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## Environmental Toxicology

# CORRELATES OF MERCURY IN FEMALE RIVER OTTERS (*LONTRA CANADENSIS*) FROM NOVA SCOTIA, CANADA

SARAH H. SPENCER,†‡ DAVE SHUTLER,*† and MICHAEL S. O'BRIEN‡
†Department of Biology, Acadia University, Wolfville, Nova Scotia, Canada
‡Nova Scotia Department of Natural Resources, Wildlife Division, Kentville, Nova Scotia, Canada

**Abstract**—Mercury (Hg) can reach toxic concentrations in aquatic habitats, sometimes as a consequence of human activity. Mercury can have deleterious effects, particularly in piscivorous mammals in which it bioaccumulates. Furs from trapper-provided female otter (*Lontra canadensis*) carcasses in Nova Scotia were analyzed for total Hg. Concentrations of total Hg in fur samples averaged 25 µg/g dry weight, ranging from 1.4 to 137 µg/g; 20 µg/g is the fur concentration at which toxic effects are expected. Mercury concentrations were greater in otters from watersheds with bedrock substrates known to contain more available Hg, from otters trapped farther inland, and from otters trapped on watersheds with hydroelectric dams. Otter reproductive potential was measured by counting the number of blastocysts in reproductive tracts. Tooth annuli were used to age otters. Reproductive potential was not related to Hg concentration, nor was Hg concentration related to age. In a general linear model, 53% of variation in fur Hg was explained by underlying bedrock, distance from the coast at which otters were trapped, and presence/absence of a hydroelectric dam. The proportion of juveniles in a population did not differ relative to bedrock Hg concentration, but was lower on watersheds with hydroelectric dams. Because we found no evidence of reduced reproductive potential from greater Hg concentrations, the low proportion of juveniles suggests that Hg reduced juvenile survival, although our evidence is circumstantial. Environ. Toxicol. Chem. © 2011 SETAC

**Keywords**—Bedrock    Hydroelectric impoundment    *Lontra canadensis*    Methyl mercury    River otter

## INTRODUCTION

Mercury (Hg) is a heavy metal that occurs naturally in the earth's crust. Inorganic Hg is released by natural processes, including erosion of bedrock, forest fires, and earthquakes, and by anthropogenic processes, including burning of fossil fuels, municipal waste incineration, and flooding to create reservoirs [1]. One of the most toxic forms of Hg, methylmercury (MeHg), is a neurotoxin that can readily transfer across the blood/brain and placental barriers, causing mental impairment, spontaneous abortion, cerebral palsy, and permanent damage to brains, livers, and kidneys [2,3]. Effects of MeHg poisoning in mink (*Neovison vison*) and river otters (*Lontra canadensis*; hereafter, otters) include lethargy, anorexia, ataxia, spinal cord degeneration, limb paralysis, tremors, convulsions, and death [3–5]. Chronic exposures to environmental Hg may affect the dopaminergic and cholinergic systems that facilitate learning and memory, motor function, thermoregulation, and cognition [6]. Reproductive effects of MeHg in mammals range from impaired fetal development and birth defects to fetal death. Impaired reproduction may lead to changes in population age structure [7]. Diets with greater than 1 mg/kg wet weight MeHg can cause neurotoxicity in adult mink and otters and can cause death at higher doses [4,5]. Here we report on concentrations and correlates of Hg in fur of otters from Nova Scotia, Canada.

Mercury concentrations in watersheds are influenced by bedrock geology [8]. Nova Scotia has two major geological zones. The Meguma Terrane encompasses all of southwestern and part of central Nova Scotia (Fig. 1) and contains the Goldenville formation (with thick layers of sandstone that have been metamorphosed to quartzite), and the Halifax formation (with gray to black slate that contains pyrrhotite, a reactive form of pyrite). When pyrrhotite weathers, it releases sulfide into runoff water [9]. Sulfide facilitates conversion of inorganic Hg to more toxic MeHg in lakes, streams, and rivers [10]. The Avalon Terrane encompasses the rest of central and northern Nova Scotia. Bedrock in this terrane is Precambrian, and consists mainly of tightly compacted sedimentary rocks that contain less Hg than the Meguma Terrane [11].

Methylmercury becomes readily available for uptake by microbes in aquatic systems, thus entering food webs [1]. Methylmercury concentration increases in watersheds with dams, probably because stagnation and siltation of water increases anaerobic methylation of elemental Hg [12], and because Hg is flushed less rapidly from watersheds. Hydroelectric dams are most common in southwestern Nova Scotia (http://www.nspower.ca/en/home/aboutnspi/bringingelectricitytoyou/ourgeneratingfacilities.aspx).

River otters become sexually mature at two years of age. Litters of two to three are normal, but as many as six pups may be produced [13]. River otters undergo delayed implantation wherein development of fertilized eggs (blastocysts) remains suspended for approximately 11 months. Thus, females give birth just before remating. Counts of blastocysts provide an index of female reproductive potential [13].

Otters have a maximum life span in the wild of 10 to 15 years. In a given year, 50% of a healthy otter population consists of juvenile animals (0–1 years). Age structure (proportion of juveniles) can be used to assess health of otter populations [13].

We used trapper-provided female carcasses collected from Nova Scotia (Fig. 1), and compared Hg in otters from the Meguma and Avalon Terranes, and from watersheds with and without dams. We also tested if blastocyst numbers were lower in otters with greater Hg, and if Hg changed with otter age.
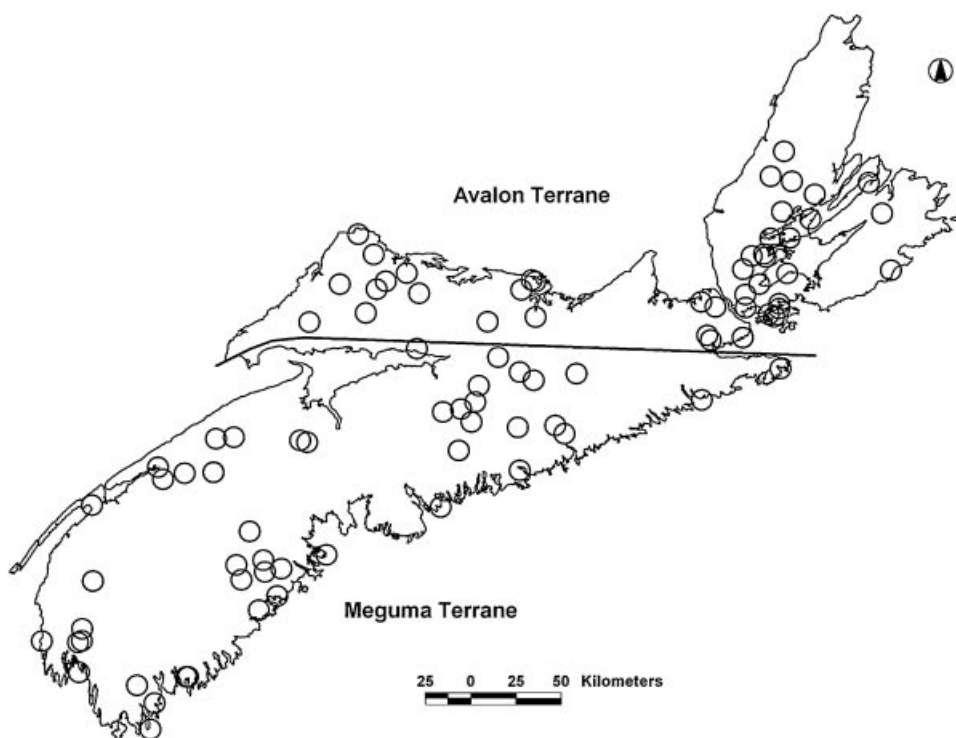
Fig. 1. Sites in Nova Scotia, Canada, where an otter carcass was trapped and collected for analysis.

Finally, we tested to determined if age structure of female populations was associated with correlates of Hg.

## MATERIALS AND METHODS

Female otter carcasses and Universal Transverse Mercator coordinates for trapping sites were collected from trappers in Nova Scotia (Figs. 1, 2) during the 2004/2005 trapping season. We identified watersheds with hydroelectric dams using data from the Nova Scotia Power website (http://www.nspower.ca/en/home/aboutnspi/bringingelectricitytoyou/ourgeneratingfacilities.aspx). Fur samples were removed from feet of carcasses and sent to Trent University (Peterborough, Ontario, Canada)

for total Hg quantification. Methylmercury is strongly correlated with total Hg [14] and highly correlated among tissues [7]. Fur samples were washed prior to analysis. A commercially available AMa254 advanced solid sample Hg analyzer (LECO) was used to determine total Hg in dry weight of otter fur. Samples were weighed in a nickel boat, inserted in a combustion/catalytic tube, and dried at 120 °C in an oxygen environment. Samples were ashed at approximately 800 °C to remove oxides of sulfur and nitrogen and to volatilize Hg, which was then captured on gold-coated silica sand. Sand was then heated to release Hg into an atomic absorption detector. Absorbance was measured as a function of Hg. Quality control was accomplished using a human hair as a reference material ([7];
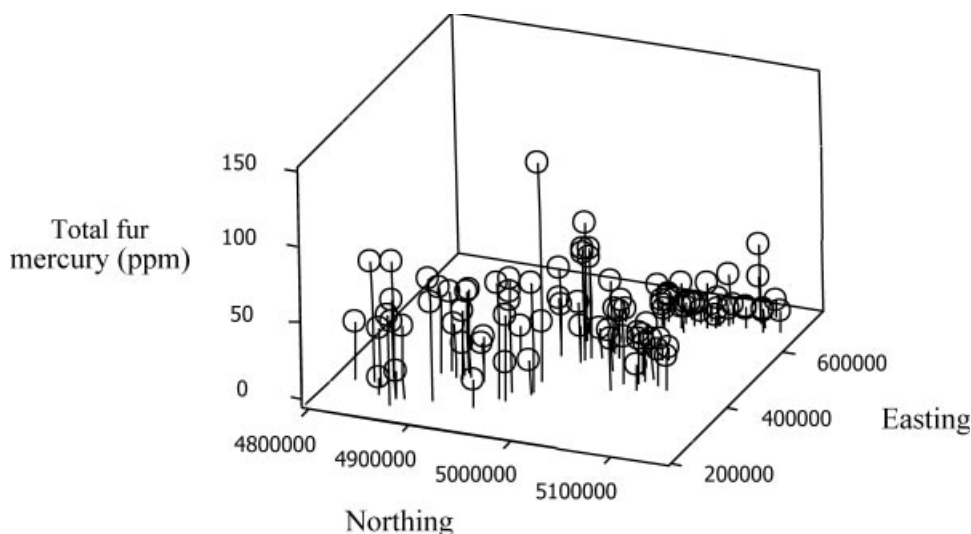


Fig. 2. Spatial variation in total fur Hg of female otter carcasses trapped in Nova Scotia, Canada. Easting is the distance in meters from the western edge of the Universal Transverse Mercator grid zone, and northing is the distance in meters from the equator.

Klenavic K, 2004, Master's thesis, Trent University, Peterborough, Ontario, Canada).

Reproductive tracts of otters were removed, placed in labeled, sealable, plastic canisters with water, and frozen within 2 h. Tracts were thawed at a later date and placed in Petri dishes. Ovaries were removed using scissors and rinsed using a syringe filled with water to ensure that no blastocysts were missed at the junction of the ovary and fallopian tube. Ovaries and runoff that remained in Petri dishes were discarded after counting blastocysts. Each fallopian tube was flushed three times with a water-filled syringe to dislodge blastocysts. Runoff was collected in Petri dishes. Contents of Petri dishes were examined under a compound microscope ($\times$10) and blastocysts were counted [15].

To age otters, two canine teeth were removed from the bottom jaw of 116 female otters; a subset of 63 teeth was sent to Matson's Lab (Milltown, MT). There, teeth were sectioned and cementum annuli counted; each annulus represents one year. The second tooth from each otter was affixed to a sheet of poster board (many teeth to a sheet) and radiographed (x-rayed) at a local veterinary clinic. Radiographs were placed on a light table and digital calipers were used to measure tooth width and pulp width in centimeters. If pulp width was more than 50% of tooth width, the animal was assumed to be juvenile (<1 year old) [13,16,17].

Statistical analyses were conducted in SAS® (SAS Institute). General linear models (GLMs) were used to test for differences in Hg between the two principal geological areas (Meguma Terrane and Avalon Terrane, Nova Scotia, Canada), and between watersheds with and without a dam. General Linear Models were used to test for a relationship between Hg and distance from the coast, number of blastocysts, and age. A GLM was used to test simultaneously for Hg relationships with terrane, damming, and age. Contingency table analyses were used to compare age structures of otter populations trapped on the Avalon and Meguma Terranes, and between otter populations trapped on undammed versus dammed watersheds. Means are reported $\pm$ standard deviation (SD) and all our Hg values are reported in $\mu$g/g dry weight.

## RESULTS

Sample sizes vary among analyses because not all data were collected on all female carcasses (Table 1). Of 116 carcasses that were collected, 51 came from the Meguma and 53 from the Avalon Terrane, with no data available for 12 carcasses. Similarly, 77 came from undammed and 31 came from dammed watersheds, with no data available for eight carcasses. Means and ranges for continuous variables are reported in Table 1. Mercury concentrations were not normally distributed

Table 1. Descriptors of variables in analyses

| Variable | $n$ | Minimum | Maximum | Mean |
|---|---|---|---|---|
| Easting | 98 | 243,480 | 715,417 | 489,692 |
| Northing | 98 | 4,814,684 | 5,137,016 | 5,001,243 |
| Distance from the coast (km) | 98 | 0.0 | 74.2 | 13.0 |
| Blastocysts (adult females only) | 20 | 0 | 4 | 0.3 |
| Age | 63 | <1 | 9 | 1.6 |
| Total mercury (ppm) | 108 | 1.4 | 137.0 | 25.0 |

Easting is the distance in meters from the western edge of the Universal Transverse Mercator grid zone and northing is the distance in meters from the equator.
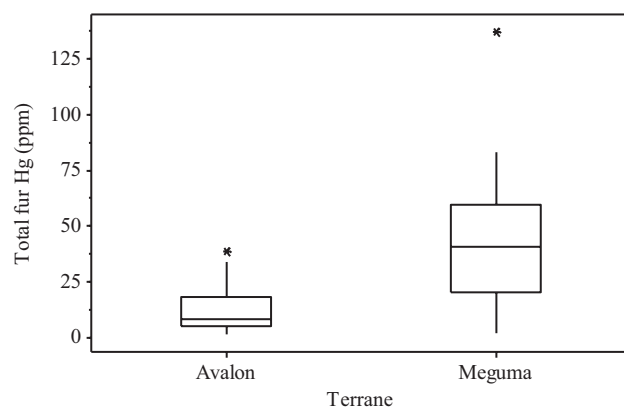


Fig. 3. Total fur Hg of otters was significantly greater in watersheds with bedrock of the Meguma Terrane than the Avalon Terrane, Canada. The top box is the 3rd quartile. The bottom box is the first quartile. Whiskers indicate the range in values, excluding outliers. Line within box indicates median; *an outlier.

(Shapiro–Wilk, $W = 0.83$, $p < 0.0001$), so analyses were performed on log-transformed data; means and SDs we report are not transformed.

Mercury concentrations were greater in otters from the Meguma ($41.3 \pm 27.0$) than the Avalon Terrane ($11.6 \pm 8.6$; Fig. 3; $R^2 = 0.40$, $F_{1,102} = 64.8$, $p < 0.0001$). A visual examination of Figure 2 suggested two other patterns for which we tested. The first was that otters farther from the coast had greater Hg concentrations; this pattern was supported (Fig. 4; $R^2 = 0.23$, $F_{1,102} = 14.6$, $p < 0.0001$). Distance from the coast was not a significant predictor of Hg concentrations within the Meguma Terrane ($R^2 = 0.07$, $F_{1,51} = 3.7$, $p = 0.06$), but was within the Avalon Terrane ($R^2 = 0.23$, $F_{1,49} = 14.6$, $p = 0.0004$). The second pattern was that otters from western Nova Scotia had greater Hg concentrations than those from eastern Nova Scotia; this was also supported (Fig. 5; $R^2 = 0.40$, $F_{1,96} = 63.8$, $p < 0.0001$). The east–west pattern was not significant within the Meguma Terrane ($R^2 = 0.04$, $F_{1,46} = 2.1$, $p = 0.15$), but was within the Avalon Terrane ($R^2 = 0.16$, $F_{1,48} = 9.1$, $p = 0.004$). Terrane and latitude were partially confounded (Fig. 1), so we only tested for Hg-latitude associations within terrane, and found neither significant (Meguma $R^2 < 0.01$, $F_{1,46} = 0.2$, $p = 0.67$; Avalon $R^2 = 0.02$, $F_{1,48} = 1.1$, $p = 0.29$). Mercury concentrations in otters from watersheds with a dam ($50.8 \pm 28.3$) were significantly greater compared to those from watersheds without a dam ($16.6 \pm 14.5$; Fig. 6;
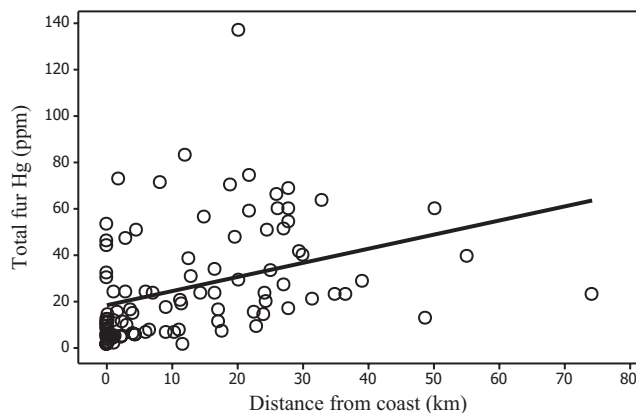


Fig. 4. Mercury concentrations were greater in otters collected farther from the coast of Nova Scotia, Canada.
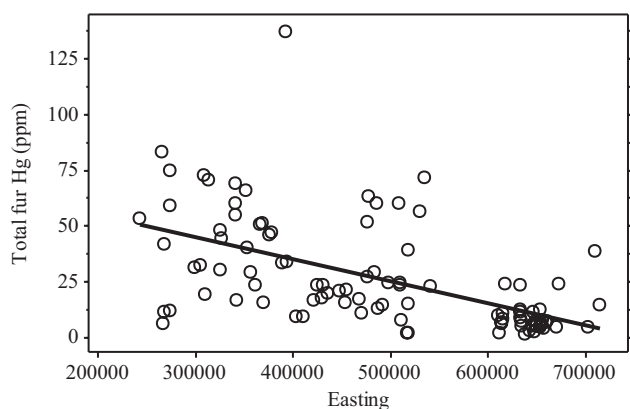
Fig. 5. Total fur Hg was significantly greater in western versus eastern Nova Scotia. Easting is the distance in meters east of the edge of the Universal Transverse Mercator grid zone.

$R^2 = 0.34$, $F_{1,106} = 51.3$, $p < 0.0001$). This relationship held within the Meguma Terrane ($R^2 = 0.15$, $F_{1,51} = 8.0$, $p = 0.007$) but could not be tested within the Avalon Terrane because no otters trapped there came from dammed watersheds. Mercury concentrations were greater in 11 otters from undammed watersheds of the Meguma ($27.8 \pm 17.2$) than it was from 50 otters from undammed watersheds of the Avalon ($11.6 \pm 8.7$; $R^2 = 0.27$, $F_{1,66} = 26.3$, $p < 0.001$), indicating that damming was not entirely responsible for the pattern in Figure 3.

There was no significant relationship between Hg concentration and number of blastocysts in adult ($\geq 2$ years old) female otter reproductive tracts ($n = 19$, $R^2 < 0.01$, $F_{1,17} < 0.1$, $p = 0.80$). Mercury concentrations did not increase significantly with age ($R^2 = 0.03$, $F_{1,61} = 1.9$, $p = 0.17$).

In a GLM with terrane, longitude, distance from the coast, damming, and age as explanatory variables entered simultaneously, we sequentially removed nonsignificant variables with the highest $p$ values until only significant associations remained [18]. Only terrane (Avalon least square [LS] mean = 25.5, Meguma LS mean Hg = 36.6; $F_{1,93} = 8.4$, $p = 0.005$), distance from coast ($F_{1,93} = 21.5$, $p < 0.0001$), and damming (LS mean undammed = 18.8, LS mean dammed = 14.1; $F_{1,93} = 14.1$, $p = 0.0003$) were retained (overall model $R^2 = 0.55$, $F_{3,93} = 37.3$, $p < 0.001$). However, this model was significantly unbalanced because of the absence of dammed watersheds in the Avalon Terrane. Following the same sequential variable
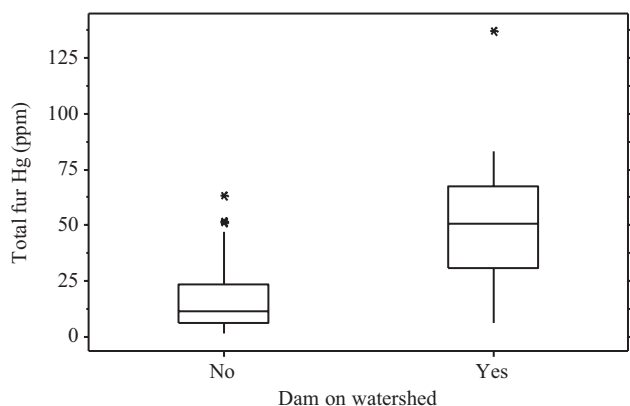


Fig. 6. Total fur Hg of otters was significantly greater in dammed versus undammed watersheds. The top box is the 3rd quartile. The bottom box is the first quartile. Whiskers indicate the range in values, excluding outliers. Line within box indicates median; *an outlier.

removal procedure described above and applied to the Meguma Terrane data, both damming ($F_{1,44} = 12.8$, $p = 0.001$) and distance from the coast ($F_{1,44} = 7.5$, $p = 0.009$) were significantly associated with Hg ($R^2 = 0.27$, $F_{2,44} = 8.3$, $p = 0.001$).

Of carcasses from the Avalon Terrane, and using teeth that were aged by pulp width, 24 were juvenile and 27 were adult, and for the Meguma Terrane where Hg in carcasses was greater, numbers were 18 juvenile and 35 adult females, but this difference in population age structure was not significant ($\chi^2_1 = 1.9$, $p = 0.17$). From undammed wetlands, we had 38 juvenile and 39 adult, and from dammed watersheds 6 juvenile and 25 adult females; this difference in population age structure was significant ($\chi^2_1 = 8.2$, $p = 0.004$).

## DISCUSSION

Bedrock, industrialism, and impoundments all contribute to variation in Hg concentrations among locations [19–21]. Beginning with bedrock, MeHg concentrations in wetlands of Kejimkujik National Park in south-central Nova Scotia were strongly dependent on lithology of underlying bedrock [8]. Bedrock in the Avalon Terrane of eastern Nova Scotia is composed of tightly compacted sedimentary rocks that contain little Hg; therefore, little Hg enters overlying lakes from geological sources [11]. Bedrock underlying lakes in the Meguma Terrane of southwestern Nova Scotia is largely granite (South Mountain batholith), shale, and sandstone. Mercury is present in each of these bedrocks, and can be leached into overlying lakes during weathering [21]. Therefore, as expected, elevated concentrations of Hg in fur of otters from southwestern Nova Scotia can, in part, be attributed to underlying bedrock.

With respect to atmospheric deposition, western Nova Scotia is closer than eastern Nova Scotia to large industrial centers, and in particular coal-fired electric utilities, located along the northeastern coast of North America. In 2005 there was a province-wide advisory for Hg in rainfall, with southwest Nova Scotia having the highest total annual average Hg concentration (National Atmospheric Deposition Program 2005; http://energy.er.usgs.gov/health_environment/mercury/mercury_network.html). Possibly, Hg deposition from this activity also partly explains greater Hg in female otters from this part of the province. However, there is presently insufficient information on geographic variation in atmospheric inputs in Nova Scotia [22], and experimental data [23,24] on how Hg arrives and moves through aquatic and terrestrial ecosystems are needed.

With respect to impoundments, Hg was at significantly higher concentrations in otters from dammed watersheds, consistent with other studies on fish [25–28]. Similarly, in Quebec, benthic insects had elevated MeHg concentrations 14 years after the LG-2 reservoir was created [28], and in Manitoba, Quebec, and Labrador, MeHg was greater in fish from bodies of water with hydroelectric dams [25–27].

Three explanatory variables (terrane, distance from the coast, and damming) explained 53% of the variation in Hg among otter carcasses in the present study. Numerous additional sources of variation existed that we could not evaluate, such as where otters traveled before being trapped and variation in the species and sizes of fish consumed. Thus, it may be surprising that we were able to detect significant associations and explain so much variation in Hg.

A previous study [14] also detected greater Hg in otters trapped further from the coast. Possibly, Hg that reaches high concentrations in terrestrial waters becomes diluted by oceanic

bodies of water, and if otters close to the coast obtain some of their fish from marine habitats, they may be exposed to less Hg. However, the relationship is not straightforward; many otters trapped close to the coast had high Hg concentrations. Because otters can have large home ranges (Spencer SH, 2006, Master's thesis, Acadia University, Wolfville, Nova Scotia, Canada), trapping locations only capture a small part of variation in Hg exposure of individual otters. However, the strong relationships we observed between explanatory variables and Hg, despite this variation, bolsters the strength of our results.

There was no relationship between Hg concentrations and the number of blastocysts in female otter reproductive tracts. Similarly, Yates et al. [29] found no correlation between Hg concentrations and corpora lutea scars in wild otters (each scar represents a past birth). In contrast, based on Hg concentration in hair samples, Hg had a negative effect on human reproduction [30]. Similarly, female mink exposed to dietary concentrations of $1.0\,\mu g/g$ Hg gave birth less often than those exposed to $0.5\,\mu g/g$ and $0.1\,\mu g/g$ [31], although this species does not appear to use selenium to sequester Hg from the body, as appears to be the case for otters [32]. Five $\mu g/g$ MeHg in the brain is used as a toxicity criterion for mink, with lowest observable adverse effects occurring at $1\,\mu g/g$ dietary Hg. Fur Hg concentrations are an order of magnitude higher than brain, liver, and kidney for both total Hg and MeHg [4,5,33]. The sample size of breeding females in the present study may not be large enough to rigorously test for consequences on reproduction, given all the other correlates involved. Moreover, blastocyst counts work best on fresh, rather than frozen, carcasses. Additional factors that may decouple fur Hg and reproductive effects include demethylation and shunting to fur. Several studies have suggested that MeHg may be demethylated (and thus detoxified) in the liver of waterbirds, and although this may occur in otters, the mechanisms are not well understood [7,14,32,33].

We found no relationship between Hg concentration and age, consistent with some studies [15,34–36], but not with Evans et al. [37], who found greater Hg concentrations in younger animals. This is consistent with females mobilizing Hg to embryos and milk. However, Mierle et al. [38] found Hg concentrations increased with otter age during the first two years, stabilized over the next several, and decreased again in older animals, the latter pattern consistent with reduced survivorship in otters with high Hg concentrations. The broad variation in results indicates that more work is needed to understand their causes.

In the present study, otters ranged in age from <1 to 9 years. However, 42 of 63 (67%) otters were juveniles ($\leq 1$ year). Similarly, both Evans et al. [37] and Mierle et al. [38] reported significantly more juveniles than adults in their populations. Expected age distribution in a healthy population is approximately 50% juveniles. Our analyses suggested that hydroelectric dams were associated with a lower proportion of juvenile otters in populations. Because we found no evidence that Hg was associated with fewer blastocysts, we have no evidence of reproductive consequences, although our sample of 19 has limited power to detect this. The low proportion of juvenile otters in dammed watersheds could indicate that juvenile otters are dying (directly or indirectly) from Hg before or shortly after they reach adulthood. This is consistent with our finding that Nova Scotia otters averaged more than the 20-$\mu g/g$-Hg concentrations at which toxic effects are expected [38]. However, damming causes significant ecological changes to watersheds; juvenile otters may be relegated to natural watersheds without large impoundments and thus fewer fish. Thus, our evidence of juvenile otters succumbing to Hg is circumstantial; more research is needed on survival of wild otters relative to mercury exposure because there are additional data for otters with Hg concentrations above toxic levels [7,39]. In addition, further research is needed to pinpoint major contributors to increased Hg in Nova Scotia otters; ultimately, this will help identify ways to mitigate effects.

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