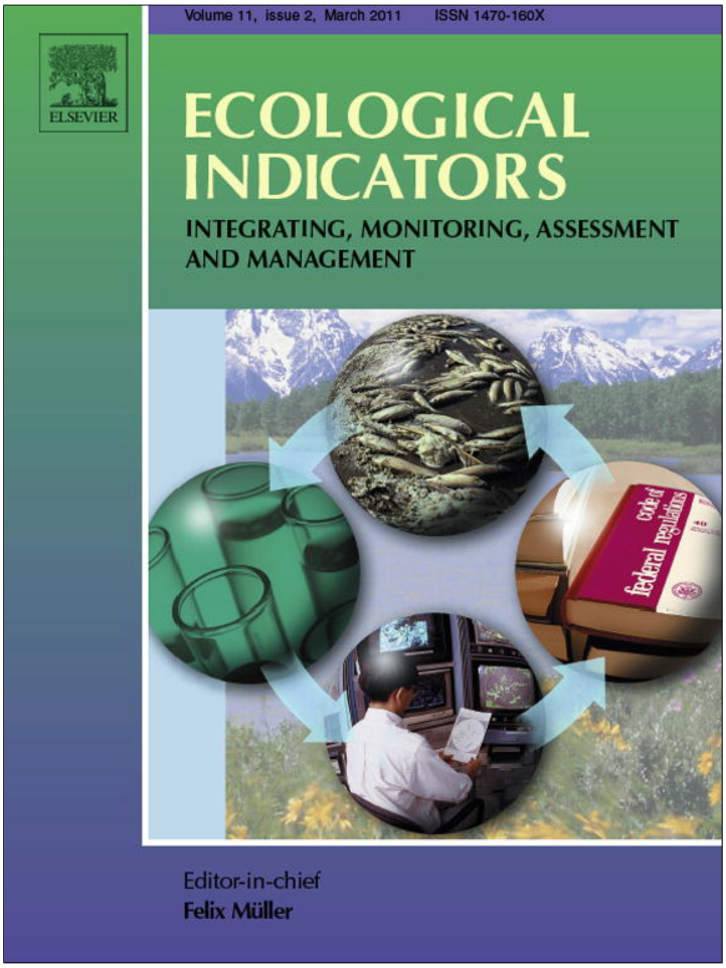
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Molted feathers indicate low mercury in bald eagles of the Chesapeake Bay, USA

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ABSTRACT

Mercury is a potent neurotoxin affecting birds and other wildlife worldwide. Bald eagles (*Haliaeetus leucocephalus*) are vulnerable to mercury bioaccumulation because they are high in the food web and associated with aquatic ecosystems prone to mercury methylation. Eagle populations, long endangered in the continental United States by contaminants and persecution, are recovering throughout their range. We used single adult eagle feathers collected near 83 occupied nests to show that mercury levels in the Chesapeake Bay eagle population are the lowest in North America (3.82 ± 5.15 μg/g dry weight). We then used 20 feathers from each of 20 salvaged eagles to calculate a confidence interval around the estimate based on single feathers from nesting eagles. Using an inexpensive and non-invasive method to assess mercury burdens we have demonstrated that few Chesapeake Bay bald eagles were above levels suspected of causing reproductive or survival effects in birds.

1. Introduction

Mercury contamination poses a threat to wildlife in ecosystems worldwide, particularly large, predatory species such as bald eagles (*Haliaeetus leucocephalus*). Mercury can affect behavior and reproduction in birds, and high levels in the diet can cause mortality (Seewagen, 2010). Bald eagles have been the focus of conservation efforts for decades, and populations are stable or increasing throughout the contiguous United States (Suckling and Hodges, 2007). Because eagles feed on large prey at the top of aquatic food chains they are prime candidates for elevated mercury contamination. Eagle tissues have frequently been monitored for contaminants, and they typically have mercury levels above published levels of concern for birds (Bechard et al., 2009). No data on mercury concentrations in adult eagle tissues have been published for the mid-Atlantic region of the United States (for eggs, see Wiemeyer et al., 1984), which includes Chesapeake Bay and is one of the bald eagle's major population strongholds. Chesapeake Bay (Fig. 1), the largest estuary in North America, has numerous tributaries that are subject to mercury fish consumption advisories and contains fish species with elevated methylmercury concentrations (Mason et al., 2006).

Recent research on avian mercury concentrations, especially for species of conservation concern, has focused on non-lethal sampling techniques, such as analysis of addled eggs, blood and feathers. Feathers accumulate mercury from the blood supply only during the few weeks that they are growing, but mercury concentration established at that time remains stable indefinitely and can be used to track historical trends in environmental mercury (Appelquist et al., 1984; Thompson et al., 1992). A feather that is grown on a breeding territory one summer, carried throughout the year, and molted near the nest the following summer will provide information about mercury in the diet during the weeks or months prior to the previous breeding season. Because eagles are highly territorial, and use the same territories for multiple years, freshly shed adult feathers collected near nests during the breeding season are likely to bear reliable information about mercury exposure on that territory. Even if the parents migrated, shifted diets, or altered their territory boundaries during the non-breeding season, feather mercury will not be affected by these changes. Sampling freshly molted feathers is a non-invasive and inexpensive way to monitor mercury exposure (Furness et al., 1986), but little has been published about the reliability of this increasingly popular technique (however, see Bond and Diamond, 2008). Thus, one objective of this study was to determine whether sampling a single adult eagle feather near a nest provides meaningful assessment of the overall mercury concentration in the parents' plumage.

The other objective of this study was to determine whether adult bald eagles breeding in the Chesapeake Bay are accumulating mercury at concentrations comparable to those reported for bald eagles in other parts of North America. These results are important because (1) they fill a major gap in the continent-wide assessment of contaminant levels in this important bioindicator

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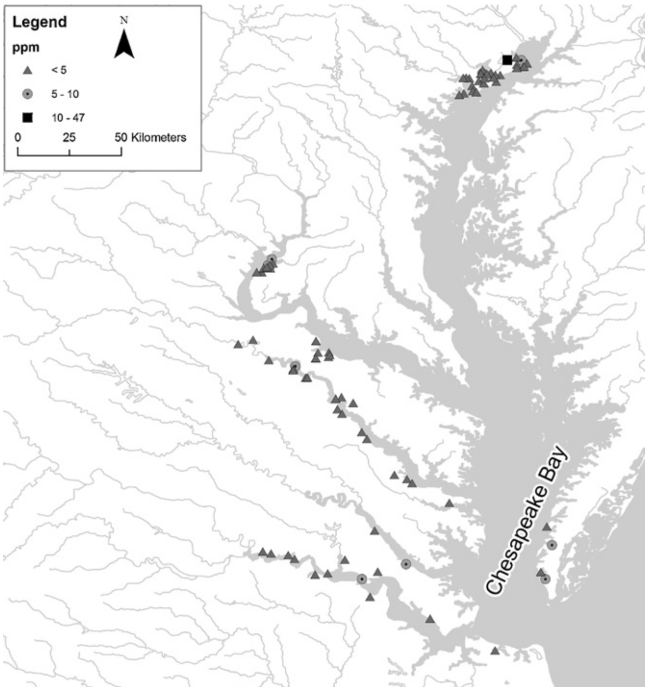

Fig. 1. Distribution of samples across Chesapeake Bay, with each symbol indicating one adult eagle feather mercury level.

species, (2) they establish a baseline against which to compare data gathered after any future change in mercury pollution policy, and (3) they are a first step in determining whether mercury is an ongoing conservation concern for this formerly endangered species.

2. Materials and methods

2.1. Sample collection

At 83 occupied eagle nests, a single body feather, determined to belong to an adult bird by wear, color and texture, was collected from the ground within 50 m of the nest in June–October 2007–2009. Chesapeake Bay bald eagles are territorial and resident year-round, and during or after nesting, adults frequently shed molted feathers while roosting or feeding on their nest tree or neighboring trees. It was not known whether the collected feather was from the male or female parent, but sampled feathers had been shed within a few weeks of collection based on condition and location. Because eagles in this population defend nest sites year-round, it is extremely unlikely that adults other than the parents would shed a feather near a nest tree. Feathers were stored at room temperature in a paper envelope until analysis for mercury concentration. Nests were located on all of the major tributaries of the Chesapeake Bay in Maryland and Virginia, USA (Fig. 1).

To determine the variance among body feathers on individual eagles, 20 body feathers were sampled from each of 20 adult eagles. These were either salvaged after accidental powerline electrocution, by the authors under permit ($n = 6$), or were injured or dead eagles ($n = 14$) found by the public and sampled by staff at The Wildlife Center of Virginia, in Waynesboro (www.wildlifecenter.org). Feathers were plucked or clipped at the base, 10 from the breast and 10 from the back, avoiding feathers from adjacent areas that likely molted simultaneously. Birds that had been in captivity for more than a few days, and thus might have molted feathers on a provisioned diet, were excluded.

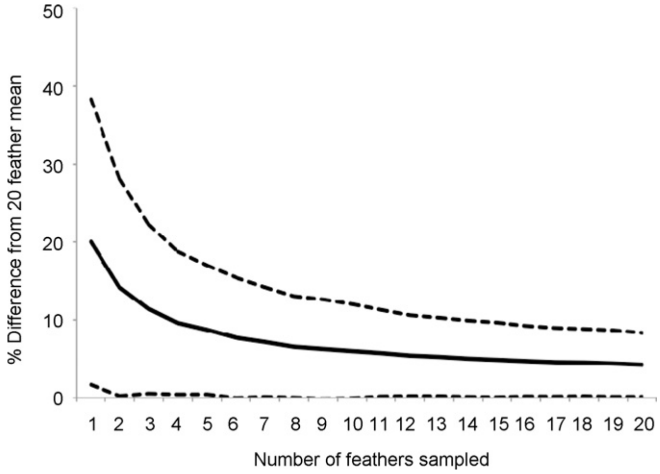

Fig. 2. Results from bootstrapping model of the accuracy of using different numbers of feathers to estimate mean feather mercury. Solid line is the average percent difference of the mean using various numbers of feathers from the 20-feather mean. Dashed lines are one standard deviation around the mean.

2.2. Mercury analysis

Samples were analyzed on a Milestone DMA-80 (Milestone, Shelton, CT). Each feather was washed with deionized water for 1 min to remove particulates, dried in a low humidity chamber for 48 h, and chopped into 1 mm pieces and mixed by hand for 1 min. Reported mercury values are technically wet weights, and it should be noted that we did not freeze-dry feathers. In most cases, two samples weighing approximately 0.02 g were run from each feather and their mercury value averaged; for smaller feathers the mercury value was based on one sample weighing approximately 0.02 g. Two blanks, two method blanks, and two samples of each of two certified reference materials (DORM-3, DOLT-4, National Research Institute, Canada) were run with each batch of 20 samples. The mercury analyzer was calibrated prior to the first samples being run and monthly thereafter. The factory calibrated minimum detection limit for the analyzer was 0.005 ng mercury. Recovery of standard reference materials was $103.0 \pm 3.9\%$ for DORM-3 ($n = 106$) and $99.3 \pm 4.8\%$ for DOLT-4 ($n = 106$) (means presented with SD throughout). When we spiked chopped domestic bird feathers with DOLT-4 ($n = 10$), recovery was $100.0 \pm 1.5\%$. The mean relative percent difference between pairs of feather samples run as duplicates was $7.0 \pm 6.6\%$ ($n = 111$).

2.3. Statistical methods

In order to assess the accuracy of using a single feather to estimate feather mercury levels in an individual eagle, we used the 20 feathers collected from each of 20 eagles found injured or freshly dead. We determined mercury concentration in each feather separately and calculated an average mercury concentration for each bird based on all 20 feathers. We then calculated the coefficient of variance (CV = standard deviation/mean) for each bird and calculated the average CV for all 20 birds. We then used the average CV to approximate a standard deviation (SD) around a single feather measurement (~SD = feather value × average CV).

To evaluate the optimal number of feathers to collect per bird in future studies we used a bootstrap resampling in which we selected a feather value at random from any of the 20 birds from which we had 20 feathers. We then calculated the percent difference of the mercury concentration of that feather from the mean feather mercury concentration based on 20 feathers from the same eagle. We repeated this process 10,000 times and determined mean

Table 1
Average adult bald eagle feather mercury (Hg) levels from North American states and provinces.

Location	Hg (ppm)	Date	Source
Virginia/Maryland	3.8	2007–2009	Body feathers; this study
Alaska	5.1	2004	Wing feathers; Burger and Gochfeld (2009)
Florida	11.5	1991–1993	Body feathers; Wood et al. (1996)
Montana	13.0	2006–2008	Body feathers; Harmata (2011)
Great Lakes	15.8	2002–2010	Body feathers; Rutkiewicz et al. (2011) [a]
Idaho	18.7	2004–2006	Mixed feathers; Bechard et al. (2009)
British Columbia[b]	21.0	2001–2002	Wing feathers, Weech et al. (2006)
Michigan	21.5	1985–1989	Mixed feathers; Bowerman et al. (1994)
New York	30.9	1998–2006	Mixed feathers; DeSorbo et al. (2008)
Maine	38.3	2001–2006	Mixed feathers; DeSorbo et al. (2009)

[a] Samples included a small percentage of nestling and immature birds.
[b] Sites included a lake impacted by a mercury mine.

difference of a single feather from the 20-feather mean for the same bird. We then repeated this process selecting 2–20 feathers at random with replacement and calculating a mean based on each number of feathers sampled and comparing that to the 20-feather mean. This allowed us to determine how much accuracy is gained by sampling each additional feather from a bird. It should be noted that this calculation was based on feathers known to be from the same bird.

3. Results and discussion

3.1. Single feathers as bioindicators

Resampling of 20 feathers from 20 eagles indicated that variation in mercury concentration among feathers on a single bird was relatively high. Only 15.1% of feathers had mercury concentrations within 5% of the mean concentration based on 20 feathers from the same bird, 38.3% of feathers were within 10% of the mean, and 71.9% of feathers were within 25% of the mean. The variation in mercury concentrations among feathers from a single individual is likely explained by the fact that bald eagles rarely, if ever, replace all body feathers within a single molt (Pyle, 2008), thus feathers collected may represent mercury burdens over several years. We calculated a 95% confidence interval around any given feather of 50.1%, which is applicable to the single feathers we collected from eagle nests in evaluating whether they are above or below levels of concern, and has applicability to any estimate based on a single feather. To produce an estimate that was within 10% of the mean based on 20 feathers one would have had to sample >4 feathers from a single bird at each nest (Fig. 2). It has long been appreciated that collecting more than one feather per individual is desirable for mercury sampling (Furness et al., 1986; Thompson et al., 1991), and high intra-individual variation was reported based on five feathers from individual seabirds (Bond and Diamond, 2008). Our results are the first statistically derived guideline for the minimum number of feathers to sample, and more importantly we are the first to estimate a population-level mercury concentration with a confidence estimate based on collecting single feathers. Because feathers sampled from beneath a nest can come from both members of the pair if they are molting simultaneously, the recommended sample size presented here is applicable when only one member of a pair is molting, when feathers can be collected under a roost used exclusively by one member of the pair, or if feathers are otherwise known to be from the same bird (e.g. collected during capture). It should also be noted that intra-individual plumage variance might be greater in areas with higher mercury exposure than experienced by the 20 injured birds and carcasses we sampled (4.27 ± 3.80 ppm; range 0.51–13.1 ppm), and thus more feathers should be sampled from each individual at such sites.

3.2. Mercury concentration

There was no indication of particular mercury hotspots or geographic trends, with the birds highest in mercury scattered around the study area (Fig. 1). However, it should be noted that sampling of the eastern shore of the Chesapeake Bay was not extensive. The average concentration of mercury in the feathers of bald eagles sampled in the Chesapeake Bay region was 3.82 ± 5.15 ppm. Based on the 95% confidence interval calculated, the mean plumage mercury concentration for the nesting eagles we sampled was between 1.91 and 5.73 ppm. Because we washed feathers in water, rather than a solvent, traces of exogenous mercury or mercury from preen gland oil may have been present and this range may be an overestimate.

In a recent study (2004–2006) from the northeastern USA, feathers from adult eagles in freshwater habitats in Maine averaged 38.3 ppm (DeSorbo et al., 2009). A less recent study from the southeastern USA reported 11.5 ppm in Florida in 1991–1993 (Wood et al., 1996). Overall, the levels we report for the Chesapeake Bay are the lowest eagle feather values yet sampled in North America and we have documented the first population in which nearly all individuals are below the typical level of concern for feather mercury (Table 1). This low mercury level, relative to other regions, is consistent with the robust recovery and rapid population growth rate observed in the region (Watts et al., 2008).

3.3. Is mercury affecting bald eagles?

It is not yet possible to set a reliable lowest observed adverse effects level for bald eagles based on literature from this or any other bird species. The lowest level commonly cited in the literature for feather concentrations is 5 ppm (fresh weight, 7.5 ppm dry weight), which is based on a widely cited review by Eisler (1987) but not on data from raptors or free-living birds. Another feather mercury value, 40 ppm, is sometimes cited as being associated with sub-lethal effects in free-living individual birds, based primarily on a study of feather asymmetry in common loons (Evers et al., 2008) and declines in populations of European raptors during a period of heavy environmental mercury exposure (usually attributed to Berg et al., 1966). In contrast, a recent dosing study in which captive raptors ate a diet containing 3 ppm mercury and attained primary feather mercury levels of 275 ppm suggests that reproduction and health were not compromised over a short time span even with exposure resulting in extremely high feather levels (Bennett et al., 2009). The range of values for possible adverse effects spans almost two orders of magnitude and is therefore nearly useless. In addition, the evidence linking effects and particular mercury levels is mostly indirect. Further work is urgently needed to establish lowest observed adverse effects levels for sub-lethal effects in birds.

Recent research on free-living tree swallows (a songbird, *Tachycineta bicolor*) with an average of 14 ppm in primary feathers molted on site suggests that the birds experienced reproductive, immunological and endocrine effects (Brasso and Cristol, 2008; Hawley et al., 2009; Wada et al., 2009). Heinz et al. (2009) compared sensitivity to injected mercury of embryos across many avian species, and found that the two raptors included in the study were highly sensitive to mercury, in contrast to tree swallows, which were moderately sensitive, and mallards (*Anas platyrhynchos*), which had low sensitivity. This suggests that a prudent estimate for the low adverse effects level in adult eagle feathers would be concentrations below the 14 ppm, as this has been shown to affect the less-sensitive tree swallow. A level of 9–11 ppm in primary ("forewing") feathers impacted mallard reproductive behavior when carefully monitored in captivity (Heinz, 1979). Because raptors are more sensitive than either tree swallows or mallards (Heinz et al., 2009), it would be reasonable to expect reproductive or health effects in eagles at levels somewhat below the 9–14 ppm range in primary feathers. Two studies of bald eagles (Bowerman et al., 1994; Rutkiewicz et al., 2011) have reported no difference in mercury value for body feathers (used in the present study) and primary feathers (used in the mallard and swallow studies cited above), although the timing of migration and location of molt would cause this to be true only for certain individuals, populations and species. A recent study that reported mercury-associated neurochemical changes in the brains of some bald eagles in a population with primary feather mercury averaging approximately 15 ppm is in accordance with the threshold suggested here (Rutkiewicz et al., 2011).

While there was variation in mercury level between individual Chesapeake Bay eagles, only one of the 83 sampled had a mercury level (47.6 ppm) clearly warranting concern based on current literature described above, and only 11 birds (13%) were above 5 ppm, the lowest level of concern ever stated in the literature (Eisler, 1987).

4. Conclusions

We found that collecting single molted feathers from near bald eagle nests provides an estimate of mercury concentration that was sufficient to assess whether the population is experiencing exposure to levels of mercury stated to be harmful in the literature on birds. Using larger samples of feathers from injured and dead birds, we showed that for this species, despite considerable variation between individual feathers, collecting five feathers from individual birds should suffice to provide an estimate that is within 10% of the value of that bird's plumage. Feathers from adult bald eagles nesting on the Chesapeake Bay, Virginia and Maryland, USA, had the lowest mercury levels ever reported for a population of this species in North America. Mercury level in most individuals was below all cited thresholds of concern for sub-lethal effects. Sampling species of conservation concern for contaminants can be expensive and invasive. Many studies have been performed in the past using feathers, typically more than one, to estimate mercury levels (Furness et al., 1986; Thompson et al., 1991, 1992). We suggest that expensive and invasive sampling of blood or other tissues for mercury is sometimes not warranted given the utility of sampling molted feathers.

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