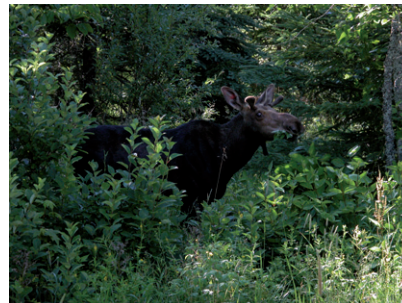


**Mercury, lead and lead isotope ratios in the teeth of moose (*Alces alces*) from Isle Royale, U.S. Upper Midwest, from 1952 to 2002**

John A. Vucetich,\* P. M. Outridge, Rolf O. Peterson, Rune Eide and Rolf Isrenn

We report a 50-year time series of Hg and Pb concentrations in the teeth of moose (*Alces alces*) from Isle Royale National Park, MI, USA.



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# Mercury, lead and lead isotope ratios in the teeth of moose (*Alces alces*) from Isle Royale, U.S. Upper Midwest, from 1952 to 2002

John A. Vucetich,<sup>\*a</sup> P. M. Outridge,<sup>b</sup> Rolf O. Peterson,<sup>a</sup> Rune Eide<sup>c</sup> and Rolf Isrenn<sup>d</sup>

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Assessing the effect of recent reductions in atmospheric pollution on metal concentrations in wildlife in North America has been difficult because of the sparse availability of historical samples with which to establish a “pre-regulation” baseline, and because many ecosystems may be affected by local point sources which could obscure broader-scale trends. Here we report a recent 50 yr annual record of Hg, Pb and Pb isotope ratios in the teeth of a resident population of moose (*Alces alces*) in Isle Royale National Park, a relatively remote island in Lake Superior, Michigan, USA. During the early 1980s, concentrations of tooth Hg abruptly declined by ~65% compared to the previous 30 years ( $p < 0.001$ ), similar to a previous study of Hg in herring gull eggs in the Great Lakes region. Lead declined at the same time, and by 2002 Pb in adult moose teeth was ~80% lower than it had been prior to the early 1980s ( $p < 0.001$ ). These trends were unaffected by normalization against the geogenic elements La and Sr, which indicates that the trends in Hg and Pb had an anthropogenic cause. Temporal patterns of Pb isotope ratios suggested that the primary sources of Pb at different times in the moose were combustion of U.S. coal and leaded gasoline. Reductions in emissions from coal combustion might explain the coincident reductions of Hg and Pb in Isle Royale moose, with elimination of alkyl Pb additives also playing a role in the continued tooth Pb reductions after 1983.

## 1. Introduction

Anti-pollution regulations enacted during the 1970s in Canada and the United States (e.g., U.S. Clean Air Act of 1970, its 1977 Amendment, and the Canadian Clean Air Act of 1971) were intended to reduce atmospheric emissions of particulates and smog-producing chemicals. Coincident with implementation of these regulations and reductions in the alkyl lead content of gasoline, atmospheric Hg and Pb deposition declined significantly throughout eastern North America during the mid-1970s to 1980s.<sup>1–4</sup> The extent to which these regional trends in atmospheric deposition were associated with lower Hg and Pb levels in animal populations is poorly known, because of the scarcity of well-preserved animal tissues collected at known times, in sufficient numbers, and over a sufficiently long period of time to establish a reliable “before and after” trend. Although several studies have documented temporal trends in Hg contamination for animal tissues (e.g., Hrabik and Watras<sup>5</sup>); to date, only two have examined such trends at sites where region-wide trends are unlikely to be obscured by local trends (i.e., Koster *et al.*,<sup>6</sup> Burger and Gochfeld<sup>7</sup>).

Here, we report a 50-year time series of Hg and Pb concentrations in the teeth of moose (*Alces alces*) from Isle Royale

National Park, MI, USA. Isle Royale is a wilderness island (544 km<sup>2</sup>) in Lake Superior (48°00' N, 89°00' W). The moose population that inhabits the island was founded in ca. 1900, is isolated from mainland populations, and is subject to predation by a resident wolf population.<sup>8</sup> This moose population is an ideal case study with which to assess temporal trends of biological Pb and Hg in a relatively remote setting with no local major pollution sources. The time-frame of the study (1952–2002) spans the decades before and after the first significant attempts to limit atmospheric pollution were enacted. Teeth preserve well when stored dry as these sample were, and are useful indicators of a mammal's exposure to and accumulation of Hg, Pb and other metals.<sup>9</sup> For additional context, we also analyzed other elements (La, Sr) known to be predominantly of geogenic origin. Finally, we assessed trends in tooth Pb isotope ratios against known regional industrial Pb sources as a means of inferring the source of Pb in the moose.

## 2. Methods

### Sample material

Since the early 1950s, the teeth and mandibles of Isle Royale moose have been collected opportunistically each year from carcasses, as a tool for assessing moose ecology.<sup>10</sup> The teeth used here were collected within a year of each animal's death. Birth year and age at death were determined by counting cementum lines in other teeth collected from the same jaws.<sup>11</sup> Samples were stored dry in paper bags inside wooden cabinets, and were never exposed to potential sources of contamination such as mercury sublimate.

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In general, the statistical power of tests for temporal trends is more influenced by the number of years of data and less so by intra-year variance.<sup>12</sup> Therefore, given the constraint of the available sample size, the number of years of data was maximized rather than the number of samples per year. We analyzed the roots of molar teeth from 102 Isle Royale moose that lived between 1946 and 2002. Because the dentine and cementum of tooth roots form throughout life, each sample represents an integrated measure of lifetime metal exposure. For statistical analysis, we associated each moose with the year corresponding to the midpoint of its life (e.g., a moose dying in 1990 at age seven years would be associated with the year 1986). Most moose ( $n = 64$ ) were <7 years old, and all moose were less than 15 years old. Using the mid-point of life to assign the tooth metal data to a specific year effectively uses the median year of each individual's lifetime as a datum point. Because the tooth metals data reported here is an integrated lifetime measure of metal intake, the median year of life is a more representative datum for lifetime intake than any other year. We checked the effect of using the mid-points on the temporal patterns in the dataset by statistically analyzing calves ( $N = 45$ ; by definition calves were <1 yr old) separately from the combine calves and adults dataset. The calves-only data gave the same temporal patterns as the combined data.

### Chemical analyses

Two subsamples incorporating complete cross-sections of cementum and dentine deposited through life were cut from the molar roots of each tooth using a rotary cutting tool. Surface contamination and any adhering tissue was removed by light grinding with a hand-held rotary tool, followed by immersion in 10% nitric acid and repeated rinses with distilled-deionized water. One subsample was analyzed for Hg and other trace elements, while the other was analysed for Pb isotope ratios. Samples were submitted to laboratories "blind" (with respect to birth year), and procedural blanks and reference materials were interspersed among the samples. For Hg determination, samples (0.2–0.5 g DW) were decomposed over heat in concentrated acid (1 mL HCl + 0.25 mL HNO<sub>3</sub>), made up to 25 mL final volume with distilled deionized water, and analyzed by cold-vapour atomic absorption spectrometry.<sup>13</sup> The calculated detection limit (D.L.), based on 10 procedural blanks with a mean  $\pm$  S.D. [Hg] of  $0.067 \pm 0.023$   $\mu\text{g/L}$  and with digest weights of 0.2–0.5 g dry weight (DW), was 0.0003–0.0009  $\mu\text{g/g}$  DW. For statistical purposes, we assigned a consistent DL of 0.001  $\mu\text{g/g}$  DW, and a value of 0.0005  $\mu\text{g/g}$  DW for samples with [Hg] below the D.L. Concurrent analyses of NIST 2976 Mussel Tissue ( $n = 3$ ) gave  $0.064 \pm 0.003$   $\mu\text{g/g}$  DW, agreeing to within 3% of the certified value. Analysis of an in-house Hg calcified reference material (NIST 1400 Bone Ash reference material mixed with DOLT-2 Liver Tissue) with a nominal Hg concentration of 0.02  $\mu\text{g/g}$  DW, gave a value of  $0.016 \pm 0.001$   $\mu\text{g/g}$  DW ( $n = 3$ ). Analysis by an independent laboratory of separate samples from five teeth (range of [Hg] 0.002–0.012  $\mu\text{g/g}$ ) showed good agreement with the original data (range of differences 0–18%, median of 13%).

For other elements (Pb, Cd, Sr, La), the same digests were analyzed by ICP-mass spectrometry and ICP-atomic emission spectrometry (see Hinners *et al.*<sup>14</sup>). Analysis of NIST Bone Ash

( $N = 5$ ) and NIST Mussel Tissue ( $N = 3$ ) gave values of  $9.22 \pm 0.22$  and  $1.31 \pm 0.12$   $\mu\text{g/g}$  DW for Pb,  $0.027 \pm 0.002$  and  $0.85 \pm 0.10$   $\mu\text{g/g}$  DW for Cd,  $290 \pm 10$  and  $65 \pm 9$   $\mu\text{g/g}$  DW for Sr, and  $0.34 \pm 0.02$  and  $0.038 \pm 0.003$   $\mu\text{g/g}$  DW for La, respectively. The Pb, Cd and Sr results for reference materials agreed to within 8, 10 and 16%, respectively, of certified or information values. Certified values for Sr in Mussel Tissue and La in both reference materials are not available, however, the La result for Bone Ash was close to an information value of 0.386  $\mu\text{g/g}$  DW.<sup>14</sup>

A subset of the moose calf teeth ( $n = 27$ ) were analyzed for Pb isotopes by thermal ionization mass spectrometry. All sample handling, dissolution, and column chemistry were performed in Class 1000 high-efficiency particle attenuation (HEPA) laboratory rooms with Class 100 fume hoods. Tooth samples were placed in Savillex Teflon® screw-cap containers and decomposed with 7N HNO<sub>3</sub>; the acid was dried down at 125 °C. The sample material was redissolved in 50% HF–12N HNO<sub>3</sub>, then with 8N HNO<sub>3</sub> and finally 6N HCl, with the residue taken up in 1N HBr for Pb column chemistry. Lead was separated from the digest solution in Bio-Rad 10-ml polyethylene columns and Dowex AG1-8X anion resin, using 1 N HBR to elute other elements and subsequently 6N HCl to elute Pb. The collected Pb solution was dried, redissolved in 1 N HBr, and the above procedure was repeated with a small volume resin bed. Total procedural blanks for Pb were <400 pg, based on two blank analyses. Samples were loaded onto single Re filaments with H<sub>3</sub>PO<sub>4</sub> and silica gel, and were run at filament temperatures of 1175–1225 °C on a ThermoFinnigan TRITON T1 mass spectrometer. All spectrometer runs were corrected for fractionation using certified values for NIST SRM 981 Common Lead Isotopic Standard. Analysis of triplicate samples of NIST 1400 Bone Ash agree to four significant figures with previous analyses of this material (see Hinners *et al.*,<sup>14</sup> Lab "C"), with an external precision of 0.02% R.S.D.

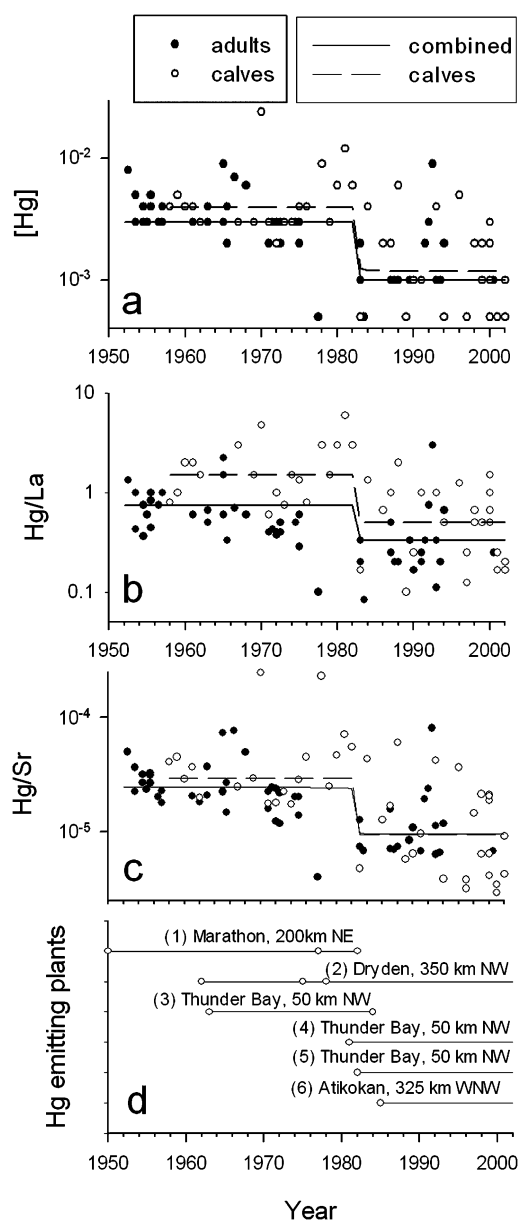
### Statistical analysis

Visual inspection of the Hg data suggested a highly nonlinear or threshold trend. To assess this possibility, we used quantile regression<sup>15</sup> to fit this model to the data (see the solid curve in Fig. 1):

$$\text{Log [Hg]} = \alpha_0 - \alpha_1(\arctan[\pi\alpha_3(\text{year} - \alpha_4)]) \quad (1)$$

This model is very flexible, and can describe declines ranging from nearly instantaneous (small, negative  $\alpha_3 \ll 0$ ) to very gradual, and nearly linear ( $\alpha_3$  close to zero). The inflection point ( $\alpha_4$ ) estimates the timing of decline, and  $\alpha_3$  estimates the steepness of decline.

We analyzed trends by building regression models based on least-squares estimation and assessing the performance and parsimony of those models with AIC<sub>c</sub>,  $\Delta_i$ , and  $W_i$ .<sup>16,17</sup> AIC<sub>c</sub> is Akaike's Information Criterion,  $\Delta_i$  equals the AIC<sub>c</sub> for the model of interest minus the smallest AIC<sub>c</sub> for the set of models being considered. By definition, the best model has a  $\Delta_i$  of zero, and models with  $\Delta_i < 2$  are generally considered worthy of consideration.  $W_i$  is the Akaike weight of model. If the ratio,  $W_i/W_j$ , is greater than one, it indicates how many times more likely model  $i$  is than model  $j$ . Methods of calculating AIC<sub>c</sub> for least squares regression and quantile regression are described by Anderson



**Fig. 1** Concentrations of Hg in the teeth of moose from Isle Royale, 1952–2002 (a), and the ratios Hg/La (b) and Hg/Sr (c) for the same moose. Units in panel (a) are  $\mu\text{g/g DW}$ . For panels (a), (b), and (c), the x-axis values are the midpoints of life of each moose (see Methods). Filled circles represent adults, open circles represent calves, solid lines represent median regression curves for adults and calves, and dashed lines represent curves for calves only. Panel (d) depicts the years of operation for Hg emitting plants closest to Isle Royale; labels indicate location of industrial plants, including direction and distance from Isle Royale. Numbers shown represent: (1) = Hg-cell chloralkali plant, discontinued in 1977, except sodium chlorate component of plant continued until 1982, (2) = Hg-cell chloralkali plant, converted to membrane process in 1975, other modifications made in 1978 to reduce Hg emissions, (3) = 100 MW coal-fired power plant, (4) = 165 MW coal-fired plant, (5) = 165 MW coal-fired plant, (6) = 230 MW coal-fired plant.

*et al.*<sup>17</sup> and Cade *et al.*<sup>18</sup> This method of model selection is becoming a standard for the environmental sciences,<sup>19</sup> additional

details about its use are provided in the Electronic Supplementary Information.

We also sought to compare concentrations between the earlier (pre-1982) and later (post-1983) portions of our study period. Some of these comparisons involved data that deviated significantly from normal distribution (*e.g.*, some of the data in Fig. 2 are right skewed). We therefore used non-parametric rank-sum tests on untransformed data to compare early and late periods of the data set. Although non-parametric tests are less powerful than comparable parametric tests, that aspect is moot in this case because the tests showed significant differences existed (see below).

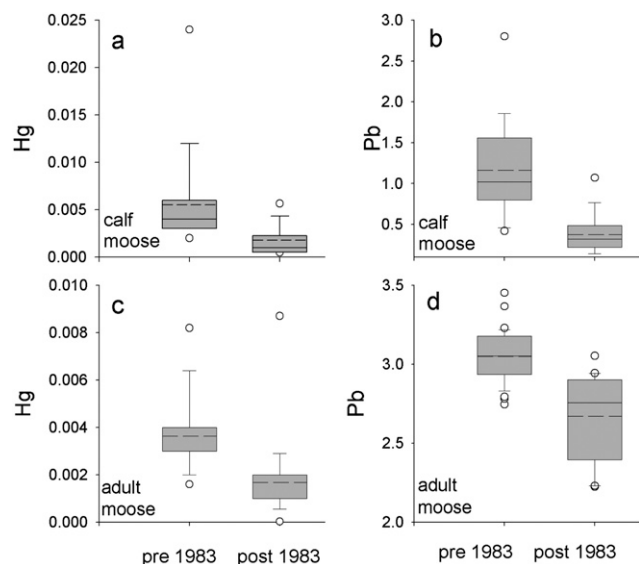
## Results

Except for a few cases, measured concentrations of Hg, Pb, La and Sr were well above detection limits. Because the median sample [Cd] equaled the detection limit ( $0.005 \mu\text{g/g DW}$ ), we will not further consider Cd in this paper.

### Temporal pattern of Hg

Tooth Hg concentrations displayed a pronounced non-linear decline during the 50 yr of record, with an apparent inflection point in the early 1980s (Fig. 1). The non-linear model represented by equation 1 (see Fig. 1a) was 280 times more likely to better describe the decline than a simple linear regression (*i.e.*,  $W_{\text{non-linear}}:W_{\text{linear}} = 280$ ). The year of most rapid decline was 1983 (*i.e.*,  $\alpha_4$  from equation 1 = 1983), and 80% of the decline took place between 1980 and 1986. This model also indicated that no significant trends occurred in Hg concentrations before or after 1983.

The median Hg concentration of all moose between 1952 and 1982 were significantly greater ( $p < 0.0001$ ) than between 1983



**Fig. 2** Box plot comparisons of Hg and Pb concentrations in calf (a, b) and adult moose (c, d) teeth for two periods of time: 1952–1982, and 1983–2002. Units are  $\mu\text{g/g DW}$ . The boxes are inter-quartile ranges, the solid line is the median, dashed line is the mean, whiskers are 10th and 90th percentiles, open circles are data outside 10th and 90th percentiles.

and 2002 (see Table 1 and Fig. 2). Also, linear regression showed there was no significant trend before 1982 ( $R^2 < 0.01$ ,  $p = 0.65$ ) or after 1983 ( $R^2 < 0.01$ ,  $p = 0.60$ ).

### Influence of age and sex on Hg concentration

To be sure the results described above were not influenced by changing sex or age of the moose sample over time, we used analysis of covariance (ANCOVA) to assess this model:

$$\log [\text{Hg}] = \alpha_0 + \alpha_1(\text{age}) + \alpha_3 I_{\text{sex}} + \alpha_4 I_{\text{time}} \quad (2)$$

where  $\alpha$ 's are coefficients and  $I$ 's are indicators for sex and time period (*i.e.*, before or after 1983). This analysis confirmed that when age and sex are taken into account, tooth Hg was significantly lower after 1983 ( $p = 0.001$ ;  $\alpha_4 = 0.385$ , which corresponds to a 59% decline in Hg). Moreover, Hg did not vary between sexes ( $p = 0.28$ ;  $n = 49$  females,  $n = 26$  males; individuals of unknown sex ( $n = 27$ ) were excluded). Although tooth Hg declined slightly with animal age ( $p = 0.02$ ;  $\alpha_1 = -1.1 \times 10^{-2}$ ), the average age of moose in the pre-1982 sample was in fact slightly older than in the post-1983 sample (5.1 *versus* 3.8 years;  $p = 0.13$ , *t*-test); therefore, age was not a factor in the observed Hg time trend.

### Temporal pattern of Hg in calves only

We repeated the above analysis on a dataset including only calves. The nonlinear model (Equation 1) was 26 times more likely to better describe the trend than was a simple linear model ( $W_{\text{non-linear}}:W_{\text{linear}} = 26$ ). Again, 1983 was the year of most rapid decline, and 89% of the decline took place between 1982 and 1983 (dashed curve in Fig. 1).

The median Hg concentrations of calves living prior to 1983 were significantly greater ( $p < 0.001$ ) than after 1983 and 2002 (Fig. 2). Mercury concentrations in calves did not show a significant trend either prior to 1982 ( $R^2 = 0.05$ ,  $p = 0.35$ ) or after 1983 ( $R^2 = 0.07$ ,  $p = 0.18$ ).

### Temporal pattern of Hg/La and Hg/Sr

If trends in tooth Hg were attributable to some unknown, non-anthropogenic environmental process, then the ratios Hg/La and Hg/Sr ought to exhibit temporal patterns different to those for

Hg. But this was not the case (see Fig. 1b and 1c). Table 1 indicates that these ratios exhibited trends similar in all respects to those of Hg.

### Temporal patterns of Pb, Pb/La and Pb/Sr

Like Hg, tooth Pb concentrations showed an overall non-linear decline during the study period, with an inflection in the early 1980s (Figs. 2 and 3). Of the models examined to assess trends in Pb, the most parsimonious was that shown in Table 2 and plotted on Fig. 3a. This model explained 86% of the variation in  $\log [\text{Pb}]$  and indicated that tooth Pb was (i) lower on average for calves than for adults, (ii) constant for adults and calves prior to 1982, and (iii) declined exponentially between 1983 and 2002 (Fig. 3a). For adult moose, the modeled median Pb concentration for 2002 was ~80% lower than it was prior to 1980 (solid line in Fig. 3a).

The pattern represented by this model is further supported by noting that Pb in calves did not significantly decline either prior to 1983 ( $R^2 < 0.01$ ,  $p = 0.90$ ) or after 1983 (slope =  $-4.33 \times 10^{-3}$ ,  $R^2 = 0.01$ ,  $p = 0.62$ ). The median calf Pb concentrations prior to 1983 were significantly greater than post-1983 (Fig. 2;  $p < 0.001$ ). For adults, tooth Pb was constant prior to 1983 ( $R^2 = 0.05$ ,  $p = 0.18$ ), but declined significantly during 1983–2002 (slope =  $-0.24$ ,  $R^2 = 0.43$ ,  $p = 0.002$ ). Trends in Pb/La and Pb/Sr were also assessed, indicating that these ratios exhibited temporal trends similar in all respects to that of Pb (Fig. 3b and 3c).

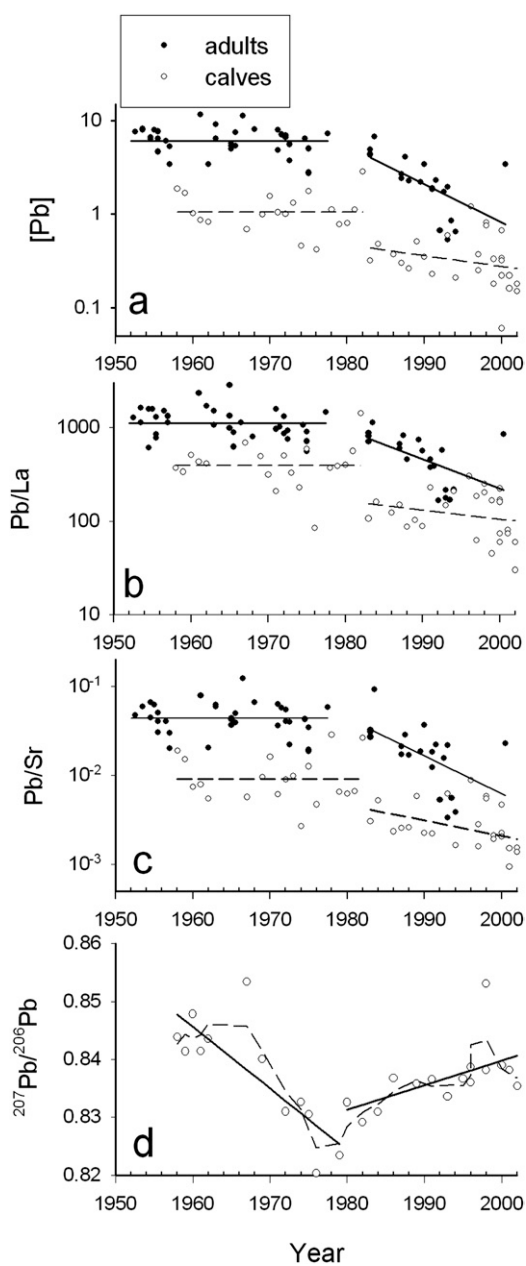
### Lead isotope ratios

For a subset of calves, we determined and statistically assessed the trend in  $^{207}\text{Pb}/^{206}\text{Pb}$  (Fig. 3d). Visual inspection (aided by a three-year moving average of the data) suggested that  $^{207}\text{Pb}/^{206}\text{Pb}$  displayed either a two-part trend (with a breakpoint at 1977) or a three-part trend (with breakpoints at 1964 and 1977). To assess the statistical justification for these patterns, we constructed and compared these models: no slope, simple linear trend, second-order polynomial trend, third-order polynomial trend, a two-part piecewise regression, and a three-part, piecewise regression. The two-part, piecewise model explained 56% of variation in  $^{207}\text{Pb}/^{206}\text{Pb}$ , and was 39 times more likely than the second-best model (a third-order polynomial).

**Table 1** Statistical analysis of the temporal trend in tooth mercury data depicted in Fig. 1

Response variable: Data set:	$\log([\text{Hg}])$		$\log(\text{Hg}/\text{La})$		$\log(\text{Hg}/\text{Sr})$	
	full	calves only	full	calves only	full	calves only
p-values for tests of equal medians	<0.0001	<0.001	<0.0001	<0.001	<0.0001	<0.0001
Ratio of medians, before and after 1983	2.8	3.3	2.3	3.0	2.6	3.1
p-values for tests of temporal trend before and after 1983	0.65	0.35	0.32	0.16	0.91	0.37
	0.60	0.18	0.52	0.37	0.25	0.16
p-value for simple linear decline	<0.0001	<0.0001	0.0005	0.0001	<0.0001	<0.0001
Weight of evidence in favor of threshold model <i>versus</i> simple linear trend <sup>a</sup>	26	326	62	1762	15	51

<sup>a</sup> Ratio of akaike weights, which indicate how many times more likely one model is than another.



**Fig. 3** Concentrations of Pb in the teeth of moose from Isle Royale, 1952–2002 (a), and the ratios Pb/La (b) and Pb/Sr (c) for the same moose. Panel (d) depicts <sup>207</sup>Pb/<sup>206</sup>Pb isotope ratios for a subset (N = 27) of the calves depicted in previous panels. In panel (d), the dotted line represents a 3 yr moving average, and the solid line represents the best-fitting piecewise linear regression. Other aspects of panels (a), (b), and (c) are described in the legend for Fig. 1.

#### 4. Discussion

Mercury concentrations in Isle Royale moose teeth decreased by about two-thirds during the early 1980s, after showing no significant trends in the previous three decades; concentrations thereafter remained constant for the following two decades. The reduction was more rapid for calves than adults, with ~90% of the decrease in calves taking place during one year (1982–83) and ~80% of the decrease in adults occurring between 1980 and 1986.

**Table 2** Results of statistical analysis of the temporal trend in tooth Pb, depicted in Fig. 3a

Model Structure	Parameters <sup>a</sup>	AIC <sub>c</sub> (Δ) <sup>b</sup>	R <sup>2</sup>
$\log([\text{Pb}])_{\text{calves}} = \alpha_1, \text{ for year} < 1983$ $\log([\text{Pb}])_{\text{calves}} = \alpha_2 + \alpha_3 \text{ year, for year} > 1983$	10	-289.5 (0)	0.86
$\log([\text{Pb}])_{\text{adults}} = \alpha_4, \text{ for year} < 1983$ $\log([\text{Pb}])_{\text{adults}} = \alpha_5 + \alpha_6 \text{ year, for year} > 1983$			

<sup>a</sup> The number of parameters is equal to the number of alphas appearing in each set of equations plus the number of equations in each set, which accounts for the parameter associated with the variance of each equation's error term. <sup>b</sup> AIC<sub>c</sub> is Akaike's Information Criterion. Δ is the AIC<sub>c</sub> for the model of interest minus the smallest AIC<sub>c</sub> for the set of models being considered. The most parsimonious model has a Δ of zero, and models with Δ < ~2 are considered worthy of consideration.

Lead concentrations in adult and calf teeth were lower by about two-thirds after 1983, like Hg, with a continuing decline in adult tooth Pb concentrations up to 2002. More rapid changes in calves reflect their short life-spans (by definition, <1 year); the calf tooth data therefore more precisely indicate the timing of changes in metal exposure of the population than do the adult data. The Pb isotopic composition of calf teeth changed in the mid-1960s (almost two decades before the marked reduction in tooth Pb concentrations in 1983) and again in the late 1970s, revealing more than one shift in the predominant Pb source in moose over the past three decades.

These results for Hg in Isle Royale moose are similar to those observed in eggs from several Great Lakes colonies of herring gulls (*Larus argentatus*) that were monitored between 1973/74 and 1992.<sup>6</sup> One of these colonies was located on islands ~40 km north of Isle Royale, while others were in eastern Lake Superior, eastern Lake Huron, and western Lake Ontario. Of the eight colonies studied by Koster *et al.*,<sup>6</sup> half displayed ~50% reductions in Hg concentrations between 1982 and 1983/85. Also, mean egg Hg levels were constant prior to 1982 and after 1983, again similar to the moose Hg trend. Taken together, this study's results from a terrestrial herbivore, and Koster *et al.*'s<sup>6</sup> results from a predator/scavenger species feeding at trophic level 3–4,<sup>20</sup> agree that the environment of northern Lake Superior experienced a substantial and sudden reduction in Hg levels around 1982/83. The present study also indicates that environmental Pb levels in this area fell at the same time as Hg, while Koster *et al.*<sup>6</sup> indicated that the sudden Hg decline was a Great Lakes-wide phenomenon. Although these sharp, abrupt declines in biotic Hg were unexpected, the probability that storage contamination, volatilization of Hg from samples, or undetected analytical errors could produce the same temporal pattern in independent studies of different biological matrices, collected and stored in different ways at different institutions, is extremely low.

The most likely explanation for the metal decline in moose was a reduction in airborne pollution, which accords with Koster *et al.*<sup>6</sup> contention that the gull egg Hg decrease was due to reduced Hg pollution. It is difficult to precisely compare between these biological time series and regional non-biological archives of atmospheric pollution such as lake sediments and peat bogs, because the temporal resolution of metal profiles in sediment and

1 peat cores is poorer (at least several years per increment) than the  
annual resolution of this study and Koster *et al.*<sup>6</sup> Nonetheless, it  
is clear that the metal decreases in moose teeth and gull eggs  
occurred several years after atmospheric deposition of Hg and Pb  
5 began to decline in the mid-1970s throughout the U.S. Midwest  
and Ontario. Sedimentary Hg profiles in rural lakes of eastern  
Minnesota and urban lakes in Minneapolis, upwind of Isle  
Royale, showed sharp declines of Hg concentrations from the  
mid- to late-1970s onwards,<sup>4</sup> while mean Hg accumulation  
10 declined by about one third between 1950–80 and 1980–91 in an  
eastern Minnesotan peat bog.<sup>2</sup> Atmospheric Pb deposition in the  
lower Great Lakes also decreased significantly from the mid-  
1970s, largely as a result of removing alkyl Pb from gasoline.<sup>3</sup>

Other possible contributing factors to the decline in moose Hg  
and Pb exposure can be ruled out. In some cases, temporal  
variations of Hg in an animal population may be caused by  
variation in diet (*e.g.*, Riget *et al.*<sup>21</sup>). However, such an expla-  
nation is unlikely in this case because moose are consistently  
herbivorous. Furthermore, the diet of Isle Royale moose has  
20 been studied on several occasions between 1946 and 2004,<sup>22</sup> and  
has not exhibited any changes in plant selection or feeding  
location coincident with the population's Hg and Pb trends. It  
also is unlikely that the abrupt declines in moose Hg and Pb were  
caused by changes in meteorological conditions, land use, or  
25 vegetation cover. For the entire study period, Isle Royale has  
been managed as a federally designated wilderness.<sup>23</sup> Meteorolo-  
gical records for the last 50 years for Thunder Bay, the closest  
meteorological station to Isle Royale, revealed no shifts or  
gradual trends in predominant wind direction, average wind  
speed, or precipitation patterns.<sup>24</sup> Finally, the patterns of decline  
30 in Hg and Pb were unaffected by normalization with the geogenic  
elements La and Sr (Figs. 1 and 3), indicating that the trends in  
moose Hg and Pb were not caused by variation in the level or  
bioavailability of geogenic (natural) Hg and Pb.

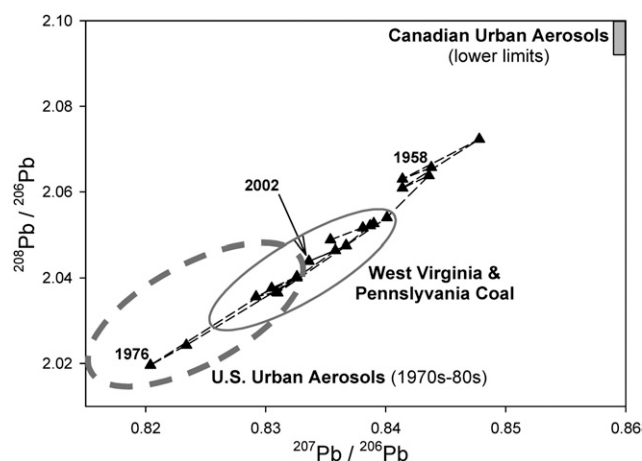
### Local versus regional sources of anthropogenic Hg and Pb

The complete explanation for why Hg and Pb concentrations  
declined so rapidly and abruptly in Isle Royale moose remains  
unclear. Several lines of evidence suggest that the abrupt decline  
cannot be explained by an abrupt reduction in emissions from  
a single, dominant, local source near Northern Lake Superior.  
Rather, the declines probably reflect a regional-scale decrease in  
anthropogenic emissions with possibly a common (coal-fired)  
45 industrial sector responsible for both Pb and Hg trends  
combined with reduced Pb deposition associated with the elimi-  
nation of gasoline Pb additives.

The first piece of evidence supporting this statement is that  
sudden reductions in Hg were detected at the same time at several  
gull colonies throughout the Great Lakes,<sup>6</sup> meaning that the  
reduction was regional in scale. This finding is consistent with  
atmospheric models which indicate that Lake Superior's Hg  
airshed is large, with more than 90% of the atmospheric Hg  
50 deposited there coming from areas more than 200 km away.<sup>25</sup>  
Second, nearby local point sources appear to have been inca-  
pable of causing the trends observed in moose and herring gull  
Hg and Pb, for several reasons. The opening and closing dates of  
some of these sources (Fig. 1d) did not correspond with signifi-  
cant changes in the Hg time trend in moose and in gulls. Even

though two local sources north of Lake Superior (*i.e.*, sources #1  
& #3 in Fig. 1d) closed or reduced operations during the late  
1970s and early 1980s, increases of tooth Hg had not occurred  
when these sources opened, suggesting that they had negligible  
effect on biotic Hg levels. Also, in the Mid-West, regional trends  
5 in Hg pollution can be detected even at sites moderately down-  
wind of very large point sources. Downwind from the Chicago/  
Gary urban area, a major Hg source in the U.S. Midwest,  
regional background levels of Hg in wet deposition, airborne  
particulates and of gaseous Hg were attained within 50–100  
10 km.<sup>26</sup> The point sources near Isle Royale were smaller and 50–  
350 km away. Finally, because the prevailing winds at Thunder  
Bay are southwesterly or westerly for most of the year and  
easterly during spring,<sup>24</sup> none of the nearby sources are directly  
upwind of Isle Royale. Therefore, although a minor local influ-  
15 ence cannot be ruled out, it seems unlikely that the relatively  
small industrial plants operating 50–350 km away could have  
significantly influenced Hg and Pb trends on Isle Royale.

Key evidence for regional-scale sources being the main  
contributor of the anthropogenic Pb in Isle Royale moose comes  
from changes in its isotopic composition over time (Fig. 4). The  
moose Pb isotope pattern is consistent with historical changes of  
atmospheric Pb pollution in eastern North America. From the  
mid-1960s to the mid-1970s, moose tooth Pb concentrations  
remained high while <sup>206</sup>Pb-based ratios declined, suggesting that  
25 while atmospheric Pb deposition had not decreased, the  
geological source of Pb had changed. At this time, increasing  
amounts of ore with relatively low <sup>206</sup>Pb-based isotope ratios<sup>27</sup>  
were introduced from the Mississippi Valley deposits in Missouri  
and Tennessee into the North American market, including alkyl  
30 Pb additive production. One result was that <sup>207</sup>Pb/<sup>206</sup>Pb values in  
U.S. leaded gasoline and in urban air decreased after the 1960s,  
attaining a consistent range of about 0.81–0.83 by the 1980s.<sup>28</sup>  
The lowest isotope ratios in moose teeth (<sup>207</sup>Pb/<sup>206</sup>Pb: 0.820–  
0.835) occurred during the 1970s–80s and corresponded to the  
35



**Fig. 4** Lead isotope ratio trends in Isle Royale moose calf teeth from 1958 to 2002, compared to the isotopic signatures of known regional atmospheric sources. (Tooth values, shown in black triangles, had analytical (internal) error bars smaller than the size of the symbols. Also shown are the range of Pb isotope ratios in West Virginian and Pennsylvanian coal deposits,<sup>32</sup> and in Canadian and U.S. urban aerosols in the 1970s–80s (dominated by lead from alkyl Pb gasoline additives<sup>28,29</sup>).

1 U.S. urban isotopic signature at that time (see Fig. 4), which were  
largely from alkyl Pb. The minimum tooth Pb isotope value was  
recorded in the mid-1970s, which agrees with the timing of Pb  
isotope profiles in regional lake sediments.<sup>3</sup> There was no  
5 evidence of Canadian Pb sources significantly impacting the  
moose trend, because the trend direction was towards U.S. urban  
air which had lower <sup>207</sup>Pb/<sup>206</sup>Pb than Canadian urban air. A  
second shift (an increase) in moose <sup>207</sup>Pb/<sup>206</sup>Pb began in the late  
1970s, accompanied 3–4 years later by abrupt declines in tooth  
10 Pb concentrations which continued until the end of the record in  
2002. Over this short time frame, these patterns can only be  
explained by a severe reduction of emissions from an anthro-  
pogenic source or sources. Analyses of lower Great Lakes sedi-  
ment cores spanning this period suggested that the elimination of  
15 alkyl Pb from U.S. gasoline after the mid-1970s was responsible  
for a rapid decline in Pb deposition throughout the region,<sup>3</sup> and  
this could have contributed to the moose Pb decline. A relatively  
significant input of Pb from coal combustion into the Isle Royale  
environment is also suggested by the limited range of moose  
20 tooth values after 1990, which fell within the range of major U.S.  
coal deposits (Fig. 3). With the elimination of alkyl Pb emissions,  
other Pb emissions to air such as from coal combustion are  
known to have become isotopically dominant even as total Pb  
concentrations in air declined in eastern North America.<sup>3,29,30</sup>

25 Unfortunately, it is not possible to quantify the contribution of  
Pb to moose from each source (coal, gasoline, general industrial  
Pb ores), because of the partial isotopic overlap between coal and  
U.S. urban air (leaded gasoline), and due to the absence of pre-  
19<sup>th</sup> Century moose tooth samples with which to establish the  
pre-industrial background Pb isotope value. Graney *et al.*'s  
30 (1995) findings point to a multiplicity of urban and industrial  
sources of Pb in the Great Lakes, which in the case of coal  
combustion (for power generation, waste incineration, and  
manufacturing) were potentially also Hg emitters. As Engstrom  
and Swain<sup>4</sup> pointed out with respect to atmospheric Hg deposi-  
35 tion in this area, given the currently available knowledge con-  
cerning relationships between emissions from various industries  
and changes in atmospheric Hg and Pb deposition, it is not  
possible to definitively assign the reductions of metals in moose  
and herring gulls to a specific source or industry.

40 Local declines of Hg and/or Pb have been detected in North  
American wildlife following reductions in pollutants emitted  
from nearby point-sources (*e.g.*, Hrabik and Watras<sup>5</sup>) or in  
relatively industrialized regions such as New Jersey.<sup>7</sup> Our study  
45 reinforces Koster *et al.*<sup>6</sup> findings that the reductions in at-  
mospheric metal deposition that occurred in the 1970s and 1980s in  
the Great Lakes region reduced Hg and Pb concentrations in  
wildlife, particularly in northern Lake Superior. Although we  
cannot identify the specific urban or industrial sources respon-  
50 sible for the biological trends, coal combustion is known to be  
a common source of Hg and Pb.<sup>31</sup> Reductions in emissions from  
this industrial sector might explain the co-incident reductions of  
Hg and Pb in Isle Royale moose, with elimination of alkyl Pb  
additives also playing a role in the continued tooth Pb reductions  
55 after 1983.

## Appendix: Model selection with Akaike's Information Criterion

The expression for AIC<sub>c</sub> is:<sup>16</sup>

$$-2[\ln(\mathcal{L}(\theta|\text{data}))] + 2K + 2K(K+1)/(N-K-1) \quad (\text{A1})$$

where  $\ln(\mathcal{L}(\theta|\text{data}))$  is the maximized log-likelihood over the  
unknown parameters (given the model and the data),  $K$  is the  
number of model parameters, and  $N$  is the sample size. The first  
term of expression (A1) represents a measure of the model's fit,  
the second term a penalty for each parameter in the model, and  
the third term accounts for statistical processes associated with  
finite sampling.  $\Delta_i$  equals the AIC<sub>c</sub> for the model  $i$  minus the  
smallest AIC<sub>c</sub> for the set of models being considered. The most  
parsimonious model has a  $\Delta_i$  of zero, and models with  $\Delta_i$  less  
15 than  $\sim 2$  are regarded as worthy of consideration.<sup>16</sup> We also  
calculated Akaike weights:<sup>16</sup>

$$W_i = \exp(-2 \Delta_i) / [\exp(-2 \Delta_i) + \exp(-2 \Delta_j)] \quad (\text{A2})$$

20 If the ratio,  $W_i/W_j$ , is greater than one, it indicates how many  
times more likely model  $i$  is than model  $j$ . Simplified versions of  
(A1) for least squares regression and quantile regression are  
described by Anderson *et al.*<sup>17</sup> and Cade *et al.*<sup>18</sup>

25 To understand the basis for using  $\Delta$  or  $W$  to assess temporal  
trends in contaminants, consider this example: Suppose  
concentrations of some contaminant,  $X$ , are measured over time.  
Is one better justified to think the mean concentration remained  
constant over time ("no trend") or to think there has been some  
positive or negative linear trend? The "no-trend" hypothesis is  
30 associated with this statistical model:  $X_t = \alpha_1 + e_t$ , where  $\alpha_1$  is  
a constant (representing the mean concentration),  $e_t$  is the error  
term with a mean value of zero and is characterized by a standard  
deviation  $\sigma^2$ , and  $t$  represents the time each observation had been  
made. Given the data and this model, one would estimate the  
parameters ( $\alpha_1$  and  $\sigma^2$ ), maximized log-likelihood, and AIC<sub>c</sub>  
35 for this model. These values would also be calculated for the "linear  
trend" hypothesis, where the statistical model is  $X_t = \alpha_2 + \alpha_3 t + e_t$ .  
The justification for concluding that  $X$  has either exhibited no  
trend or has exhibited a linear trend is determined by comparing  
40 the  $\Delta$ 's or  $W$ 's associated with each model. We employ this logic  
to make inferences about the observed trends. The advantages of  
using this approach relative to Frequentist hypothesis testing  
have been well articulated elsewhere.<sup>16,34</sup> This approach is also  
45 becoming a standard technique in many fields of science.<sup>35</sup>

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## References

- 1 W. S. Rickert and M. J. Kaiserman, *Environ. Sci. Tech.*, 1994, **28**, 924; W. L. Lockhart, R. W. Macdonald, P. M. Outridge, P. Wilkinson, J. DeLaronde and J. W. M. Rudd, *Sci. Tot. Environ.*, 2000, **260**, 171.
- 2 J. M. Benoit, W. F. Fitzgerald and A. W. H. Damman, in *Mercury Pollution: Integration and Synthesis*, ed. C. J. Watras and J. W. Huckabee, Lewis, Boca Raton, Florida, USA, 1994, sec. 2, (ch. 2), pp. 187–202.
- 3 J. R. Graney, A. N. Halliday, G. J. Keeler, J. O. Nriagu, J. A. Robbins and S. A. Norton, *Geochimica et Cosmochimica Acta*, 1995, **59**, 1715.
- 4 D. R. Engstrom and E. B. Swain, *Environ. Sci. Tech.*, 1997, **31**, 960.
- 5 T. Hrabik and C. Watras, *Sci. Tot. Environ.*, 2002, **297**, 229.
- 6 M. D. Koster, D. P. Ryckman, D. V. C. Weseloh and J. Struger, *Environ. Poll.*, 1996, **93**, 261.
- 7 J. Burger and M. Gochfeld, *Environ. Res.*, 2004, **94**, 336.
- 8 J. A. Vucetich and R. O. Peterson, *Proc. Roy. Soc., London, Series B*, 2004, **271**, 183.
- 9 G. Wesenberg, G. Fosse, P. Rasmussen and N.-P. B. Juestsen, *Int. J. Environ. Studies*, 1981, **16**, 147; R. Eide and G. B. R. Wesenberg, *Environ. Res.*, 1993, **61**, 212; P. M. Outridge, R. Wagemann and A. McNeely, *Environ. Tox. Chem.*, 2000, **19**, 1517.
- 10 J. A. Vucetich and R. O. Peterson, in *Biology and Conservation of Wild Canids*, ed. D. Macdonald and C. Sillero-Zubiri, Oxford University Press, 2004, (ch. 18), pp. 281–292.
- 11 K. L. Hamlin, D. F. Pac, C. A. Sime, R. M. DeSimone and G. L. Dusek, *J. Wildlife Management*, 2000, **64**, 441.
- 12 A. Bignert, F. Riget, B. Braune, P. Outridge and S. Wilson, *J. Environ. Monit.*, 2004, **6**, 351.
- 13 F. Armstrong and J. Uthe, *Atomic Absorption Newsletter*, 1971, **10**, 101.
- 14 T. A. Hinners, R. J. Hughes, P. M. Outridge, W. J. Davis, K. Simon and D. Woolard, *J. Anal. At. Spectrom.*, 1998, **13**, 963.
- 15 R. Koenker and G. Bassett, *Econometrica*, 1978, **46**, 33.
- 16 K. P. Burnham and D. R. Anderson, *Model selection and inference*, Springer, New York, 1998.
- 17 D. R. Anderson, K. P. Burnham and W. L. Thompson, *J. Wildlife Management*, 2000, **64**, 912.
- 18 B. S. Cade, B. Noon and C. H. Flather, *Ecology*, 2005, **86**, 786.
- 19 B. R. Missildine, R. J. Peters, G. Chin-Leo and D. Houck, *Environ. Sci. Tech.*, 2005, **39**, 6944; M. J. Vander Zanden, Y. Vadeboncoeur, M. W. Diebel and E. Jeppesen, *Environ. Sci. Tech.*, 2005, **39**, 7509; C. M. Saffron, J. H. Park, B. E. Dale and T. C. Voice, *Environ. Sci. Tech.*, 2006, **40**, 7662; J. E. Elliott, M. L. Harris, L. K. Wilson, B. D. Smith, S. P. Batchelor and J. Maguire, *Environ. Poll.*, 2007, **149**, 114.
- 20 C. E. Hebert, J. L. Shutt, K. A. Hobson and D. V. C. Weseloh, *Can. J. Fish. Aqu. Sci.*, 1999, **56**, 323.
- 21 F. Riget, R. Dietz, E. W. Born, C. Sonne and K. A. Hobson, *Marine Poll. Bull.*, 2007, **54**, 72.
- 22 L. W. Krefting, *The Ecology of Isle Royale Moose*. Technical Bulletin 297, University of Minnesota, Minneapolis, MN, USA, 1974; G. E. Belovsky, *Ecology*, 1981, **62**, 1020; T. A. Brandner, R. O. Peterson and K. L. Risenhoover, *Ecology*, 1990, **71**, 155; R. O. Peterson and J. A. Vucetich, *Ecological studies of wolves on Isle Royale, 2005–2006 Annual Report*, Michigan Technological University, Houghton, MI, 2005, www.isleroyalewolf.org.
- 23 M. P. Nelson, R. O. Peterson and J. A. Vucetich, *George Wright Soc.*, 2008, **25**, 98–113.
- 24 *Canadian Climate Data*, Environment Canada, 2008, [http://climate.weatheroffice.ec.gc.ca/climateData/canada\\_e.html](http://climate.weatheroffice.ec.gc.ca/climateData/canada_e.html).
- 25 M. Cohen, R. Artz, R. Draxler, P. Miller, D. Niemi, D. Ratte, M. Deslauriers, R. Duval, R. Laurin, J. Slotnick, T. Nettesheim and J. McDonald, *Environ. Res.*, 2004, **95**, 247.
- 26 M. S. Landis, A. F. Vette and G. J. Keeler, *Environ. Sci. Tech.*, 2002, **36**, 4508.
- 27 D. Sangster, P. M. Outridge and W. J. Davis, *Environ. Rev.*, 2000, **8**, 115.
- 28 W. T. Sturges and L. A. Barrie, *Atmos. Environ.*, 1989, **28**, 1645; A. J. Veron, T. M. Church, A. R. Flegal, C. C. Patterson and Y. Erel, *J. Geophys. Res.*, 1993, **9**, 18269; D. J. Steding, C. E. Dunlap and A. R. Flegal, *Science*, 2000, **97**, 11181.
- 29 J. Carignan and C. Garipey, *Geochimica et Cosmochimica Acta*, 1995, **59**, 4427.
- 30 A. Gallon, A. Tessier, C. Gobeil and L. Beaudin, *Geochimica et Cosmochimica Acta*, 2005, **69**, 3199.
- 31 J. M. Pacyna and E. G. Pacyna, *Environmental Reviews*, 2001, **9**, 269–298.
- 32 T. Chow and J. L. Earl, *Science*, 1972, **176**, 510.
- 33 D. J. Steding, C. E. Dunlap and A. R. Flegal, *Science*, 2000, **97**, 11181.
- 34 P. A. Stephens, S. W. Buskirk and C. M. del Rio, *Trends. Ecol. Evol.*, 2007, **22**, 192.
- 35 K. P. Burnham and D. R. Anderson, *Soc. Meth. Res.*, 2004, **33**, 261; P. M. Djuric, *IEEE Transactions on Signal Processing*, 1996, **44**, 1744; N. Hens, M. Aerts and G. Molenberghs, *Stat. Medicine*, 2006, **25**, 2502; D. Posada and K. A. Crandall, *Systematic Biol.*, 2001, **50**, 580; W. A. Schmitt and G. Stephanopoulos, *Biotech. Bioeng.*, 2003, **84**, 855; T. Utsu, *Pure Appl. Geophys.*, 1999, **155**, 509.

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