Developing a mercury exposure profile for mink and river otter in Maine

(BRI 2003-05)

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Submitted to:

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ABSTRACT

Anthropogenic releases of mercury into the environment for the past several decades have collected in aquatic ecosystems. The impact of this mercury build-up is of concern to regulators and policy makers. Maine and much of New England are especially at high risk because of local and regional emission sources, prevailing wind patterns, and certain hydrological and biogeochemical features. This study establishes an exposure profile for mercury in Maine's mink and river otter populations. A total of 36 otter and 73 mink carcasses have been collected. Mercury levels tend to be greater in mink vs. otter, interior vs. coastal populations, and females vs. males. Respective mean mercury levels in otter and mink fur, 20.08 and 20.69 ppm; based on other studies, fur mercury levels greater than 20 ppm indicate adverse effects. The proportion of sampled individuals exceeding 20 ppm in the fur was 29% for mink and 61% for otter. Mink and otter fur Hg levels ranged up to 68.5 ppm and 234 ppm, respectively. Brain and liver Hg levels were below published lethal levels. The strong and significant relationships among brain, liver, and fur Hg levels provide great flexibility in using one compartment for determining mercury exposure. Successful efforts with live-trapping are providing an ability to relate fur and blood Hg levels and also provide an effective way to target sampling areas. Ageing based on teeth indicate a significant positive relationship between otter brain Hg levels and age (n=26; mean age = 1.8 years) and no correlation among the three matrices and mink age (n=48; mean age = 0.6 years). A significant negative correlation between otter brain Hg levels and corpus luteum counts was found (n=11; mean age = 1.7 years). No relationship was found with mink and is likely explained by the majority of mink (94%) under breeding age. This investigation will soon provide (1) a geographically-relevant mercury exposure profile, (2) data that can be linked to potential mercury impacts, and (3) contributions toward a wildlife criterion value model that is protective of Maine's mink and river otter population.

INTRODUCTION

Mercury and other aquatic-based persistent bioaccumulative toxins are prevalent in Maine's freshwater and marine environments (Maine DEP 1998, NESCAUM 1998). Methylmercury (MeHg) availability to fish and wildlife varies inter-regionally (Evers et al. 1998b). Because its availability is strongly influenced by hydrology (Lucotte et al. 1999, Evers and Reaman 1998) and biogeochemical factors (Watras and Huckabee 1994) it also shows tremendous variation intra-regionally (Evers et al. 2002). To interpret environmental exposure in wildlife established benchmarks are needed. Standardized sampling of high-risk biosentinels provides a method for making informed comparisons and definitive interpretations, thereby helping assess risks to wildlife and allow landscape-level extrapolations of the hazards.

The mink (*Mustela vison)* and the river otter (*Lontra cannadensis*) are both widely distributed in New England and Maine. Both species have diets that include fish and crayfish, although mink are known prey generalists. Because of their high metabolism and piscivorous diet, both mink and river otters are highly susceptible to elevated levels of environmental MeHg (USEPA 1997).

As in 2000 and 2001, our objective in 2002 was to develop a mercury exposure profile for Maine's mink and river otter populations based on fur and tissues from carcasses provided by trappers. This profile will serve as the basis for the mammal component of the Maine-based wildlife criterion value being developed by Evers et al. (2002).

Context – Comparison with other studies

Lab-based, dose-response studies of mink (Wobeser and Swift 1976) and otter (O'Connor and Neilson 1980) have shown that terminal total Hg concentrations occur at 25–20 ppm (ww) in the liver and kidney and 15–19 ppm (ww) in the brain. Dietary MeHg concentrations > 1.8 ppm (ww) are sufficient to cause mercury intoxication (Wobeser and Swift 1976, Thompson 1996).

Although fish fillet Hg levels > 1.8 ppm are rare in Maine (Stafford and Haines 1997), fish total Hg levels > 1.0 ppm are common and these (and lower) levels may contribute to sublethal impacts. Fish species with fillets > 1.0 ppm include smallmouth bass (*Micropterus dolomieu*), yellow perch (*Perca flavescens*), chain pickerel (*Esox niger*), and land-locked salmon (*Salmo salmar*) (Stafford and Haines 1997, Evers and Reaman 1998). Nearly all Hg in fish is in the toxic methyl form and is therefore biomagnified to the next trophic level.

Table 1. Concentrations of total Hg (ppm, ww) in river otter from various study sites. All values in parentheses are ranges and single values are arithmetic means. Studies are sorted by site.

Site	Sample Size	Muscle	Brain	Liver	Kidney	Fur[1]	Source
Britain	7	-	-	(0.2-4.3)	(0.08-2.02)	-	Mason 1988
Denmark	69	-	-	(0.03-12.4)	-	-	Mason and Madsen 1992
Georgia[3]		4.4C 1.5I		7.5 C		24.3C 15.2I	Halbrook et al. 1994
Ireland	32	-	-	(0.15-17.03)	-	-	Mason and Sullivan 1993
Maine	**36**	-	**0.54** (0.08–2.01)	**1.76** (0.24-4.74)	-	**20.9** (1.1–234)	BRI, This study
Manitoba	38		(0.04-9.5)	1.3-21.7	0.03-15.1		Kucera 1983
Mass.	96			1.9 (0.5-4.8			Organ 1989
New York	34	-	-	(0.01-6.95)	-	-	Foley et al. 1988
Nova Scotia	23		(0.07-1.8)C (0.5-10.2)I				Burgess et al. 2002
Ontario-1	1	36	30	96	58	47	Wren 1985[2]
Ontario-2	-	0.9	-	2.9	1.1	-	Wren et al. 1980
Ontario-3	84	(0.1-4.3)	(0.2-7.2)	(0.2-17.4)	(0.1-12.6)		Wren et al. 1986
Ontario-4	-	-	-	(1.0-3.5)	-	-	Wren and Stokes 1988
Ontario-5	130	-	2.0	6.7	-	13.8	Mierle et al. 2000
Vermont	21	-	-	-	-	13.58 (4.91-46.5)	BRI Unpub. data 2002
Wisconsin	49	1.4	0.7	3.3	8.5	6.5	Sheffy and St. Amant 1982

[1] Fresh weight

[2] Based on one individual from the English-Wabigoon River system that contained a recently operating chlor-alkali plant; this otter was found dead due to mercury exposure.

[3] Coastal samples=C and Interior samples=I

Empirical studies conducted by BioDiversity Research Institute (BRI) in New England wildlife (e.g., Evers et al. 2002, Shriver et al. 2002) and nationwide with the Common Loon (*Gavia immer*) (Evers et al. 1998, Evers et al. 2003) indicates elevated and potentially harmful Hg levels are present in aquatic environments. Comparisons with other mammalian studies (Table 1 and 2) further indicate that mink and river otter populations in Maine are likely exposed to sufficient quantities of dietary Hg to cause sublethal impacts.

Because the prey base is similar to loons, we expected body burdens in Maine to be greater than those in other areas of the United States for river otter (Table 1) and mink (Table 2). Much of the comparative literature is based on liver total Hg levels. However, because much of the Hg in the liver is demethylated (Scheuhamer et al. 1998) the available toxicity of methylmercury is best measured in the brain, fur, or muscle tissue.

An Ontario study considered otter populations to have reduced survivorship in high Hg areas (considered > 20 ppm of Hg in the fur) (Mierle et al. 2000). While Foley et al. (1988) considered Hg exposure in New York otter and mink population did not pose a serious risk, Halbrook et al. (1994) concluded similar Hg exposure profiles in mink and otter populations from Georgia were associated with adverse effects. Irrelevant of what levels pose risk at the individual level, there is widespread agreement that these two species are subjected to elevated Hg levels (Thompson 1996, USEPA 1997). Although the otter forages at higher trophic levels, most authors note that mink appear to be more sensitive to Hg impacts.

Table 2. Concentrations of total Hg (ppm,ww) in mink from various study sites. All values in parentheses are ranges and single values are arithmetic means. Studies are sorted by site.

Site	Sample Size	Muscle	Brain	Liver	Kidney	Fur[1]	Source
CT	8	-	-	(1.1-8.5)	-	-	Major and Carr 1991
MA	4	-	-	(0.01-1.9)	-	-	Major and Carr 1991
ME	73	-	**0.57** (0.1-2.6)	**1.77** (0.3-8.0)	-	**20.69** (1.8-68.5)	BRI, This study
NY	60	-	-	(0.25-7.66)	-	-	Foley et al. 1988
OH	-	-	-	0.1	-	-	Lynch 1973
ON	94	(0.01-4.1)	(0.3-0.7)	(0.01-7.5)	(0.1-5.5)		Wren et al. 1986
PQ 1	-	1.9	0.8	9.2			Desai-Greenway and Price 1976
PQ 2	-	2.4 (0.41-6.2)	-	8.3 (2.2-20.0)	-	-	Langis et al. 1999
SK[2]	1	-	-	58.2	31.9	-	Wobeser and Swift 1976
WI	39	1.3	0.5	2.1	2.3	7.6	Sheffy and St. Amant 1982

[1]Fresh weight
[2] Mink found in wild alive but later died due to mercury exposure

STUDY AREA & METHODS

Study Area

Previous mercury-based studies in Maine provided information on "hotspots" (Welch 1994, Evers et al. 1998a), aquatic habitats prone to enhanced methylmercury availability (i.e., reservoirs with a river-based origin that have summertime water levels that fluctuate >1m; Evers and Reaman 1998), and species most at risk (Evers et al. 1998b).

We identified three focal areas for our carcasses collection efforts (Appendices 1-3): (1) Flagstaff Lake, the North Branch of the Dead River and its watershed including Chain-of-Ponds, and the Dead River outflow from the Flagstaff dam have some of the highest levels of biotic mercury in the country; (2) Seboomook and Canada Falls Lakes and neighboring areas have had reports of mink extirpations; and (3) Millinockett area was chosen because of elevated mercury levels found from 2001 opportunistic sampling. Carcasses were also opportunistically collected from other areas in the state.

Sample collection

We collected 36 river otter (8 in 2000, 18 in 2001, 10 in 2002) and 73 mink (24 in 2000, 23 in 2001, 26 in 2002) carcasses from licensed fur trappers during the 2000-02 trapping seasons. The logistics of carcass retrieval were discussed with the following trappers: Dave MacNeill of Millinocket, Dan Kusnierz of Old Town, Bobby Cercena of Eustis, Jerry Le Beau of North Anson, Lindsay Seeley of Orrington, Jim Carter of Ashland, Oscar Cronk of Wiscasset, and Bruce Connery of Acadia National Park. In 2000, the junior author met with trappers in the Boothbay area during a trapper safety course sponsored by Maine Inland Fisheries and Wildlife (where he received his trapping certificate # METS-025-00-006).

Sample Processing

Carcasses were labeled and stored in freezers. Brain, femoral muscle, liver tissue, and the lower jaw were removed using stainless-steel instruments and placed into sterilized I-CHEM® jars. The lower jaw was archived in a freezer so a canine tooth could be used in the future to accurately age individuals. Fur was taken from the foot of the animal using stainless-steel instruments, cleaned, and placed into sealed envelopes. The tissues, once harvested, were refrozen. The tissue samples and corpra lutea were harvested at the University of Southern Maine using techniques according to Tufts University Animal Wildlife clinic protocols (M. Pokras, pers. com.). Lower jaws from 76 otter and mink and ovaries from 24 females (15 mink and 9 otter) were submitted to Matson's Laboratory, LLC (P.O. Box 308, 8140 Flagler Rd., Milltown, MT 59851; 406-258-6286; www.MatsonsLab.com). Results are pending.

Sample Analysis

Fur, brain and liver tissues were analyzed for total mercury using Cold Vapor Atomic Absorption (CVAA) methods. Laboratory analysis was conducted by Texas A&M Trace Element Research Lab (TERL), College Station, Texas (2000 and 2002) and Maine Environmental Lab (MEL), Yarmouth, Maine (2001 and 2002). Femoral muscle tissue were archived for future analysis. TERL and MEL have conducted BRI's mercury analysis for bird

tissues (blood, feathers, and eggs), fish, and crayfish for the past three years. Mercury level results are given as fresh weight (fw) for fur and wet weight (ww) for liver and brain. Instead of analyzing methylmercury (MeHg) levels we focused on total Hg because it is (1) less costly, (2) generally correlated with MeHg in most tissues (Kucera 1983), and (3) reflective of fur Hg levels because >95% of the Hg is methyl (Thompson 1996).

Methods for Live Trapping

We attempted live-captures of river otter and mink at latrine sites from during the fall of 2001 and summer of 2002. One-and-one-half inch soft catch foothold traps were set at entrances and exits of the latrine sites. We sectioned off the entrance and exits of the latrine sites and set the traps where the otter and mink are forced to step on them while traveling to or from the latrine. Also, traps were set on crossing paths that otter and mink use while traveling from one water-body to another. The number of traps varied at latrine sites from two to four depending on how many entrances and exits were present.

All of the traps were set on land, using a drop of otter or mink lure. The traps were equipped with four swivels and a spring to minimize trauma to the animal's foot. One swivel at the base of the trap allowed the trap to rotate 360°. The traps were anchored to a nearby root or a three-foot stake. The traps were always set so the animal could not reach the water or a tree to pry itself free and to avoid the potential risk of injury. We dug the traps into substrate so they would be flush with the ground. We used wax paper over the pan to keep the trigger debris free. The trap was then covered by pine needles and dirt to camouflage them. We checked the traps every morning so the animals were not in the traps for more than one night.

Once the otter or mink was captured we used a catchpole to safely control the animal while placing it in a holding box for transport back to our field station. We hand injected the animals using a mixture or Ketamine (2.5 mg/kg) and Medetomidine (0.025 mg/kg). We used Atipamezole (0.100 mg/kg) as an antiseden to the Medetomidine. The maximum time the animals were anesthetized was 45 minutes before given the antiseden.

RESULTS AND DISCUSSION

A. Mercury exposure profile

Three compartments were analyzed for Hg on each individual collected in 2002.

1. River Otter

A total of 36 otter carcasses were collected from 2000 to 2002. Mean (+/- SD) length and weight for males were 71.1+/- 6.9cm and 5,712.5 +/- 1,322.5g, respectively. For females mean (+/- SD) length and weight were 67.2+/- 5.9cm and 4,956.2+/- 946.5g.

We analyzed 10 fur, liver and brain samples in 2002. Fur Hg concentrations ranged between 1.14 ppm in otter from Round Pond, on Mount Desert Island, to 234.0 ppm on Flagstaff Lake (Table 3). Otter fur Hg levels indicates individuals from several sites are elevated (>20 ppm) when compared with other studies (Table 1). Brain total Hg levels ranged from 0.08 to 2.01 ppm while liver total Hg levels ranged from 0.24 to 4.74ppm (Table 3).

Wren (1985) showed that Ontario river otters with mean fur Hg levels of 47 ppm had on average 30 ppm and 96 ppm total Hg in the brain and liver respectively. Lethal levels are

considered 25-30 ppm total Hg in the liver (Thompson 1996) and 15-19 ppm total Hg in the brain (O'Connor and Neilsen 1980, Mierle et al. 2000). Although fur Hg levels from Maine otter approach lethal levels, brain and liver Hg levels indicate lower exposure.

Table 3. Concentrations of total Hg levels (ppm, ww) in brain, liver, and fur* from river otters collected in Maine during 2000-02 trapping season.

Tissue	Year	Sample size	Mean	SD	Range
Brain	2000	8	0.45	0.24	0.08 - 0.69
Liver	2000	7	3.00	1.61	0.24 - 4.74
Fur*	2000	8	21.60	11.28	4.99 - 33.7
Brain	2001	18	0.44	0.18	0.23 - 1.03
Liver	2001	18	1.53	1.01	0.32 - 3.47
Fur*	2001	18	18.69	7.62	1.14 - 32.0
Brain	2002	10	0.77	0.56	0.26 - 2.01
Liver	2002	10	1.31	0.44	0.68 - 2.16
Fur*	2002	10	21.4	5.41	13.3 - 28.6
Total Brain	2000-02	36	0.53	0.36	0.08 - 1.03
Total Liver	2000-02	35	1.76	1.19	0.24 - 4.74
Total Fur*	2000-02	36	20.08	7.95	1.14 - 33.7

*Fresh Weight

Fur Hg levels reflects the total body burden bioaccumulated over time, particularly for individuals with high exposure. Consequently the animal's age may be a confounding factor in interpreting fur Hg results. Mierle et al. (2000) found that Hg concentrations in fur changed with age. It increased during the first four years in Ontario otters, but then declined. However, fur Hg levels in the Ontario study did not exceed 15 ppm in known age otters, and it is likely the animals were able to demethylate their Hg body burden. In our study, several otters had relatively high fur Hg levels; therefore it is not clear if these animals would be able to effectively demethylate their body burden. Blood Hg levels reflect recent dietary uptake and would help explain fur Hg concentrations.

2. Mink

A total of 73 mink carcasses were collected from 2000 to 2002. Mean (+/- SD) length and weight for females were 38.6 +/- 2.6cm and 407.3 +/- 112.5g, respectively. For males mean (+/- SD) length and weight were 38.1 +/- 2.7 cm and 617.1 +/- 139 g.

We analyzed 25 fur, brain and liver samples in 2002. Mink fur Hg concentrations ranged from 1.78 ppm on Felts Brook near Orrington to 51.8 ppm on Red Pine Brook near Daaquam (Table 4). Mink brain and liver Hg ranged from 0.22 (brain) and 0.27 (liver) to 2.55 (brain) and 6.13 ppm (liver) from Ross Stream near Daaquam and St. Johns River, respectively (Table 4).

Table 4.. Concentrations of total Hg levels (ppm, ww) in brain, liver, and fur* from mink collected in Maine during 2000-02 trapping season.

Tissue	Year	Sample size	Mean	SD	Range
Brain	2000	25	0.63	0.46	0.13 - 2
Liver	2000	25	2.46	1.92	0.49 - 8.03
Fur*	2000	25	24.32	14.24	9.2 - 68.5
Brain	2001	23	0.59	0.56	0.22 - 2.55
Liver	2001	23	1.61	1.43	0.27 - 6.13
Fur*	2001	23	19.09	13.53	1.78 - 51.8
Brain	2002	25	0.51	0.33	0.16 - 1.84
Liver	2002	25	1.24	0.73	0.16 - 2.96
Fur*	2002	25	18.56	10.27	3.7 - 53.8
Total Brain	2000-02	73	0.57	0.45	0.13 - 2.55
Total Liver	2000-02	73	1.76	1.49	0.16 - 8.03
Total Fur*	2000-02	73	20.69	12.76	1.78 - 68.5

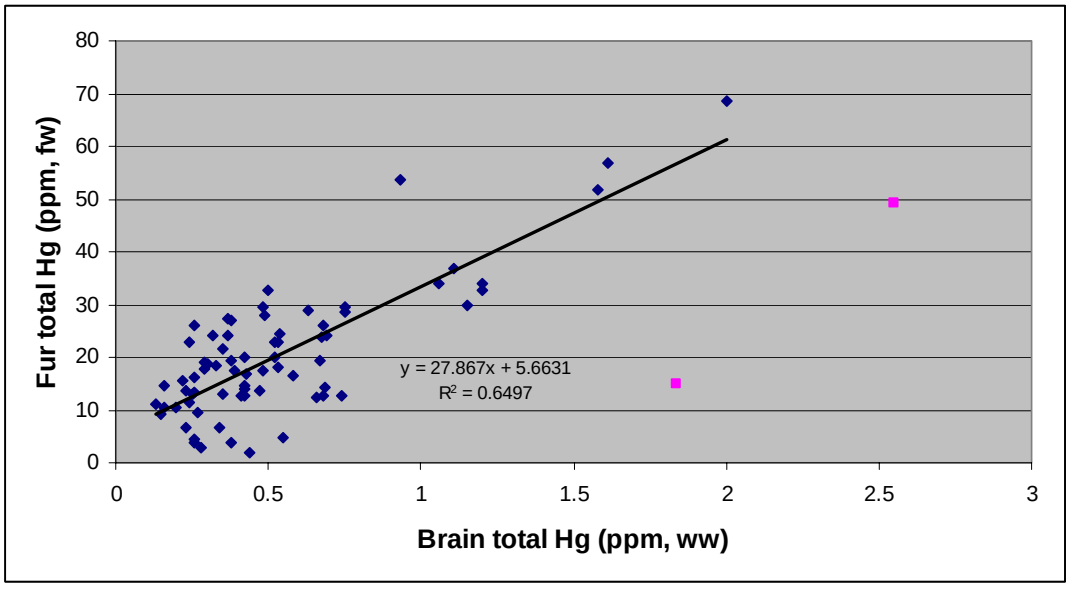
*Fresh Weight

B. Relationship Between Tissues

1. Fur vs. Brain

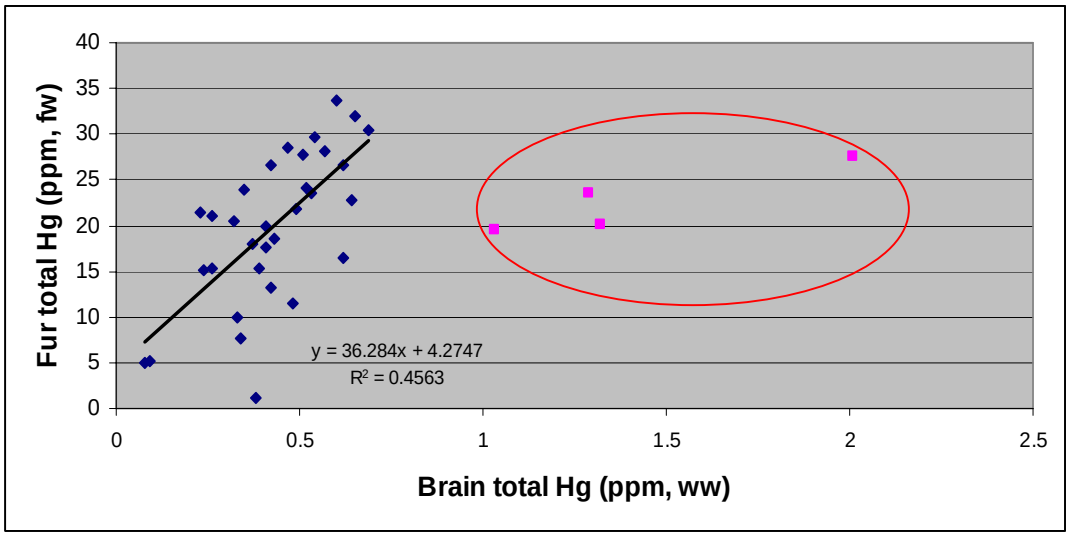
The linear relationship between fur and brain Hg levels were highly significant for mink (not including two outliers)(r^2 = 0.65, F = 80.7, df = 72, and p<0.001) (Figure 1a).

Figure 1a: Relationship between fur and brain Hg in Mink for Maine, 2000-2002.



The linear relationship between fur and brain Hg levels were significant for river otter (not including a group of four outliers) (r^2 = 0.46, F = 25.2, df = 31, p<0.001) (Figure 1b).

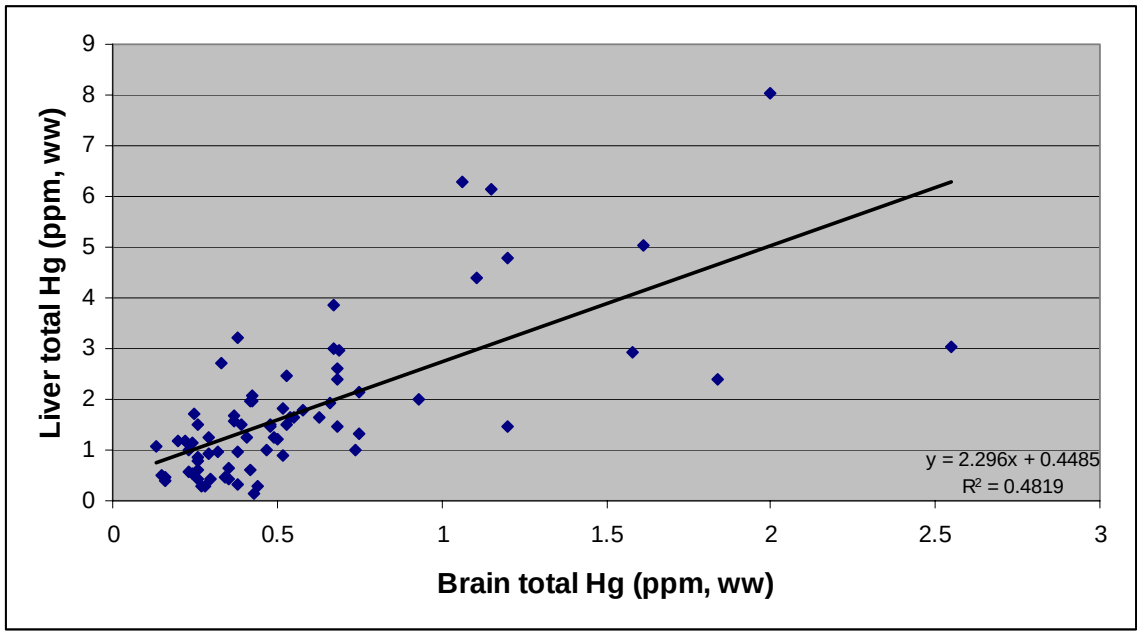
Figure 1b: Relationship between fur and brain Hg in River Otter for Maine.



2. Brain vs. Liver

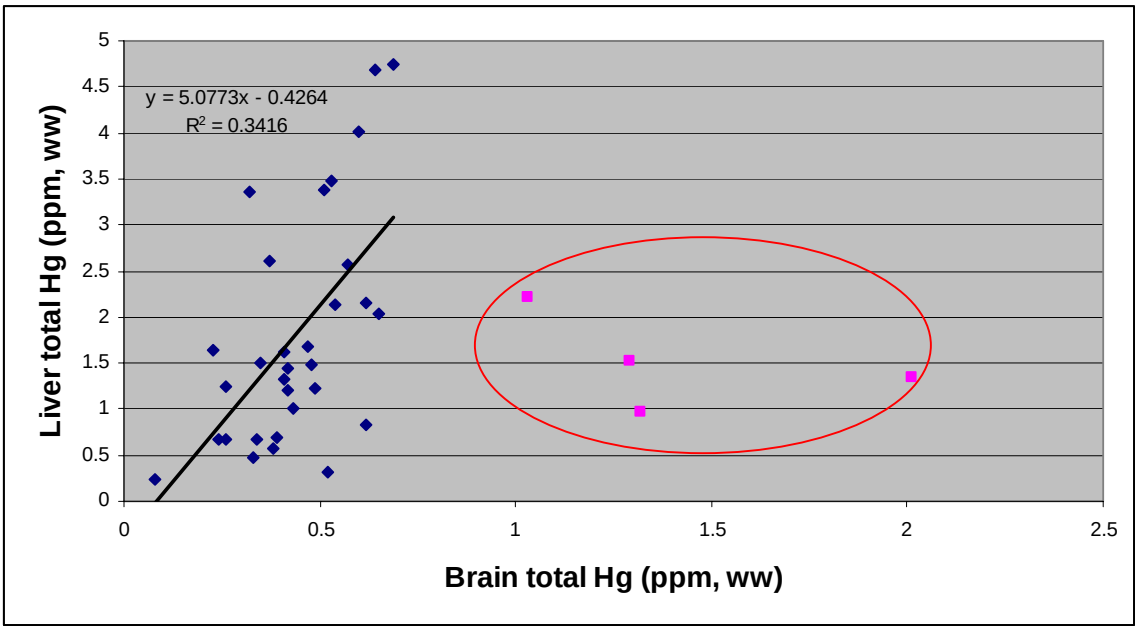
The linear relationship between brain and liver Hg levels were significant in mink (r^2 = 0.48, F = 66.0, df = 72, p <0.001) (Figure 2a).

Figure 2a: Relationship between brain and liver Hg in Mink for Maine.



The linear relationship between brain and liver Hg levels were significant in river otter (not including a group of four outliers) (r^2 = 0.34, F = 15.0, df = 30, p <0.001) (Figure 2b).

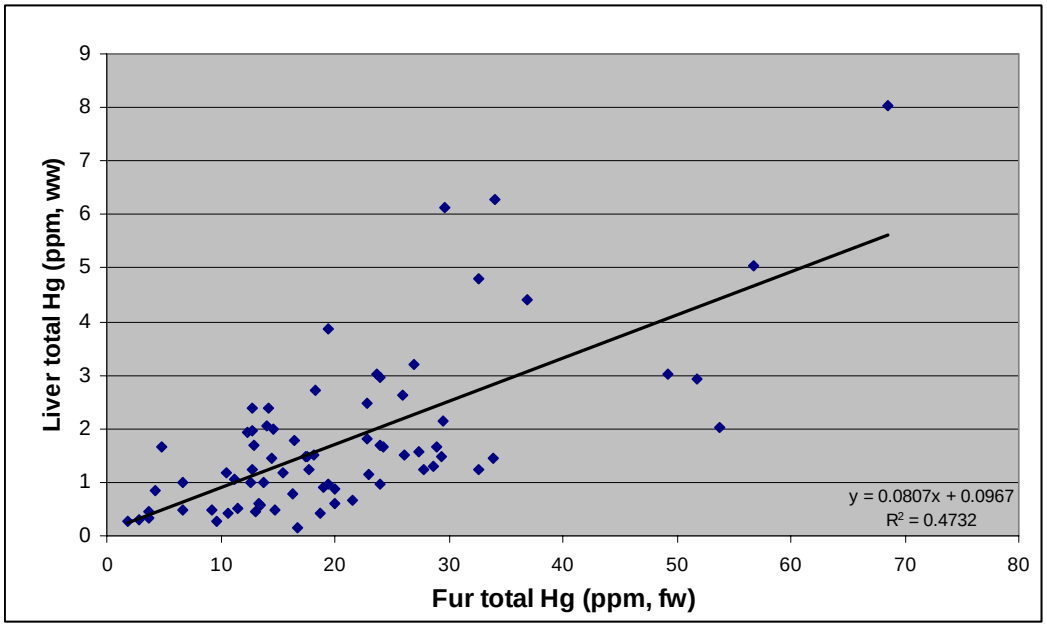
Figure 2b: Relationship between brain and liver Hg in River Otter for Maine.



3. Fur vs. Liver

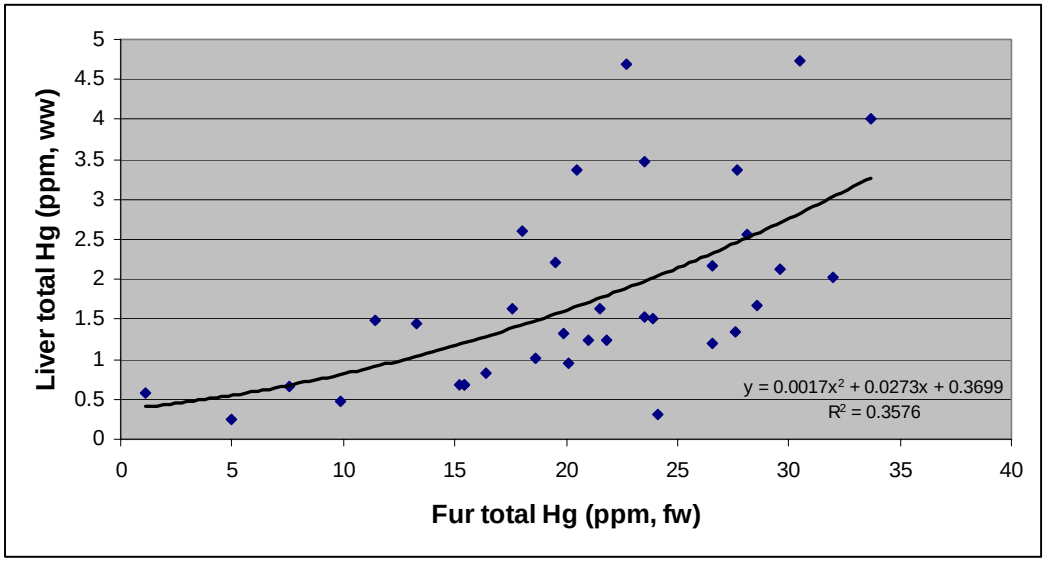
The linear relationship between fur and liver Hg levels were highly significant in mink (r^2 = 0.47, F = 63.8, df = 72, p <0.001) (Figure 3a).

Figure 3a: Relationship between fur and liver Hg in Mink for Maine.



The polynomial relationship between fur and liver Hg levels were significant in river otter (r^2 = 0.35, F = 17.5, df = 34, p<0.001) (Figure 3b).

Figure 3b: Relationship between fur and liver Hg in River Otter for Maine.



C. Live Capture

Because few trappers operate in the Flagstaff and Seboomook regions we live trapped these of know, high Hg risk. Capturing a live animal also permits blood sampling. Analysis of blood samples allow more meaningful comparisons among different sites and regions, because (1) blood Hg levels reflect a recent or short term Hg exposure of a piscivorous mammal and (2) should be independent of age. Because >95% of Hg in the blood is in the methyl form, measuring total Hg provides insight into the recent dietary uptake of MeHg. Collecting blood samples from recently killed animals is difficult because blood rapidly loses moisture after death; therefore, blood clots and whole blood Hg likely do not correlate (based on studies with loons, M. Pokras, Tufts Univ., pers. com.). Conversely, much of the Hg in organs is inorganic. By sampling and analyzing fur and blood from live individuals we hope to establish a relationship between the two matrices that can be applied to future studies for Hg interpretation of live or dead animals. Because animals can be live-trapped in areas of low density, we avoid potential population impacts and provide a comparative template for other studies that cannot afford removing animals.

Live trapping also adds another matrix of Hg measurement that can be related to other compartments such as fur, liver, kidney, and brain. Each matrix provides different information. Mercury levels in fur are an indicator of long-term body burden and organs generally demethylate Hg and do not necessarily provide an accurate assessment on toxicity to the individual. There is now evidence that the brain can demethylate Hg (particularly in the otter, D. Evans, Trent Univ., pers. com.) so that compartment may not be helpful for chronic Hg loads. Sampling certain matrices, such as muscle or fur (since fur would likely reflect remobilization of MeHg in the muscle) can provide better insights into the lifetime body burden for the animal. This is crucial part of this investigation because the bioaccumulation rate of MeHg is one of the most important aspects of toxicity to a population.

1. Capture efforts in 2002

BRI set a total of 3 leg-hold traps in the Flagstaff Lake area during July and August (Appendix 4). The traps were set out for a total of 15 nights, resulting in the successful live-capture of three otter. We had a total of 45 trap nights (Table 5) and our success was 0.07 otters per night (Table 5). In a study by Blundell G.M. et al. (1999) the trapping efficiency for river otters using leg-hold traps was 0.048.

Table 5. Live leg-hold trapping results for 2001 and 2002.

	2001	2002
Efficiency (captures/trap night)	0.001 otter/trap-night	0.07 otter/trap-night
Trapping Effort (# of traps x # of nights trapping)	952 trap-nights	45 trap-nights

We live-trapped three otters, two adult females and a juvenile female otters, they were captured at Flagstaff Lake, located in Eustis, Maine. First, the otters were removed from the trap with the aid of a catchpole and placed into a catch box, enabling transportation back to the field station to take a blood and fur sample. Once the animal was anesthetized we examined it for any obvious injuries that it may have sustained from the trapping process (no visible damage to the otter was observed).

A mixture of Ketamine (2.5 mg/kg) and Metetomidine (0.025 mg/kg) sedative was administered via hand injection to the rump of the animal. Approximately three minutes following injection, the animal was fully sedated. The otter was removed from the catch box and placed upon a padded blanket where the sampling of tissue (blood and fur) and basic measurements (weight and length) were collected. A small patch of fur was clipped from the area located just above the animal's hind foot. Using a 1cc syringe, approximately 0.3cc of whole blood was hand-drawn from the jugular vein. The animal was then placed back into the catch box and was administered the antiseden Atipamezole (0.10 mg/kg). Approximately 8 minutes preceding the injection of Atipamezole was required for the otter to fully recover. The total time the otter was anesthetized was approximately 45 minutes.

The animals were kept overnight at the field station where it was monitored for any health irregularities and then was released the following morning at the trapping site.

2. Tissue Analysis

The otter whole blood and fur sample were analyzed for total Hg at Texas A&M University, College Station, Texas. The blood mercury levels were 1.39, 1.11, and 0.176 (ppm, ww) and the fur was 234, 218, and 8.95, respectively (ppm)(Table 6). Existing studies investigating levels of Hg within otter blood, which is needed in order to make a comparison with our live-trapped otter sample, have not been found. However, the total Hg value of 234 ppm found in the otter fur is significantly higher than the existing mean of 20.09 ppm (+/-7.95) in the 36 otter carcasses collected and analyzed from Maine in 2000-02 (Table 1).

Table 6. Live Trapping Hg Results

Lake	Species	Gender	Blood Hg (ppm)	Fur Hg (ppm)
Flagstaff/ North Branch	Otter	Female/Adult	1.39	234
Flagstaff/Trout Brook	Otter	Female/Adult	1.11	218
Flagstaff/Trout Brook	Otter	Female/ Juvenile	0.176	8.95

D. Other measurements

1. Animal age

A. Otter Aging

A total of 26 otter jaws were sent to Matson's Lab to determine age. Average age of trapped otters was 1.8 years old (the oldest was 9 years old). Age and brain mercury have a significant correlation (p=0.03), while fur and liver did not (Table 7).

Table 7. Correlation of Otter Age and Mercury in Tissues.

Variable	by Variable	Correlation	Count	Signif Prob
Age	Fur Hg	0.1626	26	0.4275
Age	Brain Hg	0.4383	26	0.0251
Age	Liver Hg	-0.0459	26	0.8239

B. Mink Aging

A total of 48 mink jaws were sent to Matson's Lab to determine age. Average age of trapped mink was 0.60 years old (the oldest was 5 years old). The age and tissues of the mink did not correlate significantly (Table 8).

Table 8. Correlation of Mink Age and Mercury in Tissues.

Variable	by Variable	Correlation	Count	Signif Prob
Age	Fur Hg	-0.1803	48	0.2200
Age	Brain Hg	-0.1476	48	0.3167
Age	Liver Hg	-0.1372	48	0.3523

2. Corpus luteum

A. Otter Ovaries

A total of 11 otter ovaries were sent to Matson's Lab to quantify corpus luteum scars. Scars indicate reproductive success over time. Trapped female otters averaged 1.7 years of age (the oldest was 9 years old). Brain mercury and corpus luteum counts have a significant negative correlation (see Table 9). The other tissues do not significantly correlate with corpus luteum counts.

Table 9. Correlation of Otter Corpus luteum (CL) and Mercury in Tissues.

Variable	by Variable	Correlation	Count	Signif Prob
CL	Fur Hg	0.2686	11	0.4246
CL	Brain Hg	-0.6547	11	0.0288
CL	Liver Hg	-0.3618	11	0.2742

B. Mink Ovaries

A total of 16 mink ovaries were sent to Matson's Lab to quantify corpus luteum scars. Trapped female mink averaged 0.37 years of age (one mink was a two-year-old, 4 were one-year-olds, and the remainder were < one-year of age). No scars were detected for the 16 mink ovaries. Mink are sexually mature at 10 months of age (Chapman and Feldhammer 1982), however, known of the five individuals of breeding age showed evidence of reproductive success.

RECOMMENDATIONS

1. Continue carcass retrieval using our trapper network to fill geographic data gaps;
2. Expand live capture efforts in particularly high Hg sites, including the Flagstaff Lake and Seboomook Lake areas;
3. Add a biomarker assay (e.g., comet assays to detect genetic fragmentation) to provide insight on potential impacts from Hg with an emphasis on individuals with fur Hg levels over 20ppm;
4. Continue to submit lower jaws to the Matson Lab, Montana for aging individuals and corpus luteum counts;
5. Analyze mink and otter muscle tissue for total mercury and brains for methylmercury.

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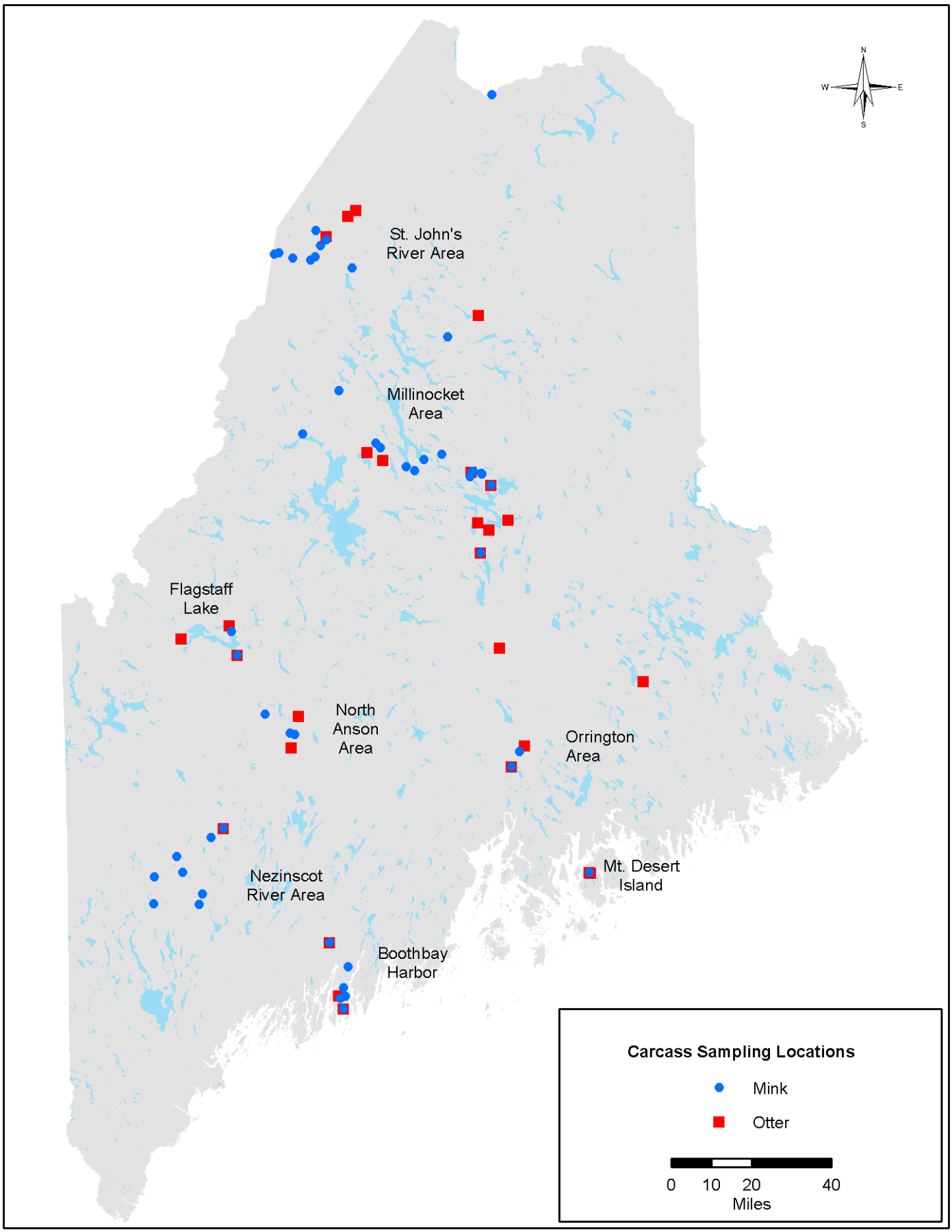
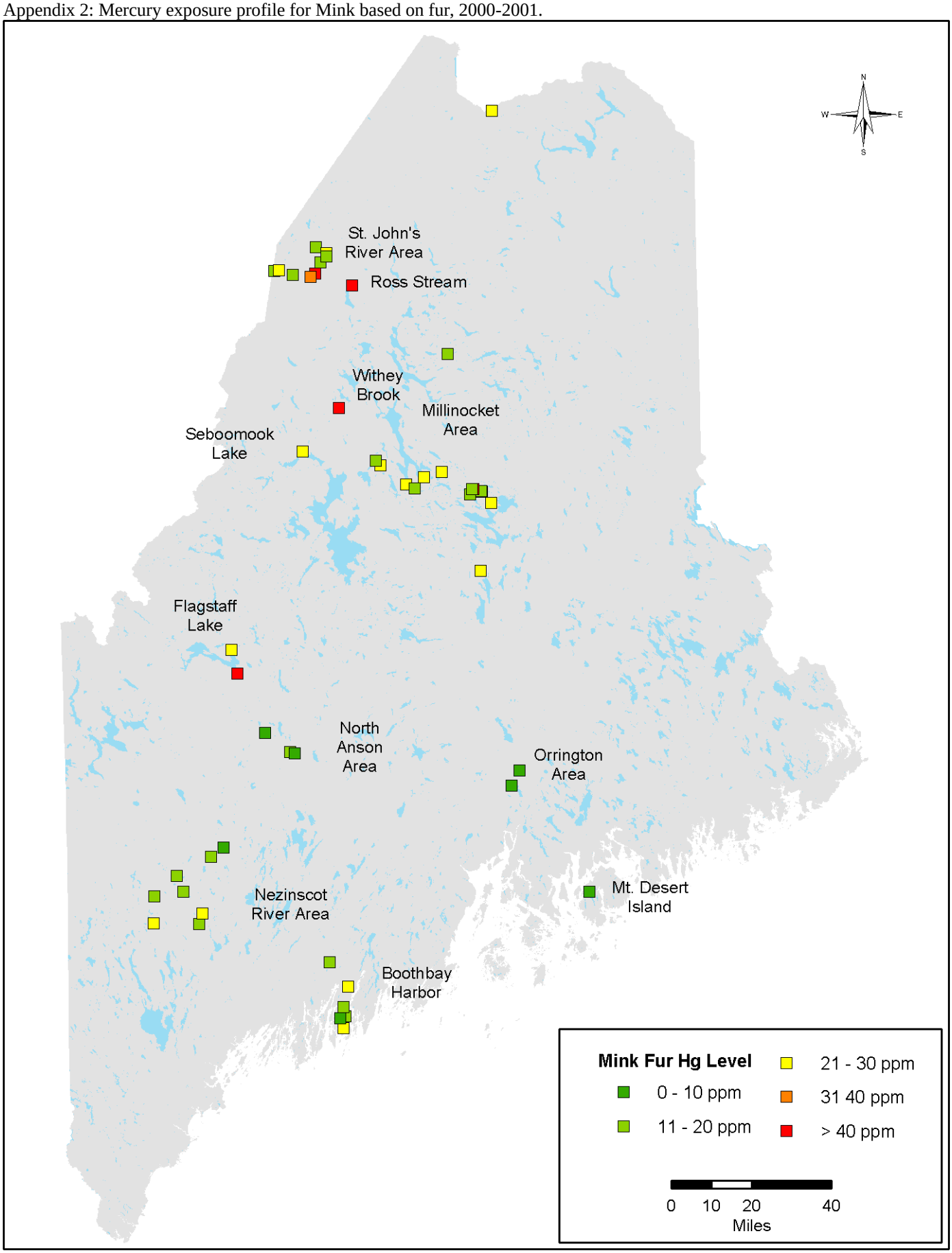
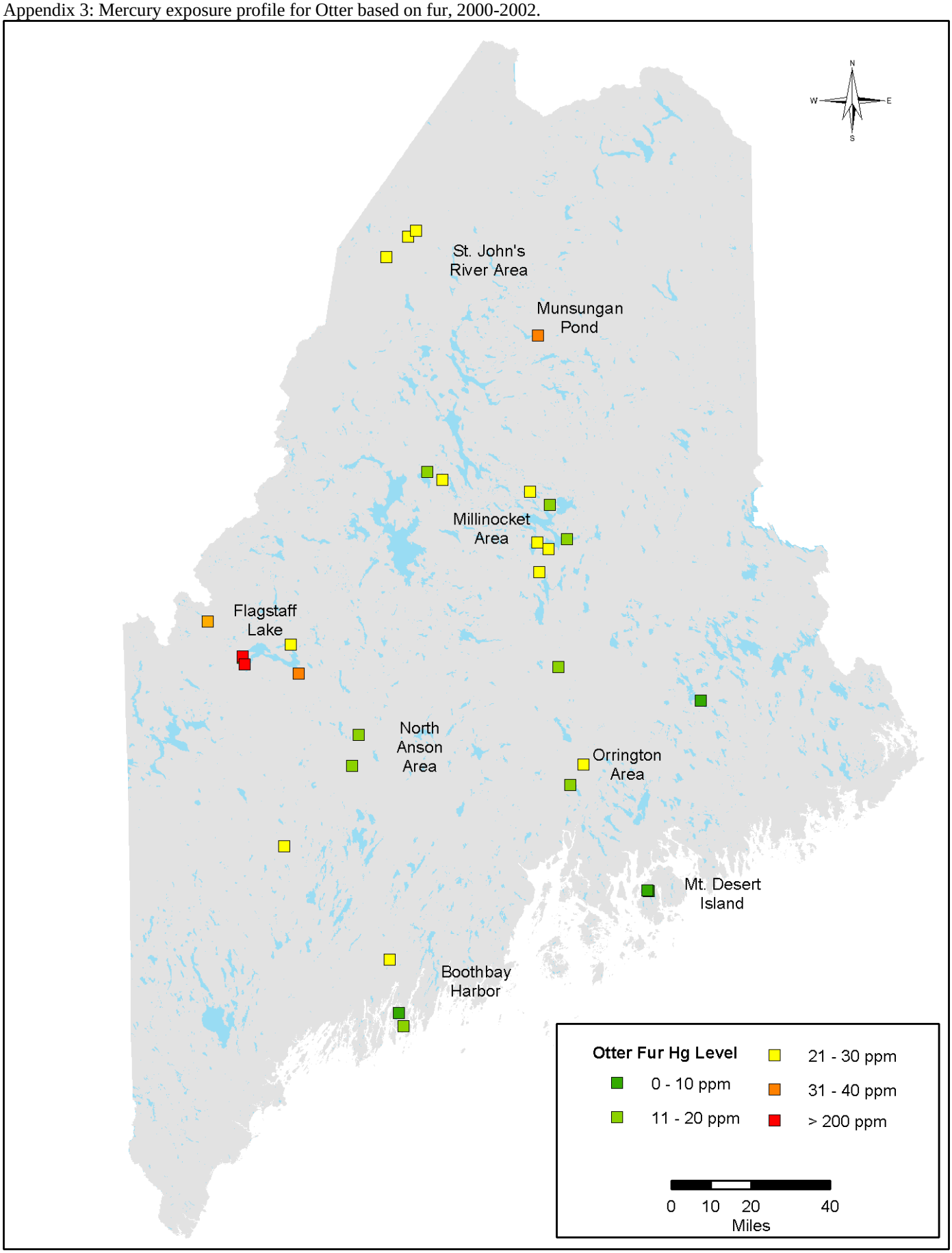
Appendix 1: Mink and River Otter carcass sampling locations, 2000-2002.



Appendix 2: Mercury exposure profile for Mink based on fur, 2000-2001.



Appendix 3: Mercury exposure profile for Otter based on fur, 2000-2002.



Appendix 4: Mink and River Otter live-trapping sites, 2001.