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Mercury Concentrations in Feathers of Adult and Nestling Osprey (*Pandion haliaetus*) from Coastal and Freshwater Environments of Florida

Darren G. Rumbold[1] · Karl E. Miller[2] · Timothy A. Dellinger[3] · Nicole Haas[1]

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Abstract We determined mercury (Hg) concentrations in feathers of osprey (*Pandion haliaetus*), both nestlings ($n = 95$) and adults ($n = 110$), across peninsular Florida and the Florida Keys during February–August 2014. Feathers plucked from nestlings, aged 3–7 weeks, contained Hg concentrations that ranged from 0.338 to 45.79 mg/kg and averaged 6.92 ± 7.58 mg/kg (mean ± 1SD). Feathers shed from adults contained significantly higher concentrations ranging from 0.375 to 93.65 mg/kg, with an average of 17.8 ± 16.1 mg/kg. These levels were in the upper range of previously reported feather Hg concentrations of osprey and clearly show that Florida continues to have Hg hotspots that are elevated compared with many other regions. While these concentrations did not exceed levels previously reported in osprey from heavily Hg contaminated areas that showed no evidence of reproductive impairments, we cannot rule out potential individual-level effects to highly exposed nestlings after fledging. Mercury concentrations in nestlings were highest in coastal habitats of Collier and Monroe counties, where ongoing declines in osprey populations also have been documented.

✉ Darren G. Rumbold
drumbold@fgcu.edu

Karl E. Miller
Karl.Miller@MyFWC.com

Timothy A. Dellinger
Tim.Dellinger@MyFWC.com

Nicole Haas
nicolefon10@gmail.com

[1] Florida Gulf Coast University, 10501 FGCU Blvd. South, Fort Myers, FL 33965, USA

[2] Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 1105 S.W. Williston Road, Gainesville, FL 32601, USA

[3] Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 27927 Tammi Drive, Tavares, FL 32778, USA

High mercury (Hg) levels were found in fish and wildlife in Florida's Everglades during the late 1980s (Ware et al. 1990; Spalding and Forrester 1991; Roelke et al. 1991). Surveys of Florida's coastal waters also found high Hg concentrations in marine fish (Hueter et al. 1995; Adams and McMichael 2001). The various physical, biogeochemical, and anthropogenic drivers responsible for Florida's Hg problem have been reviewed elsewhere (Fink et al. 1999; Guentzel et al. 2001; Orem et al. 2011). By the late 1990s and early 2000s, levels of Hg began to decrease in biota in certain regions of Florida (Rumbold et al. 2001; Frederick et al. 2002; Rumbold et al. 2002; Lange et al. 2005). These trends were initially heralded as evidence that local Hg emission reductions had succeeded (Atkeson and Axelrad 2004). However, subsequent monitoring revealed no evidence of declining atmospheric deposition in the Everglades and indicated numerous Hg "hot spots" persisted with new ones appearing (for review, see Rumbold et al. 2008). Moreover, trends in Hg levels for some biota recently have begun to increase. For example, median Hg concentrations in largemouth bass (*Micropterus salmoides*) bottomed out during the 2000s then subsequently began increasing (Axelrad et al. 2011). Fish consumption advisories are still in effect for most of the freshwater Everglades and for a large number of marine species in coastal areas (FDOH 2015). State agencies continue routinely to monitor Hg levels in fish in coastal waters and fish, wading birds, and occasionally other biota (e.g., alligators

[*Alligator mississippiensis*], panthers [*Felis concolor coryi*]) in the Everglades. There is, however, no large-scale monitoring program using a biosentinel inhabiting both Florida's freshwater and marine habitats.

Several characteristics make osprey (*Pandion haliaetus*) an excellent choice as a biosentinel in both freshwater lakes and rivers and estuaries (for review, see Grove et al. 2009). The osprey is a long-lived piscivore known to accumulate Hg, and it has a nearly worldwide distribution, which has led to its use as a biosentinel in a number of studies around the world. A considerable Hg dataset is available for comparative purposes (Grove et al. 2009).

We determined Hg levels in feathers collected in conjunction with a study of the taxonomic status of the southern Florida osprey population. The purpose of our study was twofold: (1) to use osprey as a biosentinel to assess the current status of Hg availability across the landscape, and (2) to assess whether Hg may be a possible stressor to osprey in southern Florida. Whereas most osprey populations in North America are increasing (Poole et al. 2002), the southern Florida coastal population has been in a steady decline since the 1970s (Kushlan and Bass 1983; FWC 2011). Accordingly, we are keenly interested in identifying all stressors potentially impacting this population.

Methods

The North American subspecies of osprey (*Pandion haliaetus carolinensis*) breeds throughout temperate North America and winters in the tropics. However, the population of *carolinensis* resident in southern Florida has unique characteristics not shared by other *carolinensis* populations. First, breeding phenology of osprey in southern Florida is much earlier than most of the North American population (Ogden 1977), with nesting in southern Florida beginning in late November (Bass and Kushlan 1982). Second, ospreys breeding in Monroe, Collier, and Miami-Dade counties appear to be nonmigratory (Martell et al. 2004; FWC 2011). Nonmigratory osprey in southern Florida thus can be considered good biosentinels for local environmental conditions.

We collected feather samples from focal areas along a latitudinal gradient ranging from southern Florida's nonmigratory ospreys to breeding sites of migratory osprey in north-central Florida during February–August 2014. Our goal was to sample 10–20 nests from each of the following focal areas: the lower Florida Keys (Monroe County), Florida Bay/Everglades National Park/upper Florida Keys (Monroe County), Naples/Marco Island/Ten Thousand Islands National Wildlife Refuge (Collier County), Sanibel Island (Lee County), West Palm Beach area (Palm Beach County), Lake Istokpoga (Highlands County), Blue Cypress Lake (Indian River County), St. Petersburg (Pinellas County), Indian River Lagoon (Indian River County), Leesburg (Lake County), and Gainesville (Alachua County). Within focal areas, we searched for active nests and solicited nest locations from Florida Fish and Wildlife Conservation Commission (FWC) offices, parks, refuges, land management offices, bird listservs, Audubon chapters, and rehabilitation facilities. We also collected samples opportunistically from nonfocal areas as we traveled from site to site.

We used a ladder, rope and mechanical ascenders, a climbing tree stand, or free-climbed to reach accessible nests when nestlings were well feathered but not yet able to fly (i.e., around 6 weeks of age). From these nests, we plucked 5–8 contour (body) feathers from nestlings (because their loss would not impede flying later) by grasping feather shafts as close to the skin as possible and pulling gently. It is important to note that to minimize disturbance nestlings were not removed from the nest and were handled as little as possible and, consequently, contour feathers were not always taken from the same body area (e.g., breast, shoulder). Additionally, any long flight feather clearly shed from an adult found in the nest or below the nest platform were collected; only one adult feather was analyzed per nest site (i.e., represents one adult).

We sampled multiple well-feathered nestlings from the same nest to ensure adequate samples. Plucked feathers from each individual were stored at room temperature in separate envelopes; shed feathers were stored together in one envelope. Feathers were later cleaned by pulling through a Kimwipe™ moistened with deionized water several times and then allowed to air dry. Hundreds of nest structures were located by FWC staff and volunteers, although not all were active. Among active nests, 47 were climbable and we plucked feathers from 1 to 3 chicks at these locations. Approximate age of chicks was estimated by monitoring the hatching date and growth and feather development of chicks (Poole et al. 2002). At 8 additional nests, we plucked feathers from chicks that had either died in the nest or on the ground under the structure. In the end, we analyzed 95 feather samples from chicks, including 26 broodmate pairs, one broodmate triplicate, and 40 individual chicks. We plucked feathers from 3 osprey adults found dead near nests. Finally, we received feathers collected from 30 ospreys by rehabilitation centers and feathers collected by Florida Power and Light staff from 3 electrocuted birds (age could not be determined in 18 of these dead birds, so they were not categorized as chick or adult). In total, 110 adult feather samples were analyzed.

Total-Hg concentration (includes all Hg species; hereafter designated as [THg]) was determined at Florida Gulf Coast University via thermal decomposition, gold amalgamation, and atomic absorption spectrometry (EPA method 7473) with a direct Hg analyzer (DMA-80, Milestone Srl—Via Fatebenefratelli 1/5, 24010 Sorisole, Italy), which has a working range of 0.03 ng–1500 ng Hg. It should be noted that the majority of Hg in feathers has been found to be in the form of methylmercury (Bond and Diamond 2009). Feathers (including rachis) were snipped into small pieces using stainless steel scissors and transferred to scintillation vials where they were cut into even finer pieces ($\approx$1 mm) small enough to fit within the combustion vessel (i.e., nickel sample boat) and mixed thoroughly. Calibration curves were generated using varying masses of Certified Reference Materials (CRMs): DORM-3 and DORM-4 (National Research Council Canada, Institute for National Measurement Standards, Ottawa, ON, Canada) and BCR-463 and ERM-CE464 (LGC Standards USA, 276 Abby Road, Manchester, NH). The correlation coefficient of initial calibration averaged 0.9984 ($\geq$0.9952, $n = 6$) on the low cell and 0.9998 ($\geq$0.9994, $n = 6$) on the high cell. The low cell was calibrated up to 20 ng. Initially the high cell was calibrated up to 281 ng; however, because several samples exceeded this amount, ERM-CE464 (which has a certified value of 5.24 mg/kg) was purchased and used to calibrate the high cell up to 829 ng. With the exception of two samples (which did not have sufficient mass remaining), all samples that exceeded the upper-limit of the high curve on the initial run were re-run. These same CRMs were also used for continuing calibration verification at the start and at the end of every batch of 20 samples. Percent recovery of continuing calibration verification check samples was 100.5 $\pm$ 0.04% ($n = 40$). Relative percent difference (RPD) between laboratory duplicate analyses of feathers was 12.5 $\pm$ 22% ($n = 22$).

Data analyses were conducted using Sigmaplot for Windows Version 11.0 software (Systat Software, Inc.). Assumptions of normality and equal variances were tested by the Kolmogorov–Smirnov and Levene median tests, respectively. Where necessary, Hg was log transformed to achieve normality or homogeneity of variance. Where transformations failed to achieve normality, a Mann–Whitney rank-sum test was used. Regression of Hg on estimated chick age met necessary assumptions. Results from the present study were compared to published means and standard deviations using a two-tailed *t* test (Sokal and Rohlf 1969). The *t* test, which was preceded by an *F*-test of equal variance, was performed using Microsoft EXCEL 2016 (Microsoft Corporation).

Results

Feathers plucked from nestlings all contained measureable Hg concentrations ranging from 0.338 to 45.79 mg/kg with a mean ($\pm$ 1 SD) of 6.92 $\pm$ 7.58, if based on all chick feathers individually ($n = 95$), and 7.18 $\pm$ 7.48, if calculated using average of broodmates ($n = 67$). The relative percent difference (RPD) of Hg concentrations between broodmates averaged 25.6% ($\pm$31.4%) in the 26 instances where broodmates pairs were sampled; this difference between broodmates was not statistically significant (paired *t* test; $t = -0.63$; $p = 0.5$).

Nestlings sampled from Florida Bay and the southwest coast of Florida frequently contained Hg levels that fell in the highest quartile (Fig. 1). Taken as a group, nestlings from SW Florida coastal counties (i.e., Monroe, Collier) had significantly higher Hg concentrations than nestlings from the rest of the Florida (Mann–Whitney rank-sum test; $U = 211$; degrees of freedom [df] = 33, 34; $p < 0.0.001$). The highest value (45.79 mg/kg) was from a 6-week-old nestling from Bob Key in Florida Bay; feathers from its broodmate contained 36.48 mg/kg. The minimum Hg concentration (0.338 mg/kg) was in a 3-week-old nestling in a nest located on Blue Cypress Lake in Indian River County; it is noteworthy that this was one of the youngest chicks sampled.

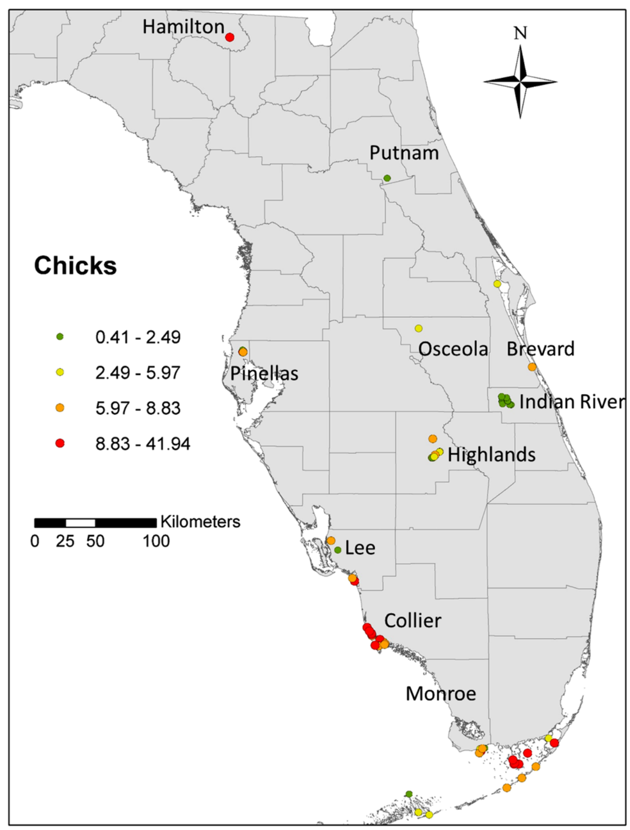

Fig. 1 Locations and Hg concentrations (mg/kg), by quartiles, of feathers plucked from osprey (*P. haliaetus*) nestlings in peninsular Florida and the Florida Keys (values are averaged for broodmates at 27 nests of the 67 data points). Names of counties where samples were collected also are shown

Variation in Hg concentrations in nestling feathers, when pooled across the state, was not explained by estimated age of the nestling (regression, $r^2 = 0.0005$, $p = 0.865$), likely due to the high spatial variability in methylmercury availability.

Not surprisingly, Hg concentrations in nestling feathers were significantly lower than levels in adult feathers (Mann–Whitney rank-sum test; $n = 110, 95$; $U = 2662$, $p < 0.001$). Feathers shed from adults had Hg concentrations ranging from 0.375 to 93.65 mg/kg (17.8 ± 16.1 mg/kg, $n = 110$). Similar to the nestlings, many of the adult feathers from Florida Bay and west coast also had levels that fell in the highest quartile of Hg concentrations; however, the geographical distribution was more widespread with more inland samples having higher concentrations (Fig. 2). Mercury concentrations in adult feathers collected from SW Florida counties thought to be nonmigratory (e.g., Monroe and Collier) did not differ statistically from other counties ($U = 778$; df = 91, 19; $p = 0.49$). The highest Hg concentration (93.65 mg/kg) occurred in an adult feather found below an active nest in Lake June-in-Winter State Park. Another adult feather shed at a nearby nest contained 21.89 mg/kg.

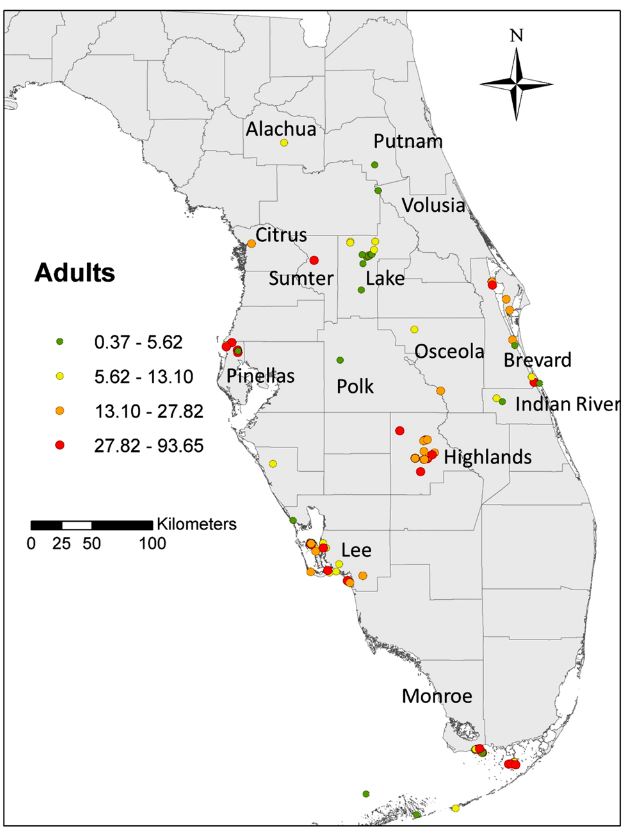

Fig. 2 Locations and Hg concentrations (mg/kg), by quartiles, in adult osprey (*P. haliaetus*) feathers shed at or near nests or taken from birds in rehabilitation centers in peninsular Florida and the Florida Keys (data points represent one adult feather per location, i.e., individual adult). Names of counties where samples were collected also are shown

An additional 18 dead birds (i.e., carcasses), which had indeterminate ages, were sampled at rehabilitation facilities and had an average Hg concentration of 9.9 ± 10.3 mg/kg.

Discussion

Our survey of Hg levels in feathers of osprey reaffirms their value as a biosentinel of both marine and freshwater systems and demonstrates that Florida continues to have Hg hotspots that are elevated compared to other regions.

Concentrations of Hg observed in the present study were in the upper range of values reported for osprey in the literature (Table 1). However, care must be taken in making these comparisons, because Hg concentrations are known to vary among feather types and age of the bird even among broodmates (Rumbold et al. 2001; Hopkins et al. 2007; Debén et al. 2012), and the present study included different feather types (contour feather from different body areas and flight feathers). Our results were consistent with a report of elevated Hg levels in feathers collected from osprey from Florida Bay during 2000–2001 (Lounsbury-Billie et al. 2008, Table 1); the samples in that study also included adult feathers that had molted and shed in and around nest. When we consider only feathers collected from Florida Bay during the present study (adult: 21.1 ± 15.9 mg/kg, $n = 17$; nestling: 11.9 ± 11.8 mg/kg, $n = 18$), Hg levels did not differ from Lounsbury-Billie et al. (2008) for either adults (t test with unequal variance, $t = -0.294$; df = 32; $p = 0.77$) or nestlings (t test with unequal variance, $t = 0.137$; df = 31; $p = 0.89$). As mentioned previously, the highest Hg concentration that we observed in a nestling feather was from Florida Bay. By comparison, concentrations reported in the present study for both adult and nestling feathers were much higher than levels reported in osprey recently sampled in Western Canada (Guigueno et al. 2012), Chesapeake Bay, Delaware Bay (Rattner et al. 2008), and Coastal South Carolina (Hopkins et al. 2007; Table 1). Although concentrations in the present study were higher than levels in osprey sampled years ago from the Great Lakes (Hughes et al. 1997; Table 1), they were lower than levels reported for osprey sampled years ago near the notorious Sulphur Bank Mercury Mine hotspot in Clear Lake (Cahill et al. 1998) and hydroelectric reservoirs in Quebec (DesGranges et al. 1998; Table 1).

Hg levels in Florida biota have long been known to be spatially highly variable (US EPA 1998). Subsequent surveys of biota have continued to document this extreme spatial variability in Hg availability (Rumbold and Fink 2006) and continue to show that Florida hotspots are

Table 1 Mercury concentrations (mg/kg) reported in osprey *(Pandion haliaetus)* feathers

Sampling Location	Year	Age Class	Feather type	N	THg (mg/kg) mean	±1SD	Source
North Central Quebec (near hydroelectric reservoirs)	1989–91	Adult		31	58.09	51.34; Max.193	DesGranges et al. 1998
North Central Quebec (near hydroelectric reservoirs)	1989–91	Nestling	Flight and body	78	37.35	20.09; Max. 101	DesGranges et al. 1998
Great Lakes and New Jersey	1991–94	Chick	Mantle	7–12	3.35–10.98	0.94–3.98	Hughes et al. 1997
Clear Lake, California	1992–96	Adult	Flight	12	20.0	11.0	Cahill et al. 1998
Clear Lake, California	1992–96	Juvenile	Flight	12	5.25	20.0	Cahill et al. 1998
Western Canada	1999–03	Adult	Breast	12	5.6	Max. 30.4	Guigueno et al. 2012
Western Canada	1999–03	Chick	Breast	12	3.9	Max 20.5	Guigueno et al. 2012
Florida Bay, Florida	2000–01	Adult	Flight and other	17	16.4	1.51	Lounsbury–Billie et al. 2008
Florida Bay, Florida	2000–01	Nestling	Breast	15	13.7	5.76	Lounsbury–Billie et al. 2008
South River, Chesapeake Bay	2001	Nestling	Body	12	0.530	Max: 1.16	Rattner et al. 2008
Inland Bays, Delaware Bay	2002	Nestling	Body	9	2.76	Max: 4.23	Rattner et al. 2008
Coastal South Carolina	2003–05	Chick	Scapular and breast	22	0.8	Max. 2.1	Hopkins et al. 2007
Coastal South Carolina	2003–05	Adult	Primary	17	5.2	Max: 21.7	Hopkins et al. 2007
Florida (coastal and inland)	2014	Adult	Flight	110	17.8	16.1, Max. 93.65	The present study
Florida (coastal and inland)	2014	Nestling	Contour	95	6.92	7.58, Max. 45.79	The present study

elevated compared with other regions (Evans and Crumley 2005; Rumbold et al. 2008; Axelrad et al. 2011; Evans et al. 2015). The higher Hg levels that we documented in osprey nestlings from Florida Bay and the southwest coast of Florida were consistent with Hg concentrations reported in fish from these areas (Evans and Crumley 2005; Rumbold et al. 2011; Thera and Rumbold 2014; Rumbold et al. 2014; Evans et al. 2015). It is important to remember that the present study did not target the freshwater marshes of the Everglades, which also is a known hotspot for Hg in fish (Axelrad et al. 2011). If osprey nesting in the Everglades had been included, the geographical distribution of concentration quartiles might have been different. As previously stated, the highest concentration quartile in adult feathers was geographically more distributed than nestling Hg (Figs. 1, 2) with the highest concentration occurring in a feather from an inland lake, Lake June-in-Winter. The lake is a known Hg hotspot in terms of average Hg levels in largemouth bass (0.72 mg/kg; T. Lange, FFWC, pers. comm.). However, nestlings sampled near Lake June-in-Winter did not have Hg levels in the highest quartile (Fig. 1). Because Hg is sequestered in the feather only during its growth, Hg levels in nestling feathers reflect local exposure and thus current, local conditions. Hg content in shed adult feathers represents local conditions only if adults used the same area at the time of the previous molt and feather replacement. Only ospreys breeding in Monroe, Collier, and Miami-Dade counties, as well as some individuals in Lee County, appear to be nonmigratory (Martell et al. 2004; FWC 2011). Therefore, high Hg concentrations that we recorded for some adults north of these counties may not be indicative of local conditions where they were sampled.

Assessing the toxicological significance of Hg levels observed in these osprey feathers based on comparisons with literature-derived toxicity reference values must be done carefully due to potential interspecific differences in sensitivity. Derivation of a critical tissue concentration in feather is further complicated because, unlike Hg in many other tissues (e.g., brain, liver, eggs), Hg bonded to keratin and sequestered in feathers no longer represents a risk to the bird (Burger and Gochfeld 1997). Thus, feather Hg has been used as an indicator of the Hg level and possible risk in the targeted organs, although it may not be the best predictor in chicks (Ackerman et al. 2011). Nonetheless, several benchmarks have been suggested. Burger and Gochfeld (1997), (2000) recommended 5 mg/kg in feathers as the benchmark for deleterious reproductive effects, such as lower clutch size and egg size. Another feather-Hg benchmark recently used (Schulwitz et al. 2015; Zillioux

2015) was derived from a study on captive birds by Frederick and Jayasena (2010). They found altered courtship behavior and depressed reproduction in white ibises (*Eudocimus albus*) dosed with Hg, even in the low-dose group of birds, which had feather Hg concentrations averaging 7.15 mg/kg (Frederick and Jayasena 2010). More than 66% of the adult osprey in the present study had feather Hg levels exceeding 7.15 mg/kg. Frederick and Jayasena (2010) speculated that the altered behavior that they observed was mediated through endocrine processes. A link between Hg and disruption of the endocrine system in birds was further strengthened by a recent study of black-legged kittiwakes (*Rissa tridactyla*) that skipped breeding (Tartu et al. 2013).

It is important to note that bald eagles (*Haliaeetus leucocephalus*) and ospreys nesting in those heavily Hg contaminated areas did not have depressed reproduction even though feather concentrations exceeded the aforementioned benchmarks (Bowerman et al. 1994; DesGranges et al. 1998; Cahill et al. 1998). DesGranges et al. (1998) reported the mean number of osprey young fledged from nests on the reservoirs in Quebec with the highest mercury exposure (Table 1) did not differ from nests with low Hg exposure. Furthermore, Cahill et al. (1998) also reported that reproduction was not depressed and population size remained stable in the highly exposed osprey on Clear Lake. Given that Hg levels in the present study and the survey of Florida Bay (Lounsbury-Billie et al. 2008) were lower than those of earlier studies that found no depressed reproduction (DesGranges et al. 1998; Cahill et al. 1998), it would appear that Hg is not a critical stressor increasing the risk of reproductive failure for the Florida osprey population. However, we cannot rule out potential individual-level effects to highly exposed nestlings after fledging, particularly after feather growth and this important Hg excretory route ceases. Mercury levels are known to increase dramatically in blood and other tissues after feather growth is complete (Spalding et al. 2000; Kenow et al. 2003; Ackerman et al. 2011), which has led some to argue risk increases immediately after fledging (Kenow et al. 2003; Rumbold 2005; Ackerman et al. 2011), particularly for naive chicks attempting to forage on their own for the first time. Unfortunately, due to the inherent difficulties of monitoring a large sample population of birds after they have fledged, data on possible impacts of Hg during this critical time are lacking.

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