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# WADING BIRDS AS BIOINDICATORS OF MERCURY CONTAMINATION IN FLORIDA, USA: ANNUAL AND GEOGRAPHIC VARIATION

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**Abstract**—Mercury contamination in wetland biota is often dynamic, difficult to predict, and costly to track. In this paper, we present results from a six-year study of growing feathers of piscivorous birds as monitors of wetland Hg exposure in Florida, USA, wetlands. Between 1994 and 2000, we collected feathers of growing great egret (*Ardea alba*) nestlings from colonies in the freshwater Everglades of southern Florida, and during 1998, feathers were collected from chicks of both great egrets and white ibises (*Eudocimus albus*) at a variety of colonies throughout peninsular Florida. Coastal colonies showed significantly lower feather Hg concentrations than did inland sites. Within the Everglades, we found significant effects of both geographic location and year on age-adjusted mean total Hg concentrations in feathers. Over the course of our study, Everglades colonies maintained their Hg concentration rankings relative to one another, but all showed strongly declining Hg concentrations (mean of 73% averaged across colonies, between 1994 and 2000). Using a previously established predictive relationship between Hg consumption in food and feather Hg for great egrets, we estimated that Hg concentrations in the aggregate diet of egrets have been reduced by an average of 67%. We conclude that the Everglades has undergone a biologically significant decline in Hg availability in the wetland food web, possibly because of decreased local inputs.

**Keywords**—Mercury Everglades Ciconiiformes Great egret *Ardea alba*

## INTRODUCTION

Populations of wild animals have often been used as bioindicators of environmental contaminants, and many studies suggest that levels of contamination in animals can serve to track fluctuations in contaminants in the environment [1]. Birds have been used as indicators of environmental contaminants, and bird feathers have been widely used for the indication of heavy-metal contamination [2–4]. Mercury has been shown to bind well with growing feather tissue, providing a history of contamination at the time that the feather was grown [2]. We previously demonstrated a direct and predictable relationship between cumulative Hg exposure in food and feathers of great egret (*Ardea alba*) young raised in captivity [5]. This work provided a firm link between Hg concentrations in growing feathers and Hg in fish.

Many aquatic ecosystems in Florida are known to be contaminated with Hg, to the extent that fish consumption advisories for humans have been issued for approximately 74% of the freshwater lakes and streams so far tested (T. Lange, Florida Fish and Wildlife Conservation Commission, personal communication). The Everglades in particular has shown very high contamination levels during the past decade, with potential effects on fish and wildlife populations, and risk to human fishers [6]. Yet predicting temporal and geographic differences in Hg contamination is difficult because Hg is known to be dynamic in the various biotic and abiotic pools of wetland systems, and particularly so in the shallow depression wetlands typical of the southeastern United States [6,7].

Piscivorous wetland birds can be important monitors of changes in Hg contamination for several reasons. First, many species are tertiary consumers, and contamination levels in birds are therefore representative of the contamination levels in the appropriate part of the aquatic wetland food web [8]. Second, the extremely high bioaccumulation of Hg in these animals may represent an important risk to the reproduction and health of these bird populations. Mercury contamination in piscivorous birds has been associated at various contamination levels with embryonic mortality and deformities [9,10] abnormal chick behavior [9,11], altered parental behavior [9,12], decreases in reproductive success [13,14], decreases in survival of adults and juveniles [15], and decreased health of young and adults [16–18]. At very high exposure levels, Hg may result in neuronal degeneration, convulsions, and death [17–20]. In herons (Ciconiiformes: Ardeidae), contamination levels similar to those measured in the Everglades have been associated with decreased health parameters [19], decreased fledging mass [5], decreased appetite and blood cell volume [20], and altered maintenance behavior and hunting behavior [21]. These sublethal effects are suggested to lead to decreased juvenile survival [5,6].

In this paper, we present the results of a seven-year study of Hg in growing feathers of piscivorous birds in Florida. We examine geographic variation in Hg exposure both within the Everglades wetland ecosystem, and within peninsular Florida, as reflected by growing feathers of nestling great egrets and, in one year, white ibises (*Eudocimus albus*). We hypothesized that Hg would be generally higher at inland sites than at coastal ones because inland freshwater marshes may be more prone to rapid recycling of Hg [7], and would have higher concentrations of Hg in fish [22]. Using established relationships between Hg consumption and contamination levels, we also were able to use the feather Hg contamination levels to estimate changes in fish contamination with Hg.

We chose great egrets as our main study animal because

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this species is a tertiary consumer in many wetland food webs (consume medium to large fish) [23,24], has a nearly worldwide distribution, and is known to exhibit high levels of Hg contamination in the Everglades wetlands of southern Florida [25]. At several colonies in 1998, we also collected feathers from young white ibises, which feed somewhat lower in the food chain (crustaceans and small fish) [26]. We used nestling birds for tissue collection because we were confident that while in the nest, their food came from a defined area around the nesting colony (~25-km radius) [27,28].

## MATERIALS AND METHODS

During April and May of each year of study (1994–2000, excepting 1996), we collected feathers from wading bird chicks of between 20 and 30 d of age (great egrets) and 14 and 20 d of age (white ibises); after that age, the chicks became too mobile to catch. Because Hg may accumulate in feathers with age of chick [5], we later standardized feather concentrations for age of chick. Because rates of bill growth are relatively invariant among individuals of a given age in ciconiiform birds [29,30], we used bill length (culmen, in mm) as an indicator of age. Using least-squares (LS) means, we then adjusted mean Hg concentrations for individual colony locations to a 7- or 8-cm culmen measurement in white ibises and great egrets, respectively (corresponding to ~ 16 and 28 d of age, respectively).

From each bird, we collected three to eight growing feathers (still erupting from sheaths, or had vascularized tissue or pulp visible on the shaft end) from the scapular region. Within any nesting colony, we collected feathers from the largest chick in each of up to 29 nests between April and June of each year. During air-conditioned storage in paper envelopes (more than three weeks in all cases), the pulp part of the feathers dried to a large extent. Although we did not dry feathers to constant mass, we have for this reason chosen to express concentrations as dry weight because it seems more accurate than fresh weight.

Individual feather samples were analyzed for total Hg concentrations by the Florida Department of Environmental Protection Chemistry Section in Tallahasee (FL, USA). Feather samples were digested with trace metal grade sulfuric acid and nitric acid, followed by 5% potassium permanganate. Samples were analyzed using a cold vapor atomic absorption spectrometer (Varian 30/40, Mulgrave, Australia; with deuterium background correction, fitted with cold vapor–hydride generator using stannous chloride reductant and automated with an SPS5 autosampler [Varian, Mulgrave, Australia]). A five-point calibration curve was created each day, and quality control samples for all runs included triplicate samples (rejection if agreement <10%), digestion blanks of deionized water, high (4 μg/L) and low (1 μg/ml) methylmercury choride sample matrix spikes, fish tissue standards (DORM-1 [National Research Council, Canada], 0.15–0.2 g), and a practical quantitation level standard inorganic Hg solution (0.25 μg/L). All Hg concentrations reported in this paper are for total Hg concentrations.

By means of the predictive relationship between Hg consumption and growing feather tissue established by Spalding et al. [5], we used feather Hg concentrations to back-estimate the average Hg per body mass consumed by nestling birds in different colonies

$$\text{cumulative Hg consumed/body mass} = \sqrt[1.1456]{\text{growing feather Hg in mg/kg dry wt}/8.0588}$$

We then used colony- and year-specific averaged body masses

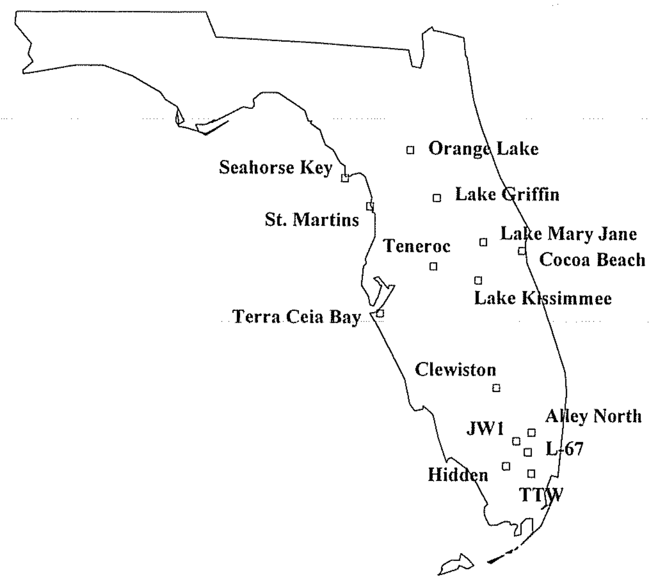


Fig. 1. Map of the state of Florida, USA, showing locations of all colonies sampled.

of chicks sampled, and body size–specific food consumption rates measured in the field [23,30] to estimate average concentrations of Hg in food items eaten by nestling great egrets.

We used analysis of covariance (SAS Institute, Cary, NC, USA) to estimate LS mean Hg concentrations within any colony or year, to adjust for the effect of age (bill length) in expressing the means. We examined colony, year, and bill length as potential sources of variation in the models. We used the same technique to assay for potential effects of colony, year, and inland or coastal location on feather Hg concentrations in the statewide sampling during 1998. We also used *t* tests in pairwise comparisons of LS mean feather Hg concentrations among colonies within years.

## RESULTS

We collected feathers from a total of 529 great egret chicks in a total of 7 colonies in the Everglades between 1994 and 2000. We also collected 43 samples from great egrets in six colonies, and 88 samples from white ibises in eight colonies outside the Everglades during 1998 (Fig. 1 and Tables 1 and 2).

Within the Everglades, mean concentrations of total Hg in colonies from all years ranged from 3.2 to 26.9 mg/kg dry weight (Table 1), with extreme values for individual birds ranging from 1.4 to 59 mg/kg. We found significant effects of colony ($F = 17.86, p < 0.0001$), year ($F = 72.97, p < 0.0001$), and colony × year interaction ($F = 7.77, p < 0.001$) on feather Hg concentrations from individual great egrets. We found no significant effect of bill length on Hg concentrations. Colony LS means were consistently different from each other within years (Table 1), suggesting local differences in exposure rate. The JW1 colony consistently showed the highest levels of Hg contamination in young birds in nearly all years, and was located in an area that also showed high (~0.4 ppm wet wt) Hg concentrations in whole mosquitofish (*Gambusia holbrooki*) [31] relative to other Everglades locations. Despite the consistent geographic variation in feather Hg concentration in nestlings, year also had a significant effect on Hg concentration, with a 73% decline in mean feather Hg concentrations in Everglades colonies between 1994 and 2000. From feather Hg, we estimated that Hg in the diet of young birds declined between 1994 and 2000 by 67% (Table 3, averaged over all

Table 1. Least-squares mean total Hg concentrations in feathers of nestling great egrets in the Water Conservation Areas of the Everglades (FL, USA) from 1994 to 2000. The LS means were standardized to an 8-cm bill size. Missing values indicate that data were not collected for that year and location, and LS means of colonies with different letters within a year are significantly different ($t$ test, $p < 0.05$)

| Colony | 1994 | 1995 | 1997 | 1998 | 1999 | 2000 |
|---|---|---|---|---|---|---|
| L-67 | | | | | | |
| LS mean | 16.28 B | 15.86 B | | 13.90 B | 5.50 BC | 3.28 AB |
| SE[a] | 0.889 | 1.178 | | 0.863 | 1.01 | 1.077 |
| *n* | 25 | 14 | | 26 | 20 | 17 |
| Tamiami | | | | | | |
| LS mean | | 12.14 C | | 6.30 A | 7.64 AB | |
| SE | | 1.10 | | 0.863 | 1.038 | |
| *n* | | 16 | | 26 | 18 | |
| Mud Canal | | | | | | |
| LS mean | 9.65 A | 6.96 A | | | | 5.93 AB |
| SE | 1.671 | 1.969 | | | | 2.887 |
| *n* | 7 | 5 | | | | 21.00 |
| 3b Mud | | | | | | |
| LS mean | | | 29.20 B | | 9.03 A | |
| SE | | | 1.97 | | 1.22 | |
| *n* | | | 12 | | 12 | |
| JW1 | | | | | | |
| LS mean | 26.87 C | 16.81 B | 25.18 B | 13.21 B | 3.97 C | 3.96 A |
| SE | 1.563 | 1.18 | 1.33 | 0.90 | 1.230 | 1.184 |
| *n* | 8 | 14 | 11 | 24 | 13 | 14 |
| Hidden | | | | | | |
| LS mean | 12.33 A | 7.67 A | 15.23 A | 6.06 A | 3.93 C | 3.80 B |
| SE | 0.924 | 0.880 | 1.665 | 0.863 | 0.941 | 3.130 |
| *n* | 23 | 25 | 7 | 26 | 22 | 16 |
| Alley North | | | | | | |
| LS mean | 13.31 AB | 7.11 A | 12.30 A | 5.93 A | 8.15 AB | 5.56 AB |
| SE | 1.392 | 1.221 | 0.889 | 0.818 | 0.985 | 3.340 |
| *n* | 10 | 13 | 25 | 29 | 20 | 21 |
| Annual CV | 42.6 | 41.3 | 45.6 | 41.7 | 40.9 | 25.9 |
| Annual number | 73 | 87 | 43 | 131 | 106 | 89 |

[a] SE = standard error of the LS mean.

colonies) and by as much as 87% in one colony (Hidden colony, 2.27 mg/kg wet wt in 1994, 0.09 mg/kg wet wt in 2000).

During the one year in which we sampled great egrets in colonies elsewhere in the Florida peninsula, we found Hg concentrations in nestling great egret feathers to be generally lower outside the Everglades than within (Table 2). With one exception, coastal colonies showed lower Hg exposure compared to inland locations. This was true when all colonies were included in the analysis ($F = 15.03$, $p = 0.0002$) but was only a marginally significant effect when locations with exceptionally high concentrations (Everglades colonies, Seahorse Key, FL, USA) were excluded from the analysis ($F = 3.62$, $p =$

Table 2. Summary of total Hg concentrations (mg/kg) in growing feathers of nestling great egrets and white ibises collected at locations in the Everglades and peninsular Florida, USA, during 1998. Least-squares (LS) means with different letters are significantly different ($t$ test, $p < 0.05$)

| | Great egrets | | | White ibises | | |
|---|---|---|---|---|---|---|
| Colony | *n* | LS mean[a] | SE[b] | *n* | LS mean[a] | SE[b] |
| Alley North | 29 | 6.068 | 0.6747 D | 13 | 1.422 | 0.3518 D |
| Terra Ceia | 7 | 2.047 | 1.3601 EF | 11 | 0.895 | 0.3886 D |
| Tamiami East | 26 | 2.257 | 0.7043 D | | | |
| Orange Lake | 8 | 3.534 | 1.2702 EF | | | |
| Cocoa Beach | 8 | 2.803 | 1.2873 E | 13 | 1.684 | 0.3838 CD |
| L-67 | 26 | 13.767 | 0.7036 B | | | |
| Hidden | 25 | 6.001 | 0.7054 D | | | |
| JW2 | 24 | 13.174 | 0.7325 C | | | |
| Lake Griffin | 6 | 2.783 | 1.4807 E | 12 | 2.037 | 0.3656 CD |
| Seahorse Key | 6 | 9.786 | 1.4660 C | 12 | 7.382 | 0.3748 A |
| St. Martin | 4 | 1.818 | 1.8182 F | | | |
| Teneroc | | | | 11 | 2.512 | 0.3819 BC |
| Lake Mary Jane | | | | 10 | 2.676 | 0.4662 B |
| Lake Kissimmee | | | | 6 | 4.023 | 0.5182 B |

[a] Concentrations are expressed as LS means, standardized to an 8-cm bill size for great egrets and a 7-cm bill for white ibises.
[b] Standard error of the LS mean.

Table 3. Estimated concentration of Hg (mg/kg food wet wt) in aggregate diet of nestling great egrets in Everglades (FL, USA) nesting colonies, as estimated from feather Hg concentrations (see methods for derivation procedure)

| Colony | 1994 | 1995 | 1997 | 1998 | 1999 | 2000 |
|---|---|---|---|---|---|---|
| L-67 | 0.3368 | 04021 | | 0.3017 | 0.1274 | 0.0872 |
| TTE | | 0.2950 | | 0.1441 | 0.2049 | |
| Mud Canal | 0.2325 | 0.1270 | | | | 0.1290 |
| 3b Mud | | | 0.4935 | | 0.2250 | |
| JW1 | 0.5464 | 0.3313 | 0.5255 | 0.2844 | 0.1044 | 0.0990 |
| Hidden | 0.2650 | 0.1683 | 0.3354 | 0.1300 | 0.1078 | 0.0950 |
| Alley North | 0.3082 | 0.1579 | 0.2613 | 0.1927 | 0.1899 | 0.1458 |

0.067). Of the coastal colonies sampled, only Seahorse Key showed high Hg concentrations (LS mean = 9.8 ± 1.47 mg/kg dry wt). Concentrations in great egret feathers from Seahorse Key were has high as in many of the Everglades colonies, and concentrations of Hg in white ibis feathers were significantly higher than the at one Everglades site at which this species was sampled in that year (Table 2).

## DISCUSSION

The main source of Hg exposure for great egrets in the Everglades is dietary [24], and very little of the total body burden of Hg in chicks is likely to come from the egg components [32]. Because a strong relationship has also been established between dietary Hg exposure and Hg in growing feathers of nestling great egrets, we are confident that the Hg concentrations we measured in the field were a reflection of Hg in the diet of young wild birds.

Our annual samplings in the Everglades and our statewide survey in 1998 indicated that Hg exposure varied significantly with geographic location of colony. In the Everglades, the geographic differences probably reflected the considerable geographic variation in methylation rates [33] and in Hg concentrations in soil [34], mosquitofish [31], and other aquatic animals [35]. In 1995, the geographic differences in mean colony feather Hg corresponded in a relative way to geographic differences in whole-body Hg found in mosquitofish [31]. An interesting feature of the great egret feather Hg concentrations in the Everglades was that the relative geographic differences were stable over time, with few changes in position of ranked colony Hg concentrations over the years. This suggests that geographic differences in concentrations of feather Hg in the Everglades are stable over time, at least when considering the scale at which great egrets are foraging from colonies (~25 km radius). However, this information by itself indicates only that relative magnitude of Hg exposure by location did not change over time and does not imply that Hg concentrations did not change over time. The period of study (1994–2001) in the Everglades was long enough to encompass considerable hydrologic variation, including a period of long inundation (1994–1998), of extremely deep water (1994–1995), and two years in which much of the marsh surface was exposed by drying, followed by reflooding (1999, 2000). Thus, the stability of geographic differences in Hg exposure for the birds was unlikely to be attributable to any particular water regime, at least at the large scale at which the birds forage.

Within peninsular Florida, the finding that coastal colonies had relatively lower exposure than inland sites fits with the general finding that small-bodied inland fishes have higher Hg concentrations than estuarine or coastal fishes [22]. Within this context, the very high Hg values encountered at Seahorse Key (a coastal site) in both great egrets and white ibises were a surprise. Birds from this colony may forage in the floodplain forests and estuarine wetlands of the Suwannee River immediately to the north of the colony, an area known to have high Hg concentrations in tissues of largemouth bass (*Micropterus salmoides*; T. Lange, personal communication). During the breeding season of 1999, we used small aircraft to visually follow 35 white ibises from the Seahorse Key colony to foraging locations. Of these, 49% flew to freshwater locations within the Suwannee basin, and the rest foraged in coastal salt marshes. This example serves to illustrate the importance of understanding foraging distributions of adults when interpreting Hg concentrations from nestling birds.

The marked decline in average Hg levels in the Everglades between 1994 and 2000 (average of 73%) was consistent in trend across the majority of colonies, suggesting that a real decline in Hg exposure in the diet of great egrets had occurred across the ecosystem. Further, this decline occurred during a variety of water conditions (as above), suggesting that the trend in exposure was not the result of water conditions alone. By extrapolating from the feather Hg dynamics, we estimated a 67% decline in Hg content of the great egret prey items during the study. Contemporaneously, Lange et al. [36; T. Lange, unpublished data] found a steady decline in concentrations of age-standardized fillets of largemouth bass in the Everglades, from 2.3 mg/kg wet weight in 1992 to 0.4 mg/kg wet weight in 2000 (82% reduction). The realization should be made that the bass were sampled in canals rather than in the marsh where the birds feed, and that great egrets rarely eat fish as large as bass [23]. Nonetheless, the Hg concentrations in a piscivorous bird and a piscivorous fish from the same ecosystem both declined by roughly the same percentage over the same time period, suggesting that some general reduction occurred in Hg concentrations in the aquatic food web.

The mechanism by which Hg concentrations declined is difficult to pinpoint. One of the most important sources of Hg deposition in the Everglades may be municipal and medical waste incineration in the metropolitan areas of Dade and Broward counties, although some debate exists about sources and mass transport [6,37]. Mercury emissions from municipal waste incineration have declined nationally since 1990 as a result of decreased Hg in waste. In Florida, state regulations phased in during the early 1990s mandated the use of scrubbing systems on incineration stacks, which may also have reduced Hg emissions. We suggest that these decreases in local emissions are one likely explanation for the decreases in Hg concentrations in Everglades biota.

The potential effects of this reduction in Hg for great egrets may be biologically important. The peak exposure levels recorded in feathers in 1994 (12–26 mg/kg dry wt, depending on colony) often exceeded the feather tissue values (5–40 mg/kg) suggested by Scheuhammer [18] to be associated with impaired reproduction. This is a necessarily conservative comparison, because the Everglades birds were measured while still in the nest, and would have likely accumulated considerably more Hg over the two years it can take to become reproductive. Adult feather values during the same period regularly exceeded the 20 mg/kg suggested by Scheuhammer [18] to result in substantial risk to the birds [38]. The average feather concentrations in 1994 were in the range of values associated with reduced packed-cell volume and loss of appetite of great egrets in a laboratory setting [5,20]. However, we have presented evidence elsewhere to suggest that effects in the field are likely to be considerably underestimated by lowest-observed-adverse-effect levels measured in stress-free captive environments [20]. By comparison, the feather concentrations we measured in 2000 (0.45–0.77 mg/kg dry wt) were well below any hepatic or feather tissue threshold so far suggested for impairment [5,9,16–20].

We believe that the evidence presented here supports the use of feather tissue from piscivorous birds for assaying Hg contamination in the upper end of the food web of wetlands, especially at a large geographic scales. The successful use of this method and its adaptation to other ecological situations rests heavily upon three things: the use of young birds that can accumulate Hg only from their immediate surroundings; an empirically measured relationship between Hg ingested and Hg concentrations in the tissue sampled; and an understanding of the distance from the nest at which food is generally obtained by parents.

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