Nalcor Energy Lower Churchill Project, Environmental Effects Monitoring Program – 2014 Ecorisk

Baseline Methylmercury (MeHg) and Total Mercury (THg) Levels in the Lower Churchill River Valley

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Executive Summary

In 2014, an Ecorisk Environmental Effects Monitoring Program (EEMP) was initiated as part of the broader EEMP that Nalcor Energy is completing, based on the requirements and commitments defined in the Lower Churchill Generation Project Environmental Impact Assessment (EIS). Field work was conducted in fall 2013 and spring/summer 2014 in order to collect Osprey (*Pandion haliaetus*) feathers and river otter (*Lontra canadensis*) hair samples from locations within the lower Churchill River valley and along the existing transmission line between Happy Valley-Goose Bay and Churchill Falls, Labrador. Amphibian (tadpole) tissues and water and sediment samples were also collected to investigate methylmercury (MeHg) contamination in species lower in the food web and in the aquatic environment in general. In addition, stable isotopes were analyzed for Osprey and amphibian samples, to further assess trophic levels (e.g., where they feed and which trophic level they belong to).

Osprey feathers were collected from 19 of the 23 active nests visited, hair samples from five of seven river otter sampling locations, and samples of northern leopard frog (*Lithobates pipiens*) and/or American toad (*Anaxyrus americanus*) from 13 locations, including 11 of water and sediment sampling sites. Total mercury (THg) levels were determined through laboratory analysis based on samples collected, and MeHg levels either estimated (for species, based on trophic level and published information), or determined through laboratory analysis (water and sediment samples only).

THg levels detected in Osprey feathers (n=18) ranged from 1.08 to 28.2 mg/kg, and averaged 9.1 ± 5.6 mg/kg in female samples (n=10) and 15.8 ± 2.4 mg/kg in male samples (n=2). Based on available literature, the expected MeHg is approximately 100 % of THg (Braune and Gaskin 1987, Odsjo et al. 2004). Stable isotope analyses indicate that Osprey feed on a number of different prey items, from two to three different tropic levels.

Results from the river otter hair sampling program yielded only one confirmed river otter sample. The average THg level along the hair sample was 3.49 ± 1.14 mg/kg. A review of the available literature suggests that MeHg is approximately 100 % of THg in river otter hair samples (Kehrig et al. 1998, Voegborlo et al. 2010).

Amphibian THg levels ranged from 0.0032 to 0.0170 mg/kg wet weight (ww) in Northern leopard frog samples (n=3) and from 0.0098 to 0.0575 mg/kg ww in American toad samples (n=6). Due to a laboratory error, MeHg could not be determined from collected samples. However, based on available literature, it is estimated that approximately 30.0 % of THg in amphibian tadpoles is made up of MeHg (Bank et al. 2007). Stable isotope analyses indicated that the two species sampled may be feeding on slightly different prey, but suggested that they feed at the same trophic level, when all results were combined.

THg and MeHg were detected in most sediment samples (10 out of 11 and 9 out of 11 samples, respectively) with THg concentrations being one to two orders of magnitude lower than the interim sediment quality guidelines (ISQGs = 0.17 mg/kg) and probable effect levels (PELs = 0.486 mg/kg) for the protection of aquatic life (Environment Canada 1999).

The current study demonstrates the bioavailability of MeHg in sediment and water and its ability to accumulate in higher trophic levels. Although mercury levels of fish, a critical prey component, were not measured as part of the EEMP, higher concentrations of mercury in higher trophic levels (i.e., 0.0227 mg THg/kg in amphibians, to 0.792 mg THg/kg in otters, to 10.6 mg THg/kg in Osprey) indicate the capabilities of mercury to accumulate in the lower Churchill River watershed.

Preliminary results suggest that, with changes in environmental conditions following inundation, hardness, conductivity and dissolved oxygen should be closely monitored to assist with predictions of MeHg levels in water and sediment.

Additional sampling may be required to complete baseline assessments for river otter and amphibians.

1.0 2014 ECORISK EEMP

The 2014 Ecorisk Environmental Effects Monitoring Program (EEMP) was completed by Stassinu Stantec Limited Partnership (Stassinu Stantec) and is part of the broader EEMP that Nalcor Energy is completing in conjunction with the Lower Churchill Generation Project (the Project). The program is based on the requirements and commitments in the Lower Churchill Generation Project Environmental Impact Statement (EIS) (Nalcor 2009a and 2009b).

The Lower Churchill River watershed and adjacent watersheds provide year-round and seasonal habitats for a variety of wildlife species in central Labrador that rely on fish and other components of the aquatic food web. The primary objectives of the Ecorisk monitoring program are to understand how the Project will affect methylmercury (MeHg) and total mercury (THg) levels in the aquatic habitat and the wildlife it supports. The 2014 Ecorisk EEMP focused specifically Osprey (*Pandion haliaetus*), river otter (*Lontra canadensis*) as top predators, and amphibians (tadpoles) and associated water and sediment as environmental condition indicators to:

- Determine existing and post-flood MeHg and THg levels in Osprey, river otter and amphibians (tadpoles) and associated water and sediment;
- Determine the trophic levels where Osprey and river otter feed, and determine where amphibians are situated in regards to trophic level. This would be done using stable isotope analyses;
- Conduct DNA analyses for Osprey and river otter to determine what influence sex and age have on dietary choices (i.e., trophic levels); and
- Determine whether environmental factors such as the pH, dissolved oxygen, hardness, and conductivity influence the existing and post-flood MeHg and THg levels in water and sediment.

This report summarizes the methods and results related to sample collection in fall 2013 (Osprey feathers) and spring/summer 2014 (river otter hair, amphibian tissues, and water and sediment). A brief summary of the laboratory methods related to THg and MeHg is also provided. The 2014 interim report for the Ecorisk EEMP represents baseline sampling of a multi-year EEMP.

1.1 Background

Mercury (Hg) occurs naturally in the environment, but significant amounts can enter the environment through anthropogenic emissions, re-emissions and discharges. As a result, Hg has become a ubiquitous contaminant. In the aquatic environment, a chemical process known as methylation can convert inorganic Hg into MeHg. Environmental conditions such as dissolved oxygen and pH are key parameters that will influence the methylation process. When land is

flooded, environmental conditions have the potential to become favorable to the process of methylation, which could result in higher MeHg levels in the aquatic environment. Thus the potential for heightened MeHg levels as a result of the Project was of concern to regulators and the public.

MeHg is the bioaccumulative form of Hg. It enters the food web primarily through fish that consume organisms below them in the food web. As these fish are eaten by larger organisms, higher levels of MeHg accumulate in species higher in the food chain (i.e., at higher trophic levels). As a result, top predators in aquatic food webs can be particularly at risk for MeHg exposure. While it is estimated that nearly 100% of THg is in the form of MeHg in the tissues of top predators, this proportion varies in the environment and little is known about the proportion of MeHg relative to THg in species lower in the food chain (e.g., amphibians).

Previously in 2006, baseline studies to assess MeHg levels in the ecosystem were initiated and an Ecological Risk Assessment (ERA) was conducted to predict the potential effects of Project activities. Two species were selected as valuable indicators of MeHg contamination in the environment: Osprey and river otter, based on their predominantly pisciviorous (fish) diet. Results from the ERA indicated a potential risk of MeHg toxicity as a result of Project activities. However, collection of additional baseline data was recommended given the small sample size.

1.2 Study Team

Planning and coordination, field surveys and report preparation components of the Ecorisk EEMP were led by Stassinu Stantec (Table 1.1). Samples were sent to Wildlife Genetics International (Nelson, British Columbia (BC)) for DNA analyses, ALS environmental (Burnaby, BC) for mercury analyses, and the University of Winnipeg (Winnipeg, MB) for stable isotope analyses. In addition, laser ablation analyses for the river otter hair was done at the University of Victoria (Victoria, BC).

Table 1.1 Ecorisk Study Team

Name	Role	Organization
Diane Ingraham	Project Manager	Stassinu Stantec
Wayne Tucker	Assistant Project Manager and Team Lead	Stassinu Stantec
Perry Trimper	Senior Technical Advisor	Stassinu Stantec
Michael Crowell	Technical Advisor	Stassinu Stantec
Jennie Christensen	Discipline Lead	Stassinu Stantec
Marie Noel	Toxicologist	Stassinu Stantec
Dustin Oaten	Field Lead - Amphibians	Stassinu Stantec
Tony Parr	Field Lead – Otter	Stassinu Stantec
Stacey Camus	Field Lead – Osprey & Reporting	Stassinu Stantec

Name	Role	Organization
Karen Rashleigh	Field Planning & Reporting	Stassinu Stantec
-	DNA Analysis	Wildlife Genetics
-	Mercury / Methylmercury Analysis	ALS Environmental
-	Laser Ablation (river otter hair)	University of Victoria

Prior to the start of the field component of the Ecorisk EEMP, all personnel reviewed the Health, Safety, and Environment (HSEQ) Plan and the Risk Management Strategy (RMS) 1 (Stassinu Stantec Limited Partnership 2014). A daily hazard assessment (RMS 2) was completed each morning. The required scientific research permits (Appendix A: IW2014-25, IW2013-66, IW2013-66 supplement via email) were acquired from the Newfoundland and Labrador Wildlife Division (NLWD), Department of Environment and Conservation, prior to the initiation of the surveys.

2.0 METHODS

The general approaches for the various species of interest are outlined below. Detailed technical methods and procedures are presented in Appendix B.

2.1 Study Area

The Study Area for the Ecorisk EEMP included the lower Churchill River valley and the proposed transmission line route between the towns of Churchill Falls and Happy Valley-Goose Bay (Figure 2-1). In particular, Osprey feather collection focused on known active nest locations in the Study Area, of which the majority were located on existing transmission line infrastructure; river otter sample collection focused on areas within the river valley where otter tracks were previously identified during 2014 winter aerial surveys; and amphibian sampling focused on accessible areas within and adjacent to the Muskrat Falls reservoir area (i.e., within or adjacent to the predicted extent of flooding).

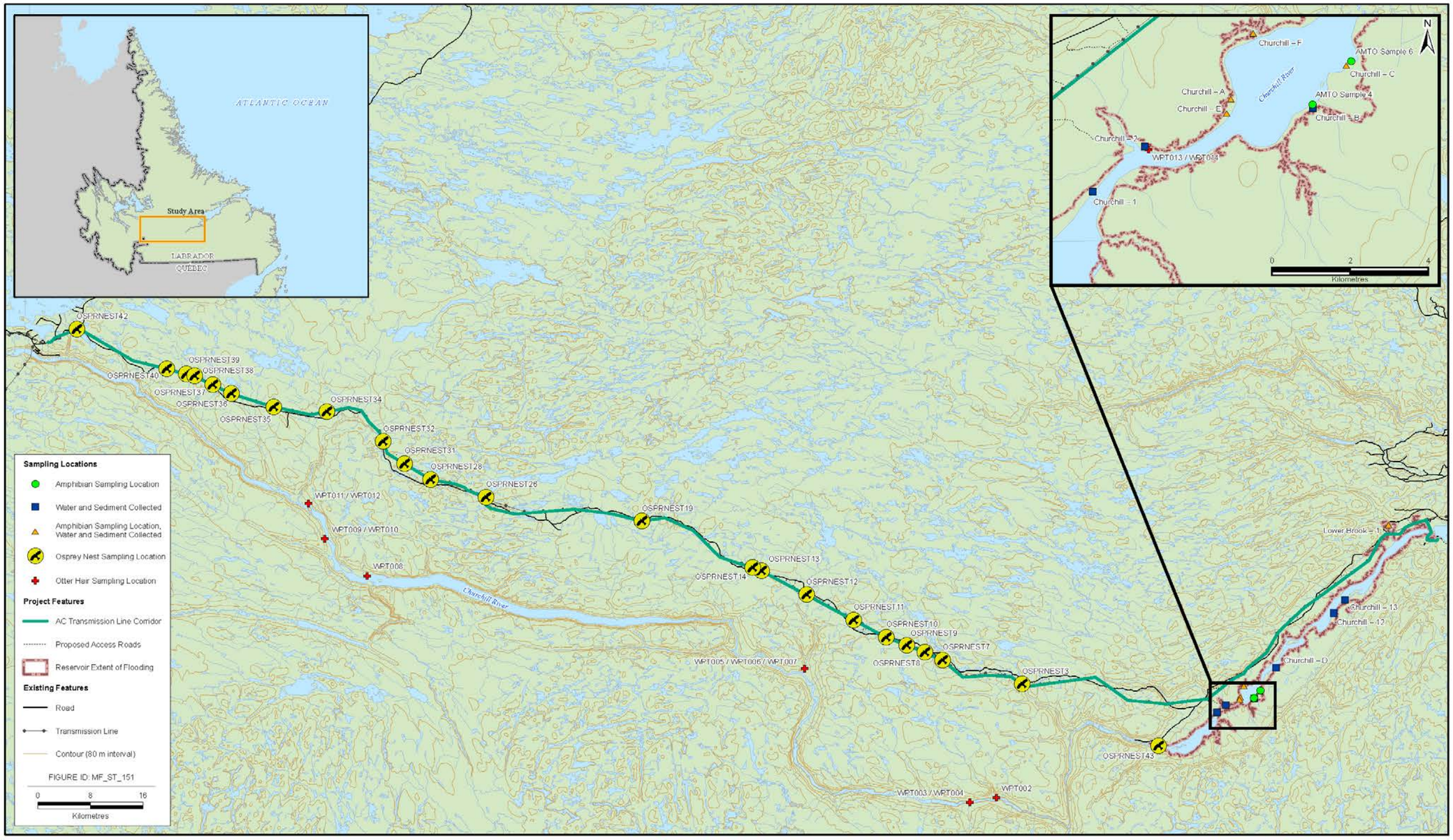


Figure 2-1 2014 Ecorisk Study Area

2.2 Osprey Sampling and Analysis

2.2.1 Sample Collection

Osprey nest in tall trees and on built structures such as power poles and artificial nesting platforms through the lower Churchill River watershed. Within the Study Area (Figure 2-1), 23 previously identified active nests[1] were visited between October 3 and October 12, 2013 (Table C-1 in Appendix C). Sites were accessed by vehicle and foot, with the exception of one nest where a helicopter was required. At each nest, an S-pattern search within a 50 m radius from the nest was conducted to collect as many feathers as possible from the ground. Feathers were stored in re-sealable bags until later analyses.

2.2.2 Total Mercury (THg) and Methylmercury (MeHg) Analysis

One Osprey feather from each nest was submitted to a certified lab for THg analyses. THg concentration was determined for two segments of the feather (Figure 2-2): the bottom first 2 cm of the shaft (piece 1), and the portion of the shaft corresponding to the first 2 cm of the vane (piece 2). THg in piece 1 represents Hg accumulated in the feather recently and is therefore more likely to represent local Hg contamination.

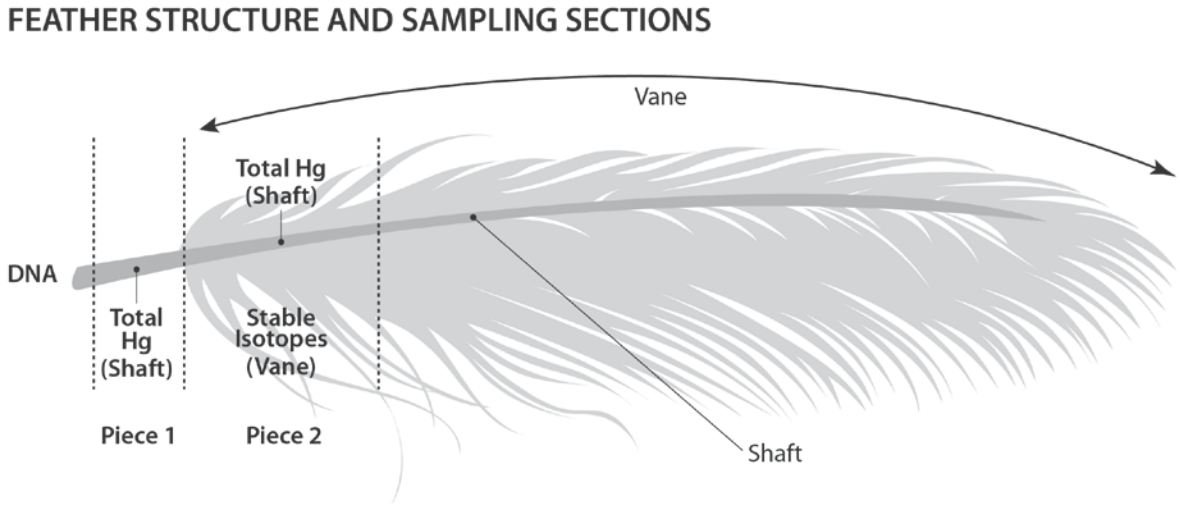


Figure 2-2 Osprey Feather Sections for Laboratory Analysis

Biota sediment accumulation factors (BSAFs) are used to estimate the ratio of a contaminant taken up into biota. A literature review was conducted on BSAFs and Osprey, and findings used

[1] Identified during aerial raptor surveys conducted as part of the Avifauna EEMP

to assess expected MeHg accumulation in Osprey. Conventional practice is to assume that for species such as Osprey (and river otter) that are high in the food chain, almost 100% of Hg is in the form of MeHg. Therefore, by analyzing for THg one can also get MeHg estimates.

2.2.3 Stable Isotopes Analysis

One Osprey feather per nest was submitted to a certified lab for Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis. Nitrogen and carbon isotopes were determined in the same portion of the feather corresponding to THg levels reported for piece 2. Stable isotope results were compared to THg in piece 2 in order to compare data from the same feather growth period.

2.2.4 DNA Analysis

Samples from two Osprey feathers per nest were submitted for DNA analysis, where possible. A total of 25 samples were sent for DNA analyses, consisting of the bottom 3 mm of the quill tips of Osprey feathers (labeled DNA in Figure 2-2).

2.3 River Otter Sampling and Analysis

2.3.1 Sample Collection

Modified body snares (Depue and Ben-David, 2007) were deployed at seven tributaries within the Study Area (Figure 2-1; Appendix C). Survey locations were selected based on sites where river otter tracks were previously identified during 2014 winter aerial surveys as a part of the Furbearer EEMP. The traps were modified so that individuals could easily escape from the trap, but hairs would be collected from each captured individual.

Trapping sites were accessed by helicopter on June 23 and one to three traps were set at each site. Traps were checked on June 29 and again on July 7. This time period was targeted as optimal for the trapping effort as it would most likely result in the collection of longer guard hairs. Traps and associated hair samples were removed and placed in paper envelopes for later analyses, and new traps were set.

2.3.2 Total Mercury (THg) and Methymercury Analysis

As river otter guard hairs grow over a period of four months, information regarding the temporal variation in THg exposure over that period can be determined by measuring THg in different parts of the hair. For this purpose, Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) analysis was used to determine THg. As the LA-ICP-MS method only requires one hair to determine Hg concentrations, the best/longest hair was selected from each sample for analysis, where possible. Information on other metals such as cadmium (Cd), lead (Pb), copper (Cu), iron (Fe) and zinc (Zn) were collected simultaneously and were used to aid the interpretation of results.

Hair samples were analyzed for THg only. MeHg accumulation in river otter was determined based on a review of the available literature. As with Osprey, this species is high on the food chain and thus MeHg is expected to comprise approximately 100% of THg.

2.3.3 DNA Analysis

All remaining hair samples collected from each trap (i.e., not selected for THg analysis) were sent for DNA analysis (species confirmation and sex/age determination, where possible).

2.4 Sampling and Analysis for Amphibians, Water and Sediment

2.4.1 Sample Collection

Eleven sites along the lower Churchill River valley were sampled for amphibians, water, and sediment samples (Figure 2-1). Sampling effort initially focused on northern leopard frog tadpoles based on the existence of well-defined methods in toxicology studies. Relatively few northern leopard frog tadpoles were captured and consequently American toad tadpoles were also collected to augment the Ecorisk EEMP.

Amphibian samples were collected from seven locations by hand and/or dipnet, from accessible wetlands in the lower Churchill River valley. Tadpoles were placed into sterile plastic bags with pond water and placed in a cooler with ice packs. Tadpoles were euthanized using a 1:1000 dilution of Eugenol and water. Tadpole measurements of total length, snout-vent length, and tail length were recorded.

Aquatic environmental parameters were recorded from eleven sampling locations in the lower Churchill River valley(Figure 2-1), including conductivity, dissolved oxygen, Oxidation Redox Potential, pH, salinity, and temperature. Water depth was recorded and samples were taken using 40 mL and 250 mL sample bottles. A Teflon spoon was used to sample the upper 2-3 cm of sediment from each site (placed in 125 ml jars), in areas of each wetland that had not recently been exposed to air.

2.4.2 Total Mercury (THg) and Methylmercury (MeHg) Analysis

Amphibian tissue, water and sediment samples were sent to a certified laboratory for analyses of THg and MeHg.

Given limited knowledge of the proportion of MeHg relative to THg in amphibians, both THg and MeHg were analyzed in water and sediment samples, and subsequently used to inform amphibian MeHg levels, along with data available in scientific literature.

The analysis of THg in water samples was carried out using procedures adapted from the American Public Health Association (APHA) method (APHA 1992) and from the United States Environmental Protection Agency (USEPA) Method SW-846 (USEPA 2007). MeHg analysis of water

samples was carried out using USEPA Method 1630 (USEPA 1998), where water samples were distilled to isolate MeHg from the sample matrix.

THg analysis in sediment samples was carried out using procedures from the Contaminated Site Regulation (CSR) (British Columbia Ministry of Environment 2009) as well as procedures adapted from the USEPA Method 200.2 (USEPA 1994). MeHg analysis in sediment samples was carried out following methods in Bloom et al. (1997).

2.4.3 Stable Isotopes Analysis

A homogenate subsample of each tadpole was removed and sent to a certified laboratory for stable isotope analyses. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic analyses were performed using continuous flow, ion-ratio, mass spectrometry (CF-IRMS) as described by Loseto et al. (2008).

2.5 Quality Assurance / Quality Control

Quality Assurance / Quality Control (QA / QC) analyses were conducted on laboratory samples sent for THg recovery and stable isotope analysis.

3.0 RESULTS

The general results related to the methods applied are summarized below. Detailed technical results and statistical analyses are presented in Appendix D. Information on QA / QC results are tabled in Appendix E.

3.1 Osprey

Osprey feathers collected from 19 of the 23 nests visited were comprised of primary, secondary, tertiary and down feathers (Appendix C). Large numbers of feathers (50+) were present at three sites (OSPRNEST14, OSPRNEST28 and OSPRNEST36), and most of the feathers appeared broken.

3.1.1 Total Mercury (THg) and Methylmercury (MeHg)

THg ranged from 1.22 to 18.6 mg/kg in piece 1 and from 1.08 to 28.2 mg/kg in piece 2 (Table 3.1). While there was no significant difference between average THg concentrations in piece 1 and piece 2 ($\alpha = 0.05$, $p = 0.781$) (Appendix E), inter-individual variations in the concentration difference between the two pieces were observed. The percentage difference in THg levels between piece 2 and piece 1 ranged from -39.9 % to 127 % with negative values suggesting that Osprey were exposed to higher concentrations more recently (Table 3.1).

Table 3.1 Total Mercury (THg) in Osprey Feathers Collected from the Lower Churchill River Valley

Sample ID	Piece 1 (mg/kg)	Piece 2 (mg/kg)	Difference (%) Between Piece 1 and Piece 2
OSP2013-07	6.99	7.93	13.45
OSP2013-08	1.22	1.08	-11.48
OSP2013-09	6.79	6.21	-8.54
OSP2013-10	8.47	8.24	-2.72
OSP2013-11	14.1	12.3	-12.77
OSP2013-12	17.5	12	-31.43
OSP2013-13	11.5	10.2	-11.30
OSP2013-14	16.5	17.5	6.06
OSP2013-19*	4.73[a]	n/a[b]	n/a[b]
OSP2013-28	4.82	4.23	-12.24
OSP2013-35	1.41	1.26	-10.64
OSP2013-36	7.29	4.38	-39.92
OSP2013-37	11.7	13.8	17.95
OSP2013-38	18.6	20.7	11.29
OSP2013-39	12.9	16.8	30.23
OSP2013-40	12.4	28.2	127.42
OSP2013-42*	0.113[a]	n/a[b]	n/a[b]
OSP2013-43	13.3	10.8	-18.80
Average ± SD	**10.3 ± 5.32**	**10.9 ± 7.30**	**2.91 ± 0.37**
Minimum	**1.22**	**1.08**	**-39.9**
Maximum	**18.6**	**28.2**	**127.4**

* These data not included in analysis as they represent down feathers only
[a] This data was from a down feather and therefore not included in the statistical analyses
[b] Not available as the feather was too small to divide into two segments
Refer to Figure 2-2 for location of Piece 1 and Piece 2 Osprey feather segments

As MeHg is estimated to comprise approximately 100 % of the THg in Osprey (Braune and Gaskin 1987, Odsjo et al. 2004), THg levels found in Osprey feathers in this study are used to represent MeHg levels.

3.1.2 Stable Isotopes

Nitrogen stable isotope ($\delta^{15}N$) values from Osprey feathers ranged from 9.1 to 15.2 ‰. Given that every 3 ‰ change in $\delta^{15}N$ corresponds to a change in trophic level (Minagawa and Wada 1984), results suggest that Osprey feed at two to three different trophic levels.

Carbon stable isotope ($\delta^{13}C$) values from Osprey feathers ranged between -32.5 and -17.5 ‰. The lighter signatures (i.e., more negative results) indicate that Osprey appear to be feeding on a number of different prey.

3.1.3 DNA

All but one of the 25 Osprey feather samples submitted produced DNA sequence profiles of suitable strength for species identification. Twenty three Ospreys were identified as well as one Spruce Grouse (*Falcipennis canadensis*) (Table 3.2). The 23 samples identified as Osprey were analyzed for gender. Despite multiple attempts, only 14 samples produced gender data strong enough to satisfy the laboratory's threshold for high confidence scoring. The low success rate related to gender identification may be, in part, due to degradation while the samples were exposed to the elements prior to collection. It had been noted that most of the feathers collected were broken. Of the 14 successfully analyzed Osprey samples, 10 were identified as female and four were identified as male (Table 3.2).

Table 3.2 DNA results (Species and Gender) from Feather Samples Collected in the Lower Churchill River Valley

Sample ID	Species	Gender
OSP2013-07	Osprey	F
OSP2013-08	Osprey	F
OSP2013-09	Osprey	U
OSP2013-10	Osprey	F
OSP2013-11	Osprey	M
OSP2013-12	Osprey	M
OSP2013-13	Osprey	F
OSP2013-14	Osprey	F
OSP2013-19	Osprey	U
OSP2013-28	Osprey	U
OSP2013-35	Osprey	F
OSP2013-36	Unconfirmed	U (failed analysis)
OSP2013-37	Osprey	U
OSP2013-38	Osprey	U
OSP2013-39	Osprey	F

Sample ID	Species	Gender
OSP2013-40	Osprey	U
OSP2013-42	Spruce Grouse	U
OSP2013-43	Osprey	F
F: Female; M: Male; U: Unidentified		

THg levels averaged 9.1 ± 5.6 mg/kg and 15.8 ± 2.4 mg/kg for the 10 females and two males, respectively. Previous studies have shown lower THg levels in female feathers compared to males and this has been attributed to maternal transfer of Hg to offspring (Braune and Gaskin 1987, Lewis et al. 1993, Becker et al. 2002). However, due to only two males being identified in the present study, no statistical analyses could be performed to evaluate the difference in THg levels between males and females.

3.2 River Otter

Hair samples were collected from five of the seven sampling locations (Figure 2-1, Appendix C). Underfur was the dominant type of hair at each hair snag station, and only a few small guard hairs were obtained (sites WPT011, WPT013 and WPT014).

3.2.1 Total Mercury (THg), Methylmercury (MeHg) and DNA Results

Nine hairs were collected from four sampling locations (Fig River area, Elizabeth River area, Metchin River, Pinus River). All samples were analyzed for THg, however of the nine hairs, only three were guard hairs, the most appropriate samples for LA-ICP-MS. THg levels among samples averaged 0.792 ± 1.08 mg/kg and ranged between 0.155 and 3.49 mg/kg (Table 3.3).

Table 3.3 Total Mercury (THg) in Hair Samples from the Lower Churchill River Valley

Otter Hair Sample	Sample Area	Hair type	Species*	THg (mg/kg)		
				Average ± SD	Min	Max
WPT008-1	Fig River area	underfur	Snowshoe Hare	0.336 ± 0.0640	0.259	0.503
WPT008-2	Fig River area	underfur	Unconfirmed	0.211 ± 0.0741	0.154	0.367
WPT008-3	Fig River area	underfur	Unconfirmed	0.155 ± 0.0552	0.068	0.238
WPT010-1	Elizabeth River area	unknown	Muskrat	0.838 ± 0.105	0.619	1.044
WPT010-2	Elizabeth River area	guard hair	Unconfirmed	0.337 ± 0.0305	0.294	0.388
WPT011	Metchin River	guard hair	Unconfirmed	0.257 ± 0.114	0.066	0.574
WPT013	Pinus River	guard hair	Snowshoe Hare	0.186 ± 0.0410	0.118	0.284
WPT014-1	Pinus River	underfur	Otter	3.49 ± 1.14	1.36	5.21
WPT014-2	Pinus River	underfur	Unconfirmed	1.32 ± 0.537	0.529	2.23
* Unconfirmed species - could not be confirmed due to inadequate samples (WPT008-2, WPT010-2 and WPT014-2) or failed test results (WPT008-3).						

Based on available literature, MeHg is expected to comprise approximately 100 % of THg in river otter hair samples (Kehrig et al. 1998, Voegborlo et al. 2010). Thus, THg levels collected as part of this study were used to represent MeHg levels in river otter.

Given the small sample size, there were challenges to successfully complete DNA analyses. Samples with no guard hair roots and with less than five underfur samples had to be excluded from analysis. Those included WPT008-2, WPT010-2 and WPT014-2 (Table 3.3). For the remaining five samples, the lab clipped the guard hair roots when available or used the entire length of finer hair. Samples were generally weak and thus the analyses had to be run twice to confirm species. As a result, gender identification could not be performed.

Results from the analysis confirmed one river otter sample (WPT014-1), as well as one muskrat (*Ondatra zibethicus*) (WPT010-1) and two snowshoe hares (*Lepus americanus*) (WPT008-1 and WPT013). WPT008-3 sample failed to produce a useable DNA sequence on either attempt at analysis (Table 3.3). The THg level along the otter hair sample averaged 3.49 ± 1.14 mg/kg. This level was in the lower range of those previously reported for river otters sampled from several locations in the United States (Halbrook et al. 1994, Strom 2008) and lower than the conservative 5.4 mg/kg neurochemical effect levels (Basu et al. 2009).

3.3 Amphibians and Water and Sediments

American toad tadpoles were collected at six sites (Appendix C): Lower Brook-1, Churchill-C, Churchill-D, Churchill-F, AMTO Sample 4 and AMTO Sample 6. Snout-vent length of tadpoles ranged from 4-10 mm and tail length from 6-11 mm (Appendix C) and did not differ significantly amongst sites ($\alpha = 0.05$, $p<0.001$; Appendix D). Total length ranged from 11-21 mm (Appendix C) and similarly did not differ amongst sites ($\alpha = 0.05$, $p<0.001$; Appendix D).

Northern Leopard frog tadpoles were collected at three sites (Appendix C): Churchill-A, Churchill-C and Churchill-F. Snout-vent length of tadpoles ranged from 4 to 21 mm (Appendix C) and did not differ amongst sites ($\alpha = 0.05$, $p=0.0750$; Appendix D). Tail length ranged from 4 to 37 mm, and tadpoles collected from Churchill-C had a tail significantly shorter than those collected at the two other sites ($\alpha = 0.05$, $p = 0.0480$; Appendix D). Total length of tadpoles from Churchill-C (range 8 – 57 mm; Appendix C) were significantly shorter compared to those collected at the two other sites ($\alpha = 0.05$, $p = 0.0460$; Appendix D).

3.3.1 Amphibians

3.3.1.1 Total Mercury (THg) and Methylmercury (MeHg)

Amphibian tissue samples were pooled within species and site, and subsampled for THg. Total mercury concentrations in amphibian tissues ranged between 0.00320 – 0.0575 mg/kg wet weight (ww) (Appendix D). The highest THg concentration (0.0575 mg/kg ww) occurred in Lower Brook 1. Due to a laboratory error, MeHg analysis of amphibian tissue samples was not possible.

The BSAF for THg in amphibians from sediment and water are factors of 1.42 and 2.27, respectively. Available literature suggests that approximately 30 % of THg in amphibian tadpoles is made up of MeHg (Bank et al. 2007). Using this estimate, the BSAFs for MeHg in amphibians based on sediment and water MeHg (see Section 3.3.2) are 42.6 for sediment, and 12.8 for water.

3.3.1.2 Stable Isotopes Analysis

American toad tadpole results suggest a variety of prey are consumed, as there was no correlation between $\delta^{15}N$ and $\delta^{13}C$ ($\alpha = 0.05$, $p = 0.944$; Appendix D). Given the overall low sample size of amphibians, site differences between stable isotopes could not be distinguished.

T-test analyses showed that the tadpoles of the northern leopard frog had lower $\delta^{13}C$ (-34.9 ± 0.264 ‰) than the American toad (-29.9 ± 2.29 ‰) ($\alpha = 0.05$, $p = 0.008$; Appendix D). $\delta^{13}C$ of northern leopard frog tadpoles were however in a similar range of two other more closely related tadpole species of the common frog (*Rana temporaria*) (Trakimas et al. 2011) and green frog (*Lithobates clamitans)* (Jefferson and Russel 2008). The $\delta^{13}C$ differences observed suggest that the two species may be feeding on slightly different prey. Importantly, the lack of difference in $\delta^{15}N$ between the American toad and northern leopard frog ($\alpha = 0.05$, $p = 0.271$; Appendix D) suggests that the two species feed at the same trophic level. When the two species are compared from the same sites (Churchill C and Churchill F) American toads appear at slightly higher trophic levels with associated higher $\delta^{13}C$ and may explain the higher THg concentrations.

3.3.1.3 Environmental and Biological Characteristics Influence on Amphibian Total Mercury (THg) and Methylmercury (MeHg)

American toad THg concentrations were significantly correlated with dissolved oxygen ($\alpha = 0.05$, $p = 0.00439$; Appendix D), supporting previous work that dissolved oxygen influences methylation and potential uptake in biota. American toad THg was also correlated with sediment MeHg ($r^2 = 0.807$, $\alpha = 0.05$, $p = 0.0525$; Appendix D) followed by water MeHg ($r^2 = 0.784$; $\alpha = 0.05$, $p = 0.0652$; Appendix D) and sediment THg ($r^2 = 0.735$, $\alpha = 0.05$, $p = 0.096$; Appendix D), where higher concentrations in the environment corresponded with higher concentrations in the tadpoles.

No correlation was observed with any of the biological variables (snout-vent length, tail length, total length, $\delta^{15}N$ or $\delta^{13}C$; Appendix D) and THg concentrations. Although previous work has found that larger animal size and higher trophic levels may drive higher mercury concentrations in amphibians (Ugarte 2005, Unrine 2007), this is not always the case (Gerstenberger 2002).

3.3.2 Water and Sediment

Water and sediment data were collected from depths ranging from 0.3 – to 1.5 m. Environmental factors were recorded as ranging from 13.3 – 24.8 °C for water temperature, 6.2 –

103.8 % for dissolved oxygen, 5.9 – 7.0 for pH, 0.018 – 0.132 s/m for conductivity, and 0.022 – 0.157 mmol/L for hardness (Appendix D).

3.3.2.1 Total Mercury (THg) and Methylmercury (MeHg) Results

All 11 water samples were below the detection limit (0.0100 µg/L) for THg (Table 3.3). Nine of 11 samples had MeHg levels above the detection limit (0.0000500 µg/L), with values ranging from <0.0000500 µg/L to 0.00215 µg/L (Table 3.3). These levels are below the water quality guidelines for THg (0.0260 µg/L) and MeHg (0.00400 µg/L) developed by Environment Canada for the protection of aquatic life (Environment Canada 2003). However, it is important to note that these guidelines do not address exposure through food or bioaccumulation to higher trophic levels. Aquatic wildlife exposed to MeHg primarily through food might not be adequately protected using these values.

Table 3.4 Total Mercury (THg) and Methylmercury (MeHg) in Water Samples from the Lower Churchill River Valley, June 2014

Site	THg (mg/L)[a]	MeHg (µg/L)	% MeHg*
Lower Brook-1	<0.000010	0.00155	15.50%
Churchill - 1	<0.000010	0.00215	21.50%
Churchill - 2	<0.000010	0.000096	0.96%
Churchill - A	<0.000010	0.00113	11.30%
Churchill - B	<0.000010	<0.000050	0.50%
Churchill - C	<0.000010	<0.000050	0.50%
Churchill - D	<0.000010	0.0001	1.00%
Churchill - E	<0.000010	0.000103	1.03%
Churchill - F	<0.000010	0.00035	3.50%
Churchill - 12	<0.000010	0.000121	1.21%
Churchill - 13	<0.000010	0.000136	1.37%
Average ± SD	**0.00001**	**0.00053 ± 0.00073**	**5.31 ± 7.35%**
Minimum	**0.00001**	**0.00005**	**0.50%**
Maximum	**0.00001**	**0.00215**	**21.50%**

[a]Analytical chemical techniques differed for THg (cold vapour atomic fluorescence spectrometry) and MeHg (gas chromatography atomic fluorescence spectrometry), resulting in different detection limits.

*When non-detected, detection limit substitution was used to allow calculation.

THg was detected in 10 of the 11 sediment samples, with levels ranging from <0.00500 mg/kg to 0.0322 mg/kg dry weight (dw) (Table 3.4). MeHg was detected in nine of the 11 samples and ranged from <0.0000500 mg/kg dw to 0.000297 mg/kg dw (Table 3.4). The THg values detected were one to two orders of magnitude lower than the interim sediment quality guidelines for THg

(ISQGs = 0.170 mg/kg) and probable effect levels (PELs = 0.486 mg/kg) for the protection of aquatic life (Environment Canada 1999a).

Percent MeHg in sediment samples ranged from 0.290% to 1.78%. Churchill – 1 and Lower Brook – 1 had the highest percentages of MeHg contribution to THg (1.78% and 1.41%, respectively) (Table 3.4).

Table 3.5 Total Mercury (THg), Methylmercury (MeHg) and Percent Methylmercury (% MeHg) in Sediment Samples from the Lower Churchill River Valley, June 2014

Site	THg (mg/kg dw)	MeHg (mg/kg dw)	% MeHg
Lower Brook - 1	0.0172	0.000243	1.41%
Churchill - 1	0.0167	0.000297	1.78%
Churchill - 2	0.0322	0.000243	0.750%
Churchill - A	0.0169	0.0000860	0.510%
Churchill - B	0.00900	<0.0000500	0.560%
Churchill - C	<0.00500	<0.0000500	n/a*
Churchill - D	0.00690	<0.0000500	0.720%
Churchill - E	0.0257	0.0000740	0.290%
Churchill - F	0.0133	0.0000780	0.590%
Churchill - 12	0.0138	0.000104	0.750%
Churchill - 13	0.0206	0.000148	0.770%
Average ± SD	**0.0160 ± 0.00800**	**0.000160 ± 0.0000900**	**0.830 ± 0.430%**
Minimum	**<0.00500**	**<0.0000500**	**0.290%**
Maximum	**0.0322**	**0.000297**	**1.78%**
***** n/a = not applicable due to values below detection limits			

3.3.2.2 Environmental Factors Influence on Water and Sediment Total Mercury (THg) and Methylmercury (MeHg)

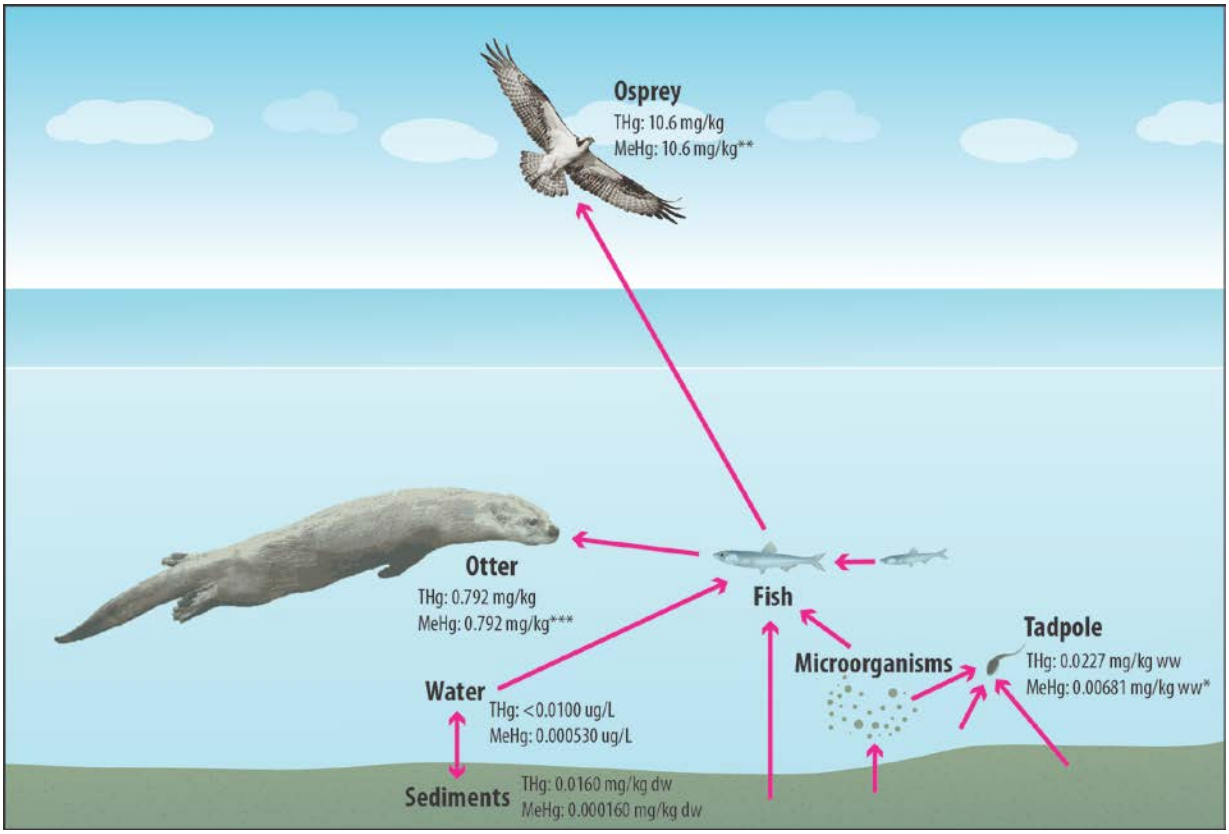
Dissolved oxygen, hardness and conductivity were significantly correlated with each other (Appendix D). Of these, hardness and conductivity appeared to be most influential in water MeHg. Hardness and conductivity were also highly correlated with sediment MeHg where greater hardness and conductivity resulted in higher concentrations of MeHg.

Sediment MeHg was most highly correlated with water MeHg (r^2 = 0.703, α= 0.05, p = 0.0108; Appendix D) in addition to a strong correlation with sediment THg (r^2 = 0.582, α= 0.05, p = 0.0471) (Appendix D).

Even though conductivity and hardness were the two parameters strongly correlated with MeHg levels in water and sediment, it is important to keep monitoring all water quality parameters to best predict the process of Hg transformation in this aquatic environment.

3.4 Ecosystem Baseline of Total Mercury (THg) and Methylmercury (MeHg) in the Lower Churchill River Valley

The BSAFs for MeHg in amphibians based on sediment MeHg levels in the current study (i.e., from sampling locations in the lower Churchill River valley) is estimated to be 42.6, and based on water MeHg is 12.8. These accumulation factors demonstrate the bioavailability of MeHg and its ability to accumulate in higher trophic levels. Figure 3-1 presents a schematic of potential pathways of mercury transfer in an ecosystem. Although mercury levels of fish, a critical prey component, were not measured as part of the EEMP, higher concentrations of mercury in higher trophic levels (i.e., 0.0227 mg THg/kg in amphibians, to 0.792 mg THg/kg in river otter, to 10.6 mg THg/kg in Osprey) indicate the capabilities of mercury to bioaccumulate in the Lower Churchill Muskrat Falls Project area.



* Values based on expected MeHg concentrations (~30% of THg for tadpoles (Bank et al., 2007) and ~100% of THg concentrations for river otter and Osprey (Braune and Gaskin 1987, Odsjo et al. 2004, Kehrig et al. 1998, Voegborlo et al. 2010))

Figure 3-1 Ecosystem Schematic of Mercury Trophic Transfer

4.0 SUMMARY

The 2014 Ecorisk EEMP was designed to collect baseline levels of THg and MeHg levels in the Lower Churchill River valley, using information from Osprey feathers, river otter hair, amphibian (tadpole) tissues, and water and sediment samples. In addition, stable isotopes were analyzed for Osprey and amphibians, to assess the trophic levels (e.g., where they feed and which trophic level they belong to).

Osprey feathers were collected from 19 of the 23 active Osprey nests visited, hair samples from five of the seven sampling locations, and samples of northern leopard frog and/or American toad from 13 locations in the Study Area, including 11 of water and sediment sampling sites.

THg levels detected in Osprey feathers (n=18) ranged from 1.08 to 28.2 mg/kg. THg levels averaged 9.1 ± 5.6 mg/kg in female samples (n=10) and 15.8 ± 2.4 mg/kg in male samples (n=2). Based on available literature, the expected MeHg is approximately 100 % of THg (Braune and Gaskin 1987, Odsjo et al. 2004). Stable isotope analyses indicate that Osprey feed on a number of different prey items, from two to three different tropic levels.

Results from the river otter hair sampling program yielded only one confirmed river otter sample. The average THg level along the hair sample was 3.49 ± 1.14 mg/kg. A review of the available literature suggests that MeHg is approximately 100 % of THg in river otter hair samples (Kehrig et al. 1998, Voegborlo et al. 2010).

Amphibian THg levels ranged from 0.0032 to 0.0170 mg/kg ww in Northern leopard frog samples (n=3) and from 0.0098 to 0.0575 mg/kg ww in American toad samples (n=6). Based on available literature suggesting that approximately 30.0 % of THg in amphibian tadpoles is made up of MeHg (Bank et al. 2007), the BSAF for MeHg in amphibians from sediment MeHg is 42.6 and from water MeHg is 12.8. Stable isotope analyses indicated that the two species sampled may be feeding on slightly different prey, but suggested that they feed at the same trophic level, when all results were combined. When the analyses looked at only the two sites where they were both sampled, American toad appeared at a slightly higher trophic level compared to Northern leopard frog.

THg and MeHg were detected in most sediment samples (10 out of 11 and 9 out of 11 samples, respectively) with THg concentrations being one to two orders of magnitude lower than the interim sediment quality guidelines (ISQGs = 0.17 mg/kg) and probable effect levels (PELs = 0.486 mg/kg) for the protection of aquatic life (Environment Canada 1999). Hardness and conductivity were highly correlated with MeHg levels in water ($r^2 = 0.84$, $p < 0.001$ and $r^2 = 0.85$, $p < 0.001$, respectively) and sediment ($r^2 = 0.81$, $p < 0.001$ and $r^2 = 0.83$, $p < 0.001$, respectively).

The current study illustrates the bioavailability of MeHg in sediment and water and its ability to accumulate in higher trophic levels. Although mercury levels of fish, a critical prey component,

were not measured as part of the EEMP, higher concentrations of mercury in higher trophic levels (i.e., 0.0227 mg THg/kg in amphibians, to 0.792 mg THg/kg in river otter, to 10.6 mg THg/kg in Osprey) indicate the capabilities of mercury to accumulate in the Project area.

Additional sampling may be required to complete baseline assessments for river otter and amphibians.

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APPENDIX A

Research Permit

Newfoundland Labrador

GOVERNMENT OF NEWFOUNDLAND AND LABRADOR
Dept of Environment & Conservation

Scientific Research Permit

(as under Section 86 of the Wildlife Regulations, Consolidated Newfoundland and Labrador Regulation 1156/96)

Permit #: IW2013-66

Project Title: *Wildlife Environmental Effects Monitoring During Construction of the Lower Churchill Hydroelectric Development*

Issued to:
Perry Trimper, Stassinu Stantec Limited Partnership
P.O. Box 482, Station C, Happy Valley-Goose Bay, NL A0P 1C0
Tel: (709) 896-5860

Permit to:
1) ***Winter Research***: Undertake winter aerial and ground track surveys for moose, otter, marten, porcupine and other wildlife;
2) ***Spring Summer Research***: Undertake spring/summer breeding bird point count surveys, otter and black bear hair snag trapping, and directed surveys for spring peeper and salamanders;
3) ***Fall Research***: Undertake fall aerial surveys for beaver colonies and deploy specialized traps to determine presence of water and pygmy shrews.

The objectives of these studies are to collect additional baseline information and to monitor potential environmental effects during construction of the Lower Churchill Hydroelectric Development.

Date of research: March 1 to October 1, 2014.

Date of Permit Expiration: November 1, 2014.

Location: All field investigations will occur primarily within the lower Churchill River watershed of Labrador. Of interest is a 20 km radius around the Project footprint in the lower Churchill River valley and the AC transmission line from Muskrat Falls to Churchill Falls (Figure 1). The intent is to establish a monitoring grid throughout the Study Area where cells become permanent monitoring stations. Where possible and appropriate, pre-existing transects and grids will be resurveyed and supplemented.

Conditions:
1) The permit holder may designate other individuals to perform these actions on his behalf, with suitable supervision. The permit holder is responsible for the training of any designated individuals and must ensure that designated individuals follow all conditions of this permit.

2) Names and contact information for all individuals participating in research activities shall be provided to the Wildlife Division, Department of Environment and Conservation prior to commencement of field work. Additional names or deletion of names can be provided to Wildlife Division on an ongoing basis.

3) Prior to initiation of the field program for effects monitoring and baseline investigations, a digital copy of the shape files of all survey routes must be provided to the Wildlife Division.

4) This permit is only valid for work within the indicated study area (Figure 1).

5) With the exception of activities covered under this permit, no wildlife species, including the study species, will be unduly harassed, injured or killed as a result of activities performed under this permit. The Wildlife Division advises applicants to operate under established regulations and guidelines with respect to wildlife and wildlife habitat to minimize adverse impacts (Section 106 of the Wild Life Regulations under the *Wild Life Act* (O.C. 96-809)).

6) Disturbance of all wildlife should be minimized during helicopter and ground transportation. Whenever possible, aircraft should not descend lower than 100 meters (above ground level) during surveys.

7) The field program will be conducted using accepted wildlife research techniques and targeted species will be disturbed as little as possible. The methods and survey dates described in the application will be followed as closely as possible. Any changes to the survey design or methodology outlined in the initial permit request will require prior approval before implementation.

8) A detailed protocol should be provided to the Wildlife Division for approval prior to any sampling of small mammals or amphibians. Any samples that are collected must be turned into the Wildlife Division following identification. A permit is required and must be obtained prior to transporting any samples or specimens out of the province.

9) To avoid the introduction of non-native species all research equipment should be new and unused, or equipment that has not been previously used outside of Labrador.

10) Final reports should be submitted for each of the components of the work proposed and permitted. Reports should provide a synopsis of the location of surveys, methods employed, number of samples/specimens taken, location of samples/specimens, additional relevant ecological information and a summary of next steps. The raw data and coordinates should be submitted in digital format along with the final reports for each component and for the following: small mammals, amphibian, otter, marten, moose, black bear, porcupine, beaver, breeding birds, mercury level analysis and all sightings of wildlife and sign. The permit holder is responsible to obtain any and all permissions which may be required to release this information to the Wildlife Division. Final reports are to be remitted by the following dates to the Wildlife Division:

Winter Research: May 1, 2014
Spring/Summer Research: October 1, 2014
Fall Research: December 1, 2014

11) Any unusual wildlife observations or any adverse effects observed during the Project are to be reported immediately to the Wildlife Division.

12) This permit does not absolve or relieve the permit holder from any other laws, permits, regulations or orders.

13) This permit does not relieve the permit holder from the requirement to acquire permission to access private property.

14) All conditions of this permit must be adhered to and data and results from this project submitted to the Wildlife Division prior to another permit being issued.

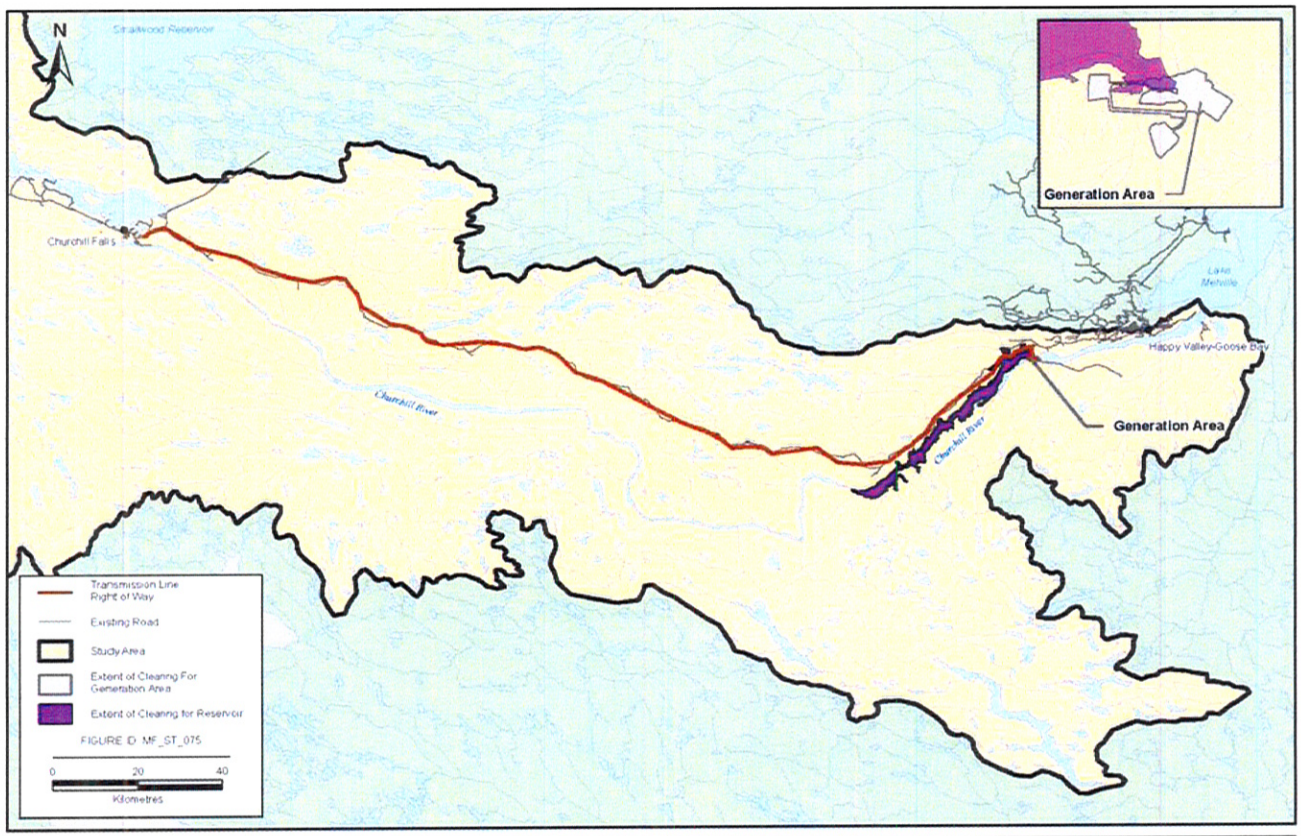
15) Under the discretion of the Director of Wildlife, this permit can be revoked without notice.

March 12, 2014
Date:

Director of Wildlife

PO Box 2007
Corner Brook, NL
A2H 2L7
Phone: (709) 637-2008
Fax: (709) 637-2004

Figure 1.1 **Study Area Overview: Happy Valley – Goose Bay to Churchill Falls**



nalcor energy	**FIGURE 1.1**
	Study Area Overview: Happy Valley-Goose Bay to Churchill Falls

Newfoundland Labrador

GOVERNMENT OF NEWFOUNDLAND AND LABRADOR
Dept of Environment & Conservation

Scientific Research Permit

(as under Sections 82, 85 and 86 of the Wildlife Regulations, Consolidated Newfoundland and Labrador Regulation 1156/96)

Permit #: IW2014-25

Supplement to Permit #: IW2013-66

Project Title: *Wildlife Environmental Effects Monitoring During Construction of the Lower Churchill Hydroelectric Development*

Issued to:
Perry Trimper, Stassinu Stantec Limited Partnership
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Permit to:
Collect and euthanize Northern Leopard Frog (*Rana pipiens*) tadpoles to assess Methylmercury (MeHg) levels for the investigation of potential health effects and eco-risk as part of ongoing Environmental Effects Monitoring (EEM) studies in association with the Lower Churchill Hydroelectric Generation Project. The specimens collected will be transported to ALS Laboratory in Burnaby, B.C.

Date of Research: June 23 to July 31, 2014.

Date of Permit Expiration: August 31, 2014

Location: All field investigations will occur primarily within the Lower Churchill River watershed in Labrador, primarily in the area expected to be flooded as a result of Project activities. The intent is sample eleven (11) wetland sites representative of the area of inundation and collect eighteen (18) Northern Leopard Frog (*Rana pipiens*) tadpoles within each site.

Conditions:

1) The permit holder may designate other individuals to perform these actions on his behalf, with suitable supervision. The permit holder is responsible for the training of any designated individuals and must ensure that designated individuals follow all conditions of this permit.

2) Names and contact information for all individuals participating in research activities shall

be provided to the Wildlife Division, Department of Environment and Conservation prior to commencement of field work. Additional names or deletion of names can be provided to the Wildlife Division on an ongoing basis.

3) Prior to initiation of the field program for effects monitoring and analysis, a digital copy of the shape files of all survey routes must be provided to the Wildlife Division.

4) This permit is only valid for work within the indicated study area.

5) With the exception of activities covered under this permit, no wildlife species, including the study species, will be unduly harassed, injured or killed as a result of activities performed under this permit. Disturbance of all wildlife should be minimized during helicopter and ground transportation. The Wildlife Division advises applicants to operate under established regulations and guidelines with respect to wildlife and wildlife habitat to minimize adverse impacts (Section 106 of the *Wild Life Regulations* under the *Wild Life Act* (O.C. 96-809)).

6) The field program will be conducted using accepted wildlife research techniques and targeted species will be disturbed as little as possible. The methods and survey dates described in the application will be followed as closely as possible. Any changed to the survey design or methodology outlined in the initial permit request will require prior approval before implementation.

7) To avoid the introduction of non-native species all research equipment should be new and unused, or equipment that has not been previously used outside of Labrador.

8) A final report should be submitted upon completion of the work proposed and permitted. Report should provide a synopsis of the location of surveys, maps showing locations of each wetland and each sample site, methods employed, number of samples/specimens taken, location of samples/specimens, additional relevant ecological information, analysis results, and a summary of next steps. The raw data and coordinates should be submitted in digital format along with the final report. The permit holder is responsible to obtain any and all permissions which may be required to release this information to the Wildlife Division. The final report is to be remitted to the Wildlife Division by: **September 30, 2014**

9) Any unusual wildlife observations or any adverse effects observed during this survey are to be reported immediately to the Wildlife Division.

10) This permit does not absolve or relieve the permit holder from any other laws, permits, regulations or orders.

11) This permit does not relieve the permit holder from the requirement to acquire permission to access private property.

12) All conditions of this permit must be adhered to and data and results from previous projects submitted to the Wildlife Division prior to another permit being issued.

13) Under the discretion of the Director of Wildlife, this permit can be revoked without notice.

June 20, 2014

Date:

Director of Wildlife

Wildlife Division
PO Box 2007
Corner Brook, NL A2H 7S1
Ph (709) 637-2383
Fax (709) 637-2004

Trimper, Perry

From: Trimper, Perry
Sent: Monday, June 30, 2014 9:38 AM
To: Miller, Kirsten (kirstenmiller@gov.nl.ca)
Cc: Rashleigh, Karen; Oaten, Dustin; Ingraham, Diane (diane.ingraham@stantec.com); Christensen, Jennie; Tucker, Wayne
Subject: Amendment to Permit #IW2014-25 Supplement to IW2013-66

Hello Kirsten

As per our conversation this morning, our field team has searched (for three days) the majority of suitable locations for leopard frog tadpoles in the reservoir area, with only locating them at two sites. We have noted this species is commonly associated with warmer waters near the Trans-Labrador Highway, but not so in the reservoir area. Therefore, we would like to also collect **American Toad tadpoles** which are common throughout the reservoir area. We would then have a duplicate sampling of a variety of tadpoles.

As discussed we will attach this email to our permit.

Thanks
PGT

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APPENDIX B

Technical Methods

B.1 Osprey

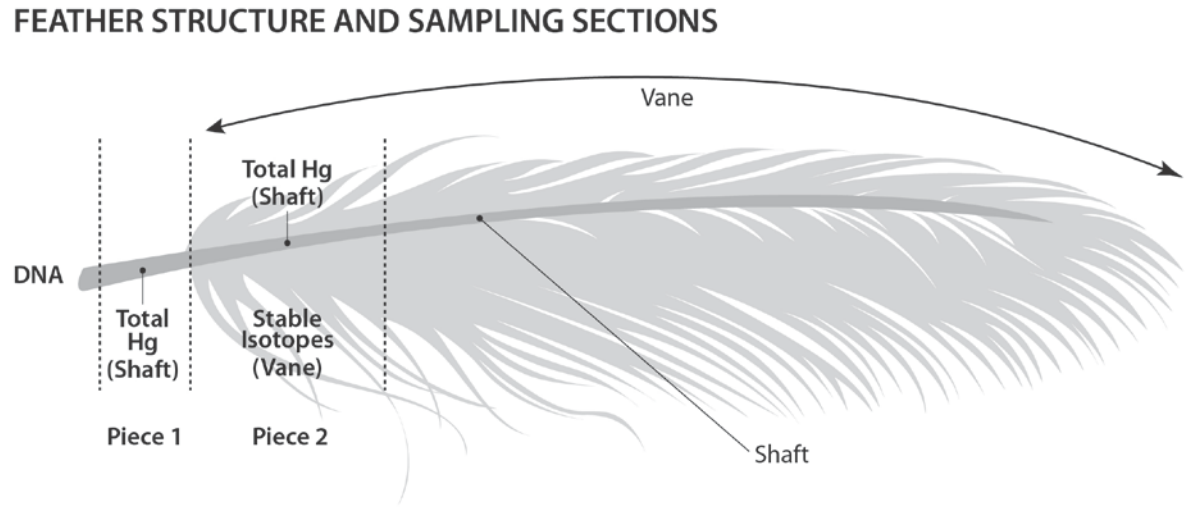
B.1.1 Sample Collection

Twenty-three active nests were visited between October 3 and October 12, 2013. Nests had been previously located during spring 2013 aerial raptor surveys, as part of the Avifauna Management Plan. Most nests were located less than 1 km from the Trans Labrador Highway (TLH) and were accessed by hiking, however one nest required helicopter (BA helicopter) access. At each nest site, the area within a 50 m radius from the nest was searched in a S-shaped pattern. On average, three nests were searched per day with a maximum of five nests and a minimum of one nest (weather caused field day to be called off). At each nest, as many feathers as possible were collected from the ground in the immediate vicinity. Feathers were placed in re-sealable bags until later analyses.

B.1.2 Total Mercury (THg) and Methylmercury (MeHg) Analysis

Osprey feathers were sent to a certified lab for Total Mercury (THg) analyses. Samples from only one feather per nest were submitted for THg analyses. THg was determined in the bottom first 2 cm of the shaft (piece 1) as well as in the portion of the shaft corresponding to the first 2 cm of the vane (piece 2) (Figure B-1). THg in piece 1 therefore represents Hg accumulated in the feather recently and is more likely to represent local Hg contamination.

Figure B-1 Osprey Feather Sections for Laboratory Analysis



Analyses of THg were carried out using a method adapted from the United States Environmental Protection Agency (USEPA) Method 200.2 (USEPA 1994). Tissue samples were homogenized and sub-sampled prior to hotblock digestion with nitric and hydrochloric acids, in combination with repeated additions of hydrogen peroxide. The extracts were then analyzed using cold vapor atomic fluorescence spectrophotometry (CVAFS), adapted from USEPA Method 245.7 (USEPA 2005).

Biota sediment accumulation factors (BSAFs) indicates the ratio of a contaminant taken up into biota. The BSAF was determined for the accumulation of MeHg in Osprey based on published results available in the literature. The expected MeHg is approximately 100 % of THg (Braune and Gaskin 1987, Odsjo et al. 2004). Thus MeHg levels in Osprey feathers are determined by analyzing for THg.

B.1.3 Stable Isotopes Analysis

Samples from one feather per nest were submitted for stable isotope analysis. The first two cm of the Osprey feather vane (both sides) were removed (Figure B-1) and sent to a certified lab for analyses. Nitrogen and carbon isotopes were determined in the first 2 cm portion of the feather shaft used in the analysis of the THg levels for piece 2. Stable isotope results will therefore only be compared to THg in piece 2 in order to compare data from the same feather growth period.

Carbon and nitrogen isotopic analyses were performed using continuous flow, ion-ratio, mass spectrometry (CF-IRMS) as described by Loseto et al. (2008). The standards used for carbon and nitrogen analyses were Vienna PeeDee Belemnite (VPDB) and International Atomic Energy Agency, Vienna, Austria (IAEN-N1), respectively.

The standard procedure for presenting results for carbon and nitrogen isotypes is to express them using standard delta (δ) notation in units of per mil (‰). The delta values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) represent a deviation from a standard:

$$\textbf{Equation 1: } \delta_{sample}‰ = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio in the sample and the standard.

B.1.4 DNA Analysis

The bottom quill tips (~ 3mm) of Osprey feathers collected were sent for DNA analyses (Figure B-1). These included samples from two feathers per nest, when possible. All DNA was extracted from all samples using QIAGEN DNeasy kits, according to the manufacturer's instructions.

Extracted Osprey samples were analyzed for species using a sequence-based analysis of the mitochondrial 16S rRNA gene (Johnson and O'Brien 1997). The sequence profiles generated were compared to the laboratory reference data of over 60 bird species as well as to reference sequences on Genbank, where applicable. Samples were analyzed for gender using the chromo-helicase-DNA-binding (CHD) marker (Griffiths et al. 1998).

B.2 River Otter

B.2.1 Sample Collection

Modified body snares (Depue and Ben-David 2007) were established at seven tributaries within the Study Area. Survey locations were selected based on sites where river otter tracks were previously identified during 2014 winter aerial surveys as part of the Furbearer EEMP. The traps

were modified such that individuals could easily escape from the trap, but hairs would be collected from each individual that came in contact with the trap. Fifteen or more hairs were expected at each successful trap.

Sites were accessed by helicopter on June 23 and one to three traps set at each site. Traps were checked July 29 and again July 7. This time period was targeted as optimal for the trapping effort as it would most likely result in the collection of longer guard hairs. From May through August, otters shed and replace underfur and from August to November they shed and replace guard hair (Ben-David et al. 2000, 2005). Sampling of fully grown guard hair could therefore provide a four-month window of mercury exposure. Traps and associated hair samples were removed and placed in paper envelopes until later analyses, and new traps established.

B.2.2 Total Mercury (THg) and Methylmercury (MeHg) Analysis

Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) analysis was used to provide information on the temporal variation in THg exposure over the four month period represented by the full-grown river otter guard hair. Information on other metals such as cadmium (Cd), lead (Pb), copper (Cu), iron (Fe) and zinc (Zn) collected simultaneously aid the interpretation of the Hg results.

The longest hair collected at each active hair snag station was removed and washed according to the standard procedure for human hair developed by the IAEA (Ryabukin 1978). Individual hairs were mounted onto a glass slide using double-sided tape and shipped for LA-ICP_MS analysis at the University of Victoria (Victoria, BC). The analysis used a New Wave UP-213 (213 nm) laser coupled to a Thermo-X2 ICP-MS following a method developed previously by the Stantec team (Noel et al. 2014). Hair samples were ablated with a spot diameter of 30 µm and a frequency of 20 Hz, and in a series of 2000 µm line scans along the middle line of the hair at a rate of 50 µm/s. Each line scan was followed by a 50 second break to allow cell washout between each scan. Similar to previous studies, sulfur and DOLT 2 (National Research Council of Canada, Ottawa, ON) were used for internal and external standards, respectively (Rodushkin et al. 2003, Stadlbauer et al. 2005, Noel et al. 2014).

Otter hair sample size was insufficient to test for MeHg. However, the expected MeHg is calculated as approximately 100 % of THg in the river otter hair samples (Kehrig et al. 1998, Voegborlo et al. 2010). Thus, MeHg levels in otter hair can be assumed by analyzing for THg.

B.2.3 DNA Analysis

Extracted hair samples were analyzed for species using a sequence-based analysis of the mitochondrial 16S rRNA gene (Johnson and O'Brien, 1997). The sequence profile generated was compared to the laboratory reference data of over 130 mammal species. Genotyping started with the analyses of up to 15 microsatellites markers (including gender) that have been used for individual ID in northern BC river otters and marker variability was assessed using Cervus 3.0. Based on this assessment, a group of strong and variable markers were to complete the analysis

of individual ID. All data were error checked, as per Paetkau (2003) and mismatched markers in similar genotypes reanalyzed to check for genotyping error.

B.3 Amphibians

Adults and juveniles of six amphibian species have been previously noted as occurring within the lower Churchill River watershed including: American toad (*Anaxyrus americanus*), blue-spotted salamander (*Ambystoma laterale*), mink frog (*Lithobates septentrionalis*), northern leopard frog (Lithobates pipiens), northern two-lined salamander (*Eurycea bislineata*)and wood frog (*Lithobates sylvaticus*)(Minaskuat Inc. 2008; Nalcor Energy 2009c). Sampling effort for the Ecorisk EEMP initially focused on northern leopard frog given there are well-defined methodologies for using northern leopard frog tadpoles in studies of toxicology, thus making interpretation of the results from this work more comparable to other research on effects of MeHg on amphibians. As a result of a lack of collection for northern leopard frog, additional samples of American toad were also collected to augment the Ecorisk EEMP.

B.3.1 Sample Collection

Seven sites along the lower Churchill River valley were sampled for amphibians. Amphibian samples were collected by hand and/or dipnet from accessible wetlands in the lower Churchill River valley. Tadpoles were placed into sterile plastic bags with pond water and placed in a cooler with ice packs. Tadpoles were euthanized using a 1:1000 dilution of Eugenol and water. Tadpole measurements of total length, snout-vent length, and tail length were recorded.

B.3.2 Total Mercury (THg) and Methylmercury (MeHg) Analysis

Amphibian tissue, and water and sediment samples were sent to a certified laboratory for Total Mercury (THg) analyses. While nearly 100% of THg is in the form of MeHg in the tissues of top predators, this proportion varies in the environment and little is known about the proportion of MeHg relative to THg in amphibians. As a result, both THg and MeHg were analyzed in amphibian water and sediment samples. Due to a laboratory error, MeHg levels in amphibians are not available.

THg analysis in amphibians was carried out using a method adapted from the United States Environmental Protection Agency (USEPA) Method 200.2 (USEPA 1994). Tissue samples were homogenized and sub-sampled prior to hotblock digestion with nitric and hydrochloric acids, in combination with repeated additions of hydrogen peroxide. The extracts were then analyzed using cold vapor atomic fluorescence spectrophotometry (CVAFS), adapted from USEPA Method 245.7 (USEPA 2005).

Biota sediment accumulation Factors (BSAFs) indicate the ratio of a contaminant taken up into biota. The BSAF was calculated for the accumulation of MeHg in amphibian tadpoles based on the literature. The expected MeHg is calculated as approximately 30 % of THg in the amphibian tadpole sample (Bank et al. 2007).

B.3.3 Stable Isotopes Analysis

A subsample of each tadpole were removed and sent to a certified lab for analyses. Carbon and nitrogen isotopic analyses were performed using continuous flow, ion-ratio, mass spectrometry (CF-IRMS) as described by Loseto et al. (2008). The standards used for carbon and nitrogen analyses were Vienna PeeDee Belemnite (VPDB) and International Atomic Energy Agency, Vienna, Austria (IAEN-N1), respectively.

The standard procedure for presenting results for carbon and nitrogen isotopes is to express them using standard delta (δ) notation in units of per mil (‰). The delta values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) represent a deviation from a standard:

Equation 2: $$\delta_{sample}‰ = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio in the sample and the standard.

B.4 Water and Sediment Samples

B.4.1 Sample Collection

Aquatic environmental parameters including conductivity, dissolved oxygen, Oxidation Redox Potential, pH, salinity, and temperature, were measured *in situ* from eleven wetlands in the lower Churchill River valley. Water depth was also recorded. Water samples were also taken using 40 mL and 250 mL sample bottles. A 1:1 HCL solution was added to each water sample as a preservative. Sediment samples (125 mL jar) were also taken using a Teflon spoon from each site in areas of each wetland that had not recently been exposed to air and at a maximum depth of 2-3 cm.

B.4.2 Total Mercury (THg) and Methylmercury (MeHg) Analysis

The analysis of THg in water samples was carried out using procedures adapted from the American Public Health Association (APHA) method (APHA 1992) and from the USEPA Method SW-846 (USEPA 2007). The procedure involved a cold-oxidation of the acidified sample using bromine monochloride prior to reduction of the sample with stannous chloride. The extracts were then analyzed using CVAFS, adapted from USEPA Method 245.7 (USEPA 2005).

MeHg analysis of water samples was carried out using USEPA Method 1630 (USEPA 1998), where water samples were distilled to isolate MeHg from the sample matrix. A portion of each extract was analyzed by aqueous phase ethylation and purge and trap, followed by capillary gas chromatography (GC). Highly selective and sensitive detection was achieved using Atomic Fluorescence Spectrometry (AFS) after pyrolytic decomposition of the GC eluent, an instrumental method adapted from USEPA Method 1630 (USEPA 1998).

THg analysis in sediment samples was carried out using procedures from the Contaminated Site Regulation (CSR) (BC Ministry of Environment 2009) as well as procedures adapted from the USEPA Method 200.2 (USEPA 1994). Samples were manually homogenized, dried at 60ºC, sieved

through a 2 mm sieve, and a representative subsample of the dry material weighed. Each sample was then digested at 95ºC for two hours by block digester using concentrated nitric and hydrochloric acids. The extracts were then analyzed using CVAFS, adapted from USEPA Method 245.7 (USEPA 2005).

MeHg analysis in sediment samples was carried out as per Bloom et al. (1997). Sediment samples were treated with sulphuric acid, potassium bromide, and copper sulphate prior to extraction with dichloromethane. A portion of the sample was back extracted into water and analyzed by aqueous phase ethylation and purge and trap followed by capillary GC. Highly selective and sensitive detection was achieved using AFS after pyrolytic decomposition of the GC eluent, an instrumental method adapted from USEPA Method 1630 (USEPA 1998).

Interim sediment quality guidelines (ISQGs) and probable effect levels (PELs) are used to evaluate the degree to which adverse biological effects are likely to occur in aquatic biota as a result of exposure to THg in sediments (Environment Canada 1999a). There are no values available for MeHg.

B.5 Statistical Analysis

In instances where sample values were below detection limits, the detection limit of the machine was substituted in order to calculate summary statistics as well as to conduct statistical analysis.

Pearson correlation coefficients and related *p*-values for environmental factors, water and sediment THg and MeHg, American toad THg, and Osprey THg and MeHg and distances from the reservoir area were analyzed using SigmaPlot Version 12.3 (Systat Software, San Jose, CA). Values were considered statistically significant at $p < 0.05$ and with Bonferroni correction at $p < 0.005$.

LA-ICP-MS data was integrated for each 1000 µm line representing an average of over 10 data points using Thermo Electron PlasmaLab Software 2003, version 2.6. Concentration data were filtered according to a method previously used for LA-ICP-MS data derived from corals, fish otoliths and grizzly bear hair (Sinclair et al. 1998, Sandborn et al. 2003; Noel et al. 2014). The precision of the analysis (RSD% = [standard deviation (SD) / average] x 100) was calculated for a total of 25 replicates of DOLT2 reference material. The accuracy was also calculated (RD% = [(average DOLT value – reference value) / reference value] x 100) and provides an indication of the relative deviation of the average concentration obtained in this study from the reference value (D'Oriano et al. 2008).

Normality and homogeneity of variances were tested on amphibian morphometrics data with the Kolmogorov-Smirnov test and Levene's test, respectively. If the data did not meet the assumption of normality and homogeneity of variances, the data was log-transformed. Analyses of variances (ANOVA) followed by a Dunnett's test were performed to determine potential differences in amphibian morphometrics amongst sites.

B.6 Quality Assurance / Quality Control

Quality Assurance / Quality Control (QA / QC) analyses were performed for THg, MeHg, and stable isotope analyses.

THg and MeHg

A variety of samples were analyzed as part of the QA/QC for the determination of THg and MeHg. These included control reference materials (CRM), internal reference materials (IRM), laboratory control samples (LCS), and method blanks (MB). In addition, for water and sediment samples, replicates were run.

Carbon and Nitrogen Stable Isotopes

Replicate analyses were performed as part of QA/QC for Osprey feathers only, as tadpole samples were too small to allow for replication.

APPENDIX C

Survey Results and Biological Information

C.1 Sample Locations and Survey Conditions

Table C.1 2013 Osprey Feather Collection

Date	Osprey Nest	Easting	Northing	Feathers Collected	Evidence of Nesting Activity	Weather Conditions
October 3	OSPRNEST 13	546132	5896607	3 feathers (2 feathers and 1 smaller feather)	Scat at the base of the pole; no birds present	5^{0}C, winds 10 – 40 km/h, light drizzle
October 3	OSPRNEST 12	553034	5892923	2 feathers (1 feather and a smaller one)	No birds present	5^{0}C, winds 10 – 40 km/h, light drizzle
October 3	OSPRNEST 3	585926	5879333	Little pile of down feathers	Scat at the base of the pole; No birds present	5^{0}C, winds 10 – 40 km/h, light drizzle
October 5	OSPRNEST 11	560224	5889093	17 feathers (2 large feathers, 4 medium feathers, and 11 small feathers)	Bird scat at the base and around the nest; fish vertebrae found near nest; no birds present	6^{0}C, winds < 10 km/h, showers
October 5	OSPRNEST 10	565214	5886447	3 feathers (2 large feathers and 1 small feather)	Bird scat at the base and around the nest; pellet found near nest; no birds present	6^{0}C, winds < 10 km/h, showers
October 5	OSPRNEST 9	568322	5885257	2 feathers (1 medium feather and 1 small feather)	Bird scat at the base of the pole; fish bone; no birds present	6^{0}C, winds < 10 km/h, showers
October 6	OSPRNEST 7	573823	5882965	16 feathers (small)	Bird scat at the base of the pole; pellet at base of pole; no birds present; dead young found at base of pole where nest was located (15 feathers from this individual) ; looked like a piece of cloth hanging from the base of the nest	6^{0}C, winds < 10 km/h, no precipitation
October 6	OSPRNEST 8	571115	5884193	2 feathers (large)	Scat at the base of the pole; No birds present; huge nest structure	6^{0}C, winds < 10 km/h, no precipitation

Date	Osprey Nest	Easting	Northing	Feathers Collected	Evidence of Nesting Activity	Weather Conditions
October 6	OSPRNEST 14	544780	5897053	44 feathers (medium)	Scat at the base of the pole; No birds present	6^{0}C, winds < 10 km/h, no precipitation
October 6	OSPRNEST 19	527906	5904170	2 feathers (small)	Scat at the base of the pole; No birds present; huge nest structure found on the ground next to the pole where a remaining nest base was found	6^{0}C, winds < 10 km/h, no precipitation
October 7	OSPRNEST 26	504143	5907813	No feathers collected	Scat at the base of the pole; No birds present	5^{0}C, winds 40 km/h, no precipitation
October 10	OSPRNEST 28	495675	5910518	107 (41 small feathers, 60 medium, and 6 large)	Scat at the base of the pole; No birds present	4^{0}C, winds 20 km/h, flurries
October 10	OSPRNEST 31	491689	5912923	No feathers collected	Scat at the base of the pole; No birds present; Very limited nesting structure but no signs of a fallen nest structure, only a few sticks on the ground. May have been abandoned?	4^{0}C, winds 20 km/h, flurries
October 10	OSPRNEST 32	488447	5916322	No feathers collected	No birds present	4^{0}C, winds 20 km/h, flurries
October 10	OSPRNEST 40	455406	5927385	1 feather (large)	Scat at the base of the pole; No birds present; old fallen nest structure on the ground near the pole	4^{0}C, winds 20 km/h, flurries
October 10	OSPRNEST 42	441650	5933429	1 feather (small)	Scat at the base of the pole; No birds present	4^{0}C, winds 20 km/h, flurries
October 11	OSPRNEST 39	458388	5926637	1 feather (medium) and some down	Scat at the base of the pole; No birds present	-2^{0}C, winds 15 km/h, flurries
October 11	OSPRNEST 38	459675	5926312	1 feather (medium)	Scat at the base of the pole; No birds present	-2^{0}C, winds 15 km/h, flurries

Date	Osprey Nest	Easting	Northing	Feathers Collected	Evidence of Nesting Activity	Weather Conditions
October 11	OSPRNEST 37	462438	5924965	3 feathers (small) and some down	Scat at the base of the pole; No birds present	-2^{0}C, winds 15 km/h, flurries
October 11	OSPRNEST 35	471707	5921612	25 feathers (13 small, 10 medium, and 2 large)	Scat at the base of the pole; No birds present	-2^{0}C, winds 15 km/h, flurries
October 12	OSPRNEST 43	606768	5869902	1 feather (large)	Scat at the base of the pole; No birds present; natural nest (in a tree)	-3^{0}C, winds 20 km/h, no precipitation
October 12	OSPRNEST 34	479825	5920889	No feathers collected	Scat at the base of the pole; No birds present	-3^{0}C, winds 20 km/h, no precipitation
October 12	OSPRNEST 36	465243	5923612	65 (29 small, 30 medium, and 6 large)	Scat at the base of the pole; No birds present	-3^{0}C, winds 20 km/h, no precipitation

Table C.2 Otter Hair Sampling Locations (Body Snares) in the Lower Churchill River Valley

Sample Site	Site Reference(s)	UTM Coordinates (20 U)	
		Easting	Northing
Diver Brook	WPT002	582024	5861912
Beaver Brook	WPT003 WPT004	577926	5861217
Cache River	WPT005 WPT006 WPT007	552762	5881615
Fig River Area	WPT008	485929	5895745
Elizabeth River Area	WPT009 WPT010	479487	5901431
Metchin River	WPT011 WPT012	477023	5906770
Pinas River	WPT013 WPT014	617114	5875989

Table C.3 Amphibian, Water and Sediment Sampling Locations in the Lower Churchill River Valley

Site	Sample Type			UTM coordinates	
	Water Quality	American Toad	Northern Leopard Frog	Easting	Northing
Lower Brook – 1	✓	✓		641856	5903503
Churchill – 1	✓			615686	5874918
Churchill – 2	✓			617024	5876070
Churchill – A	✓		✓	619219	5877283
Churchill – B	✓			621319	5877046
Churchill – C	✓	✓	✓	622175	5878144
Churchill – D	✓			624681	5881751
Churchill – E	✓	✓		619121	5876911
Churchill – F	✓	✓	✓	619797	5878952
Churchill – 12	✓			633471	5890084
Churchill – 13	✓			635201	5892097
AMTO Sample 4*	✓	✓		621309	5877150
AMTO Sample 6*	✓	✓		622297	5878255

*Water and sediment samples were not collected at this site

C.2 Amphibian Biological Information

Table C.4 Biological Information from Tadpole Samples, 2014

Site	Species	Animal No.	Length (cm)		
			Snout-Vent	Tail	Total
Lower Brook - 1	American Toad	1	5	12	17
Lower Brook - 1	American Toad	2	6	12	18
Lower Brook - 1	American Toad	3	7	11	18
Lower Brook - 1	American Toad	4	5	9	14
Lower Brook - 1	American Toad	5	7	7	14
Lower Brook - 1	American Toad	6	6	7	15
Churchill - A	N. Leopard Frog	1	17	16	43
Churchill - A	N. Leopard Frog	2	21	19	40
Churchill - A	N. Leopard Frog	3	21	25	46
Churchill - C	N. Leopard Frog	1	4	4	8
Churchill - C	American Toad	2	7	7	14
Churchill - C	American Toad	3	9	12	21
Churchill - C	American Toad	4	10	11	21
Churchill - C	American Toad	5	9	12	21
Churchill - C	American Toad	6	9	12	21
Churchill - C	N. Leopard Frog	1	10	8	18
Churchill - C	N. Leopard Frog	2	10	13	23
Churchill - C	N. Leopard Frog	3	11	12	23
Churchill - E	American Toad	1	6	7	13
Churchill - E	American Toad	2	7	6	13
Churchill - E	American Toad	3	6	7	13
Churchill - E	American Toad	4	7	7	14
Churchill - E	American Toad	5	6	7	13
Churchill - E	American Toad	6	6	7	13
Churchill - F	American Toad	1	5	8	13
Churchill - F	American Toad	2	6	8	14
Churchill - F	American Toad	3	5	10	15
Churchill - F	American Toad	4	4	10	14
Churchill - F	American Toad	5	7	8	15
Churchill - F	American Toad	6	6	8	14
Churchill - F	N. Leopard Frog	1	20	37	57
Churchill - F	N. Leopard Frog	2	8	17	25

Site	Species	Animal No.	Length (cm)		
			Snout-Vent	Tail	Total
Churchill - F	N. Leopard Frog	3	9	16	25
AMTO Sample 4	American Toad	1	4	7	11
AMTO Sample 4	American Toad	2	5	6	11
AMTO Sample 4	American Toad	3	4	7	11
AMTO Sample 4	American Toad	4	4	7	11
AMTO Sample 4	American Toad	5	4	8	12
AMTO Sample 4	American Toad	6	4	7	11
AMTO Sample 6	American Toad	1	10	11	21
AMTO Sample 6	American Toad	2	5	6	11
AMTO Sample 6	American Toad	3	5	6	11
AMTO Sample 6	American Toad	4	5	6	11
AMTO Sample 6	American Toad	5	5	6	11
AMTO Sample 6	American Toad	6	5	6	11

Table C.5 American Toad and Northern Leopard Frog Tadpoles collected during Sampling in the Lower Churchill River Valley, June 2014

Site		American Toad Lengths (mm)			Northern Leopard Frog Lengths (mm)		
		Snout-Vent	Tail	Total	Snout-Vent	Tail	Total
Lower Brook - 1	n	6	6	6	0	0	0
	Average ± SD	6.0 ± 0.8	9.7 ± 2.3	16.0 ± 1.9	n/a	n/a	n/a
	Minimum	5	7	14	n/a	n/a	n/a
	Maximum	7	12	18	n/a	n/a	n/a
Churchill - A	n	0	0	0	3	3	3
	Average ± SD	n/a	n/a	n/a	19.7 ± 2.3	20.0 ± 4.6	43.0 ± 3.0
	Minimum	n/a	n/a	n/a	17	16	40
	Maximum	n/a	n/a	n/a	21	25	46
Churchill - C	n	5	5	5	4	4	4
	Average ± SD	8.8 ± 1.1	10.8 ± 2.2	19.6 ± 3.1	8.8 3.2	9.3 ± 4.1	18.0 ± 7.1
	Minimum	7	7	14	4	4	8
	Maximum	10	12	21	11	13	23
Churchill - E	n	6	6	6	0	0	0
	Average ± SD	6.3 ± 0.5	6.8 ± 0.4	13.2 ± 0.4	n/a	n/a	n/a
	Minimum	6	6	13	n/a	n/a	n/a
	Maximum	7	7	14	n/a	n/a	n/a
Churchill - F	n	6	6	6	3	3	3
	Average ± SD	5.5 ± 1.1	8.7 ± 1.0	14.2 ± 0.8	12.3 ± 6.7	23.3 ± 11.8	35.7 ± 18.5
	Minimum	4	8	13	8	16	25
	Maximum	7	10	15	20	37	57
AMTO Sample 4	n	6	6	6	0	0	0
	Average ± SD	4.2 ± 0.4	7.0 ± 0.6	11.2 ± 0.4	n/a	n/a	n/a
	Minimum	4	6	11	n/a	n/a	n/a
	Maximum	5	8	12	n/a	n/a	n/a
AMTO Sample 6	n	6	6	6	0	0	0
	Average ± SD	5.8 ± 2.0	6.8 ± 2.0	12.7 ± 4.1	n/a	n/a	n/a
	Minimum	5	6	11	n/a	n/a	n/a
	Maximum	10	11	21	n/a	n/a	n/a

APPENDIX D

Detailed Results and Statistical Analysis

D.1 Osprey

D.1.1 Sample Collection

Feathers collected from 19 of the 23 active nests visited (refer to Figure 2-1 in the main report and Appendix C) were comprised of primary, secondary and tertiary feathers as well as down feathers (Appendix B). Large numbers of feathers (50+) were present at three sites (OSPRNEST14, OSPRNEST28 and OSPRNEST36) and most of the feathers appeared broken.

D.1.2 Total Mercury (THg) and Methylmercury (MeHg)

Quality control analyses of THg recovery (for certified reference material and blanks) were within the acceptable range of 70.0 – 130% (81.8 ± 2.82 %). All submitted samples were above laboratory detection limits (ranging between 0.0100 – 0.800 mg/kg dry weight (dw); Appendix E).

As only down feathers were available for OSP2013-19 and OSP2013-42, THg results for these two nests were not included in the statistical analyses. THg ranged from 1.22 to 18.6 mg/kg in piece 1 and from 1.08 to 28.2 mg/kg in piece 2 (Table D.1). While there was no significant difference between average THg concentrations in piece 1 and piece 2 ($\alpha = 0.05$, $p = 0.781$; Table D.2), inter-individual variations in the concentration difference between the two pieces were observed. The percentage difference in THg levels between piece 2 and piece 1 ranged from -39.9% to 127% with negative values suggesting that Ospreys were exposed to higher concentrations more recently (Table D.1).

Table D.1 Total Mercury (THg) in piece 1 and piece 2 of Osprey Feathers Collected from the Lower Churchill River Valley

Sample ID	Piece 1 (mg/kg)	Piece 2 (mg/kg)	Difference between the 2 pieces (%)
OSP2013-07	6.99	7.93	13.45
OSP2013-08	1.22	1.08	-11.48
OSP2013-09	6.79	6.21	-8.54
OSP2013-10	8.47	8.24	-2.72
OSP2013-11	14.1	12.3	-12.77
OSP2013-12	17.5	12	-31.43
OSP2013-13	11.5	10.2	-11.30
OSP2013-14	16.5	17.5	6.06
OSP2013-19	4.73[a]	n/a[b]	n/a[b]
OSP2013-28	4.82	4.23	-12.24
OSP2013-35	1.41	1.26	-10.64
OSP2013-36	7.29	4.38	-39.92
OSP2013-37	11.7	13.8	17.95
OSP2013-38	18.6	20.7	11.29

Sample ID	Piece 1 (mg/kg)	Piece 2 (mg/kg)	Difference between the 2 pieces (%)
OSP2013-39	12.9	16.8	30.23
OSP2013-40	12.4	28.2	127.42
OSP2013-42	0.113[a]	n/a[b]	n/a[b]
OSP2013-43	13.3	10.8	-18.80
Average ± SD	**10.3 ± 5.32**	**10.9 ± 7.30**	**2.91 ± 0.37**
Minimum	**1.22**	**1.08**	**-39.9**
Maximum	**18.6**	**28.2**	**127.4**

[a]this data was from a down feather and therefore not included in the statistical analyses
[b]not available as the feather was too small to divide into two segments

Table D.2 Summary Statistics and ANOVA Analysis between THg in Piece 1 and Piece 2 of Osprey Feathers Collected in the Lower Churchill River Valley

Analyses	p-value
Normality	0.479
Equality of Variances	0.136
ANOVA	0.781

An analysis of the distance of the Osprey nests from the Reservoir Area versus THg levels in feathers shows no relationship for both piece 1 ($\alpha = 0.05$, $p = 0.699$) and piece 2 ($\alpha = 0.05$, $p = 0.178$) of the feather (Table D.3, Figure D.1). A stronger relationship would indicate increased mercury exposure in Ospreys nesting closer to the reservoir area (or vice versa). In subsequent years of sampling after inundation, THg in relation to nest locations will be a valuable comparator for the exposure and health of Osprey nesting in the lower Churchill River valley. THg levels in piece 1 and piece 2 were however significantly correlated with each other ($\alpha = 0.05$, $p = 0.000407$; Figure D-2) with a slope value close to 1 (slope = 1.11). This correlation between the youngest (piece 1) and slightly older (piece 2) sections of the feathers suggest the mercury exposure in the animals is consistent over time and likely from the same area.

Table D.3 Distances to Reservoir Area for Total Mercury (THg) Analyzed Osprey Feathers

Sample ID	Easting	Northing	Distance to Nearest Reservoir Area (km)
OSP2013-07	573823	5882965	34.0
OSP2013-08	571115	5884193	37.0
OSP2013-09	568322	5885257	40.0

Sample ID	Easting	Northing	Distance to Nearest Reservoir Area (km)
OSP2013-10	565214	5886447	43.3
OSP2013-11	560224	5889093	48.9
OSP2013-12	553034	5892923	57.1
OSP2013-13	546132	5896607	64.9
OSP2013-14	544780	5897053	66.3
OSP2013-19	527906	5904170	84.6
OSP2013-28	495675	5910518	117
OSP2013-35	471707	5921612	143
OSP2013-36	465243	5923612	150
OSP2013-37	462438	5924965	153
OSP2013-38	459675	5926312	156
OSP2013-39	458388	5926637	157
OSP2013-40	455406	5927385	160
OSP2013-42	441650	5933429	176
OSP2013-43	606768	5869902	0.0775

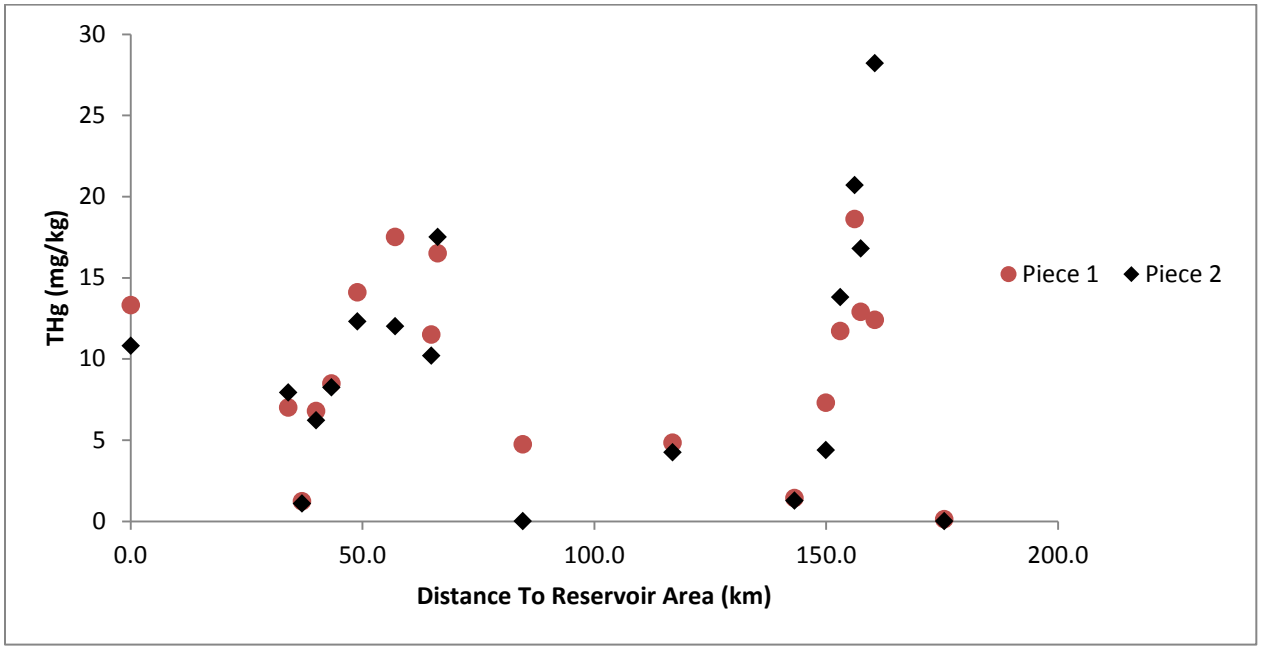


Figure D-1 Total Mercury (THg) in relation to the Distance from the Reservoir Area from Osprey Feathers in the Lower Churchill River Valley

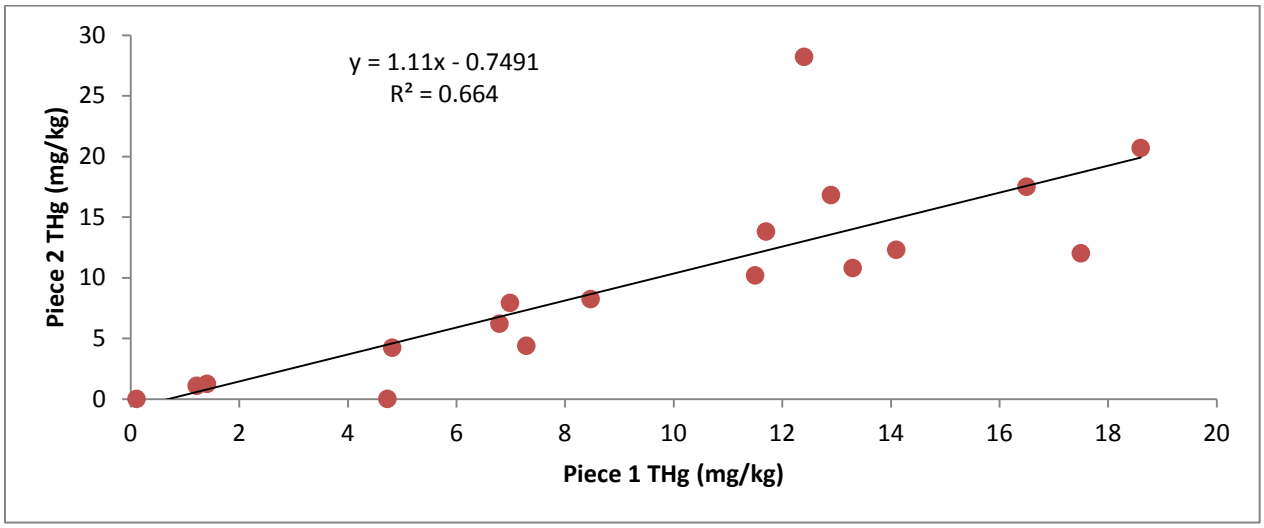


Figure D-2 Linear Regression of Total Mercury (THg) in pieces 1 and 2 of Osprey Feathers from the Lower Churchill River Valley

As MeHg is estimated to comprise approximately 100 % of the THg in Osprey (Braune and Gaskin 1987, Odsjo et al. 2004), THg levels found in Osprey feathers in this study are used to represent MeHg levels.

D.1.3 Stable Isotopes

Quality control analyses revealed low variability between replicates with a percentage of 0.801 ± 0.850 % and 0.543 ± 0.402 % for δ15N and δ13C, respectively (Appendix E)

$\delta^{15}N$ results suggest that the Ospreys feed at two to three different trophic levels. $\delta^{15}N$ ranged from 9.1 to 15.2 %, where every 3 ‰ change in $\delta^{15}N$ corresponds to a change in trophic level (Minagawa and Wada 1984). Osprey appear to be feeding on prey from various sources with lighter signatures (i.e., more negative as $\delta^{13}C$ ranged between -32.5 and -17.5 ‰) suggesting stronger reliance on terrestrial prey, or in an aquatic environment that has greater sedimentation from terrestrial sources (Table D.4). There was further evidence of Osprey feeding on various prey as $\delta^{13}C$ and $\delta^{15}N$ were not significantly correlated ($\alpha = 0.05$, $p = 0.412$; Figure D-3). THg in piece 2 was not correlated with $\delta^{15}N$ ($\alpha = 0.05$, $p = 0.789$; Figure D-4) or $\delta^{13}C$ ($\alpha = 0.05$, $p = 0.550$; Figure D-5). Although Hg concentrations in Osprey and other seabirds have been found to correlate with trophic level and food web structure (as indicated by $\delta^{15}N$ and $\delta^{13}C$) (Nisbet et al. 2002, Guigueno et al. 2012) variation in feather rates of assimilation of Hg, nitrogen isotopes, and carbon isotopes can occur (Bond 2010). THg is also known to vary with sex in seabird feathers (Becker et al. 2002) (refer to Section D.1.4 for results of DNA analysis).

Table D.4 Nitrogen (δ15N) and Carbon (δ13C) Stable Isotope Ratios in the First 2 cm of the Vane of Sampled Osprey Feathers

Sample ID	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰
OSP2013-07	15.2	-17.5
OSP2013-08	10.2	-29.6
OSP2013-09	12.9	-22.7
OSP2013-09 (rep)	12.9	-22.5
OSP2013-10	9.60	-24.4
OSP2013-10 (rep)	9.80	-24.4
OSP2013-11	10.0	-25.5
OSP2013-12	9.80	-24.1
OSP2013-13	10.6	-25.0
OSP2013-14	13.1	-22.0
OSP2013-19	11.2	-30.5
OSP2013-28	9.60	-23.9
OSP2013-28 (rep)	9.60	-23.8
OSP2013-35	10.4	-30.0
OSP2013-35 (rep)	10.5	-30.3
OSP2013-36	9.80	-24.2
OSP2013-36 (rep)	9.90	-24.1
OSP2013-37	9.90	-22.1
OSP2013-38	10.9	-28.4
OSP2013-39	13.9	-31.3
OSP2013-40	10.2	-24.9
OSP2013-42	2.7[a]	-25.6[a]
OSP2013-43	9.10	-24.6

[a] this data was from a down feather and therefore not included in the statistical analyses

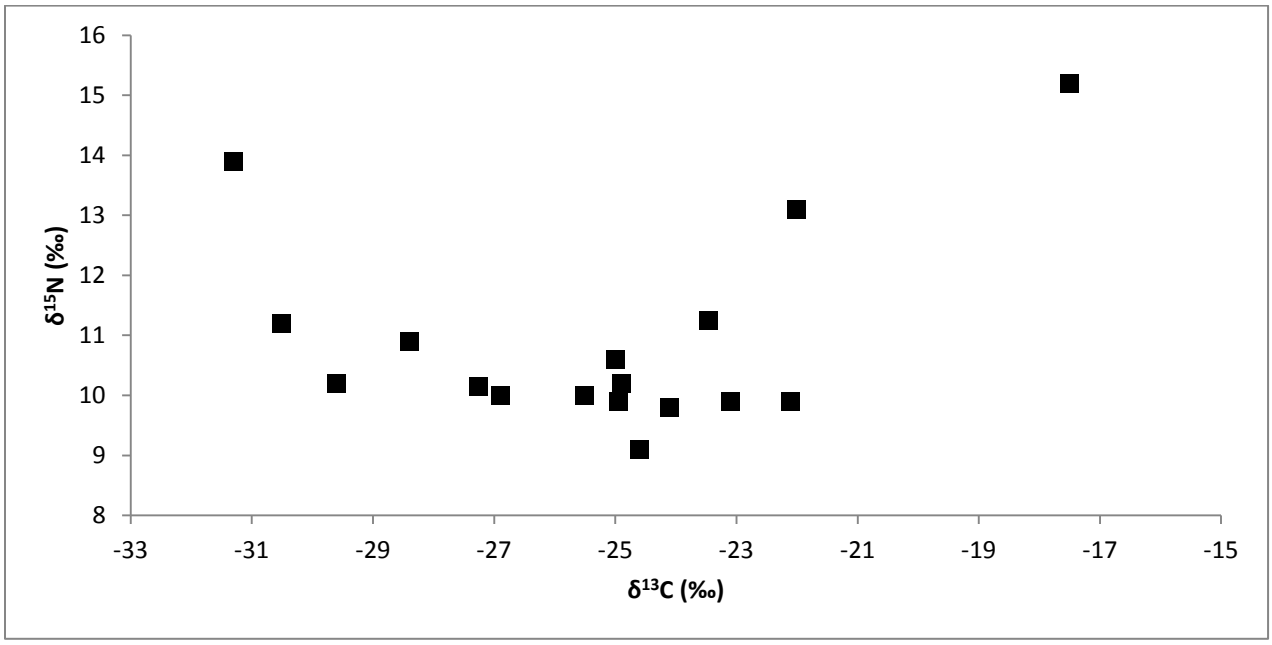


Figure D-3 $\delta^{13}C$ in relation to $\delta^{15}N$ in Osprey from Lower Churchill River Valley

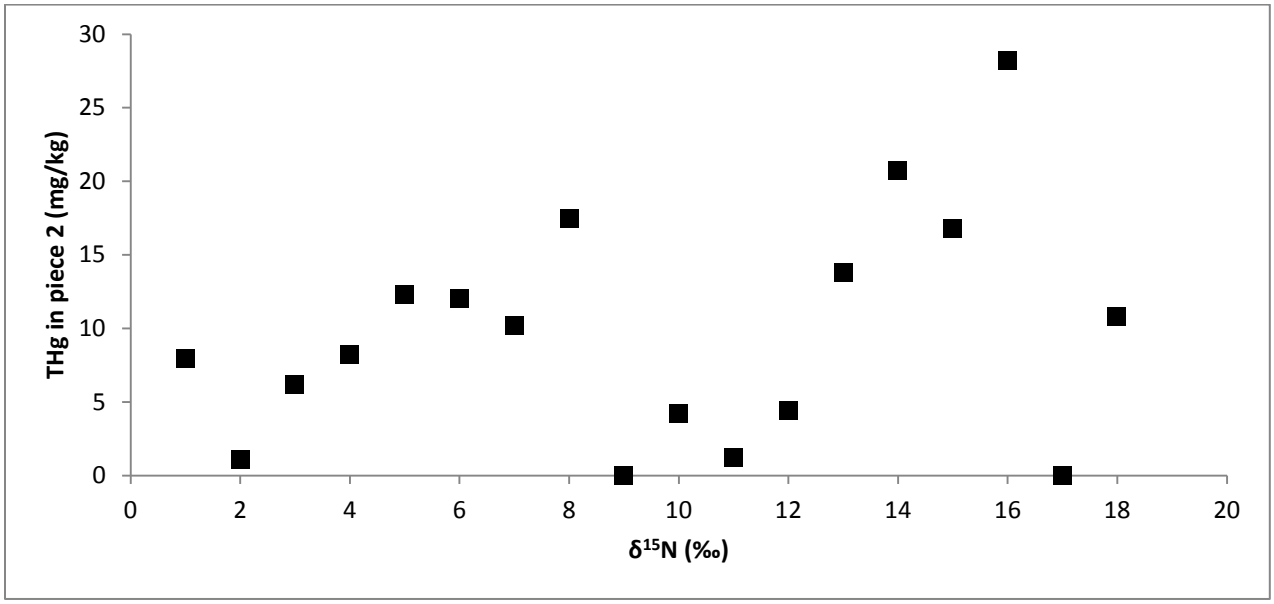


Figure D-4 $\delta^{15}N$ Corresponding to Total Mercury (THg) from Osprey in the Lower Churchill River Valley

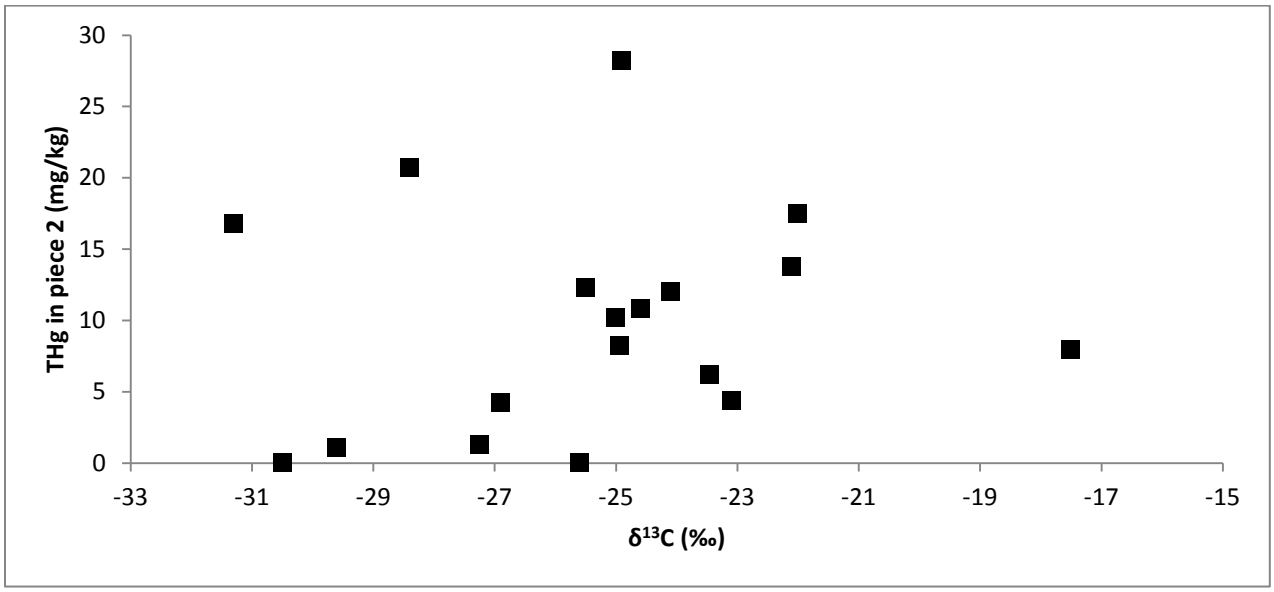


Figure D-5 δ13C Corresponding to Total Mercury (THg) from Osprey in the Lower Churchill River Valley

D.1.4 DNA

All but one of the 25 feather samples submitted produced DNA sequence profiles of suitable strength for species identification. Twenty three ospreys were identified as well as one spruce grouse (Table 1). The 23 samples identified as Osprey were analyzed for gender. Despite multiple attempts, only 14 samples produced gender data strong enough to satisfy the laboratory's threshold for high confidence scoring. The low success rate related to gender identification may be, in part, due to degradation while the samples were exposed to the elements prior to collection. It had been noted that most of the feathers collected were broken. Of the 14 successfully analyzed Osprey samples, 10 were identified as female and four were identified as male (Table D.5).

Table D.5 DNA results (Species and Gender) from Feather Samples Collected in the Lower Churchill River Valley.

Sample ID	Species	Gender
OSP2013-07	Osprey	F
OSP2013-08	Osprey	F
OSP2013-09	Osprey	U
OSP2013-10	Osprey	F
OSP2013-11	Osprey	M
OSP2013-12	Osprey	M

Sample ID	Species	Gender
OSP2013-13	Osprey	F
OSP2013-14	Osprey	F
OSP2013-19	Osprey	U
OSP2013-28	Osprey	U
OSP2013-35	Osprey	F
OSP2013-36	Unknown	U (failed test)
OSP2013-37	Osprey	U
OSP2013-38	Osprey	U
OSP2013-39	Osprey	F
OSP2013-40	Osprey	U
OSP2013-42	Spruce Grouse	U
OSP2013-43	Osprey	F
F: Female; M: Male; U: Unidentified		

THg levels in female Osprey feathers (n=10) averaged 9.1 ± 5.6 mg/kg and in male Osprey feathers (n=2) averaged 15.8 ± 2.4 mg/kg. Previous studies have shown lower THg levels in female feathers compared to males and this has been attributed to maternal transfer of Hg to offspring (Braune and Gaskin 1987, Lewis et al. 1993, Becker et al. 2002). However, due to only two males being identified in the present study, no statistical analyses could be performed to evaluate the difference in THg levels between males and females.

D.2 River Otter

Hair samples were collected from five of seven sampling locations (refer to Figure 2-1 in the main report and Appendix C). Underfur was the dominant type of hair at each hair snag station, and only a few small guard hairs were obtained (sites WPT011, WPT013 and WPT014).

As the LA-ICP-MS method only requires one hair to determine THg concentrations, the best/longest hair was selected from each sample for analysis (with the exception of sample WPT003 where there was not enough sample to successfully undertake any analyses). Remaining samples were sent for DNA analyses.

D.2.1 Total Mercury (THg) and Methylmercury (MeHg) Analysis

Nine hairs were collected from four sampling locations (Fig River area, Elizabeth River area, Metchin River, Pinas River). Of those nine hairs, only three were guard hairs, the most appropriate samples for LA-ICP-MS. As only one hair per sample was available for analyses, it was not possible to perform any QA/QC for THg analyses.

THg levels in all nine hairs averaged 0.792 ± 1.08 mg/kg and ranged between 0.155 and 3.49 mg/kg (Table D.6). These levels were in the lower range of those previously reported for river otter sampled from several locations in the United States (Halbrook et al. 1994, Strom 2008) and lower than the conservative 5.4 mg/kg neurochemical effect levels (Basu et al. 2009). While diet can explain some of the inter-individual variations, THg levels in river otter have also been shown to be influenced by age and sex. There are suggestions, however, that mercury-age and mercury-sex relationships are specific to tissue or region (Yates et al. 2005).

Table D.6 Total Mercury (THg) in Hair Samples from the Lower Churchill River Valley

Hair Sample	Sample Area	Hair type	Suspected Species	THg (mg/kg)		
				Average ± SD	Min	Max
WPT008-1	Fig River area	underfur	otter	0.336 ± 0.0640	0.259	0.503
WPT008-2	Fig River area	underfur	otter	0.211 ± 0.0741	0.154	0.367
WPT008-3	Fig River area	underfur	otter	0.155 ± 0.0552	0.068	0.238
WPT010-1	Elizabeth River area	unknown	beaver	0.838 ± 0.105	0.619	1.044
WPT010-2	Elizabeth River area	guard hair	beaver	0.337 ± 0.0305	0.294	0.388
WPT011	Metchin River	guard hair	otter	0.257 ± 0.114	0.066	0.574
WPT013	Pinas River	guard hair	rabbit	0.186 ± 0.0410	0.118	0.284
WPT014-1	Pinas River	underfur	otter	3.49 ± 1.14	1.36	5.21
WPT014-2	Pinas River	underfur	otter	1.32 ± 0.537	0.529	2.23

LA-ICP-MS results were also plotted as distance from the root vs. THg (Figure D-6), such that THg levels at x = 0 represent THg at the root of the hair and therefore the most recent exposure. As there is currently no data on river otter hair growth rate, it was not possible to convert distances from the hair root into time. THg patterns revealed variation in THg levels along the length of the river otter hairs (Figure D-6), particularly for WPT014-1 and WPT014-2. Intra-hair variation between minimum and maximum THg levels ranged between 32 % and 770 % (Table D.5; Figure D-6). Maximum levels along the length of the hair (indicating transient THg exposures) ranged from 0.238 – 5.21 mg/kg. The LA-ICP-MS results show a double peak in THg at two time points in the samples from Pinas River (WPT014-1 and WPT014-2; Figure 3-6). These peaks suggest possible changes in seasonal exposure. Notably, sample WPT014-1 transiently peaks at 5.21 mg/kg, very close to the neurochemical effect level of 5.4 mg/kg. This spike in THg would have been missed if whole otter hair had been analyzed and averaged (i.e., average THg for WPT014-1 is 1.32 THg mg/kg).

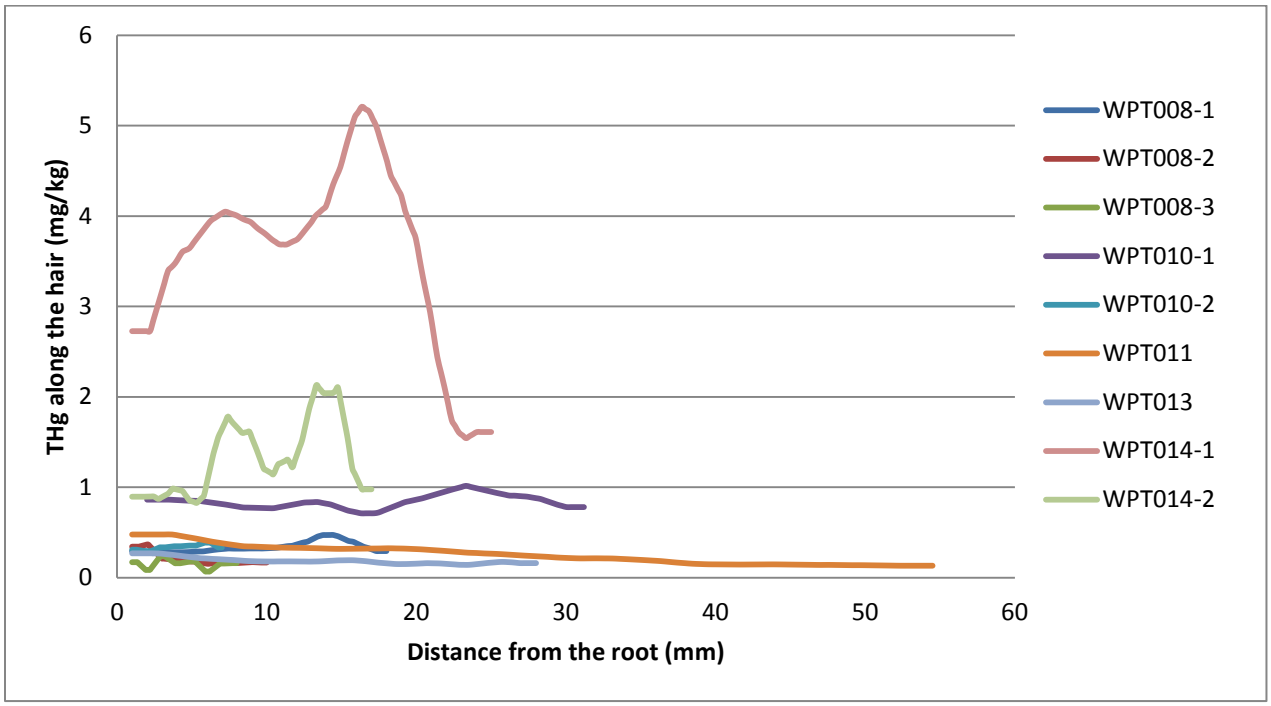


Figure D-6 Total Mercury (THg) Levels along the Length of Hairs Collected in the Lower Churchill Valley

Based on available literature, MeHg is expected to comprise approximately 100 % of THg in river otter hair samples (Kehrig et al. 1998, Voegborlo et al. 2010). Thus, THg levels collected as part of this study were used to represent MeHg levels in river otter.

D.2.2 DNA

Given the small sample size, there were challenges to successfully complete DNA analyses. Samples comprised of no guard hair roots and with less than five underfur samples had to be excluded from analysis as not enough DNA could be extracted. Those included WPT008-2, WPT010-2 and WPT014-2 (Table 2). For the other five samples, the lab clipped the guard hair roots when available or used the entire length of finer hair.

As the samples were generally weak, the analyses were run twice to confirm the species results and unfortunately, as a result, the gender identification could not be performed. One river otter sample was identified (WPT014-1) as well as one muskrat (WPT010-1) and two snowshoe hares (WPT008-1 and WPT013). WPT008-3 sample failed to produce a useable DNA sequence on either attempt at analysis (Table D.7).

THg level along the hair of the one river otter identified averaged 3.49 ± 1.14 mg/kg. This level was in the lower range of those previously reported for river otters sampled from several locations in the United States (Halbrook et al. 1994, Strom 2008) and lower than the conservative 5.4 mg/kg neurochemical effect levels (Basu et al. 2009). As only one otter sample was

identified, the investigation of the Hg-sex relationship in this particular river otter population was not possible.

Table D.7 DNA Results (Species) from Hair Samples collected in the Lower Churchill River Valley

Hair Sample	Sample Area	Hair type	Species
WPT008-1	Fig River area	underfur	Snowshoe Hare
WPT008-2	Fig River area	underfur	Inadequate
WPT008-3	Fig River area	underfur	Failed
WPT010-1	Elizabeth River area	unknown	Muskrat
WPT010-2	Elizabeth River area	guard hair	Inadequate
WPT011	Metchin River	guard hair	Failed
WPT013	Pinas River	guard hair	Snowshoe Hare
WPT014-1	Pinas River	underfur	Otter
WPT014-2	Pinas River	underfur	Inadequate

D.3 Amphibians and Associated Water and Sediment Samples

D.3.1 Amphibians

American toad tadpoles were collected at six sites including Lower Brook-1, Churchill-C, Churchill-D, Churchill-F, AMTO Sample 4 and AMTO Sample 6 (refer to Figure 2-1 in the main report and Appendix C). Snout-vent length ranged from 4 to 10 mm and tail length from 6to 11 mm and did not differ significantly amongst sites ($\alpha = 0.05$, $p<0.001$; Table D.6). Similarly, total length ranged from 11-21 mm and did not differ amongst sites ($\alpha = 0.05$, $p<0.001$; Table D.8).

Northern Leopard frog tadpoles were collected at three sites including Churchill-A, Churchill-C and Churchill-F (refer to Figure 2-1 in the main report and Appendix C). Snout-vent length ranged from 4 to 21 mm and did not differ amongst sites ($\alpha = 0.05$, $p=0.0750$; Table D.8). Tail length ranged from 4 to 37 mm and tadpoles collected at the Churchill-C site had a tail significantly shorter than those collected at the two other sites ($\alpha = 0.05$, $p = 0.0480$; Table D.8). Finally, total length ranged from 8 to 57 mm of tadpoles collected at the Churchill-C site (Appendix B) were significantly shorter compared to those collected at the two other sites ($\alpha = 0.05$, $p = 0.0460$; Table D.8).

Table D.8 Summary Statistics and ANOVA Analysis between Sample Locations for Amphibian Morphometrics in the Lower Churchill River Valley

Analyses	Snout-Vent Length	Tail Length	Total Length
American Toad			
Normality	0.0130	0.00900	0.00300
Equality of Variances	0.156	0.0110	0.0110
ANOVA	0.000	0.000	0.000
N. Leopard Frog			
Normality	0.000	0.000	0.000
Equality of Variances	0.731	0.692	0.607
ANOVA	0.0750	0.0480	0.0460
P-values presented (statistical significance at $p < 0.05$)			

D.3.2 Total Mercury (THg) and Methylmercury (MeHg) Analysis

Amphibian tissue samples were pooled within species and site, subsampled and submitted for THg analysis. THg in amphibian tissues ranged between 0.00320 – 0.0575 mg/kg wet weight (ww) (Table D.9). Due to low amphibian sample numbers, differences between THg concentrations at sample sites could not be distinguished using statistical analysis. Of note however, the highest THg concentration (0.0575 mg/kg ww) occurred in the Lower Brook 1.

Table D.9 Total Mercury (THg) for Amphibian Tissues Collected

Site	THg American Toad (mg/kg ww)	THg Northern Leopard Frog (mg/kg ww)
LOWER BROOK-1	0.0575	-
CHURCHILL-A	-	0.0170
CHURCHILL-C	0.0115	0.00320
CHURCHILL-E	0.0389	-
CHURCHILL-F	0.0421	0.0147
AMTO SAMPLE 4	0.00980	-
AMTO SAMPLE 6	0.00980	-
Missing samples not available due to minimal species presence		

Quality control analyses of THg recovery (for laboratory controls, references, and blanks) were within the acceptable range of 70.0 – 130% (84.1 ± 0.350 %). All submitted samples were above lab detection limits (ranging between 0.00100 – 0.00700 mg/kg wet weight (ww); Appendix E).

Due to a laboratory error in the handling of amphibian tissue samples, it was not possible to analyze for MeHg. Available literature suggests that approximately 30 % of THg in amphibian tadpoles is made up of MeHg (Bank et al. 2007). This estimate was used to estimate the BSAFs for MeHg in amphibians based on sediment and water MeHg collected as part of this study (results presented below in Section D.4). The BSAFs for MeHg in amphibians based on sediment MeHg is 42.6, and based on water MeHg is 12.8.

D.3.3 Stable Isotopes Analysis

Overall, $\delta^{15}N$ ranged between 1.9 and 5.1‰ while $\delta^{13}C$ ranged between -35.1 and -27.5‰ (Table D.10). T-test analyses showed that the tadpoles of the northern leopard frog had lower $\delta^{13}C$ (-34.9 ± 0.264 ‰) than the American toad (-29.9 ± 2.29 ‰) ($\alpha = 0.05$, $p = 0.008$). $\delta^{13}C$ of northern leopard frog tadpoles were however in a similar range of two other more closely related tadpole species of the common frog (*Rana temporaria*)(Trakimas et al. 2011) and green frog (*Lithobates clamitans)* (Jefferson and Russel 2008). The $\delta^{13}C$ differences observed suggest that the two species may be feeding on slightly different prey. A lack of correlation between $\delta^{15}N$ and $\delta^{13}C$ in American toad tadpoles suggests they are likely feeding on a variety prey ($\alpha = 0.05$, $p = 0.944$; Figure D-7). Importantly, the lack of difference in $\delta^{15}N$ between the American toad and northern leopard frog ($\alpha = 0.05$, $p = 0.271$) suggests that the two species feed at the same trophic level. When the two species are compared from the same sites (Churchill C and Churchill F), however, American toads appear at slightly higher trophic levels with associated higher $\delta^{13}C$; this may explain the higher THg concentrations. Due to low amphibian sample number, site differences between stable isotopes could not be distinguished.

Table D.10 Nitrogen (δ15N) and carbon (δ13C) for Amphibian Tissues Collected

Sample ID	American Toad		Northern Leopard Frog	
	$\delta^{15}N$‰	$\delta^{13}C$‰	$\delta^{15}N$‰	$\delta^{13}C$‰
LOWER BROOK-1	5.10	-30.1	-	-
CHURCHILL-A	-	-	4.10	-34.6
CHURCHILL-C	3.60	-33.6	2.30	-35.1
CHURCHILL-E	3.10	-29.4	-	-
CHURCHILL-F	2.60	-31.2	1.90	-35.0
AMTO SAMPLE 4	3.60	-27.5	-	-
AMTO SAMPLE 6	3.40	-27.7	-	-
Missing samples not available due to minimal species presence				

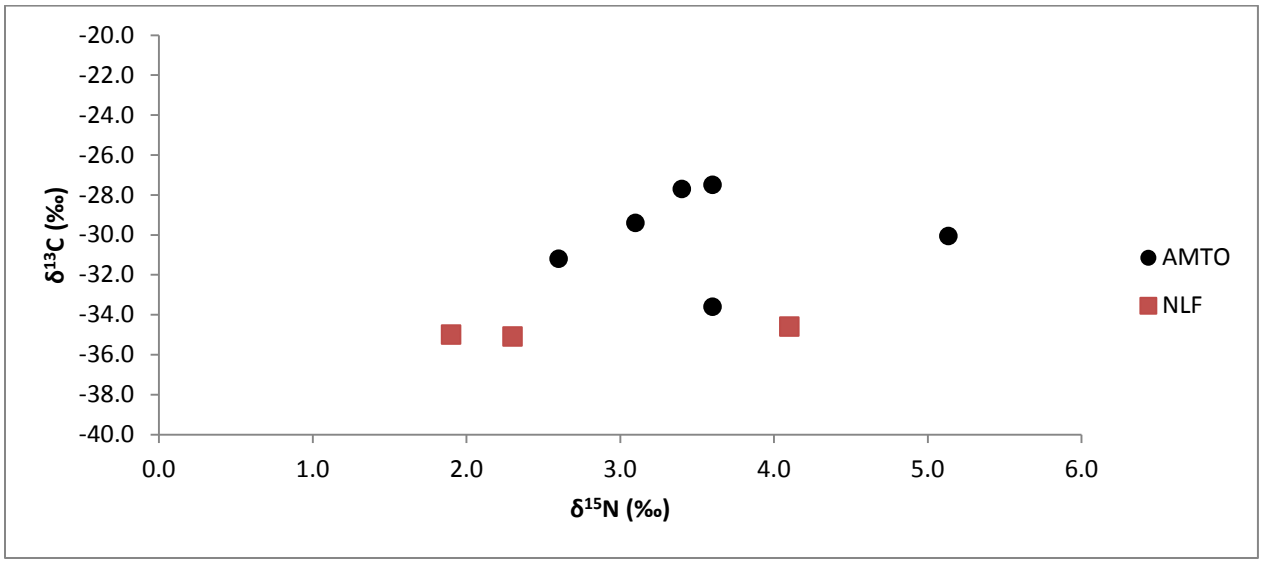


Figure D-7 $\delta^{15}N$ in relation to $\delta^{13}C$ for American Toad (AMTO) and Northern Leopard Frog (NLF)

Due to limited amount of samples available for amphibians, it was not possible to perform replicate analyses as part of the QA/QC.

D.4 Water and Sediment

Environmental characteristics of water (water quality data) were collected from depths ranging from 0.3 to 1.5 m, from 11 sites in the lower Churchill River valley. Water temperature ranged from 13.3 to 24.8°C, dissolved oxygen from 6.2 to 103.8%, pH from 5.9 to 7.0, conductivity from 0.018 to 0.132 s/m, and hardness from 0.022 to 0.157 mmol/L (Table D.11).

Table D.11 Water Quality Data from Sampling Locations in the Lower Churchill River Valley, June 2014

Site*	Water Temperature (°C)	Dissolved Oxygen (%)	pH	Conductivity (µs/m)	Hardness (mmol/L)	Depth (m)
Lower Brook-1	13.3	94.0	6.47	0.0550	0.0550	0.700
Churchill - 1	16.6	6.20	6.71	0.132	0.157	0.400
Churchill - 2	17.3	82.0	6.19	0.0180	0.0220	1.00
Churchill - A	19.5	28.6	5.94	0.0380	0.0440	0.400
Churchill - B	16.7	54.8	6.30	0.0300	0.0370	0.350
Churchill - C	21.0	56.0	6.54	0.0470	0.0470	0.450
Churchill - D	18.9	66.4	6.54	0.0240	0.0280	0.650
Churchill - E	22.9	89.1	6.81	0.0260	0.0270	0.650
Churchill - F	23.8	92.0	6.60	0.0360	0.0360	0.400

Site*	Water Temperature (°C)	Dissolved Oxygen (%)	pH	Conductivity (µs/m)	Hardness (mmol/L)	Depth (m)
Churchill - 12	16.4	98.5	6.41	0.0200	0.0240	1.50
Churchill - 13	24.8	104	6.95	0.0270	0.0270	0.500
Average ± SD	**19.2 ± 3.60**	**70.1 ± 31.2**	**6.50 ± 0.300**	**0.0410 ± 0.0320**	**0.0460 ± 0.0380**	**0.640 ± 0.340**
Minimum	**13.3**	**6.20**	**5.94**	**0.0180**	**0.0220**	**0.350**
Maximum	**24.8**	**104**	**6.95**	**0.132**	**0.157**	**1.50**
*Refer to Figure 2.2 in the main report for sampling locations.						

D.4.1 Total Mercury (THg) and Methylmercury (MeHg)

All 11 water samples were below the detection limit (0.0100 µg/L) for THg (Table D.12). Nine of 11 samples had MeHg levels above the detection limit (0.0000500 µg/L), with values ranging from <0.0000500 µg/L to 0.00215 µg/L (Table D.12). These levels are below the water quality guidelines for THg (0.0260 µg/L) and MeHg (0.00400 µg/L) developed by Environment Canada for the protection of aquatic life (Environment Canada 2003). However, it is important to note that these guidelines do not address exposure through food or bioaccumulation to higher trophic levels. Aquatic wildlife exposed to MeHg primarily through food might not be adequately protected using these values.

Percent MeHg in water samples ranged from 0.5% to 21.5%. Highest MeHg contributions were associated with three sites in particular: Churchill – 1 (21.5%), Lower Brook – 1 (15.5%) and Churchill – A (11.3%) (Table D.12).

Table D.12 Total Mercury (THg), Methylmercury (MeHg) and Percent Methylmercury (% MeHg) in Water Samples from the Lower Churchill River Valley, June 2014

Site	THg (mg/L)[a]	MeHg (µg/L)	% MeHg*
Lower Brook-1	<0.000010	0.00155	15.50%
Churchill - 1	<0.000010	0.00215	21.50%
Churchill - 2	<0.000010	0.000096	0.96%
Churchill - A	<0.000010	0.00113	11.30%
Churchill - B	<0.000010	<0.000050	0.50%
Churchill - C	<0.000010	<0.000050	0.50%
Churchill - D	<0.000010	0.0001	1.00%
Churchill - E	<0.000010	0.000103	1.03%
Churchill - F	<0.000010	0.00035	3.50%
Churchill - 12	<0.000010	0.000121	1.21%

Site	THg (mg/L)[a]	MeHg (µg/L)	% MeHg*
Churchill - 13	<0.000010	0.000136	1.37%
Average ± SD	**0.00001**	**0.00053 ± 0.00073**	**5.31 ± 7.35%**
Minimum	**0.00001**	**0.00005**	**0.50%**
Maximum	**0.00001**	**0.00215**	**21.50%**

[a]Analytical chemical techniques differed for THg (cold vapour atomic fluorescence spectrometry) and MeHg (gas chromatography atomic fluorescence spectrometry), resulting in different detection limits.

*When non-detected, detection limit substitution was used to allow calculation.

QA / QC analyses revealed that the difference in MeHg concentrations between water sample replicates (3.90%) was well below the maximum relative percent difference (RPD = 20.0%). All recovery percentages (for laboratory controls, reference materials and blanks) were within the acceptable range of 70.0% to 130% (104 ± 6.70% for THg and 90.2 ± 4.10% for MeHg) (Appendix E). These values together indicate a high quality of the samples being tested and analyzed.

THg was detected in 10 of the 11 sediment samples, with levels ranging from <0.00500 mg/kg to 0.0322 mg/kg dry weight (dw; Table D.13). MeHg was detected in nine of the 11 samples and ranged from <0.0000500 mg/kg dw to 0.000297 mg/kg dw (Table D.13). The THg values detected were one to two orders of magnitude lower than the interim sediment quality guidelines for THg (ISQGs = 0.10 mg/kg) and probable effect levels (PELs = 0.486 mg/kg) for the protection of aquatic life (Environment Canada 1999a).

Percent MeHg in sediment samples ranged from 0.290% to 1.78%. Churchill – 1 and Lower Brook – 1 had the highest percentages of MeHg contribution to THg (1.78% and 1.41%, respectively) (Table D.13).

Table D.13 Total Mercury (THg), Methylmercury (MeHg) and Percent Methylmercury (% MeHg) in Sediment Samples from the Lower Churchill River Valley, June 2014

Site	THg (mg/kg dw)	MeHg (mg/kg dw)	% MeHg
Lower Brook - 1	0.0172	0.000243	1.41%
Churchill - 1	0.0167	0.000297	1.78%
Churchill - 2	0.0322	0.000243	0.750%
Churchill - A	0.0169	0.0000860	0.510%
Churchill - B	0.00900	<0.0000500	0.560%
Churchill - C	<0.00500	<0.0000500	n/a*
Churchill - D	0.00690	<0.0000500	0.720%
Churchill - E	0.0257	0.0000740	0.290%
Churchill - F	0.0133	0.0000780	0.590%
Churchill - 12	0.0138	0.000104	0.750%
Churchill - 13	0.0206	0.000148	0.770%

Site	THg (mg/kg dw)	MeHg (mg/kg dw)	% MeHg
Average ± SD	**0.0160 ± 0.00800**	**0.000160 ± 0.0000900**	**0.830 ± 0.430%**
Minimum	**<0.00500**	**<0.0000500**	**0.290%**
Maximum	**0.0322**	**0.000297**	**1.78%**
***** n/a = not applicable due to values below detection limits.			

QA / QC analyses revealed that the difference in THg and MeHg concentrations between sediment sample replicates (2.00% and 4.80%, respectively) was well below the maximum relative percent difference (RPD = 40.0% for THg and 30% for MeHg). All recovery percentages (for laboratory controls, reference materials and blanks) were within the acceptable range of 70.0% to 130% (97.4 ± 10.3% for THg and 97.7 ± 5.24% for MeHg) (Appendix D: Table D.2). These values together indicate a high quality of the samples being tested and analyzed.

D.4.2 Influence of Environmental Characteristics on Water and Sediment Total Mercury (THg) and Methylmercury (MeHg)

Dissolved oxygen, hardness and conductivity were significantly correlated with each other (Table D.14, Table D.15). Of these, hardness and conductivity appeared to be most influential in water MeHg (hardness $r^2 = 0.842$, $\alpha = 0.05$, $p = 0.000588$; and conductivity $r^2 = 0.855$, $\alpha = 0.05$, $p = 0.000398$; Figure D-8). Hardness and conductivity were also highly correlated with sediment MeHg (hardness $r^2 = 0.612$, $\alpha = 0.05$, $p = 0.0314$; and conductivity $r^2 = 0.615$, $p = 0.033$; Figure D-9) where greater hardness and conductivity resulted in higher concentrations of MeHg.

Sediment MeHg was however most highly correlated with water MeHg ($r^2 = 0.703$, $\alpha = 0.05$, $p = 0.0108$; Figure D-10) in addition to a strong correlation with sediment THg ($r^2 = 0.582$, $\alpha = 0.05$, $p = 0.0471$; Figure D-11).

Even though conductivity and hardness were the two parameters strongly correlated with MeHg levels in water and sediment, it is important to keep monitoring all water quality parameters in order to best predict the process of Hg transformation in this aquatic environment.

Table D.14 Correlation Matrix for Environmental Characteristics and Total Mercury (THg) and Methylmercury (MeHg) in Water and Sediment Samples

Parameters	Water Temperature	Oxygen	pH	Conductivity	Hard-ness	Water MeHg	Water Contribution MeHg	Sediment THg	Sediment MeHg	Sediment Contribution MeHg
Water Temperature	1									
Oxygen	.251	1								
pH	.486	.319	1							
Conductivity	-.285	**-.692***	.215	1						
Hardness	-.309	**-.730***	.176	**.994****	1					
Water MeHg	-.462	-.569	-.059	**.851****	**.839****	1				
Water Contribution MeHg	-.461	-.569	-.059	**.851****	**.839****	**1.000****	1			
Sediment THg	.060	.260	.008	-.108	-.092	.065	.065	1		
Sediment MeHg	-.468	-.159	.073	.602	**.602***	**.692***	**.692***	.545	1	
Sediment Contribution MeHg	-.525	-.387	.190	**.834****	**.811****	**.763****	**.763****	-.115	**.751****	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

THg in water was not included as all samples were below the detection limit

Table D.15 p-values of Correlation Matrix for Environmental Characteristics and Water and Sediment Total Mercury (THg) and Methylmercury (MeHg)

	Water Temp	Oxygen	pH	Conduct-ivity	Hard-ness	Water MeHg	Sediment THg	Sediment MeHg
Water Temp	NA							
Oxygen	0.430	NA						
pH	0.111	0.315	NA					
Conductivity	0.384	0.0157	0.524	NA				
Hardness	0.340	0.00864	0.603	5.39E-11	NA			
Water MeHg	0.142	0.0620	0.839	3.98E-04	5.88E-04	NA		
Sediment THg	0.841	0.419	0.982	0.881	0.902	0.715	NA	
Sediment MeHg	0.147	0.654	0.857	0.0332	0.0344	0.0108	0.0471	NA
*NA = not applicable; MeHg = MethylMercury; THg = Total Mercury								

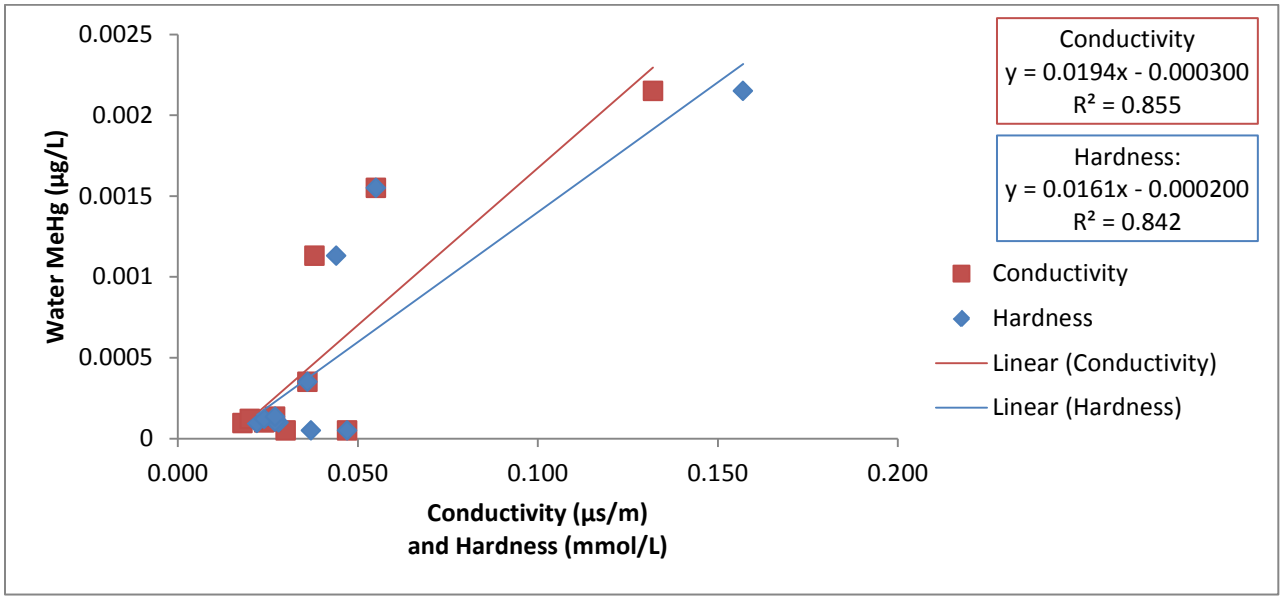


Figure D-8 Conductivity and Hardness in Relation to Methylmercury (MeHg) Levels in Water Samples

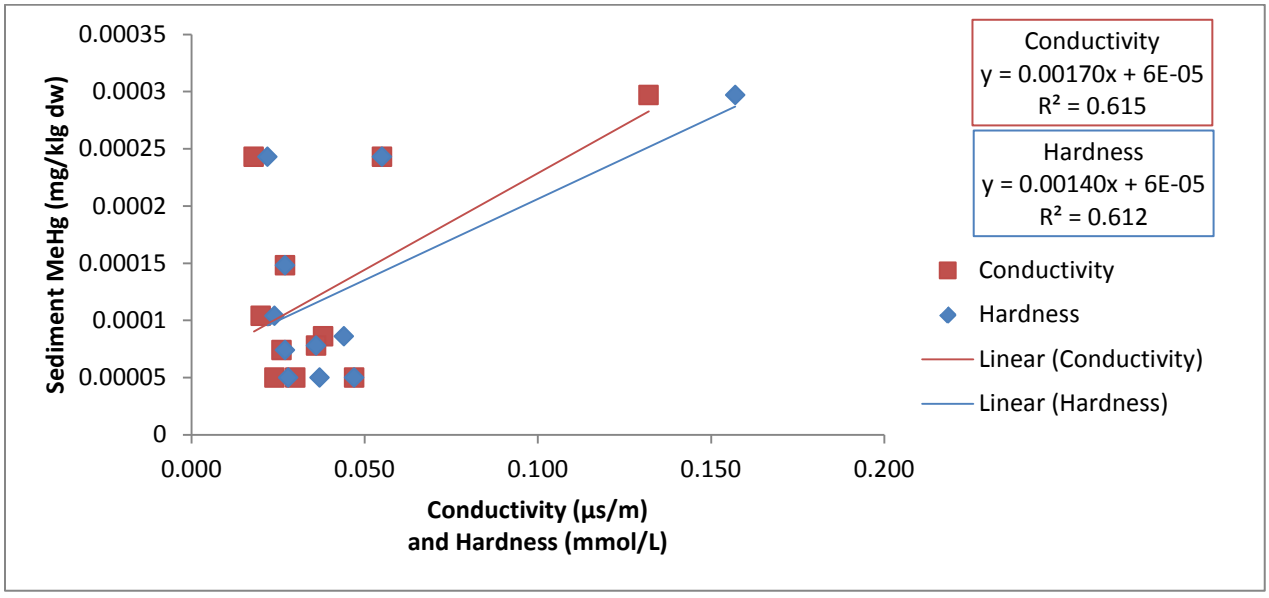


Figure D-9 Conductivity and Hardness in Relation to Methylmercury (MeHg) in Sediment Samples

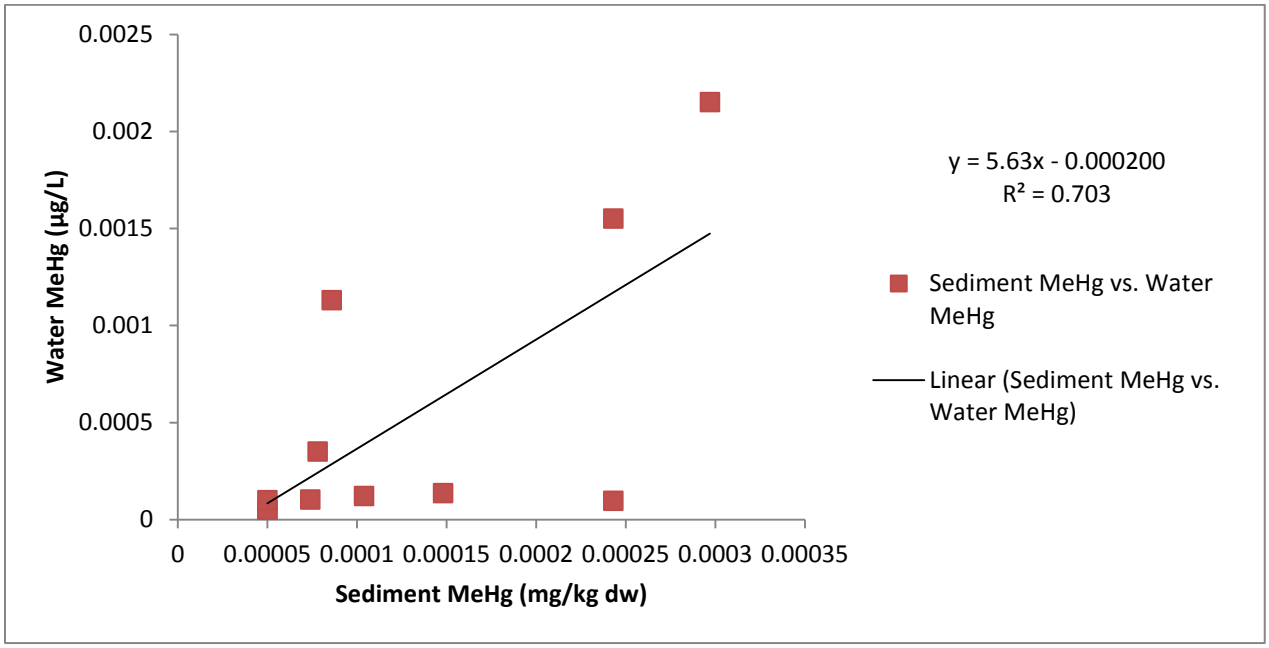


Figure D-10 Linear Regression of Sediment Methylmercury (MeHg) in Relation to Water Methylmercury (MeHg)

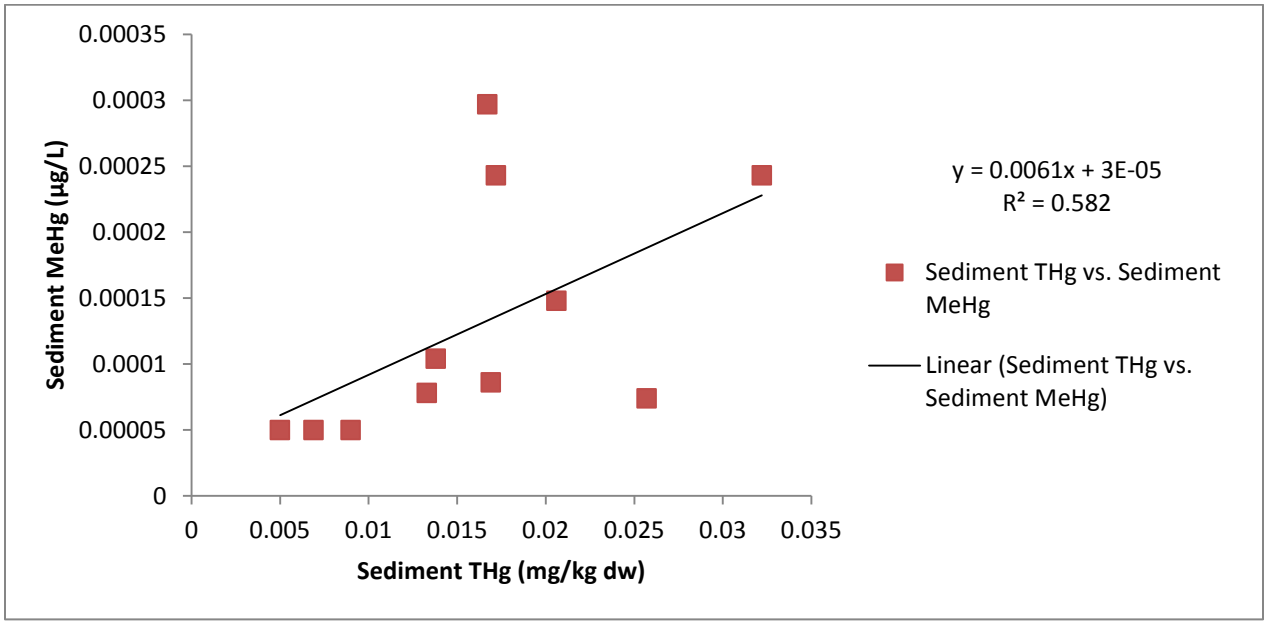


Figure D-11 Linear Regression of Sediment Total Mercury (THg) in Relation to Sediment Methylmercury (MeHg)

D.4.3 Influence of Environmental and Biological Characteristics on Amphibian Total Mercury (THg) and Methylmercury (MeHg)

American toad THg concentrations were significantly correlated ($\alpha = 0.05$, $p = 0.00439$; Table D.16; Figure D-12) with dissolved oxygen, supporting previous work that dissolved oxygen influences methylation and potential uptake in biota. American toad THg was also correlated with sediment MeHg ($r^2 = 0.807$, $\alpha = 0.05$, $p = 0.0525$; Table D.16; Figure D-13) followed by water MeHg ($r^2 = 0.784$; $\alpha = 0.05$, $p = 0.0652$; Table D.16; Figure D-14) and sediment THg ($r^2 = 0.735$, $\alpha = 0.05$, $p = 0.096$; Table D.16; Figure D-15) where higher concentrations in the environment corresponded with higher concentrations in the tadpoles.

Table D.16 Correlation Values for American Toad Total Mercury (THg) with Biological Variables, Stable Isotopes, Environmental Characteristics, and Water and Sediment THg and Methylmercury (MeHg)

Parameter	American Toad THg Correlation	
	R value	p-value
Snout-Vent Length	0.138	0.702
Tail Length	0.357	0.330
Total Length	0.552	0.126
$\delta^{15}N$	0.0710	0.846
$\delta^{13}C$	-0.414	0.251
Water Temp	-0.116	0.827
Dissolved Oxygen	0.945	4.39E-03
pH	0.354	0.491
Conductivity	0.470	0.347
Hardness	0.360	0.483
Water MeHg	0.784	0.0652
Sediment THg	0.735	0.0960
Sediment MeHg	0.807	0.0525

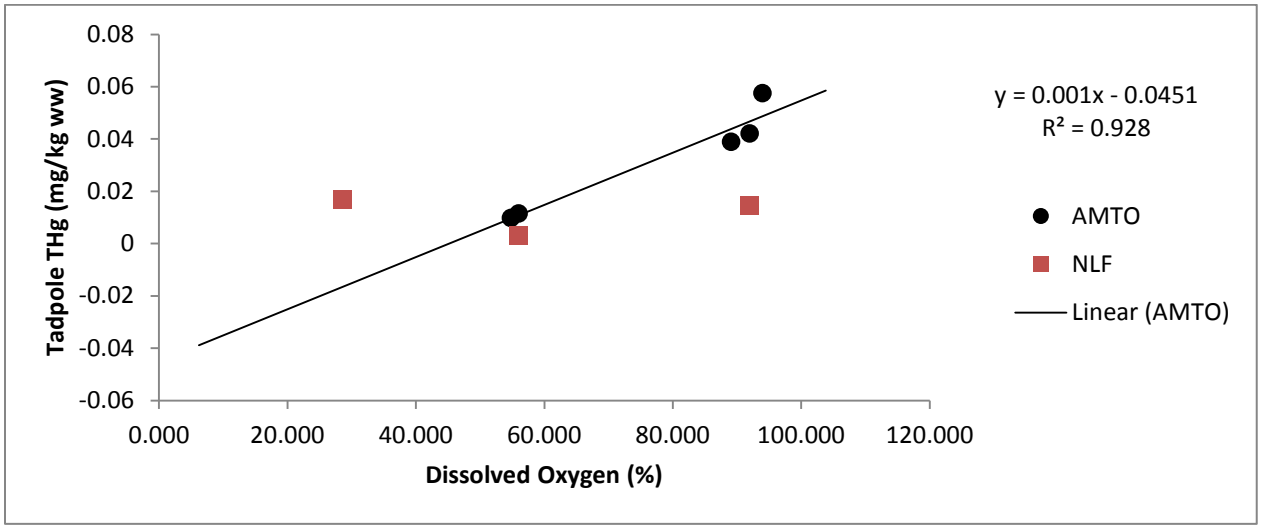


Figure D-12 Linear Regression of Dissolved Oxygen in Relation to the Total Mercury (THg) in American Toad (AMTO) and Northern Leopard Frog (NLF) Tadpoles

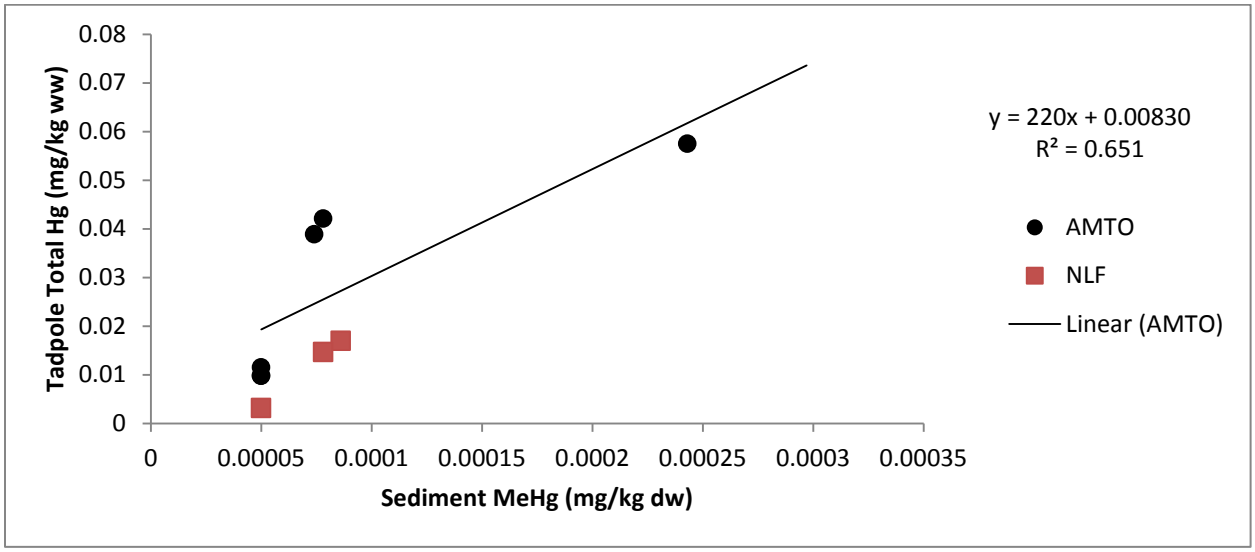


Figure D-13 Linear Regression of Sediment Methylmercury (MeHg) in Relation to the Total Mercury (THg) in American Toad (AMTO) and Northern Leopard Frog (NLF) Tadpoles

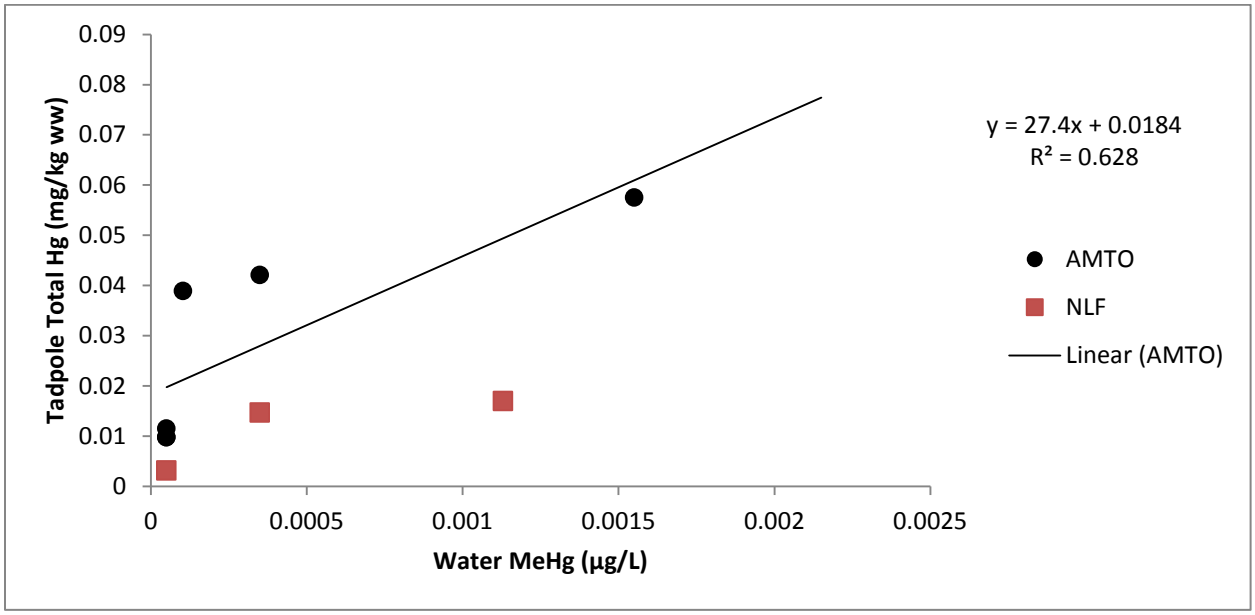


Figure D-14 Linear Regression of Water Methylmercury (MeHg) in Relation to the Total Mercury (THg) in American Toad (AMTO) and Northern Leopard Frog (NLF) Tadpoles

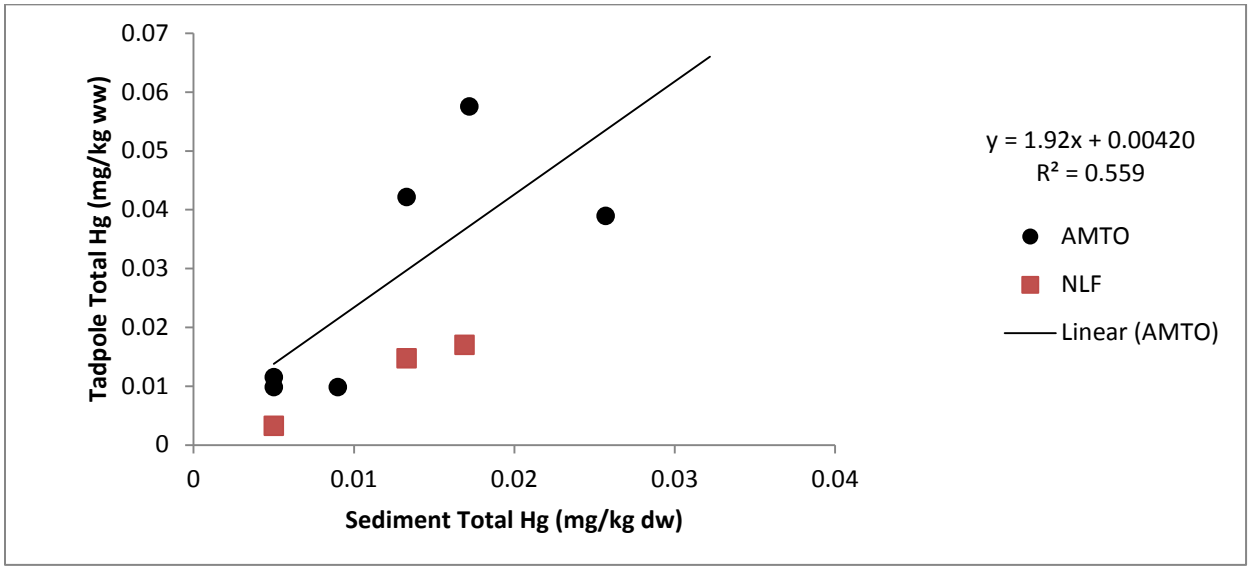


Figure D-15 Linear Regression of Sediment Total mercury (THg) in Relation to the Total Mercury (THg) in American Toad (AMTO) and Northern Leopard Frog (NLF) Tadpoles

No correlation was observed with any of the biological variables (snout-vent length, tail length, total length, $\delta^{15}N$ or $\delta^{13}C$; Table D-16) and THg concentrations. Although previous work has found that larger animal size and higher trophic levels may drive higher mercury concentrations in amphibians (Ugarte 2005, Unrine 2007), this is not always the case (Gerstenberger 2002). The lack of correlation observed here between THg and trophic level or average amphibian length (Figures D-16 and D-17; Table D-16) could be explained by the low sample size and/or the fact that mainly MeHg is known to increase with those variables. During the 2014 sampling year, MeHg concentrations were not analyzed in amphibian tissues, however, Bank et al. (2007) found MeHg to make up 7.60-40.0% of total Hg in green frog and bullfrog (*Lithobates catesbeiana*) tadpoles.

Due to low sample numbers, analyses of northern leopard frog THg concentrations were not statistically possible in the current sampling year. Nevertheless northern Leopard frog data are presented in Figures D-12 to D-17 for reference. Differences of mercury uptake in tadpole species may occur but amphibian diet, trophic level, and life stage (i.e., herbivorous tadpole vs. carnivorous adult amphibians) are likely to play a larger role in mercury uptake. Stable isotope analysis (discussed in Section D.3.3) suggests that both northern leopard frog and American toad tadpoles feed at the same trophic level although, at Churchill-C and Churchill-F, American toad seems to feed at a slightly higher trophic level, which could explain why at those locations American toad has consistently higher THg concentrations than northern leopard frog.

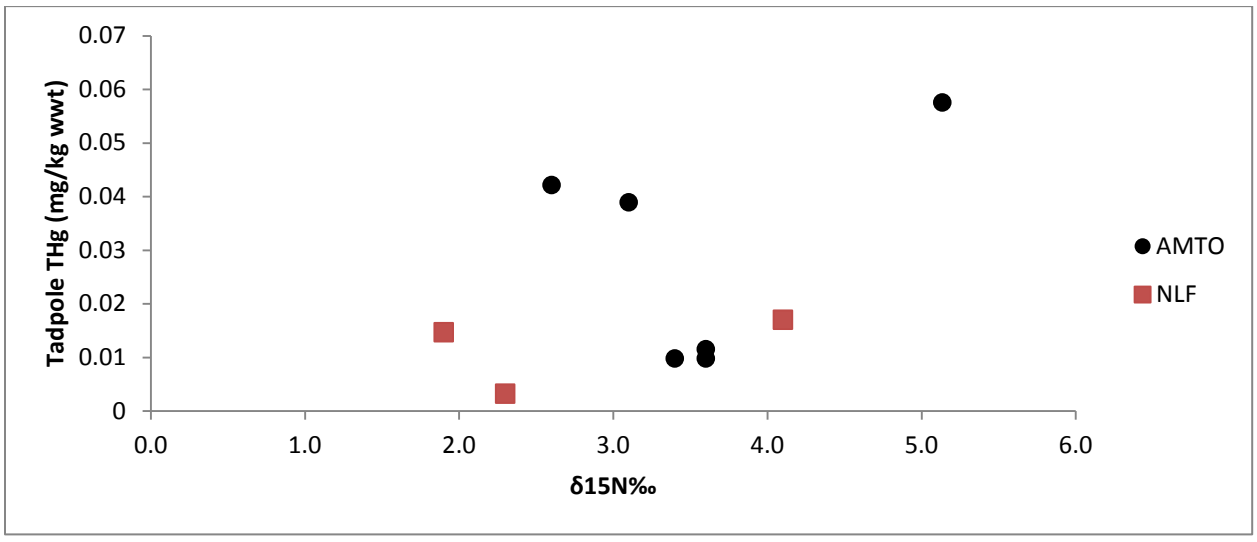


Figure D-16 δ15N Stable Isotope in Relation to the Total Mercury (THg) in American Toad (AMTO) and Northern Leopard Frog (NLF) Tadpoles

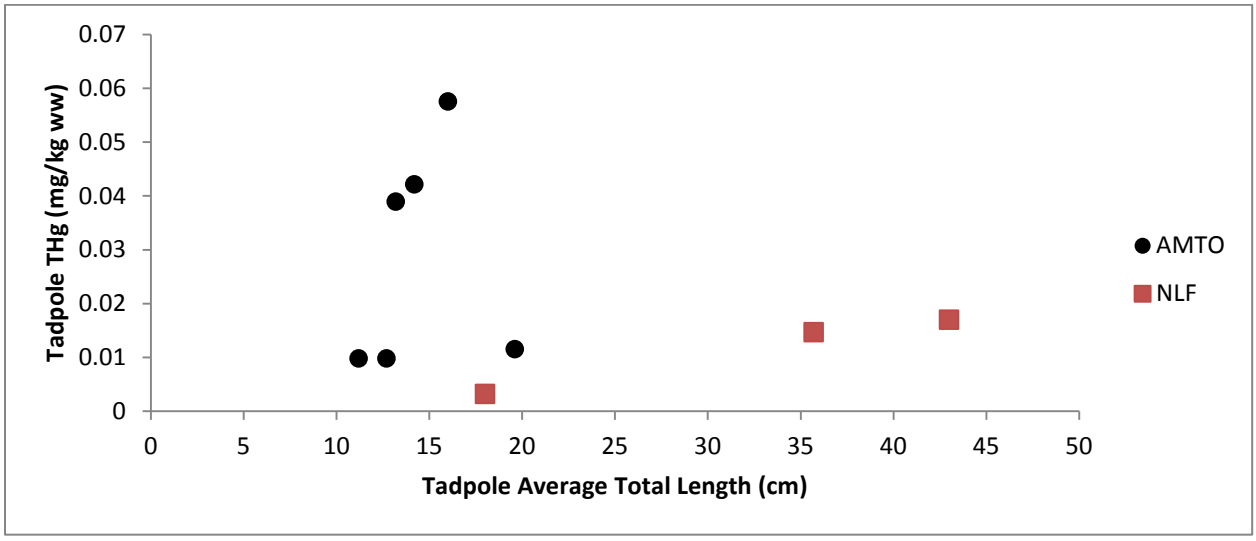
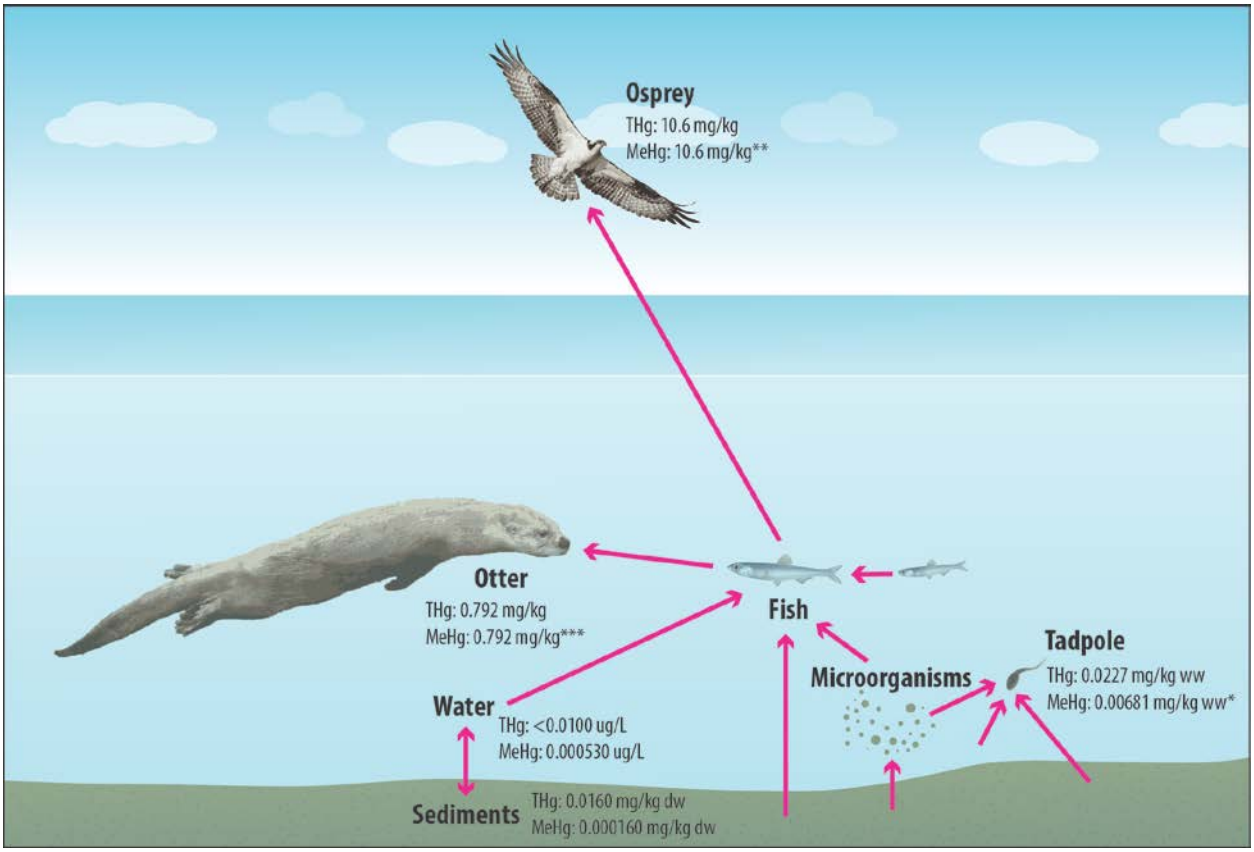


Figure D-17 American Toad (AMTO) and Northern Leopard Frog (NLF) Tadpoles Total Length in Relation to the Total Mercury (THg) in American Toad (AMTO) and Northern Leopard Frog (NLF) Tadpoles

D.4.4 Ecosystem Baseline of Total Mercury (THg) and Methylmercury (MeHg) in the Lower Churchill River Valley

The biota sediment accumulation factors (BSAFs) for THg in amphibians from sediment THg and water THg are factors of 1.42 and 2.27, respectively. The literature suggests that approximately 30.0% of THg in amphibian tadpoles is made up of MeHg (Bank et al., 2007). Using this estimate, the BSAF for MeHg in amphibians from sediment MeHg is 42.6 and from water MeHg is 12.8. These accumulation factors in the current study exemplify the bioavailability of MeHg and its ability to accumulate in higher trophic levels. Although mercury levels of fish, a critical prey component, were not measured as part of the EEMP, higher concentrations of mercury in higher trophic levels (i.e. 0.0227 mg THg/kg in amphibians, to 0.792 mg THg/kg in otters, to 10.6 mg THg/kg in osprey) indicate the capabilities of mercury to accumulate in the Lower Churchill Muskrat Falls Project area. Figure D-18 depicts a schematic drawing of the possible pathways for mercury transfer.



* Values based on expected MeHg concentrations (~30% of THg for tadpoles (Bank et al., 2007) and ~100% of THg concentrations for river otter and Osprey (Braune and Gaskin 1987, Odsjo et al. 2004, Kehrig et al. 1998, Voegborlo et al. 2010))

Figure D-18 Ecosystem Schematic of Mercury Trophic Transfer

APPENDIX E

Quality Control

Table E.1 Quality Assurance/Quality Control Data for Osprey Feather Total Mercury (THg) Lab Analyses

Matrix	QC Type	Analyte	Reference	Result	Target	Units	%	Limits
Tissue	CRM	THg	VA-NRC-TORT3	0.233	0.292	mg/kg	79.8	70-130
Tissue	CRM	THg	VA-NIST-1566B	0.0311	0.0371	mg/kg	83.8	70-130
Tissue	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
Tissue	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
Tissue	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
Tissue	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
Tissue	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
Tissue	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
CRM = Control Reference Material, MB = Method Blank								

Table E.2 Quality Assurance/Quality Control Data for Osprey Feather Stable Isotope Analysis

Sample ID	Analyte	Replicate 1	Replicate 2	Units	Percentage difference
OSP2013-09	$\delta^{15}N$	12.9	12.9	‰	0.00%
	$\delta^{13}C$	-22.7	-22.5	‰	0.889%
OSP2013-10	$\delta^{15}N$	9.6	9.8	‰	2.041%
	$\delta^{13}C$	-24.4	-24.4	‰	0.000%
OSP2013-28	$\delta^{15}N$	9.6	9.6	‰	0.000%
	$\delta^{13}C$	-23.9	-23.8	‰	0.420%
OSP2013-35-01	$\delta^{15}N$	10.4	10.5	‰	0.952%
	$\delta^{13}C$	-30	-30.3	‰	0.990%
OSP2013-36	$\delta^{15}N$	9.8	9.9	‰	1.01%
	$\delta^{13}C$	-24.2	-24.1	‰	0.415%

Table E.3 Quality Assurance/Quality Control Data for Amphibian Total Mercury (THg) Lab Analyses

Matrix	QC Type	Analyte	Reference	Result	Target	Units	%	Limits
Tissue	CRM	THg	VA-NRC-TORT3	0.246	0.292	mg/kg	84.3	70-130
Tissue	CRM	THg	VA-NIST-1566B	0.0311	0.0371	mg/kg	83.8	70-130
Tissue	MB	THg		<0.0010	<0.001	mg/kg		0.001
Tissue	MB	THg		<0.0010	<0.001	mg/kg		0.001
CRM = Control Reference Material, MB = Method Blank								

Table E.4 Quality Assurance/Quality Control Data for Water and Sediment Lab Analyses

Matrix	QC Type	Analyte	Reference	Result	Target	Units	%	Limits
Soil	CRM	THg	VA-CANMET-TILL1	0.0911	0.0980	mg/kg	93.0	70-130
Soil	CRM	THg	VA-NRC-STSD1	0.110	0.110	mg/kg	100.1	70-130
Soil	CRM	THg	VA-CANMET-TILL1	0.0962	0.0980	mg/kg	98.2	70-130
Soil	CRM	THg	VA-NRC-STSD1	0.108	0.110	mg/kg	98.5	70-130
Soil	IRM	THg	ALS MET IRM1	0.862	1.04	mg/kg	82.9	70-130
Soil	IRM	THg	ALS MET IRM1	0.920	1.04	mg/kg	88.5	70-130
Soil	IRM	THg	ALS MET IRM1	1.04	1.04	mg/kg	99.9	70-130
Soil	IRM	THg	ALS MET IRM1	0.961	1.04	mg/kg	92.4	70-130
Soil	IRM	THg	ALS MET IRM1	1.02	1.04	mg/kg	97.6	70-130
Soil	IRM	THg	ALS MET IRM1	1.34	1.04	mg/kg	128.4	70-130
Soil	LCS	THg		0.311	0.300	mg/kg	103.7	70-130
Soil	LCS	THg		0.289	0.300	mg/kg	96.4	70-130
Soil	LCS	THg		0.255	0.300	mg/kg	85.0	70-130
Soil	LCS	THg		0.271	0.300	mg/kg	90.4	70-130
Soil	LCS	THg		0.312	0.300	mg/kg	103.8	70-130
Soil	LCS	THg		0.297	0.300	mg/kg	99.0	70-130
Soil	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
Soil	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
Soil	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
Soil	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
Soil	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
Soil	MB	THg		<0.0050	<0.005	mg/kg	-	0.005
Water	LCS	THg		0.000112	0.000100	mg/L	112.5	80-120

Matrix	QC Type	Analyte	Reference	Result	Target	Units	%	Limits
Water	LCS	THg		0.000103	0.000100	mg/L	102.5	80-120
Water	MB	THg		<0.000010	<0.00001	mg/L	-	0.00001
Water	MB	THg		<0.000010	<0.00001	mg/L	-	0.00001
Water	MS	THg	Anonymous	0.0000975	0.000100	mg/L	97.5	70-130
Water	MS	THg	Anonymous	0.0000993	0.000100	mg/L	99.3	70-130
Water	LCS	MeHg		0.00211	0.00250	ug/L	84.2	80-120
Water	LCS	MeHg		0.00231	0.00250	ug/L	92.5	80-120
Water	MB	MeHg		<0.000050	<0.00005	ug/L	-	0.00005
Water	MB	MeHg		<0.000050	<0.00005	ug/L	-	0.00005
Water	MS	MeHg	L1482388-12	0.00241	0.00265	ug/L	90.6	70-130
Water	MS	MeHg	L1482388-2	0.00448	0.00465	ug/L	93.3	70-130
Soil	CRM	MeHg	SQC-MEHG-RM	0.00917	0.0100	mg/kg	91.7	70-130
Soil	LCS	MeHg		0.00506	0.00500	mg/kg	101.2	70-130
Soil	MB	MeHg		<0.000050	<0.00005	mg/kg	-	0.00005
Soil	MB	MeHg		<0.000050	<0.00005	mg/kg	-	0.00005
Soil	MS	MeHg	L1482388-17	0.00502	0.00500	mg/kg	100.3	60-140

CRM = Control Reference Material, IRM = Internal Reference Material, LCS = Laboratory Control Sample, MB = Method Blank, MS = Matrix Spike, CRM = Control Reference Material