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Mercury concentrations in deer mouse (*Peromyscus maniculatus*) tissues from Isle Royale National Park

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"Capsule": *Mercury from an unknown source was detected in deer mice from Isle Royale National Park, a protected area in Lake Superior.*

Abstract

We used deer mice (*Peromyscus maniculatus*) to investigate variation in mercury exposure across the terrestrial ecosystem of Isle Royale National Park (Michigan, USA). Although previous work suggested that mercury (Hg) levels may be higher inside the Sargent Lake watershed of Isle Royale than outside the watershed, Hg concentrations in livers were higher outside the Sargent Lake watershed (100.13 ng Hg/g dry tissue) than inside the watershed (35.50 ng Hg/g dry tissue; $P=0.06$). Mercury levels in kidneys did not differ significantly ($P=0.57$) between samples collected outside (443.23 ng Hg/g dry tissue) and inside (360.62 ng Hg/g dry tissue) the Sargent Lake watershed. Mean Hg concentrations in the livers of mice at some sites in Isle Royale are not significantly lower ($P=0.62$) than Hg concentrations considered by some government agencies to be unhealthy for human consumption. Although Hg concentrations in mouse tissues were not remarkably high (compared to heavily polluted sites), concern is warranted because: (1) Isle Royale National park is a protected area in a remote location; (2) any exposure in deer mice represents a path for biomagnification in the terrestrial food web; and (3) the source of this mercury remains unidentified.

Keywords: *Peromyscus*; Mercury; Isle Royale; Biomagnification; Terrestrial bioaccumulation

1. Introduction

Sediment cores from Michigan and Wisconsin suggest that mercury (Hg) levels in the Lake Superior region are currently 3–4 times greater than they were in 1850 (Swain et al., 1992). Awareness of elevated levels of Hg in biotic and abiotic components of the ecosystem has increased over the past decade. For example, the USA and Canada have posted fish consumption advisories because of elevated Hg (United States Geological Survey, 1996). Hg is also one of nine environmental toxins of concern addressed by the United States–Canadian 'Binational Program to Restore and Protect the Lake Superior Basin'. Furthermore, the US EPA (1996) 'Conference on Mercury in the Midwest' called for monitoring the biota to identify areas with elevated Hg levels.

This increased awareness and concern over Hg motivated an investigation of Hg levels on Isle Royale National Park (Michigan, USA), a wilderness island in Lake Superior (Fig. 1A). Because of its remote location and its status as a national park and a Biosphere Reserve (recognized by the United Nations Educational, Scientific, and Cultural Organization [UNESCO]), Isle Royale has been expected to serve as a baseline ecosystem which has been only minimally affected by human sources of pollution (United States Department of Interior, 1998, see also Sinclair 1998). However, preliminary investigations from inland lakes of Isle Royale revealed that Hg concentrations in northern pike (*Esox lucius*) from some lakes exceed the Michigan consumption advisory of 0.50 ppm (Kallemeyn, unpublished data; Michigan Department of Natural Resources, 1994, 1997). Moreover, not only has Hg been found in feathers and blood of common loons (*Gavia immer*) from Isle Royale (Evers et al., 1998), but blood Hg concentrations in loon chicks from inland lakes are higher than in chicks from Lake Superior waters

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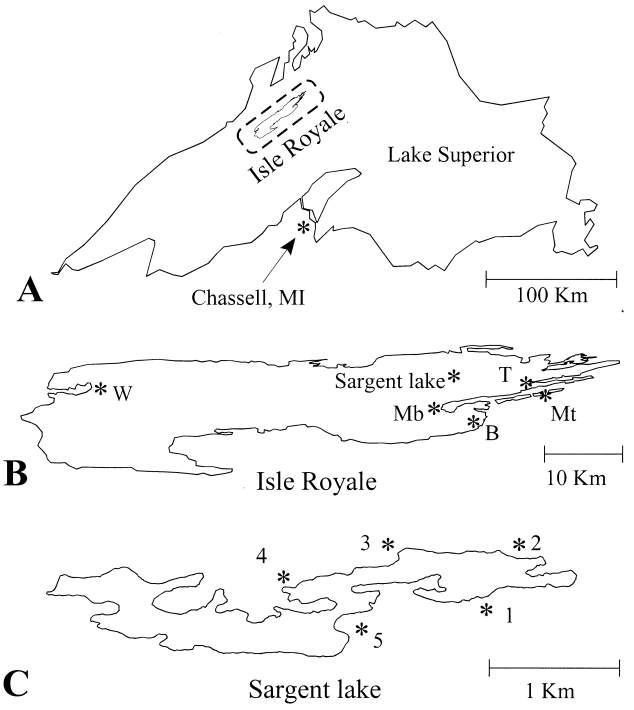

Fig. 1. Locations of deer mouse collection sites. Panel B shows the locations of Sargent Lake (SL) on Isle Royale and five sample sites outside the SL watershed: W, Windigo; Mb, Moskey Basin; B, Bangsund; T, Three Mile; and Mt, Mott Island. Panel C shows the locations of five sample sites inside the Sargent Lake watershed.

surrounding Isle Royale (Kaplan and Tischler, 2000). Hg concentrations in the teeth of moose (*Alces alces*) from Isle Royale have also increased from the 1940s to the 1970s (Eide and Peterson, 1997). These findings justify further investigation of Hg in the Isle Royale National Park ecosystem. Following this justification, the National Park Service identified the watershed of Sargent Lake (lake area = 143 ha, watershed area = 1035 ha), located near the northeast end of Isle Royale, to be the focus of detailed Hg studies (Fig. 1). The Sargent Lake (SL) area was selected as a focal point because preliminary studies indicated that northern pike in SL (and five other adjacent lakes) had Hg concentrations that were 50–500% higher than northern pike from most other sampled lakes in Isle Royale (Kallemeyn, unpublished data; Michigan Department of Natural Resources, 1994, 1997).

Because most Hg research focuses on aquatic systems, less is known about Hg in terrestrial systems. However, small mammals are known to accumulate heavy metals (Jeffries and French, 1976; Anthony and Kozlowski, 1982; Smith and Rongstad, 1982; Talmage and Walton, 1990). More specifically, deer mice (*Peromyscus maniculatus*) should be important bio-indicators of heavy metal pollution in the terrestrial ecosystem of Isle Royale for several reasons. First, deer mice are predators of insects and other invertebrates, so they are likely to bioaccumulate environmental toxins more rapidly than terrestrial herbivores. Also, deer mice have relatively short life spans (usually < 2 years in the wild) and small home ranges (typically 0.2–2.5 ha), thus providing fine-scale temporal and spatial resolution of the distribution of heavy metals in terrestrial environments. Finally, because deer mice are the only small mammal (i.e. < 150 g) inhabiting Isle Royale, they are a primary prey item for foxes, owls, and weasels. Thus, deer mice on Isle Royale should be an important link in the transport of heavy metals through the terrestrial food chain. Here, we report the Hg concentrations in tissues of adult deer mice (*P. maniculatus*) from Isle Royale National Park.

2. Materials and methods

2.1. Sample collection

We began by conducting a pilot study including four trial mice (two from the SL area on Isle Royale and two from the utensil drawer of our [LMV & JAV] home in Chassell, MI, USA [Fig. 1]) to confirm which tissues are most sensitive to Hg accumulation. Then, to address the hypothesis that the SL watershed contains high Hg concentrations (relative to other portions of Isle Royale), we live-trapped three adult deer mice from each of five locations outside (Fig. 1B) and inside (Fig. 1C) the SL watershed. Mice were sacrificed by cervical dislocation and frozen immediately for storage. Animal handling was conducted in accordance with methods approved by the Michigan Technological University Institutional Animal Care and Use Committee.

In the lab, samples were thawed for processing. All lab equipment (glass, Teflon, or stainless steel) was washed with at least 10% nitric acid and triple rinsed with distilled, deionized water. We recorded the sex, full body weight, liver weight and kidney weight of each specimen. Each tissue type was homogenized and refrozen for storage in separate Teflon vials until analysis for total Hg. Because the carcasses of the trial mice had very low Hg levels and because kidneys and livers have been used as representative organs in previous studies (Jeffries and French, 1976; Bull et al., 1977; Talmage and Walton, 1990), we limit our focus to Hg concentrations of livers and kidneys for the remainder of this study. We collected tissues from a total of 30 mice on Isle Royale for Hg analysis. Three mice were collected from each of five sites inside and five sites outside the SL watershed (Fig. 1). Tissue samples from each site were pooled and analyzed as a single composite sample. Although this pooling precludes our ability to examine intrasite variability, this design permits the analysis of intersite variability, which is the focus of our analysis. Livers and kidneys were analyzed separately. A subset of each composite was weighed wet to determine wet

concentrations, and then dried at 40°C for 48 h to determine dry concentrations.

2.2. Sample digestion

Digestion was performed in two batches: (1) the trial samples collected from Chassell and SL; and (2) study samples from inside and outside the SL watershed. For Hg determinations, a known weight of homogenized sample was placed into a clean Teflon bomb with 7 ml of a nitric and sulfuric trace metal grade acid mixture (5:2 HNO_3: H_2SO_4; Cleckner et al., 1998). Bombs were wrench tightened, placed into a 900 W microwave oven, and heated for two cycles (2 min each) at 30% power. Each bomb was then vented to release acid fumes. Approximately 23 ml of Milli-Q (Millipore) water (volume recorded to nearest 0.1 ml) and 1 ml of BrCl were added. Each bomb was wrench-tightened and placed in a 60°C oven overnight. Before digestion, 20% of the homogenized samples were split into replicates. The average difference between the replicated samples was 17%. Each batch included at least three blanks (bombs containing reagents but no sample) to determine detection limits and monitor contamination. Each batch also included three TORT-2 standard reference samples (lobster hepatopancreas, from the National Research Council of Canada) to quantify Hg recovery. Estimated mercury concentrations (mean±S.E.) from TORT-2 reference samples for the two digestion batches were 293.4±14.4 ($n=3$) and 293.0±8.9 ($n=3$) ng/g. Both values are within the certified value of 270±60 ng/g for TORT-2.

2.3. Mercury analysis

Mercury analysis was performed using established protocols for cold vapor atomic fluorescence spectroscopy techniques (Gill and Fitzgerald, 1987; US EPA, 1996; Olson et al., 1997; Cleckner et al., 1998). Briefly, standards and blanks were run on analysis day to establish a standard curve and to ensure adequate detection of expected sample concentrations. NH_2OH was added to each sample bomb to neutralize the BrCl. A known aliquot of sample was pipetted into a bubbler with 0.5 ml of $SnCl_2$. Gold traps were placed on the bubblers and the aliquots were purged for 20 min with N_2. Each sample was analyzed twice, and the average value of each duplicate sample was used for our statistical analysis (no duplicate measurement differed by more than 10%). Check standards and blanks were run for every six samples and following analysis.

2.4. Statistical analysis

We based our statistical analyses on nanograms of Hg per gram of *dry* tissue to avoid confounded results due to differences in moisture content of the tissues. However, we also report nanograms of Hg per gram of *wet* tissue to facilitate comparison with previous studies. We used a two-sample *t*-test assuming unequal variance to compare mean Hg concentrations in livers versus kidneys and to compare mean Hg concentrations inside versus outside the SL watershed. Because inferences about Hg concentrations inside and outside the SL watershed could be confounded by differences in the age or size of *P. maniculatus* collected from the various sites, we used a two-sample *t*-test to compare mean weights of body, kidney, and liver inside and outside the SL watershed. To further understand the potential for body weight to confound our inferences, we also calculated the Pearson correlation coefficient between mean body weight of mice represented by each composite sample and Hg concentrations in livers or kidneys. To understand the potential for variation in sex ratio to confound our inferences, we compared the proportion of males collected inside and outside the SL watershed using a chi-squared test. Finally, the relationship between Hg concentrations in livers and kidneys was examined using Pearson correlation. All statistical analyses were performed using Microsoft Excel.

3. Results

In the trial samples, Hg concentration was greatest in the kidneys, intermediate in the livers, and least in the carcasses. Specifically, for the SL trial samples the dry carcass Hg concentration was 39.23, the dry liver Hg concentration was 52.94, and dry kidney Hg concentration was 372.41 (see also Table 1).

In the Isle Royale study specimens, kidneys also had significantly higher Hg concentrations than livers both inside ($t=-3.81$, $P<0.01$) and outside ($t=-2.97$, $P=0.02$) the SL watershed (Table 1). Sites outside the SL watershed had higher ($t=-1.90$, $P=0.06$) liver Hg concentrations than sites inside the SL watershed (Table 1). However, sites inside and outside the SL watershed did not differ ($t=-0.59$, $P=0.57$) for Hg concentrations of kidneys (Table 1). Total Hg in liver and kidney tissue combined did not differ ($t=-0.87$, $P=0.42$) inside and outside the SL watershed.

The proportion of males in the samples from inside (6/15) and outside (7/15) the SL watershed was not significantly different ($\chi^2=0.54$, $P>0.25$). Thus, variation in Hg concentrations between samples is unlikely biased by an uneven sex ratio. Based on the mean weights of the three mice represented in each of the 10 composite samples, the mean (±S.E.) weight of mice from inside the SL watershed (17.03±0.41 g) did not differ significantly ($t=-1.50$, $P=0.17$) from that outside the watershed (18.01±0.50 g). However, because $P=0.17$ is not a convincing failure to reject the null hypothesis, we

Table 1
Mercury concentrations in the tissues of *Peromyscus maniculatus* from Isle Royale, MI, USA (ng Hg/g tissue ± S.E.)[a]

Location	Wet liver	Wet kidney	Dry liver	Dry kidney
Inside Sargent Lake watershed	10.99 (±2.36)	102.55 (±25.73)	35.50 (±7.05)	360.62 (±85.06)
Outside Sargent Lake watershed	29.98 (±9.81)	125.56 (±31.34)	100.13 (±33.23)	443.23 (±110.78)
Trial mice from mainland	21.41	155.13	58.83	294.75

[a] See Fig. 1 for map of collection sites.

investigated correlations between mean body weight and Hg concentration of each composite sample. These calculations indicate that body weight is not significantly associated with Hg concentration in livers ($r = 0.014$, $P = 0.97$) or kidneys ($r = 0.18$, $P = 0.62$).

We found a weaker correlation between kidney and liver Hg inside ($r = 0.786$, $P = 0.12$) than outside ($r = 0.998$, $P < 0.01$) the SL watershed (Fig. 2). In addition, the relationship between kidney and liver Hg had a significantly steeper slope outside than inside the SL watershed ($P < 0.05$). Alternatively, these patterns could be interpreted in terms of differences in levels of variation. That is, variation in the Hg concentration of kidneys inside (coefficient of variation, CV = 0.53) and outside (CV = 0.56) the SL watershed were similar. In contrast, variation in Hg concentration of livers inside the SL watershed (CV = 0.44) was about 50% less than outside the SL watershed (CV = 0.74). These patterns are unlikely to be spuriously associated with systematic variation in organ weights, because neither mean kidney weights nor mean liver weights differed with respect to location (i.e. inside vs. outside the SL watershed; $t = 0.93$, $P = 0.66$ for kidneys; $t = 0.29$, $P = 0.78$ for livers).

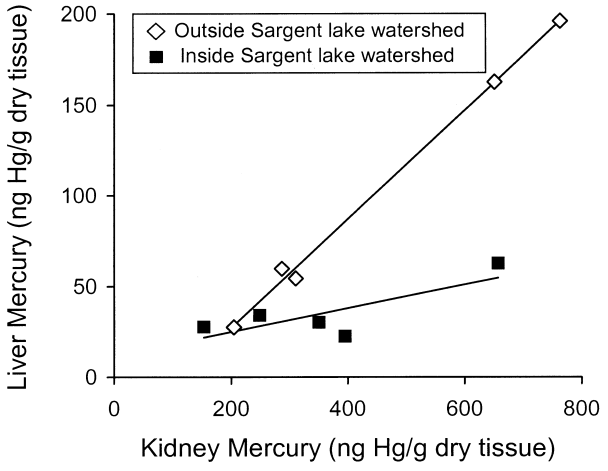

Fig. 2. The relationship between mercury concentrations in liver tissues and kidney tissues for samples inside (filled symbols) and outside (open symbols) the Sargent Lake watershed. Trend lines are provided to facilitate comparison.

4. Discussion

Consistent with the results of previous studies (Jeffries and French, 1976; Bull et al., 1977; Talmage and Walton, 1990), Hg levels throughout Isle Royale were higher in kidney than in liver tissue. However, contrary to expectation, neither mean Hg concentration in kidneys nor mean Hg concentration in livers were higher inside than outside the SL watershed. Moreover, we have no certain explanation for why the relationship between kidney and liver Hg is characterized by a much steeper slope for mice outside the watershed compared with mice inside the watershed. Because various tissues within an animal's body respond differently to different forms of mercury (e.g. elemental Hg, methyl-Hg; Ulfvarson, 1970), these differences may be associated with variation in levels of various forms of Hg across the Isle Royale landscape. However, additional consideration and research are required to fully understand these patterns. Mean (±S.E.) dry weight Hg concentrations of Isle Royale kidneys (361±85 ppb inside and 443±110 ppb outside the SL watershed; 1 ppb = 1 ng/g) were higher than that reported for laboratory raised control *Peromyscus* (200 ppb; Talmage and Walton, 1990; Table 2). Given that Isle Royale National Park is a remote wilderness and a UNESCO Biosphere Reserve, it is disturbing that mean Hg concentrations in the livers of mice outside the SL watershed are not significantly lower ($P = 0.62$) than Hg concentrations considered unfit for human consumption (i.e. 0.5 ppm; United States Geological Survey, 1962). The highest mercury concentration on Isle Royale was found in mice from the Windigo site (Fig. 1). The most obvious difference between these mice and others in our sample is that the Windigo mice were trapped in and around a permanent human settlement. Perhaps, by means of an undetermined mechanism, this difference is associated with the high Hg concentrations.

New world mice of the genus *Peromyscus* are regarded as ecological equivalents to old world mice of the genus *Apodemus* (Montgomery, 1989). The mean wet weight Hg concentrations in livers of *Apodemus* found near a polluted chlor-alkali site in Great Britain and at an associated control site were higher than Hg concentrations in Isle Royale *Peromyscus* livers inside

Table 2
Comparisons of mercury concentrations (ng Hg/g tissue ± S.E.) between Isle Royale, MI, USA and other related studies[a]

Species	Location	Wet liver	Wet kidney	Dry kidney
Peromyscus maniculatus	Isle Royale, inside SL watershed[b]	11 (± 2)	103 (± 26)	361 (± 85)
	Isle Royale, outside SL watershed[b]	30 (± 9.8)	126 (± 31)	443 (± 110)
	Mainland (Michigan, USA)[b]	21.41 (± 3.66)	155.13	294.75
	Lab-raised control[c]			200
Apodemus sylvaticus	Chlor-alkali site[d]	230 (± 70)	520 (± 16)	
	Control site[d]	40 (± 10)	120 (± 20)	
	Hg-treated wheat field[e]	2220 (± 1070)	3300 (± 1840)	

[a] See Fig. 1 for Isle Royale collection sites.
[b] This study.
[c] Talmage and Walton (1990).
[d] Bull et al. (1977).
[e] Jeffries and French (1976).

and outside the SL watershed (Bull et al., 1977; Table 2). The mean wet weight Hg concentration in kidneys of *Apodemus* found near the same chlor-alkali site (Bull et al., 1977) was also higher than the mean wet weight Hg concentration in Isle Royale *Peromyscus* kidneys inside and outside the SL watershed (Table 2). However, the mean wet weight Hg concentration in Isle Royale *Peromyscus* kidneys was similar to that of *Apodemus* kidneys from the associated control site (Bull et al., 1977; Table 2). Compared with *Apodemus* trapped in a wheat field near Monks Wood Experimental Station in Southern England 2 months after the field was planted with wheat seeds coated in Hg-containing compounds (Jeffries and French, 1976), the mean wet weight Hg concentrations of Isle Royale tissues were much lower (Table 2).

Evidence suggests that total Hg concentrations tend to be greater for organisms positioned at higher trophic levels. For example, near a smelter in northern Sweden, Hg concentrations in Tengmalm's owl (*Aegolius funereus*) nestlings (43–48 ppb) equaled or exceeded that of their primary prey, the bank vole (*Clethrionomys glareolus*) (10–43 ppb; Hornfeldt and Nyholm, 1996). In another example of Hg bioaccumulation, total Hg concentrations were high (270–1700 ppb) for samples from the top 5 cm of sediments in a polluted salt marsh adjacent to a chlor-alkalai plant near Brunswick, Georgia, USA (Gardner et al., 1978). Hg concentrations of primary producers in this marsh were greater than in sediments and Hg concentration increased with trophic level. For example, herbivorous cotton rats (*Sigmodon hispidus*) had dry weight concentrations of total liver Hg of only 3800 ppb. However, the Hg concentrations were substantially higher for tissues from omnivorous species such as the raccoon (*Procyon lotor* = 8000 ppb), opossum (*Didelphis marsupialis* = 13,000 ppb), and Norway rat (*Rattus norvegicus* = 15,000 ppb).

Despite Hg contamination being substantially lower than in these highly polluted ecosystems, we have found some evidence of Hg bioaccumulation across terrestrial trophic levels on Isle Royale. Specifically, the mean Hg concentration in the kidneys of deer mice within the SL watershed (360±62 ppb dry wt.) was significantly higher ($t = -3.0$, $P < 0.01$, d.f. = 11) than in the soils within the SL watershed (164±30 ppb; Woodruff and Cannon, 2000). Future studies of Hg concentration in predators of Isle Royale (specifically, red fox, *Vulpes vulpes*) could further reveal a pattern of Hg bioaccumulation across the terrestrial ecosystem of Isle Royale.

Although Hg concentrations in the tissues of Isle Royale deer mice may not be remarkably high (relative to heavily polluted sites), the Hg concentrations are, nevertheless, high relative to standards that might be expected for protected areas in remote locations. Moreover, any exposure in this mid-trophic level species represents a path for more dangerous levels of biomagnification at higher trophic levels of the terrestrial ecosystem. Additional research in the Isle Royale ecosystem is required to understand: (1) the extent of bioaccumulation in higher trophic levels; (2) the sources of mercury; and (3) the nature of fine-scale spatial variation in mercury concentrations. Continued monitoring is also required so that temporal trends in Hg concentrations may be detected should they exist.

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