichog were present (6 of the 14 sites; Figure 6.1).

Mussels

In late April of each year, 5,200 blue mussels, half with shell lengths of between 2 and 3 cm (juveniles), the other half with shell lengths of between 6 and 8 cm (adults), were collected from the reference site and divided among 14 cages. Each cage, made of ABS plumbing-grade tubing, carried 20 socks with juveniles (10 individuals per sock) and 20 socks with adults for a total of 400 mussels per cage. The cages were launched in early May and sampling of adults commenced 30 days later, in early June and monthly thereafter until early October. Each month, twenty adults (two socks) were chosen randomly from each site and brought to the lab where the assays were performed. Each cage was equipped with a Vemco sensor, which recorded ambient temperature periodically throughout the summer.

Upon arrival to the laboratory, haemolymph (blood) was extracted from each adult mussel and the following immunological measures were taken:

- phagocytic activity;
- lysosome retention;
- nitro blue tetrazolium reduction, a measure of the oxygen super-anion production;
- hydrogen peroxide production;
- *p*-nitrophenyl acid glucosaminide (NAG) assay, which measures the production of bactericide; and
- total protein.

In addition, the following morphological measures of condition were made for each adult mussel:

- wet-weight;
- length;
- soft-tissue (meat) weight; and
- glycogen content.

At the end of the exposure, adult mussels from each cage were inoculated with bacteria and their clearance rate was measured. The ability to clear foreign bacteria is another measure of how well the immune system is functioning.

Mussel sampling sites were ranked according to stress categories, as measured by a battery of immune system responses (Appendix E, Table E.5). Increased stress, relative to reference, was observed in mussels deployed at Boat Harbour, although overall the stress response measured in Boat Harbour Estuary mussels was less than for mussels exposed to municipal effluents (e.g., Pictou sewage, ERPAS), a thermal discharge (e.g., Power

Plant) and relatively strong wave and current action (e.g., Monroe Island). The spatial pattern representing stress in mussels was most clearly evident for the measure of phagocytic activity. When compared with reference sites, phagocytic activity was lowest at near-field sites at Boat Harbour Estuary intermediate at the far-field sites suggestive of a gradient response to effluent exposure.

Juvenile mussels were used for growth, condition and survival comparisons between sites. No differences among Boat Harbour Estuary mussels and mussels deployed in nearby reference sites were noted. Some limited enhancement of juvenile mussel growth and condition was noted at Boat Harbour Estuary.

Fish

Sampling of the Mummichog commenced in April 2000. At each of the six Mummichog collection locations, 10 fish were collected once a month with either a beach seine or minnow traps (Figure 6.1). Water temperature and salinity were measured coincident with the fish collections. Upon arrival at the lab, each animal was anaesthetized, weighed and measured and then killed by spinal severance. The liver and gonads were weighed for analyses of energy stores and reproduction. The spleen was collected and from it, the leukocyte (white blood cells) extracted and subsampled for immunological assays. Each assay developed for mussels, except for glycogen, was adapted for the fish blood and was performed in triplicate for each fish. In addition, five fish from each of: Power plant, ERPAS, Boat Harbour Estuary and Caribou, were sampled in each of June, July and August 2000 for a study of histopathology and parasites of the gills and livers. In 2001, special care was taken to ensure a minimum of 10 males and females were taken for a total of 20 fish from each sampling.

Similar patterns in immunological response to those observed in mussels were observed in Mummichog. Mummichog collected at Boat Harbour Estuary had higher stress than those at reference locations but the stress levels was intermediate within the overall station-by-station ranking (Appendix E, Table E.6).

The frequency of liver necrosis in Mummichog at Boat Harbour Estuary was about two-thirds (i.e., about two thirds of the fish investigated had some degree of liver necrosis) (Appendix E, Table E.7). Liver necrosis was seen in about 47% of the fish at the Caribou reference site and about 73% of the fish collected near the ERPAS site.

Discussion

The results of caged-bivalve and fish studies completed over the period 1999 through 2003 would seem to be fairly consistent. That is, the response seen in each of the study species was similar over the duration of the sampling program. For example, increased stress levels (as measured by immunological markers) relative to reference were measured in fish and mussels exposed to mill effluent. Overall stress levels (as measured by immune function) in fish and mussels at Boat Harbour Estuary were greater than background but

intermediate within the local study area and less than those measured in specimens exposed to other effluents, as well as those exposed to more harsh natural conditions (strong currents). Overall, the data indicated that mill effluent elicited a response in both mussels and fish that was statistically detectable relative to reference.

6.2.2 Fisheries Resources Use

EEM Cycle 1

In Cycle 1, an analysis of chlorinated dioxin and furan concentrations in the edible portion of blue mussel (*Mytilus edulis*) tissue was completed. A composite sample of tissue from ten adult blue mussels was obtained from the mouth of Boat Harbour Estuary (exposed) and a small estuary on Caribou Island (reference). Analysis revealed no detectable concentrations of furans in either sample. Low levels of specific dioxin congeners (e.g., hexa- and octa- congeners) were detected in tissues from both locations, but these levels were below consumption advisory limits. It was concluded that exposure to mill effluent did not result in bioaccumulation of chlorinated dioxins and furans in the tissues of blue mussels.

An evaluation of the propensity of NPNS effluent to taint lobster flesh was also conducted as part of the Cycle 1 EEM program. This study was based on a local fisherman's speculation of lobster tainting in the vicinity of the effluent discharge. The tainting evaluation indicated that lobsters exposed to 10 to 20% effluent did taste different from the controls, as well as, those exposed to lower effluent levels; however, the change was not considered unacceptable. Additionally, the dilution factor of the receiving environment is so great that the effluent concentration required to taint far exceeds that which would be encountered under natural conditions.

EEM Cycles 2 through 6

Dioxin and furan analysis in fish tissue was not a requirement of the Cycles 2 through 6 EEM programs at NPNS.

Over the first three cycles of EEM, fish usability was also evaluated on the basis of the potential for mill effluents to taint fish. As tainting was not identified as an issue of national concern, the requirement for tainting evaluations as part of EEM has been discontinued. However, any complaints of fish tainting must be reported directly to the Authorization Officer.

6.3 EEM Cycle 7 Study Design Methods

6.3.1 Overview and Rationale

6.3.1.1 Fish Survey

The Cycle 4 study suggested the most conspicuous difference between Mummichog in the Boat Harbour estuary and the reference area was related to age distribution – that is, exposure area fish tended to be younger than their reference area counterparts. The Cycle 5 EEM study at NPNS followed the same design as the Cycle 4 study and this age difference in reference and exposure fish populations was confirmed. In Cycle 6, no significant difference in age was found between the reference and exposure area fish populations. During Cycle 6, fish that were generally older than those previously caught were captured in the Boat Harbour Estuary. This finding was contrary to Cycles 4 and 5 and all other previous work. As a result, the Cycle 7 collections focused on confirming the lack of age difference between the Boat Harbour estuary and Little Lake Mummichog adults.

Enlarged liver size in Boat Harbour estuary Mummichogs was also confirmed through the Cycle 4 and 5 results. In Cycle 6 the cause of the enlarged livers was investigated through dietary analysis and a preliminary histopathogical assessment. The dietary analysis results did not suggest that fish diet was likely related to the enlarged liver size in exposure fish. In contrast, the results of the tissue analysis component of the study suggested that this was an avenue that warranted further study. Therefore, the Cycle 7 fish survey focused on further investigating the cause of liver effects.

6.3.1.2 Fisheries Resource Use

Dioxin and furan analysis in fish tissue was not required for the NPNS Cycle 6 EEM because:

- NPNS does not presently use chlorine-based bleaching in its operations;
- chlorophenol-contaminated wood chips are no longer used at the mill; and
- NPNS has been currently in compliance with CEPA regulations for dioxins and furans in final mill effluent over the last twelve months.

6.3.2 Sample Collection

6.3.2.1 Sampling Location

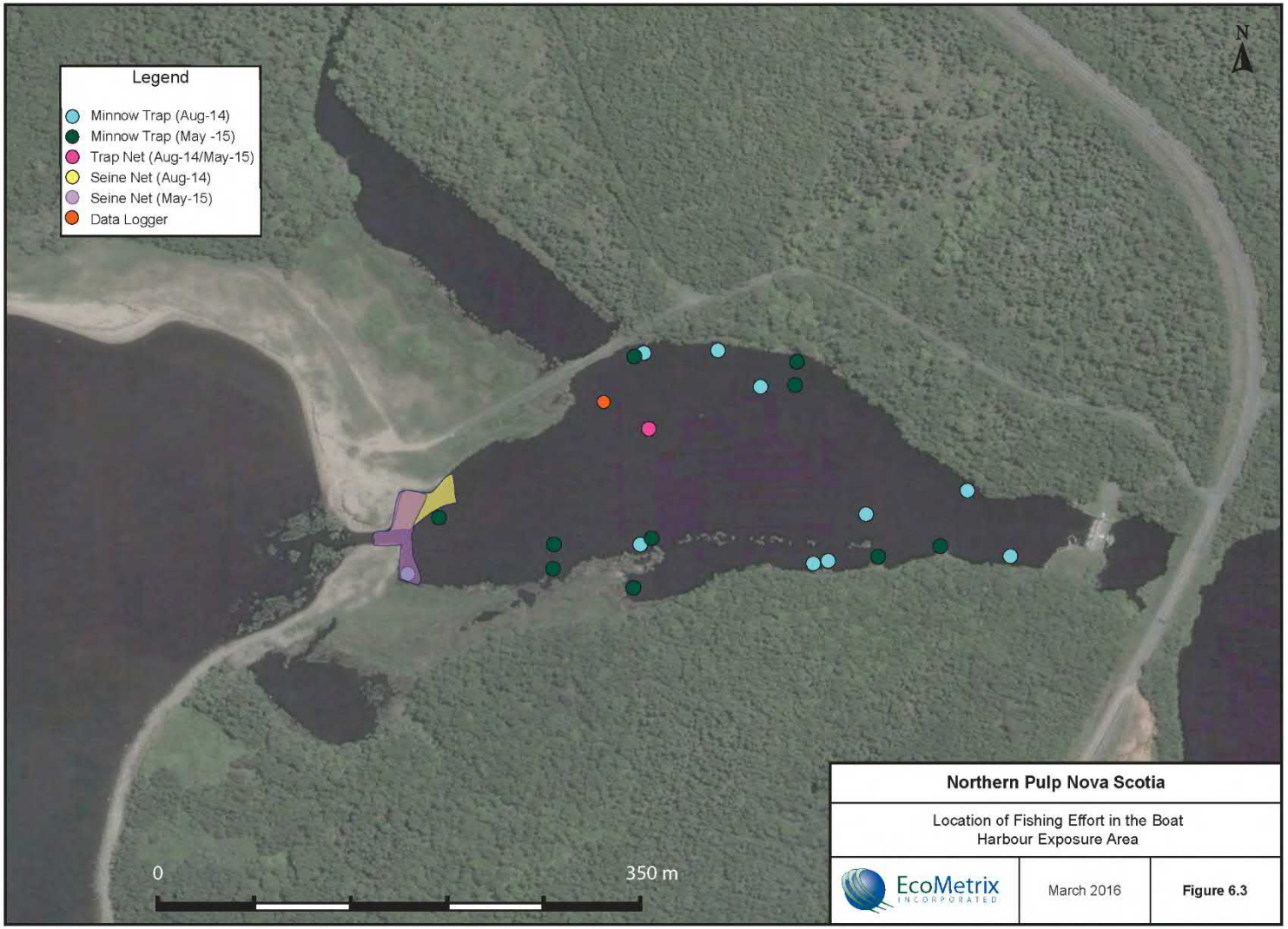
Exposure area fish were collected in August 2014 and May 2015 at the downstream end of Boat Harbour Estuary (Figure 6.3). The fish collected in May 2015 were used for measurement and analysis of the standard EEM effect endpoints, whereas the August fish collections were used for liver lipid and glycogen analyses, liver histopathology and a population structure assessment. Little Lake was used again as the reference area with

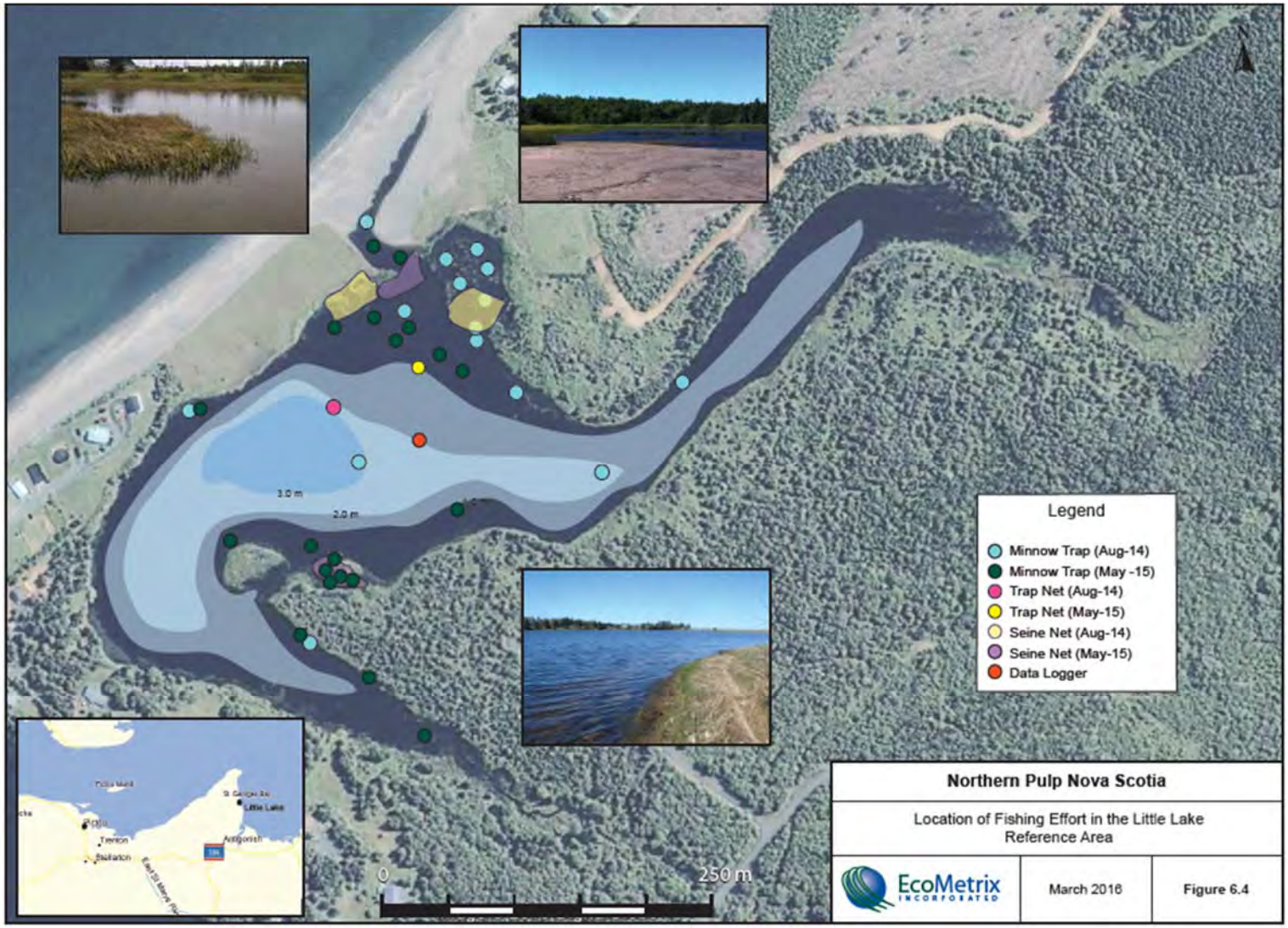
collections also occurring in August 2014 and May 2015 (Figure 6.4). Fish were analyzed in the same manner as the exposure area fish.

6.3.3 Fish Species

Cycles 4 through 6 indicated that Mummichog were abundant within the greater Pictou area, were collected in numbers sufficient for the purposes of EEM and were suitable EEM sentinel species. With this in mind, Mummichog were again targeted for collection in Cycle 7.

Cycle 4 through 6 fish collections also indicated that only Mummichog were sufficiently abundant in the exposure area for the purposes of EEM and therefore no specific contingency was proposed for collection of a second sentinel species.


Legend
Minnow Trap (Aug-14)
Minnow Trap (May -15)
Trap Net (Aug-14/May-15)
Seine Net (Aug-14)
Seine Net (May-15)
Data Logger
N
0
350 m
Northern Pulp Nova Scotia
Location of Fishing Effort in the Boat Harbour Exposure Area
EcoMetrix INCORPORATED
March 2016
Figure 6.3


Legend
Minnow Trap (Aug-14)
Minnow Trap (May -15)
Trap Net (Aug-14)
Trap Net (May-15)
Seine Net (Aug-14)
Seine Net (May-15)
Data Logger
3.0 m
2.0 m
0
250 m
Northern Pulp Nova Scotia
Location of Fishing Effort in the Little Lake Reference Area
EcoMetrix INCORPORATED
March 2016
Figure 6.4

6.3.4 Fish Collections and *in situ* Water Quality Measures

Fish were collected in both areas via active (e.g., 40' by 4' beach seine with 3/16" mesh) and passive (i.e., baited minnow traps and trapnets) means. Trapnets and minnow traps allowed sampling of deeper, offshore (i.e. unwadeable) habitats. Captured Mummichog were kept separate based on their habitat of capture, specifically to determine if the offshore catches were comprised of older fish. All fish collected were identified and enumerated. By-catch and excess Mummichogs not needed for the Cycle 7 study were released into the area in which they were collected.

During the Cycle 7 field program *in situ* measurements of temperature, dissolved oxygen, pH and salinity were taken at each collection area using a hand-held meter. Water samples for the analysis of colour, TKN, TOC, and DOC were collected within the Boat Harbour Estuary and Little Lake sampling areas. Potential exposure to effluent at the Boat Harbour Estuary mouth was also confirmed by visual characteristics of the water (e.g., colour) and by chemical analysis of water samples that indicated elevated levels of nitrogen and carbon relative to background. The water quality/chemistry data collected coincident with the fish survey were discussed in detail in Section 4.0 of this report (See Table 4.3).

In addition to laboratory measures and field spots measurements, an HOBO optical Dissolved oxygen logger and a HOBO conductivity logger were placed in both Boat Harbour and Little Lake to aid in the interpretation of potential differences from the fish survey. Details of the results of the logger deployments are provided in Figure 4.1 and Table 4.4

6.3.5 Field Processing

6.3.5.1 August 2014

Captured fish were removed from fishing gear and transferred into appropriately sized holding containers. Fish in the containers were identified and enumerated and non-sentinel fish were released. Mummichog needed for further processing were kept alive in aerated containers. Processing for all required measurements was completed within a few hours of the time of capture and all data were recorded on field data sheets. In addition to fish for tissue analyses, in August, 555 Mummichogs from Boat Harbour and 473 from Little Lake were weighed and measured. All other Mummichog were slotted into size classes based on 1 cm length intervals and ages estimated subsequently based on the length-frequency distribution determined for fish whose age was determined from otolith analysis (see below).

Fish were measured on a standard measuring board and total length (TL) was recorded to the nearest millimetre. Abnormalities such as external parasites, tumours, or scarring were documented, consistent with a Health Assessment Index (adapted from Hunn, 1988). This information provided a qualitative measure of the external condition of each fish.

Whole fish were weighed to the nearest 0.001 g using an Ohaus® Adventurer Pro electronic balance, which permitted measurement precision of ±1%. Balance accuracy was assessed

each day using standard 0.01 g and 0.1 g aluminium weights and if necessary, the balance was re-calibrated.

Mummichogs in August were also checked for marks applied during previous studies.

Liver was extracted using forceps and weighed to the nearest 0.001 g using the same balance as used for body weight. Each liver was divided into three similar size fractions. The two fractions for lipid and glycogen analysis were placed in 1.5 mL microcentrifuge vials and frozen immediately. The third fraction was placed in a microcentrifuge tube and preserved for histology using formalin.

Otoliths were collected from all Mummichogs retained for processing for subsequent ageing. Otoliths (two from each fish) were removed using a fillet knife and forceps. The otoliths of each fish were dried and packaged together in a labeled 1.5 mL microcentrifuge tube. All ageing structures were packaged by area in labeled Ziploc™ bags and shipped to EcoMetrix.

6.3.5.2 May 2015

Captured fish were removed from fishing gear and transferred into appropriately sized holding containers. Fish in the containers were identified and enumerated and non-sentinel species were released at the location of capture. Mummichog needed for further processing were kept alive in aerated containers. Processing for all required measurements was completed within a few hours of the time of capture and all data were recorded on field data sheets. Recorded characteristics and measurements of each fish included gender, length (total), body weight, gonad weight and liver weight. All measures were made in accordance with Environment Canada guidelines (Environment Canada, 2010).

Fish were measured on a standard measuring board and total length (TL) was recorded to the nearest millimetre. Abnormalities such as internal and external parasites, tumours, or scarring were documented, consistent with a Health Assessment Index (adapted from Hunn, 1988). This information provided a qualitative measure of the external condition of each fish.

Whole fish were weighed to the nearest 0.001 g using an Ohaus® Adventurer Pro electronic balance, which permitted measurement precision of ±1%. Balance accuracy was assessed each day using standard 0.01 g and 0.1 g aluminium weights and if necessary, the balance was re-calibrated.

Liver and gonad were extracted using forceps and weighed to the nearest 0.001 g using the same balance as used for body weight. Discussions with Environment Canada indicated that fecundity and egg weight measurements were not required as part of the Cycle 7 EEM program because they were not related to the confirmed effects being investigated. Liver samples were preserved in the same manner as the August 2014 samples.

Otoliths were collected from all Mummichogs retained for processing for subsequent ageing. Otoliths (two from each fish) were removed using a fillet knife and forceps. The otoliths of

each fish were dried and packaged together in a labeled 1.5 mL microcentrifuge tube. All ageing structures were packaged by area in labeled Ziploc™ bags and shipped to EcoMetrix.

6.3.6 Laboratory Processing

6.3.6.1 Aging Structures

Upon arrival, EcoMetrix Incorporated employees crosschecked the ageing structures to ensure that all of the structures collected in the field were present and properly labelled. Aging structures from August and May were processed by Jon Tost of Northshore Environmental services, an experienced fisheries biologist with over 25 years of experience aging fish. All otoliths were aged by the same technician. QA/QC was performed on a random subsample of Mummichogs via independent blind testing.

Aging was conducted on all Mummichogs sampled for the conventional EEM endpoints in May. Thirteen and 17 individuals representing the range of captured lengths were aged from the Little Lake and Boat Harbour Estuary collections in August.

6.3.6.2 Histopathology Analysis

Ten and 15 fish collected from the reference and exposure areas August 2014, respectively, were used for the histopathology analysis. Histopathology analysis was be conducted by J. S. Lumsden DVM, PhD, ACVP of the Ontario Veterinary College at the University of Guelph. Dr. Lumsden has many years of experience dealing with histopathology issues in both domestic and wild fish.

Liver tissues were examined to investigate any differences in the tissue compositions between the reference and exposure areas. Up to three sections were completed for each fish. To date fish from the May samples have not been completed as a result of time constraints with the laboratory doing the lipid analysis. It is our intention to submit and addendum to the report in the form of additional liver investigation following receipt of this data.

6.3.6.3 Liver Glycogen/Lipid Profiles

Determination of energy partitioning in reference and exposure fish was investigated through analysis of the glycogen and lipid concentrations in liver tissue. The intended purpose was that potential differences in the ratios of glycogen and lipids between the two populations could be associated with or related to the cause of liver enlargement in exposure area fish. That is, is the enlarged livers the result of increased food availability or the result of chronic effluent exposure?

<u>Glycogen Analysis</u>

EPBI Laboratory of Mississauga, Ontario conducted the glycogen analysis using the Glycogen Assay Kit II purchased from Abcam ®. This assay kit is colorimetric and is able

to detect glycogen at levels less than 4 µg/mL. All procedures followed were in accordance with the Abcam instructions and no problems were encountered during the extractions or analysis.

The full report prepared by EPBI is provided in Appendix 3.

Lipid Analysis

Lipid analysis of liver tissues were conducted by Dr. Stephanie Hixson of Ryerson University in Toronto, Ontario. Samples were initially weighed to obtain a wet mass then freeze-dried and subsequently weighed to obtain a dry mass. Dried liver tissue was then homogenized with a mortar and pestle, in liquid nitrogen, to a fine powder. Total lipids were extracted using a modified Folch et al. method (Folch et al. 1957). Total lipid was presented as a % of wet weight and dry weight.

6.3.7 Conventional Statistical Analyses

Prior to analysis all field data was transcribed from field sheets into an electronic spreadsheet (*Microsoft® Excel* 2010). The data were then subjected to QA/QC checks and screened for any outliers. Descriptive statistics (sample size, mean, median, maximum, minimum, standard error, standard deviation) were determined for key fish survey endpoints in the Microsoft Excel spreadsheets. These descriptive statistics are provided in Appendix 3. Catch data were summarized to generate comparable measures of fishing effort and success (i.e., catch-per-unit-effort [CPUE]) between the sampling areas.

All statistical procedures used were consistent with Environment Canada guidance (Environment Canada, 2010). Specific effect endpoints used for the fish survey included those recommended by Environment Canada (2010), and may be broadly grouped into the following response categories: survival, energy use, reproduction and condition. Note that fecundity measures and egg weight were not considered during the Cycle 7 analysis.

Detailed statistical analyses were performed using SPSS for Windows version 17.0 (SPSS Inc., 2007) on fish measurements representing the four primary response categories (Table 6.13). Comparisons for all endpoints were made individually between the exposure area and the reference area. Age data were compared using a non-parametric Mann-Whitney U-Test due to non-normality of the data. The remaining response variables were compared with Analysis of Covariance (ANCOVA) or ANOVA (Analysis of Variance), as appropriate. The statistical analyses utilized were consistent with the procedures recommended in the EEM technical guidance (Environment Canada, 2010).

Sexually immature fish (if any) were removed from the data set prior to statistical analyses. Testing was done on log-transformed data. Scatter plots for each variable and covariate combination (9 for males and females – per area) were examined to ensure that there was adequate covariate overlap between reference and exposure groups. For example, if age was to be used as a covariate, and it was found that the exposure and reference fish age

distributions did not overlap, then ANCOVA would not be performed. In this example, if the ANCOVA was to proceed, it would involve extrapolation outside the data range for both groups. Scatter plots for all ANCOVA combinations are provided in Appendix E, Figures E.1 to E.4.

When there was adequate overlap of the two populations, then the covariate relationships (slopes) were compared between reference and exposure groups by inserting a variable*covariate interaction term into the ANCOVA model. If the interaction term was significant, the analysis of that particular variable-covariate combination was stopped. In these cases the comparison of the two sites was completed following the three parametric approaches for resolving significant interaction terms, as outlined in the EEM technical guidance (Environment Canada, 2010). If there was no significant interaction (i.e., homogeneous slopes between the two populations), then the ANCOVA model was run without the interaction term. If the covariate was not statistically significant in the ANCOVA, it was also dropped from the analysis, and the variable was tested for significance using ANOVA.

Statistical outliers were identified at each stage using the "Explore" function in SPSS. According to the criteria used by SPSS, significant outliers have values greater than 3 times the interquartile distance (i.e., the distance between the 25^{th} and 75^{th} percentiles). When outliers were identified, statistical analyses were performed and reported with and without the outliers present.

Other measures of fish health including mean condition factor, gonad somatic index and liver somatic index were calculated as part of the fish survey supporting data. No statistical comparisons of the gonad somatic index and liver somatic index were performed as these were addressed using liver and gonad weights adjusted for body weight as described in Table 6.13.

The condition factor (CF) for each fish was calculated as follows:

$$\text{condition factor} = \frac{100 \times \text{body weight}}{(\text{total length})^3}$$

The gonad somatic index (GSI) was calculated as follows:

$$\text{gonad somatic index} = \frac{100 \times \text{gonad weight}}{(\text{body weight} - \text{gonad weight})}$$

The liver somatic index (LSI) was calculated as follows:

$$\text{liver somatic index} = \frac{100 \times \text{liver weight}}{(\text{body weight} - \text{gonad weight} - \text{liver weight})}$$

Table 6.13: Summary of Fish Survey Statistical Analyses and covariates Combinations

Response Characteristic	Morphological Measure	Covariate
Survival	Mean Age[1]	None
Energy Use	Weight[1]	Age
	Length[2]	Age
Reproduction	Gonad Weight[1]	Adjusted Body Weight
	Gonad Weight[2]	Length
	Fecundity[3, 4]	Adjusted Body Weight
	Fecundity[3, 4]	Length
	Egg weight[4]	Length
Condition	Adjusted Body Weight[2]	None
	Length[2]	None
	Weight[1]	Length
	Liver Weight[1]	Adjusted Body Weight
	Liver Weight[2]	Length

[1] Denotes an EEM Effect Endpoint

[2] Denotes an EEM Supporting Response Variable

[3] Fecundity is measured for females only (as number of eggs).

[4] Not required as part of Cycle 7 Investigation of Cause Study

6.3.8 Study Specific Data Analyses

Lipid and glycogen analytical results from the August samples were plotted versus liver weight, body, total length and condition. Lipids were also plotted versus glycogen. Plots were made using the whole dataset, with the data divided by area and with the data divided by fish gender and area. Plots were visually inspected for differences and/or patterns. A two-way anova was conducted for glycogen and lipids using area and sex as the test variables. This test was used to determine if there was a statistical difference in lipid or glycogen concentrations in the Boat Harbour Estuary compared to the Little Lake reference area Mummichog livers for either sex. .

The length of Mummichogs collected in August and May in Boat Harbour were compared based on their depth of capture. Fish collected in gear set in 0.75 m or less were assigned to the “shallow” depth strata, whereas fish captured in gear at depths over 0.75 m were assigned the “deep” depth strata. The total lengths of the fish capture in the two depth strata were then compared using a two-sample Kolmogorov-Smirnov Test.

Length frequency distributions for all of the Mummichog captured in Little Lake and Boat Harbour were created for both the August and May sampling events. The population structure in Little Lake and Boat Harbour were then compared for both seasons using a two-sample Kolmogorov-Smirnov Test.

6.3.9 Power Analysis

Power analyses were completed to identify the sample sizes required to detect differences of these magnitudes between reference and effluent-exposed fish for subsequent surveys. *A priori* power analysis is based on the measurement of variability in the sample population and assumes that the variability measured in the sample population is representative of the entire population. For the purposes of the power analyses conducted here, sample sizes for male and female fish were determined such that it would be possible to detect a difference of 25% in relative gonad size or a 10% difference in condition factor, using a power level of 0.90 (1-β) and the following relationship:

$$n = 2\,(t_{\alpha}(2) + t_{\beta})^2\,(SD/\delta)^2$$

where: n is the number of samples
SD is the sample standard deviation (or MSE from ANOVA or ANCOVA model)
δ is the specified effect size (between reference and near field area)
$t_{(2)}$ (two tailed test) and t (one tailed test) are critical values of the Student's t-test statistic (given Type I error probability and Type II error probability as taken from Student's tables with 2(n-1) degrees of freedom).

6.4 Results

Tables containing the May 2015 raw fish data used for the conventional EEM effect endpoint calculations are provided in Appendix E, Table E.8 to E.11. Covariate plots for the various fish endpoints are also provided in Appendix E. Fish collection data for August and May are summarized in Table 6.14 and Table 6.15. Measurement data for August and May are provided in Table 6.16 and Table 6.17. All supplementary measurement data from August are provided in Appendix E Tables E.12 and E.13. All supplementary measurement data from May are provided in Appendix E Tables E.14 to E.15. Set and lift times for all fishing effort in August and May is provided in Appendix E Table E.16 and E.17.

6.4.1 Fish Community Composition

In both August and May Mummichogs were the most abundant fish species in the Boat Harbour exposure area. Nearly 3,000 Mummichogs were captured in August. In addition, four Ninespine Stickleback (*Pungitius* pungitius), five Banded Killifish (*Fundulus diaphanous* diaphanous) and three American Eel (*Anguilla rostrata*) were captured. A total of four species were also captured in May in Boat Harbour with Threespine Stickleback (*Gasterosteus aculeatus*) being captured in addition to Mummichogs, Banded Killifish and

Ninespine Stickleback. In total 749 Mummichogs, 13 Threespine Stickleback, 96 Ninespine Stickleback, a single White Perch (*Morone americana*) and a single Banded Killifish comprised the Boat Harbour May catches.

Table 6.14: Summary of Fishing Effort for NPNS EEM Cycle 7 Fish Survey – August 2014

Common name	Scientific name	Little Lake	Boat Harbour
Ninespine Stickleback	*Pungitius pungitius*	0	4
White Perch	*Morone americana*	5	0
Striped Bass	*Morone saxatilis*	2	0
Alewife	*Alosa pseudoharengus*	21	0
American Eel	*Anguilla rostrata*	5	3
Banded Killifish	*Fundulus diaphanus diaphanus*	2,224	5
Atlantic Silverside	*Menidia menidia*	619	0
Mummichog	*Fundulus heteroclitus heteroclitus*	653	2,906
	Total	**3,529**	**2,918**
	Gear Soak Time1	**330.3**	**331.2**
	CPUE2	**1.04**	**0.13**

1 Only includes minnow traps

2 CPUE (fish/hour) calculated using only Mummichogs and minnow traps

Table 6.15: Summary of Fishing Effort for NPNS EEM Cycle 7 Fish Survey – May 2015

Common name	Scientific name	Little Lake	Boat Harbour
Ninespine Stickleback	*Pungitius pungitius*	2	96
Threespine Stickleback	Gasterosteus aculeatus	148	13
Blackspotted Stickleback	*Gasterosteus wheatlandi*	40	0
Fourspine Stickleback	*Apeltes quadracus*	61	0
Atlantic Silverside	*Menidia menidia*	2,916	0
Rainbow Smelt	*Osmerus mordax*	1	0
Brook Trout	*Salvelinus fontinalis*	1	0
Winter Flounder	*Pseudopleuronectes americanus*	1	0
Striped Bass	*Morone saxatilis*	3	0
Tomcod	*Microgadus tomcod*	2	0
White Perch	*Morone americana*	1,118	1
Banded Killifish	*Fundulus diaphanus diaphanus*	2,701	1
Mummichog	*Fundulus heteroclitus heteroclitus*	939	749
	Total	**7,933**	**860**
	Gear Soak Time [1]	**307.4**	**224.9**
	CPUE [2]	**1.51**	**0.35**

[1] Only includes minnow traps

[2] CPUE (fish/hour) calculated using only Mummichogs and minnow traps

The fish community in Little Lake is reflective of its seasonal connection to the ocean. In August the most abundant species captured in Little Lake was Banded Killifish (2,224) with Mummichogs (653) and Atlantic Silverside (*Menidia menidia*, 619) the next most numerous species. White Perch (5), Striped Bass (*Morone saxatilis*, 2), Alewife (*Alosa pseudoharengus*, 21) and American Eel (5) were also captured in Little Lake in August (Table 6.14). Little Lake catches in May were comprised of 2,916 Atlantic Silverside, 2,701

Banded Killifish, 1,118 White Perch, 939 Mummichog, 148 Threespine Stickleback, 61 Fourspine Stickleback (*Apeltes quadracus*), three Striped Bass, two Atlantic Tomcod (*Microgadus tomcod*), two Ninespine Stickleback and one Rainbow Smelt, Winter Flounder (Pseudopleuronectes americanus) and Brook Trout (*Salvelinus fontinalis*) (Table 6.15). Northern Pipefish (*Syngnathus fuscus*) have also been captured in previous surveys of Little Lake.

A total of seven and four species were captured at Little Lake and the Boat Harbour Estuary in August compared to 14 and five species captured in these areas in May.

6.4.2 Catch-Per-Unit Effort (CPUE)

Fish collections utilized both active and passive gear including beach seine, minnow traps and trapnets. CPUE was calculated using only Mummichogs captured in minnow traps.

In August CPUE was 1.04 and 0.13 Mummichog per hour in Little Lake and Boat Harbour minnow traps, respectively.

In Boat Harbour the trapnet was far more effective at capturing Mummichog than in Little Lake. A total of 333 Mummichog were captured in 46.8 hours of trapnet effort in Boat Harbour' whereas none were capture in Little Lake. It is suspected that the location of the trapnet in Little Lake had low oxygen levels as a number of fish were dead upon retrieval of the net. Also Little Lake has a large number of predatory fish (i.e., White Perch), and Mummichog may only inhabit the nearshore areas where shelter is more readily available.

In May, the CPUE of minnow traps was only five times higher in Little Lake than in Boat Harbour with values of 1.51 and 0.35 Mummichog captured per hour compared to the 10-fold difference in August. In May, 541 Mummichog were captured in the 22.5 hours of trapnet effort in Boat Harbour, whereas in Little Lake only a single Mummichog was captured in the in 16.8 hours of trapnet deployment.

According to minnow trap data, capture success was much lower in August 2014 and May 2015 when compared to the same seasonal catches in 2012 in both areas. However, beach seines in both areas had similar catches. In August and May three and four seines were conducted in Boat Harbour in similar areas to past EEM cycles resulting in 2,538 and 130 Mummichog. In Little Lake in May, three seine pulls resulted in the capture of around 574 Mummichog and in August 2014 three seine pulls captured 304 Mummichog. Despite some of the seining effort being reduced compared to previous cycles the results of Cycle 7 still far exceeded other historic surveys in Boat Harbour. For example 18 beach seines during the 2002 Environment Canada survey yielded 59 Mummichogs (St-Jean, 2002). Changes in the catches are more a result of different targeted effort to address other effects rather than from a lack of Mummichog in Boat Harbour.

6.4.3 Fish Health

Overall, the majority of Mummichog and incidental species captured in the study area appeared to be in good physical health. Unlike in Cycle 4 and 5, liver necrosis was not observed in Mummichog as they were being processed. In May, 20% and 30% of adult fish used for EEM endpoints had liver parasites in Boat Harbour and Little Lake, respectively. One Little Lake fish was also entirely devoid of ovaries.

In August, there were no signs of internal parasites in either area. One fish from Boat Harbour in August did have scoliosis (i.e., curvature of the spine). No indication of any mill-related effect was evident by the external health assessments of individual fish.

In August 2014, the 489 Mummichog measured from Little Lake ranged in size from 2.0 to 11.3 cm, with a mean length of 6.1 cm. Body weight for 488 of these Little Lake Mummichog ranged in weight from 0.1 g to 14.6 g, with a mean of 3.6 g. In Boat Harbour, the length of the 577 Mummichog measured in August 2014 ranged from 1.7 to 11.7 cm, with a mean length of 5.7 cm, whereas the mean weight was 4.1 g, with a range of 0.1 to 25.9 g.

Table 6.16: Fish Metric Summary Data for Fish Collected at NPNS EEM Cycle 7 – August 2014

Location	Metric	unit	n	Mean	Median	Minimum	Maximum	SD[1]	SE[2]
Little Lake	Total length	cm	489	6.1	6.4	2.0	11.3	1.6	0.1
	Body weight	g	488	3.6	3.4	0.1	14.6	2.47	0.11
	Condition[3]	-	488	1.3	1.3	0.1	2.2	0.15	0.01
Boat Harbour Estuary	Total length	cm	577	5.7	5.1	1.7	11.7	2.4	0.1
	Body weight	g	575	4.1	1.7	0.1	25.9	4.51	0.19
	Condition[3]	-	577	1.39	1.39	0.00	1.88	0.16	0.01

[1] SD is standard deviation

[2] SE is standard error

[3] Condition is 100 * (body weight/length^3)

In May 2015, the 627 Mummichog measured from Little Lake ranged in size from 2.8 to 10.3 cm, with a mean length of 6.9 cm. In May the 781 Mummichog measured in Boat Harbour had a mean length of 7.9 cm, with a range of 3.9 to 14.7 cm. Mean body weight for the May Little Lake and Boat Harbour Mummichog were 4.7 and 7.8 g, respectively, ranging from 0.2 to 14.5 g and 0.7 to 41.1 g, respectively.

Table 6.17: Fish Metrix Summary Data for Fish Collected at NPNS EEM Cycle 7 – May 2015

			Little Lake Reference							Boat Harbour Estuary						
Gender	Metric	unit	n	Mean	Median	Min.	Max.	SD[1]	SE[2]	n	Mean	Median	Min.	Max.	SD[1]	SE[2]
Male	Age	years	25	2.0	2.0	2	3	0.2	0.0	25	2.9	3.0	2	5	0.8	0.2
	Body weight	g	25	7.225	7.003	5.535	8.507	0.721	0.144	25	13.573	13.348	8.032	21.686	4.11	0.82
	Total length	cm	25	8.2	8.3	7.5	8.8	0.25	0.05	25	10.0	9.9	8.6	11.9	0.95	0.19
	Condition[3]	-	25	1.3	1.3	1.1	1.4	0.1	0.0	25	1.3	1.3	1.2	1.5	0.1	0.0
	Liver weight	g	25	0.143	0.132	0.095	0.309	0.045	0.009	25	0.546	0.552	0.237	0.882	0.176	0.035
	LSI[4]	-	25	2.0	1.9	1.4	4.3	0.6	0.1	25	4.3	4.3	2.7	6.8	0.9	0.2
	Gonad weight	g	25	0.113	0.106	0.079	0.158	0.021	0.004	25	0.244	0.254	0.094	0.410	0.082	0.016
	GSI[5]	-	25	1.6	1.6	1.1	2.1	0.3	0.1	25	1.9	1.9	0.9	3.2	0.6	0.1
Female	Age	years	25	2.4	2.0	2	3	0.5	0.1	25	3.2	3.0	2	5	1.0	0.2
	Body weight	g	25	10.840	10.402	8.769	14.536	1.565	0.313	25	17.203	14.725	9.536	41.095	6.884	1.377
	Total length	cm	25	9.3	9.2	8.6	10.3	0.49	0.10	25	10.7	10.4	9.0	14.7	1.34	0.27
	Condition[3]	-	25	1.3	1.3	1.2	1.6	0.1	0.0	25	1.3	1.3	1.2	1.4	0.1	0.0
	Liver weight	g	25	0.324	0.321	0.204	0.494	0.073	0.015	25	0.916	0.885	0.446	2.000	0.364	0.073
	LSI[4]	-	25	3.2	3.3	2.2	4.9	0.6	0.1	25	6.0	5.5	3.5	9.4	1.4	0.3
	Gonad weight	g	24	0.359	0.337	0.218	0.718	0.103	0.021	25	0.625	0.585	0.321	1.232	0.243	0.049
	GSI[5]	-	25	3.3	3.2	0.0	5.2	1.1	0.2	25	3.8	3.7	2.7	4.9	0.5	0.1
All Fish[6]	Body weight	g	627	4.691	4.478	0.225	14.536	2.348	0.094	781	7.811	6.944	0.663	41.095	5.626	0.201
	Total length	cm	627	6.9	7.1	2.8	10.3	1.19	0.05	781	7.9	8.1	3.9	14.7	2.00	0.07
	Condition[3]	-	627	1.3	1.3	1.0	1.6	0.1	0.004	781	1.3	1.3	0.9	1.8	0.1	0.004

[1] SD is standard deviation
[2] SE is standard error
[3] Condition is 100 * (body weight/length^3)
[4] LSI is liversomatic index which equals 100 * liver weight/(body weight-liver weight-gonad weight)
[5] GSI is gonadsomatic index which equals 100 * gonad weight/(body weight-gonad weight)
[6] All fish includes fish used for conventional EEM endpoints and length frequency distribution

6.4.4 Histopathology Analysis

The histopathogical analysis of a total of 25 fish (15 from Boat Harbour and 10 from Little Lake) collected in August 2014 was undertaken. There were a number of conditions or parasites identified including minor cell necrosis, metazoans, paripancreatitis, and perihepatitis (i.e., inflammation of the peritoneal covering of the liver). Dr. Lumsden's report is provided in Appendix E.

Generally, the majority of the observations were reported to be of minimal significance. Three of the 15 fish from Boat Harbour had signs of parasites, higher than the percentage noted during the May 2015 adult survey. Conversely, only one of the 10 fish from Little Lake had signs of parasites. Two of the 15 Boat Harbour fish has perihepatitis, whereas this was not observed in the Little Lake fish. Two Little Lake fish had paripancreatitis (i.e., inflammation of the pancreas whereas this was not observed in Boat Harbour fish. Overall, conditions noted were those expected in wild fish populations and the prevalence of the conditions were considered mild.

The exception to the aforementioned is that Boat Harbour fish appeared to have a higher degree of hepatic lipidosis compared to Little Lake fish. This was a qualitative observation by an experienced histologist. The qualitative observation of lipidosis may suggest effluent exposure causes a different liver composition. To quantify the amount of glycogen in the liver tissue special stains may be used for the May samples.

As noted the May adult fish samples were not submitted for histology until it can be confirmed that the analysis of lipid and glycogen can also be completed.

6.4.5 Liver Glycogen/Lipid Profiles

In all samples the amount of lipid in the liver profiles was much higher than the amount of glycogen on a wet weight basis. A summary of lipid and glycogen measures, as well as associated meristic measures of sampled fish are provided in Table 6.18. Wet weight lipid percent ranged from 15% to 34.8% with a mean of 23.4% in Boat Harbour females and ranged from 7.5% to 12.9% with a mean of 10.6% in Little Lake females. For males, mean lipid percentages were 21.7% and 11.8% in Boat Harbour and Little Lake ranging from 14.2% to 32.5% and 6.5% to 23.3%, respectively. With the genders combined Boat Harbour fish had a range of wet weight lipid percentage that ranged from 14.2% to 34.8% with a mean of 22.7% whereas Little Lake liver lipid percentage had a mean of 11.3% and range from 6.5% to 23.3%.

Wet weight glycogen percent ranged from 0.08% to 0.52% and from 0.17% to 0.67% in Boat Harbour and Little Lake fish with a mean of 0.33% for both areas. Little Lake female liver glycogen percentage ranged from 0.23% to 0.35% with a mean of 0.26% whereas Boat Harbour ranged from 0.08% to 0.52% with a mean of 0.33%. Boat Harbour males had a mean liver glycogen percentage of 0.34% whereas Little Lake males had a mean of 0.38%. The range of glycogen in male Mummichog liver tissue was 0.24% to 0.40% and 0.17% to 0.67% in Boat Harbour and Little Lake, respectively.

Table 6.18: Summary of Lipid and Glycogen Percentages in Liver Tissues of Mummichog Collected in Little Lake and Boat Harbour – August 2014

	Age	Total Length (cm)	Body Weight (g)	Condition	Liver Weight (g)	Dry Matter Ratio	Dry Weight Lipid %	Wet Weight Lipid %	Wet Weight Glycogen %	Glycogen µg/mg
Boat Harbour - Females	2	11.7	25.932	1.6	0.717	0.42	53.2	22.1	0.075	0.75
	2	10.1	13.119	1.3	0.419	0.48	52.3	25.3	0.314	3.14
	2	10.2	13.474	1.3	0.407	0.39	38.0	15.0	0.515	5.15
	3	10.1	12.843	1.2	0.473	0.44	43.0	18.9	0.203	2.03
	2	9.6	11.684	1.3	0.347	0.47	46.5	22.0	0.263	2.63
	2	8.2	7.538	1.4	0.391	0.55	53.6	29.2	0.382	3.82
	1	6.7	3.431	1.1	0.102	0.48	49.8	23.7	0.467	4.67
	2	11.7	22.838	1.4	0.725	0.49	40.5	19.8	0.331	3.31
	2	7.3	5.563	1.4	0.202	0.51	67.6	34.8	0.418	4.18
Sample Size	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**9**
Mean	**2**	**9.5**	**12.9**	**1.3**	**0.420**	**0.47**	**49.4**	**23.4**	**0.33**	**3.30**
Median	**2**	**10.1**	**12.8**	**1.3**	**0.407**	**0.48**	**49.8**	**22.1**	**0.33**	**3.31**
Minimum	**1**	**6.7**	**3.4**	**1.1**	**0.102**	**0.39**	**38.0**	**15.0**	**0.08**	**0.75**
Maximum	**3**	**11.7**	**25.9**	**1.6**	**0.725**	**0.55**	**67.6**	**34.8**	**0.52**	**5.15**
Standard Deviation	**0.5**	**1.68**	**7.01**	**0.13**	**0.1941**	**0.045**	**8.38**	**5.54**	**0.129**	**1.288**
Standard Error	**0.2**	**0.56**	**2.34**	**0.04**	**0.0647**	**0.015**	**2.79**	**1.85**	**0.043**	**0.429**
Little Lake - Females	1	8.3	7.235	1.3	0.226	0.51	24.0	12.4	0.239	2.39
	1	7.2	4.981	1.3	0.086	0.46	27.9	12.9	0.346	3.46
	1	6.7	4.303	1.4	0.085	0.46	26.7	12.4	0.234	2.34
	2	10.1	12.545	1.2	0.207	0.31	25.9	8.1	0.243	2.43
	2	9.0	9.416	1.3	0.150	0.32	23.7	7.5	0.245	2.45
Sample Size	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Mean	**1.4**	**8.3**	**7.7**	**1.3**	**0.151**	**0.41**	**25.7**	**10.6**	**0.26**	**2.61**
Median	**1**	**8.3**	**7.2**	**1.3**	**0.15**	**0.46**	**25.9**	**12.4**	**0.24**	**2.43**
Minimum	**1**	**6.7**	**4.3**	**1.2**	**0.085**	**0.31**	**23.7**	**7.5**	**0.23**	**2.34**
Maximum	**2**	**10.1**	**12.5**	**1.4**	**0.226**	**0.51**	**27.9**	**12.9**	**0.35**	**3.46**
Standard Deviation	**0.5**	**1.22**	**3.02**	**0.07**	**0.0589**	**0.083**	**1.59**	**2.34**	**0.042**	**0.425**
Standard Error	**0.2**	**0.55**	**1.35**	**0.03**	**0.0263**	**0.037**	**0.71**	**1.05**	**0.019**	**0.190**
Boat Harbour - Males	1	8.3	6.943	1.2	0.204	0.40	46.6	18.8	0.400	4.00
	1	7.6	5.821	1.3	0.214	0.53	61.4	32.5	0.243	2.43
	1	6.5	3.41	1.2	0.099	0.54	50.6	27.5	0.376	3.76
	4	11.4	18.249	1.2	0.500	0.45	31.3	14.2	0.336	3.36
	2	9.4	10.801	1.3	0.321	0.43	38.3	16.5	0.312	3.12
	2	11.4	21.38	1.4		0.46	46.0	21.0	0.374	3.74
Sample Size	**6**	**6**	**6**	**6**	**5**	**6**	**6**	**6**	**6**	**6**
Mean	**1.83333**	**9.1**	**11.1**	**1.3**	**0.268**	**0.47**	**45.7**	**21.7**	**0.34**	**3.40**
Median	**1.5**	**8.9**	**8.9**	**1.3**	**0.214**	**0.45**	**46.3**	**19.9**	**0.36**	**3.55**
Minimum	**1**	**6.5**	**3.4**	**1.2**	**0.099**	**0.40**	**31.3**	**14.2**	**0.24**	**2.43**
Maximum	**4**	**11.4**	**21.4**	**1.4**	**0.5**	**0.54**	**61.4**	**32.5**	**0.40**	**4**
Standard Deviation	**1.1**	**1.84**	**6.60**	**0.08**	**0.1358**	**0.050**	**9.43**	**6.35**	**0.052**	**0.521**
Standard Error	**0.4**	**0.75**	**2.69**	**0.03**	**0.0607**	**0.021**	**3.85**	**2.59**	**0.021**	**0.213**
Little Lake - Males	1	8.2	6.957	1.3	0.114	0.40	58.0	23.3	0.408	4.08
	1	7.1	4.806	1.3	0.085	0.35	18.8	6.5	0.389	3.89
	1	6.6	3.406	1.2	0.053	0.39	23.3	9.1	0.672	6.72
		7.8	6.701	1.4	0.138	0.47	32.1	15.0	0.320	3.20
	2	10.1	14.579	1.4	0.613	0.37	17.6	6.5	0.172	1.72
	2	8.9	10.076	1.4	0.235	0.34	29.7	10.2	0.348	3.48
Sample Size	**5**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
Mean	**1.4**	**8.1**	**7.8**	**1.3**	**0.206**	**0.39**	**29.9**	**11.8**	**0.38**	**3.85**
Median	**1**	**8.0**	**6.8**	**1.4**	**0.126**	**0.38**	**26.5**	**9.6**	**0.37**	**3.685**
Minimum	**1**	**6.6**	**3.4**	**1.2**	**0.053**	**0.34**	**17.6**	**6.5**	**0.17**	**1.72**
Maximum	**2**	**10.1**	**14.6**	**1.4**	**0.613**	**0.47**	**58.0**	**23.3**	**0.67**	**6.72**
Standard Deviation	**0.5**	**1.15**	**3.68**	**0.09**	**0.1905**	**0.042**	**13.59**	**5.89**	**0.149**	**1.494**
Standard Error	**0.2**	**0.47**	**1.50**	**0.04**	**0.0778**	**0.017**	**5.55**	**2.40**	**0.061**	**0.610**

6.4.6 QA/QC

6.4.6.1 Fish Aging

No issues were identified with the initial age estimates. All fish were assigned the same age in the blind aging events. Results of the QA check on the ages are detailed in Appendix E, Table E.18.

6.4.7 Conventional Statistical Comparisons

A summary of the statistical comparisons of EEM effect endpoint data for Little Lake vs. Boat Harbour Estuary is provided in Table 6.19. Detailed results for statistical comparisons including plots of all analyzed effect and support endpoints are provided in Appendix E.

6.4.7.1 Exposure to Reference Area Comparisons

Effect Endpoints

Male and female Mummichog were both significantly different with respect to age in the Boat Harbour Estuary and the Little Lake reference area with older fish being from Boat Harbour (Table 6.19). Mature male and female fish at Boat Harbour both ranged in age from 2+ to 5+. The majority of Boat Harbour males and females were both 3+. There were more 5+ females than males in Boat Harbour. In Little Lake the majority of males and females were 2+ with only one 3+ male and nine 3+ females. Unlike in Cycle 6 there were no fish aged more than 3+ in Little Lake in May 2015.

Boat Harbour males were not significantly different than Little Lake males with respect to condition. There was a significant interaction for condition in female fish. Using the second method in the hierarchal tiers for dealing with interactions outlined in EC technical guidance (Environment Canada, 2010), that compares the coefficient of determination for the full and reduced regression model, indicated that there was no significant difference between the condition of females from Little Lake and Boat Harbour. Liver weight at adjusted body weight, another indicator of “condition” was not significantly different for males but was significantly different for females. Boat Harbour females had larger livers than the Little Lake fish. The magnitude of this difference was 90%, exceeding the critical effect size (CES) outlined by Environment Canada (Table 6.19).

Body weight at age was significantly different for both sexes. Body weight at age for age 2+ males and females were both larger in Boat Harbour compared to Little Lake. The magnitude of the difference was 28% and 17% for males and females respectively. Age 3+ females in Boat Harbour were also significantly heavier compared to their Little Lake counterparts by a magnitude of 25%. The magnitude of these differences were comparable to the CES of 25% indicating potential ecological relevance.

Table 6.19: Summary of the Statistical Analyses for the Boat Harbour (exposure) and Little Lake (reference) Mummichog – May 2015

Gender	Type of Response	Endpoint	Endpoint Effect or Support Analysis	Primary Analysis					Power Analysis	Interaction Resolution Method[5]
				Interaction Statistic[1] (P)	Intercept Statistic[2] (P)	SSD[3]	Direction	Magnitude[4] (%)	25% except Condition = 10%	
Male	Survival	Age[6]	Effect	n/a	**4.56E-06**	yes	Exp > Ref	89	nc	-
	Energy Storage	Condition (body weight @ total length)[7]	Effect	0.350	0.333	no	-	-	2	-
		Relative liver size (liver weight @ adjusted body weight)[8]	Effect	0.447	0.718	no	-	-	-	-
		Relative liver size (liver weight @ total length)[8]	Support	0.143	0.687	no	-	-	-	-
	Energy Use	Size (Body Weight) @ Age = 2	Effect	n/a	**9.62E-07**	yes	Exp > Ref	28	4	-
		Size (Total Length) @ Age = 2	Support	n/a	**5.99E-07**	yes	Exp > Ref	9	-	-
		Body Weight[7,9]	Support	n/a	**1.89E-09**	yes	Exp > Ref	69	-	-
		Total Length	Support	n/a	**2.50E-09**	yes	Exp > Ref	18	-	-
Female	Survival	Age[6]	Effect	n/a	**0.050**	yes	Exp > Ref	34	nc	-
	Energy Storage	Condition (body weight @ total length)	Effect	**0.085**	0.480	no	-	-	7	2
		Relative liver size (liver weight @ adjusted body weight)[7]	Effect	0.780	**4.91E-11**	yes	Exp > Ref	90	38	-
		Relative liver size (liver weight @ total length)[7]	Support	0.498	**5.25E-10**	yes	Exp > Ref	347	-	-
	Energy Use	Size (Body Weight) @ Age =2	Effect	n/a	**0.003**	yes	Exp > Ref	17	4	-
		Size (Body Weight) @ Age =3[9]	Effect	n/a	**0.029**	yes	Exp > Ref	25	-	-
		Size (Total Length) @ Age = 2	Support	n/a	**0.006**	yes	Exp > Ref	7	-	-
		Size (Total Length) @ Age = 3[9]	Support	n/a	**0.048**	yes	Exp > Ref	7	-	-
		Body Weight	Support	n/a	**9.78E-04**	yes	Exp > Ref	27	-	-
		Total length	Support	n/a	**9.56E-04**	yes	Exp > Ref	9	-	-

[1] test for equal slopes
[2] test for equality of test groups
[3] statistically significant difference
[4] difference between reference and exposure relative to reference mean
[5] Interaction resolution approach applied according to Environment Canada TGD (2010) - Section 7
[6] used Mann-Whitney U-test due to non-normality
[7] calculated using $\log_{10}$-transformed data
[8] Used non-parametric ANCOVA due to severe violation of assumption of normality
[9] T-test assuming unequal variance
n/a - not applicable
nc - not calculable

Support Endpoints

The support endpoint of liver weight at length for males was similar between the two sampling areas. Total length at age, body weight and total length were significantly higher in Boat Harbour males by a magnitude of 9%, 69% and 18%, respectively. Exposure females had 347% heavier liver weight at length compared to reference females. References females were also significantly longer by 7% for both 2+ and 3+ aged fish (i.e., size at age). Additionally, Boat Harbour females were 27% heavier and 9% longer than Little Lake females.

6.4.8 Study Specific Statistical Comparisons

Two-way ANOVA was used to determine if there was a significant difference in the lipid or glycogen concentrations in the liver tissue of male or female Mummichog collected in Boat Harbour and Little Lake. There was not effect of either area or sex for glycogen with p-values of 0.776 and 0.226, respectively (Table 6.20). For lipids there was also no significant difference between the sexes (p value = 0.949). However, area did show a significant difference with a p-value of <0.001 and indicated that Boat Harbour fish had higher lipid concentrations.

Table 6.20: Results of the Two-way ANOVA for Glycogen and Lipids in Liver Tissue from Fish Captured in Little Lake and Boar Harbour in August 2014

Dependent Variable:Glycogen %					
Source	Type III Sum of Squares	df	Mean Square	F	p-value
Area	0.118	1	0.118	0.083	0.776
Sex	2.211	1	2.211	1.549	0.226
Error	32.833	23	1.428		
Dependent Variable:Dry Weight Lipid					
Source	Type III Sum of Squares	df	Mean Square	F	P-value
Area	2459.689	1	2459.689	23.719	**6.44E-05**
Sex	0.442	1	0.442	0.004	0.949
Error	2385.163	23	103.703		

significant P <0.10

There was a statistical difference in mean size of fish collected in deep strata gear compared to shallow strata gear in May. The mean length in the deep strata catches was 8.3 cm whereas in the shallow sets it was 7.0 cm. There was no statistical difference in the size of the catches in the two depth strata in August 2014 (Table 6.21). Figure 6.5 provides the length frequency histograms for Boat Harbour and Little Lake in both August 2014 and May 2015. There was no significant difference between the population structures in the two areas in either sampling season.

Table 6.21: Results of the Kolomogorov-Smirnov Tests of Fish Capture at Shallow and Deep Depths and between the Population Structures in Little Lake and Boar Harbour in August 2014 and May 2015.

	Shallow Vs Deep Boat Harbour		Boat Harbour versus Little Lake	
	KS-Statistic	p-values	KS-Statistic	p-values
Aug-14	0.429	0.111	0.214	0.844
May-15	0.356	0.055	0.214	0.477

Significant difference at p <0.10

6.4.9 Power Analyses

Power analysis was conducted on each of the effect endpoint measures to determine sampling requirements (i.e., collection targets) for subsequent fish surveys. The analysis was based on identifying the number of fish required for the study to have the ability to detect a 25% difference in relative liver and gonad size and a 10% difference in condition. The number of fish required was generally low for condition and gonad weight and larger for the liver weight endpoints. Detailed power analysis results are provided in Appendix E and are summarized briefly below.

Using the Little Lake as the reference, the collection targets in any future cycles that would require calculation of the conventional EEM endpoints would be a minimum of 20 males and 38 females. The relatively large numbers for the females required is driven by the variability seen in relative liver size within the individual sampling areas. Overall, the use of around 25 fish for each sex for each area would be considered adequate sampling to confidently assess the effect endpoints.

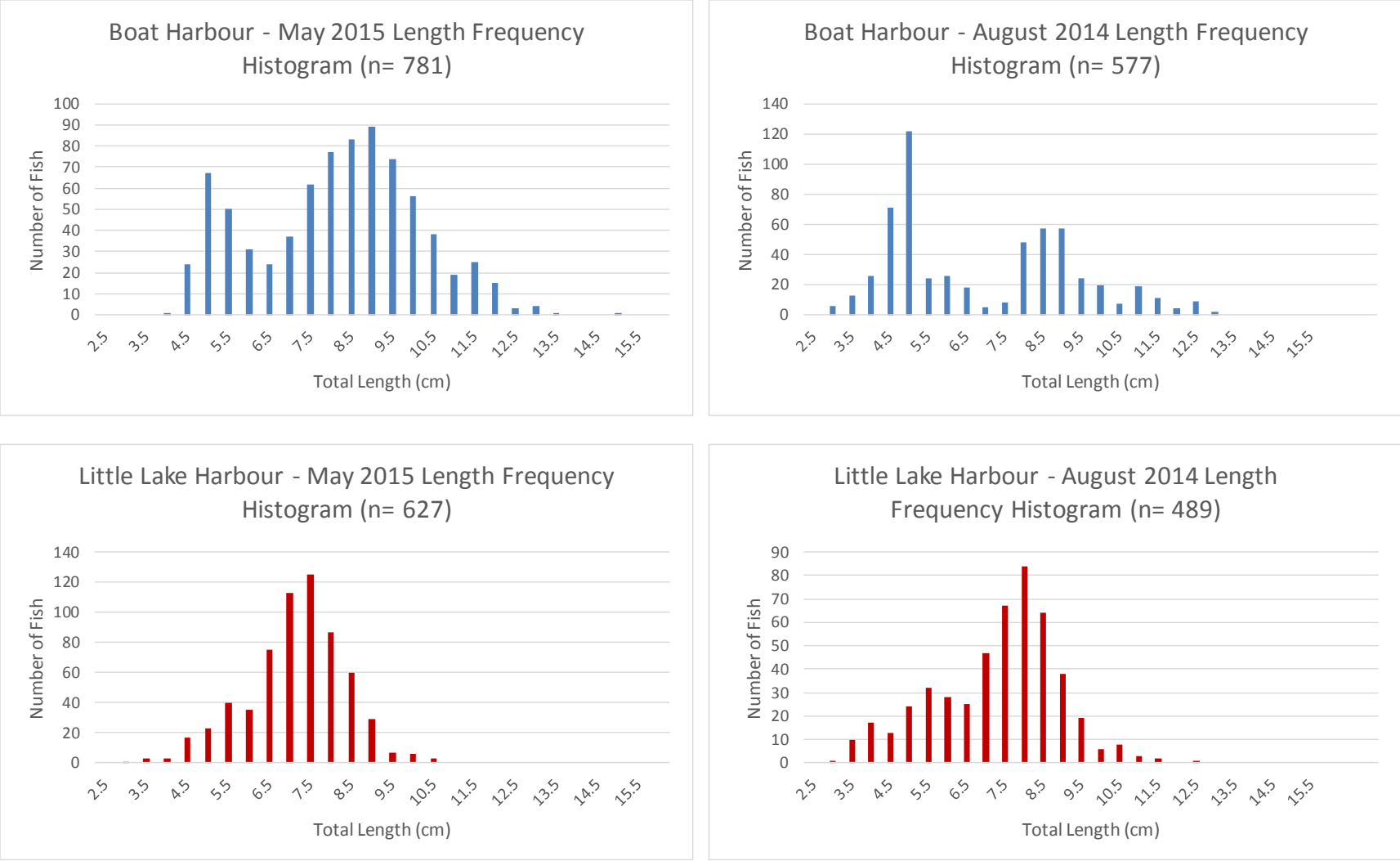


Figure 6.5: Length Frequency Histogram of Mummichog Capture in Little Lake and Boat Harbour in August 2014 and May 2015.

6.5 Discussion

The purpose of the NPNS Cycle 7 fish survey was to continue to investigate the potential cause(s) of the confirmed effects on fish liver reported in the Cycle 4 and 5 EEM programs and to confirm the lack of an age effect reported in Cycle 6. Attempts were made to assess the potential cause of the increased liver size in the Boat Harbour estuary Mummichog compared to the Mummichog from Little Lake. To this end the Cycle 7 fish survey used histological and glycogen and lipid quantification of liver tissue to determine the potential cause(s) of increased size of the livers in Boat Harbour fish.

Contrary to Cycle 4 and 5 when Boat Harbour Mummichog were significantly younger than reference fish this was not the case in Cycle 6. Although a new primary reference area was established in Cycle 6 the lack of an age effect was more the result of the capture of older fish in Boat Harbour rather than an overall younger age in the reference and may be due to a change in fishing technique.

One of the hypotheses from Cycle 6 was that the age effects seen previously were because the gear used in the Cycle 4 and 5 field surveys did not sample all habitats/areas and therefore did not capture all the life stages present. That is, there were older fish present in Boat Harbour but they were simply not collected using the methods employed because Cycles 4 and 5 used a beach seine and targeted nearshore, shallow, vegetated habitats easily accessible from shore. Within the Boat Harbour Estuary there are offshore habitats with and without vegetation with depths up to 3.0 m. In the Cycle 6 EEM these habitats were sampled with a trapnet and minnow traps to determine if the previous sampling bias precluded the capture of older fish that inhabit some of the offshore areas.

In Cycle 6 the catch-per-unit-effort (CPUE), data from May in Little Lake and the Boat Harbour Estuary indicated that older Boat Harbour Estuary fish do not inhabit the offshore area. Only two Mummichogs were captured in 65 hours of trapnet effort in these two areas. However, in Cycle 7 a contrary results were found. In May 2015, 541 Mummichog of all sizes were capture in just over 22 hours of effort. Comparison of the lengths of fish collected in shallow (<0.75 m) gear sets and deep (>0.75 m) gear sets indicated that the deep sets captured significantly larger fish. This result only occurred in May and not in August indicating that the difference in habitat preference may be seasonal and may have been the reason for some earlier findings. The difference in the length of fish capture in the deep and shallow sets was only 1.3 cm, however inspection of the histogram indicates that this may be the divide between age 1 and older fish, as is indicated by the somewhat tri-modal distribution of the 781 fish measured during the May sampling event.

The other confirmed effect during the Cycle 4 and EEM fish surveys was adjusted liver weight. Boat Harbour Estuary Mummichog had increased liver size compared to the three reference areas. In Cycle 6, to test the hypothesis that liver size is related to food availability or food type rather than effluent exposure stomach contents were analyzed from fish collected in August from the same areas and on the same individuals used for the

histopathology analysis. There was no conclusive evidence from the stomach content analysis that indicated that the quantity or type of food available in the Boat Harbour Estuary was related to the enlarged livers. The histopathology of the liver tissue also did not indicate that food was the cause for the higher liver weights. As a result of the diet data being inconclusive, another avenue of investigation was undertaken in Cycle 7. Lipid and glycogen concentrations and associated liver histology were completed on a subset of fish collected from August 2014 when the majority of fish are done spawning. The results indicated that lipid concentrations were significantly higher in both sexes of Boat Harbour Mummichog, whereas glycogen was similar between the two areas. Additionally, the histopathologist noted that there was larger amount of hepatic lipidosis in Boat Harbour liver tissues corroborating the results of the lipid quantifications. Conducting lipid analyses on such small quantities of liver tissue is very specialized and the second set of samples, from spawning condition fish collected in May 2015, are not completed to date. The analysis this set of livers from during spawning time may allow for further consideration as to the reason for increase livers in the exposure area.

It has been shown that the Boat Harbour area has higher nutrient levels and water there is generally warmer than it would be naturally because of the presence of the mill effluent. Together these factors likely contribute to a relatively highly productive ecosystem that likely affords fish there more available energy through a more plentiful diet. Higher total lipid levels in the liver, greater liver mass, and larger size of Boat Harbour Mummichog compared to Little Lake may indicate Boat Harbour fish have a different or more plentiful diet. However, to date diet study results have been inconclusive. Regardless of the cause it does appear that exposure fish are storing more lipids than other benthivorous fish of similar size. It is possible that lipophilic organics are being stored in the livers as a result of exposure to mill effluent. Testing for the presence of these organic compounds in the liver tissue should be further investigated to determine if the increased liver size is a direct result of effluent exposure (i.e., organics) or indirect exposure better diet based on increased nutrients in the Boat Harbour Estuary. As mentioned preliminary results of the dietary tests indicated that diets did not vary between areas but further study may be needed.

The conventional endpoints for the EEM program were conducted on adult fish from Boat Harbour and Little Lake. Boat Harbour males and females were both significantly older than fish from the Little Lake population. The change in the age difference from being significantly younger in Cycle 4 and 5 to not significantly different in Cycle 6 and now significantly older in Cycle 7 calls into question if the observed difference is real and ecologically meaningful or just represents year to year variation. The lack of an age difference in Cycle 6 and then significantly older fish in the exposure area in Cycle 7 are both contrary to the age results of most previous studies in the Boat Harbour Estuary, including those involving Mummichogs (see Stantec, 2004a, EcoMetrix, 2007a and 2010) and Winter Flounder (*Pleuronectes americanus*) (Beak, 1998). A reduction in age due to exposure to pulp mill effluent has also been reported at other maritime mills where Mummichogs have been used for EEM (Stantec, 2004c). Additionally, age effects have been seen in other fish species used in the EEM program including Fallfish (*Semotilus*

corporalis), Yellow Perch (*Perca flavescens*), Cunner, White Sucker (*Catostomus commersonii*), Rock Bass (*Amblopites rupestris*), Mottled Sculpin (*Cottus bairdi*), Pumpkinseed (*Lepomis gibbosus*), Common Shiner (*Luxilus cornutus*) and Walleye (Sander vitreus). In Cycles 4 and 5 approximately 30% of mills in Canada had a similar response to effluent exposure. Conversely, there have also been occurrences of age distributions of other species not being negatively influenced by effluent exposure in some EEM studies and there was one occurrence of exposure area Mummichog being significantly older than the reference area Mummichog similar to this current study.

In Cycle 6 when exposure and reference area fish were not significantly different with respect to age, the fact that exposure fish being significantly younger in Cycle 4 and 5 did not appear to be the artifact of gear bias. However, the results of Cycle 7 appear that gear bias is a potential that cannot be eliminated. It has been reported that changes in the age structure of a fish population are sometimes reported to be the result of some stress related event or set of circumstances in receiving environments (Gibbons and Munkittrick, 1994). Installation of the dataloggers provided a longer time series of the changing environment that the Little Lake and Boat Harbour Mummichog endure throughout the year. Boat Harbour Mummichog do appear to be subject to wide swings in dissolved oxygen and long period of low oxygen that could potentially cause stress related shifts in the population. However, Mummichog are known to be one of the most tolerant species of low oxygen and changing salinity and temperatures. Therefore, if these changes are part of the cause of stress if may be being exacerbated by exposure to the mill effluent.

Relative liver weight provides an indication of fish condition or energy storage. Fish primarily store energy in the form of lipids in the mesenteric cavity, muscle tissues and liver, or as glycogen in the liver (Busacker *et al.,* 1990; Goede and Barton, 1990). Increased liver size in effluent-exposed fish may indicate:

- more available energy in the receiving environment (i.e., increased food resources) (Novinger, 1973; Tyler and Dunn, 1976);
- an adaptive response increasing the capacity of the liver to detoxify contaminants (Addison, 1984; Heath, 1987); and/or
- greater allocation of energy to storage, potentially at the expense of growth or reproduction, related to altered metabolic pathways (Munkittrick *et al.,* 1991).

In Cycle 6, Boat Harbour Estuary and outer male and female Mummichogs had significantly larger livers (when adjusted to account for body weight) than their Little Lake area counterparts. This trend was consistent with the national average response pattern and it is suggested that the increased liver size is a response to greater food availability associated with increased benthic productivity in nutrient- or organically-enriched areas (Environment Canada, 2005b). However, in Cycle 7 there was no significant difference in the adjusted liver weight of males between the two sampling areas. This is all despite a significantly higher

concentration of lipids in exposure males compared to females. Exposure females had significantly larger livers by a magnitude of 90% far exceeding the CES. The difference in this response may indicate a difference in energy allocation by the two genders. Further analysis of the sample from the May spawning period may shed light on the reason for this difference. In Cycle 6 the Boat Harbour Estuary and Boat Harbour Outer male and female liver weights were not significantly different from each other but were significantly different from Little Lake fish and hence may indicate effluent exposure as a cause. Additionally, the diet analysis did not show a difference between reference and exposure. This means that it is possible that persistent organic compounds in the effluent may in fact be the cause of liver enlargement. As stated in previous EEM studies two reasons confound the separation of food and effluent exposure. Firstly, Mummichog captured outside the immediate Boat Harbour estuary (i.e., Boat Harbour Outer) are only exposed to high levels of effluent intermittently according to the tidal cycle and secondly, past studies have resulted in highly variable difference in livers (i.e., up to 54%).

Overall, based on the investigation of the cause of decreased age and increased liver size in Boat Harbour Estuary it is suggested that further studies be designed based on the information learned from the current study. In Cycle 6, there was no significant difference in the ages between Boat Harbour Estuary and Little Lake fish whereas in Cycle 7 Boat Harbour fish were significantly older. This may indicate previous results were an artefact of sampling gear bias, that previous results may have not be a true reflection of the population structure present or simply be yearly variation. Results of the statistical tests on the population structure indicate Boat Harbour had a similar population in two both sampling seasons.

Liver weights continued to be higher in the Boat Harbour Estuary female Mummichog similar to previous reports. However, there was no significant difference in male liver weight. Lipid concentrations were significantly higher in Boat Harbour and increased hepatic lipidosis was also observed. Analysis of the spawning condition liver tissue for histology, glycogen and lipids may provide additional insight into the difference in the effect endpoint for liver during this current cycle and may also point to the potential cause(s) for enlarged livers generally seen in the exposure area. Increased liver size being a result of effluent exposure cannot be discounted at this time and requires further investigation.

There were some other significant differences in the EEM endpoints. Exposure males and females were significantly longer and heavier and had larger gonads at adjusted body weight than their reference area counterparts. Boat Harbour fish were also heavier and longer at age than in the reference areas. Despite this, condition was not significantly different between the two areas. The size of the fish of similar ages in Boat Harbour being bigger for other endpoints suggests that differing diets or amount of food available still needs to be considered.

7.0 INTEGRATION OF STUDY COMPONENTS

Geometric mean IC25s for sublethal toxicity test completed over the duration of the Cycle 7 EEM were as follows:

- *Lytechinus pictus* GMIC25 = 2.44 %; and
- *Champia parvula* GMIC25 = 0.62%.

The results of the seventh-cycle EEM sublethal toxicity testing indicate that there are chronic effects seen in laboratory test species at relatively low effluent concentrations, well within the range of effluent concentrations seen in Boat Harbour Estuary. Cycle 7 results for *Champia* and sea urchin showed little difference compared to Cycle 6 and the GM IC25s for *Champia* for the past three cycles remain an order of magnitude lower than the previous four cycles. Sea urchin results were also similar to previous cycles and showed a similar trend. Under the nearshore effluent dispersion scenario, the potential effects zone based on the results of the sublethal toxicity testing is within 323 m of the discharge at Boat Harbour. Under the offshore effluent dilution scenario, the potential effects zone extends to greater lengths (to ~ 7.3 km). The extent to which these effluent dispersion predictions hold true is unclear and directed field programs may be warranted to determine if they translate into ecologically relevant effects. Investigation into potential correlation between more frequent parameter measurement and the sublethal toxicity results did not indicate that any conventional analytes were related to the slight improvement in toxicity results. Further work on additional and potentially non-typical parameters could be incorporated into the effluent collections for the Cycle 8 tests.

The investigation of cause survey indicated that Boat Harbour Mummichog were significantly older than the Little Lake reference Mummichog for both sexes after being comparable in Cycle 6 and significantly younger in Cycle 4 and 5. This is somewhat similar to Cycle 6 however, when Boat Harbour Outer fish were significantly older than Little Lake for both sexes. Liver size in Boat Harbour Estuary females was significantly larger than in Little Lake whereas liver size in males was not different. Condition was also similar between the reference and exposure area fish. There were significant differences in size with larger body weight, length and body weight at age and gonad size for male and female exposure fish compared to the reference.

Overall, the results of the EEM Cycle 7 field program at NPNS indicate that:

- Age differences previously reported for the Boat Harbour area may be the result of the gear type bias. There were significantly larger fish captured in deeper gear sets, a result contrary to Cycle 6. Stressors such as the wide ranging oxygen levels seen in Boat Harbour may work in unison with some factor caused by effluent exposure to have contributed to the changes in population structure between cycles.

- Exposure area female Mummichogs had enhanced liver growth and both sexes had significantly higher liver tissue lipid concentrations that Little Lake fish. The reason for the differences between the sexes is unknown and may be illuminated following further analysis of the spawning condition fish from the May 2015 season. Previous stomach content analysis results did not indicate a difference between the reference and exposure areas diets. However, results of the diet investigation were generally inconclusive. It is possible enlarged liver are a result of organic constituents present in the mill effluent. However, without similar patterns in both sexes this seems unlikely. Further investigation is warranted potentially including more dietary work and possible an investigation into any potential compounds that may be accumulating in the liver.

8.0 RECOMMENDATIONS FOR FUTURE MONITORING

Decisions regarding nature of future monitoring (Cycle 8) at NPNS will be made within the decision making framework available at the time that the study design is developed and in response to comments provided by Environment Canada regarding the Cycle 7 EEM study.

An addendum to this report will be provided in the form of an update fish section once the laboratories are able to analyze the liver tissues from the May 2015 spawning period for lipids, glycogen and histology.

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