Appendix 2B-4: A Retrospective Study of Mercury Contamination in Avian Tissues from South Florida

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Final Phase

Final Report
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Background and Problem Statement:

The south Florida environment and particularly the Everglades ecosystem is known to be heavily contaminated with mercury, as evidenced by very high levels in virtually all of the high trophic level consumers. The source of mercury is atmospheric in nature, although the relative contributions of local and distant sources are a subject of some debate. In addition, a study of sediment cores has demonstrated that local mercury deposition in the Everglades has increased by a factor of at least three over the course of the 20th century. It is less clear whether mercury in tissues of biota have increased concomitantly. Studies of mercury contamination in other food webs have suggested that concentrations in Everglades biota can be expected to be in rough proportion to deposition. However, the lag time between deposition and accumulation in biota is poorly known, and for highly mobile animals such as migratory birds, there is always the possibility of contamination or depuration at distant sites. Thus a study of past mercury contamination in Everglades predatory wildlife could firmly establish whether or not mercury exposure has increased during this century, and if mercury contamination has increased, by approximately how much.

In addition, a solid database of historical contamination levels could be instrumental in providing a basis for setting acceptable contamination rates. Although acceptable contamination criteria for pollutants in animal tissues should be empirically determined based on effects, there are numerous logistical problems in determination. These criteria involve large uncertainties in determining interspecific differences in response, picking appropriate endpoints, and in guessing at the individual importance of multiple stressors. In contrast, if "background" (= prior to widespread human

contamination) levels can be established, this gives contaminant managers a "safe", though possibly conservative number to aim for. Given limited time and resources for reducing the uncertainties of effects-driven criteria, an understanding of contamination rates from some period when pollution levels were known to be acceptable would be of great value.

Bird feathers are excellent tissues for measuring mercury accumulation rates, over both short and long time scales. When taken up by growing feather tissue, the chemical bonds are known to be extremely strong (=covalent), and mercury is known to be stable for very long periods in feather tissue. Contamination of feather tissue with mercuric substances used in preparation of museum specimens is an obvious problem, which can be avoided by measuring methylmercury. Methylmercury was never used in skin preparation practices since its source until recently has been entirely biogenic. Methlymercury usually constitutes a naturally high proportion of total mercury in avian feathers. This means that methylmercury can be used as an honest indicator of total mercury load in feathers, and that it is highly unlikely to have come from post-mortality sources.

Historical records of feather mercury can therefore be obtained from museum specimens, and can be compared with feather mercury samples from recent years. This provides a means to look at trends in contamination rates, and to establish mercury concentrations that are representative of an earlier, less polluted period. Feathers from wading birds (Ciconiiformes) are of particular value for this work in south Florida, since there exists a sizeable and geographically diverse sample of feathers from the past ten years (1990 – 2000). In addition, there is a reasonable understanding of the effects of

age, sex, geographic location, and mercury exposure on feather mercury for some species of birds in the south Florida area. Thus there is some understanding of the effects of many of the variables that could otherwise confound comparisons with historical specimens.

This report concludes the final phase of research initiated in 2000, aimed at identifying and collecting feathers from museum specimens of birds from south Florida in an effort to derive historical mercury trends and dynamics in the Everglades ecosystem. Phase I concentrated on identifying museum specimens that were likely to provide scientifically defensible comparisons for mercury in various historical periods. That work was also a feasibility study because it was not initially clear that enough specimens existed to justify these comparisons. Although there did not appear to be enough specimens in one of the historical periods for most species (pre-1900), there are enough in our judgement to proceed with comparisons during various parts of this century.

Phase II of this project was initiated in February 2001. This phase was directed at the actual collection of feather samples from museum collections, followed by submission of samples for analysis by the DEP chemistry section. The final phase (begun in October 2002) has been to complete analysis of the dataset and interpretation of results, as reported here.

Methods

Study Species: We chose four species as our study animals: Great Blue Heron (*Ardea herodias*), Great Egret (*Ardea albus*), White Ibis (*Eudocimus albus*) and Anhinga (*Anhinga anhinga*). As tertiary consumers in the Everglades food web, Great Blue Herons and Great Egrets are ideal indicator species for mercury contamination, since we have the best understanding of Hg dynamics, bioaccumulation rates, and effects on these species. We have also collected feathers from White Ibises and Anhingas, as these species have historically been common in the Everglades and are abundant in museum collections. Also, Anhingas are typically located only in freshwater, so any specimens collected from the area of interest were most likely feeding on freshwater fish from the Everglades system. Only adult specimens were considered in this study.

Geographic Location: We accepted specimens with records from any locations within the freshwater Everglades ecosystem, including previous inland freshwater sites that may have been developed into drier urban areas since the time of collection (e.g. western Miami locations). These inland sites include Big Cypress and the Everglades slough and tree island habitat (currently Water Conservation Areas and Everglades National Park. We also included some areas that were once freshwater (Cape Sable) but have since become saline through the action of hurricanes. Specimens were only considered for sampling if valid museum tags were attached, with collection locations specific to at least the county level. Florida County locations were matched with contemporaneous boundaries, since county designations have changed considerably during the 20th century.

Searching for specimens: Specimens were identified in museum collections by searching on-line museum collection lists as well as visiting museums for a direct examination of their archives. A list of museum specimens previously identified by Bill Robertson and the EPRI team (previous study carried out under DEP contract) was also used.

Specimen handling: Generally, museum policies prohibited removal of feathers from the scapular region, where most of the field-collected feathers from 1990 – 2000 were taken from. When collecting from museum specimens we therefore collected body contour feathers from roughly the middle of the abdominal region. Individual feathers were collected by isolating them with forceps, and snipping them as close to the skin as possible with a clean pair of surgical scissors. We wore surgical gloves when collecting and handling feathers, and the feather sample (5 – 10 feathers) was placed into a clean zip-lock plastic bag and individually labeled. Samples were stored at room temperature for a period of up to several months prior to mercury determinations.

Mercury determination: Mercury determinations were performed on samples from individual birds at the Florida Department of Environmental Protection Chemistry Section in Tallahassee Fl. For each sample, the feathers were first rinsed in a dilute acid solution to remove any particulate matter and any mercury used as a preservative that might be clinging to the surface of the feather. The rinsate samples were then analyzed individually for total mercury concentration. The rinsed feathers were then weighed and digested with reagent grade nitric acid, and the resulting solution analyzed for methylmercury using the XXXX technique.

The sample of feathers collected from museum specimens was compared with samples collected more recently from the Everglades. In all cases, the recent samples were from adult birds from the freshwater Everglades region. These feathers were taken from roadkills and birds in rehabilitation centers (scapular feathers, from Great Egrets and Great Blue Herons). In the case of Great Blue Herons, breeding plume feathers (head and neck region) were also collected (1994) from underneath individual active nests in WCA 3 of the Everglades. In the case of ibises, feathers were collected from the scapular region from adult birds trapped at feeding locations in the Everglades (Heath 2002). Anhinga feathers were taken from the scapular region from adults trapped on the nest in 2002, or from adult feathers collected from underneath individual nests.

All samples collected during the 1990's were analyzed for total mercury at the Florida Department of Environmental Protection Chemistry section in Tallahassee. 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, Palo Alto California USA, with deuterium

background correction, fitted with cold vapor/hydride generator using stannous chloride reductant and automated with an SPS5 autosampler). 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 ug/l), low (1 ug/ml) methylmercury choride sample matrix spikes, fish tissue standards (DORM –1, 0.15 – 0.2g), and a practical quantitation level (PQL) standard inorganic Hg solution (0.25 ug/l). All Hg concentrations reported in this paper are for total Hg concentrations.

Results

In aggregate, we collected feather samples from a total of 73 individual birds of the four species between the period 1900 – 1980, and 191 birds from post-1990 (Table 1).

Table 1. Numbers of individual birds sampled for feather tissue during this study.

	Pre-1980	Post-1990	Total
Great Egret	7	37	**44**
Great Blue Heron	12	49	**61**
White Ibis	33	98	**131**
Anhinga	21	7	**28**
Total	**73**	**191**	**264**

Generally, we found no acceptable specimens in museum collections collected after the late 1970's, and usually no specimens at all of the study species from this period. The

reason for this gap is unclear, but it was almost universal in the museums we visited, suggesting a cultural phenomenon among museum curators, or perhaps that collections were considered largely complete at that point. In any case, it is clear that the gap in the history of specimens was a function of the museum collections, and not our sampling regime.

We found very little evidence that the feathers were contaminated with inorganic mercury from preservatives. The rinsate from feathers (all species combined) contained an average of only 0.135% of the total mercury found in the feathers (methylmercury plus mercury in rinsate, see Table 2).

Table 2. Museum feather mercury concentrations, comparing mercury in rinsate with the total of all mercury in the feather sample (methyl plus total in rinsate).

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	Mean total Hg, ppm	S.D. Total Hg	Mean Hg in rinsate, ppm	S.D. Hg in rinsate	Proportion of total Hg in rinsate
Great Blue H.	3.3456	3.2022	0.0084	0.0058	0.0025
White Ibis	1.0422	0.7616	0.0029	0.0021	0.0028
Great Egret	2.7738	3.3744	0.0038	0.0019	0.0014
Anhinga	1.8627	2.6520	0.0018	0.0010	0.0010

The very small proportion of inorganic mercury found on the surface of feathers suggests that the feathers were not generally contaminated with mercury picked up in the preservation or storage process, such as mercuric preservatives. We therefore felt confident that the mercury we measured in the methylated mercury in the feathers

constituted an overwhelming proportion of all mercury present, and was a good measure of feather mercury concentrations at the time the bird was collected.

Our sample of feathers from museums represented only body contour feathers (= abdominal feathers), while our samples from the 1990's contained mostly feathers from the scapular region. Since mercury concentration might be affected by location on the body, this factor might bias any comparison of museum and 1990's specimens.

To test for consistent bias in concentrations of mercury in scapular and abdominal feathers, we used a group of 17 adult birds (9 White Ibises, 4 Anhingas, 3 Great Egrets and one Great White Heron) for which we had feathers from both abdominal and scapular regions. These birds were sampled in 2002 from either a rehabilitation center (Great Egrets, White Ibises, Great White Heron) or from adults captured on their nests in WCA 3 (Anhingas). Because sample sizes were small for all species, we pooled all species together for analysis. We compared the magnitude of difference in total mercury concentration between abdominal and scapular feathers in two different cases: those where abdominal feathers from an individual had the higher mercury concentration, and those where the abdominal feather had the higher mercury concentration (Table 3).

Table 3. Comparison of mercury concentrations in scapular and abdominal feathers.

Case	N	Mean difference In Hg concentration, ppm	Standard deviation Of difference in Hg, ppm
Abdominal > Scapular	7	2.62	3.27
Scapular > Abdominal	10	1.81	1.40

The numbers of cases in which abdominal feathers had higher concentrations than than scapulars from the same bird, was similar to the numbers in which the opposite was the case (7 vs 10 cases). We also found no consistent difference in the mean difference in concentrations between the two cases (abdominal>scapular and vice versa). This suggests that while there may be differences in mercury concentration of feathers depending on where they are located on the body, the direction of those differences seem to be randomly distributed among individuals. Further, the magnitude of differences seems to be no greater in cases where abdominal feathers had higher concentrations, than in cases where the opposite were true.

While this does not constitute a rigorous rejection of the hypothesis that there is a bias in mercury concentration depending on feather location, it is clear that none of the evidence that we have supports the hypothesis. More importantly, the variation in Hg concentration that seems due to feather location (means of 1.8 – 2.6 ppm) do not seem large enough to explain the differences we found when comparing museum feathers with feathers collected during the 1990's (see below).

The concentrations of methylmercury (museum specimens) and total mercury (1990's specimens) are compared by species in Figures 1 – 4. These plots suggest a common pattern among the four species. Generally, mercury concentrations in the post-1990 era were considerably higher than during the pre-1980 era. Further, although sample sizes are small for some species, the pre-1980 samples showed no obvious trend in any of the species, either declining or increasing. This does not appear to be an effect of sample size. The lack of trend seemed most convincing in fact, for White Ibises, which had the largest sample size pre-1980.

Figure 1. Concentrations of total mercury (mg/kg dry weight) in feathers of individual Anhingas from freshwater areas of the Everglades.

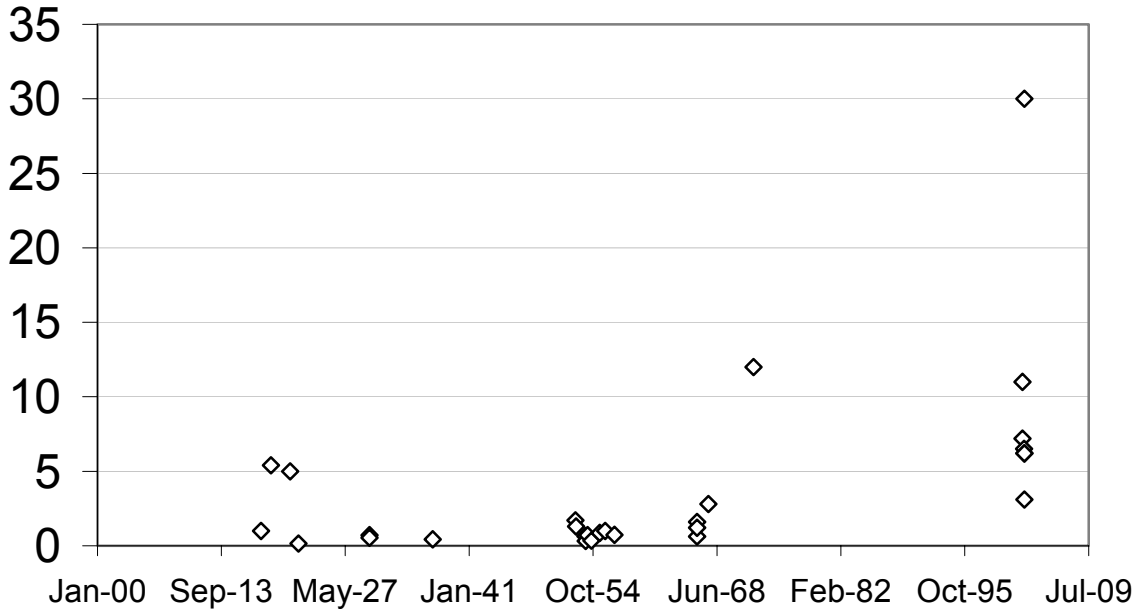


Figure 2. Concentrations of total mercury (mg/kg dry weight) in feathers of individual White Ibises from freshwater areas of the Everglades.

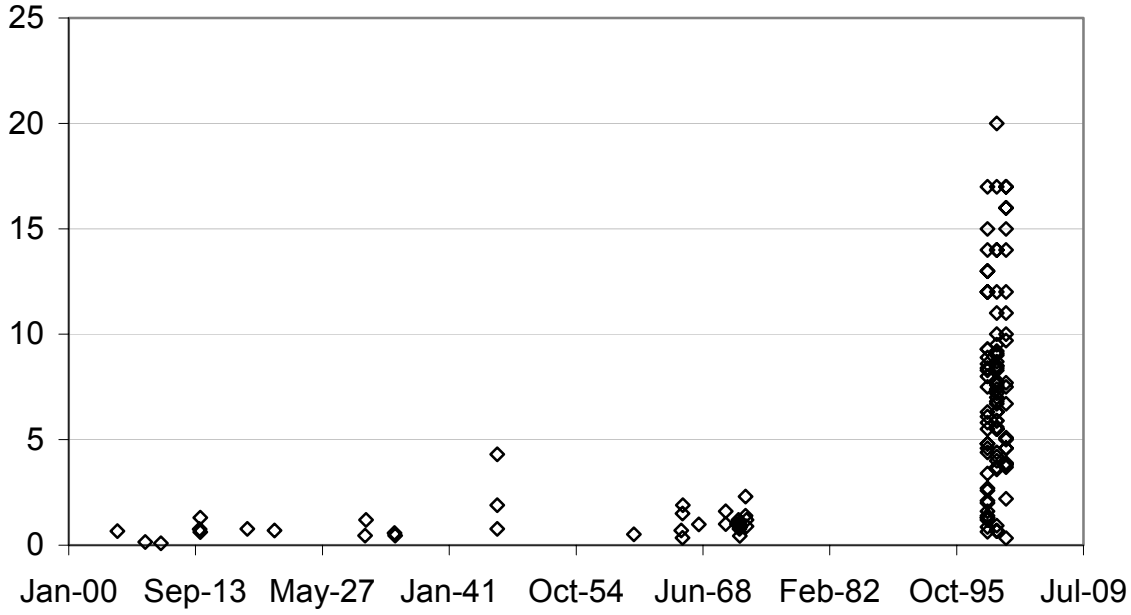


Figure 3. Concentrations of total mercury (mg/kg dry weight) in feathers of individual Great Egrets from freshwater areas of the Everglades.

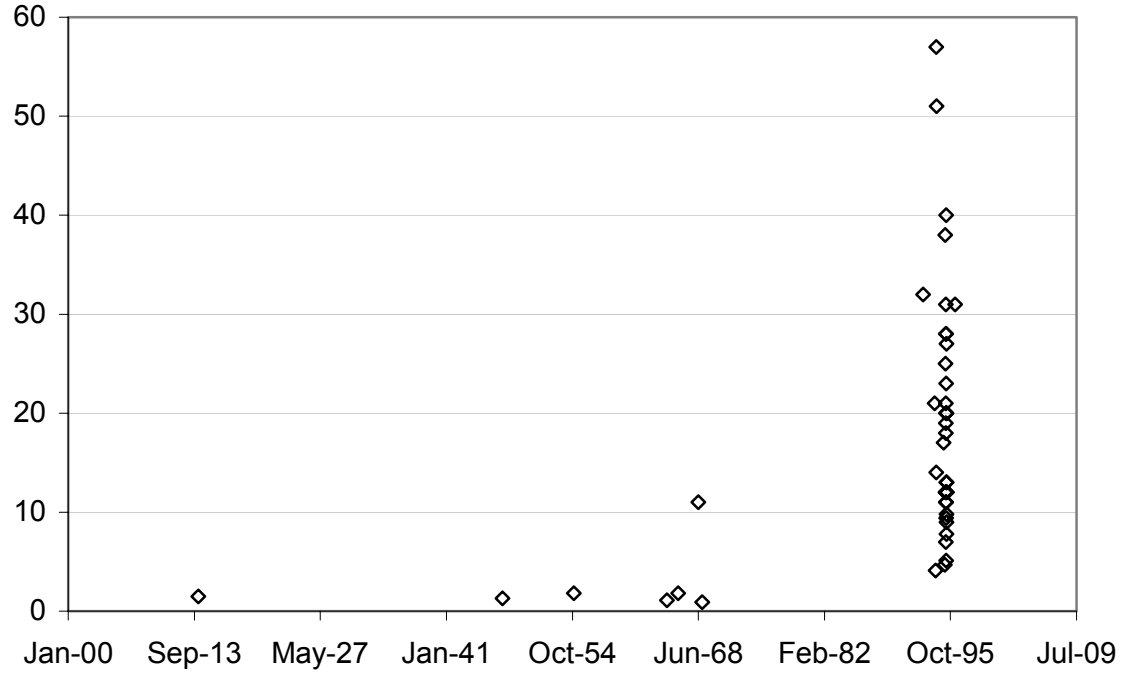
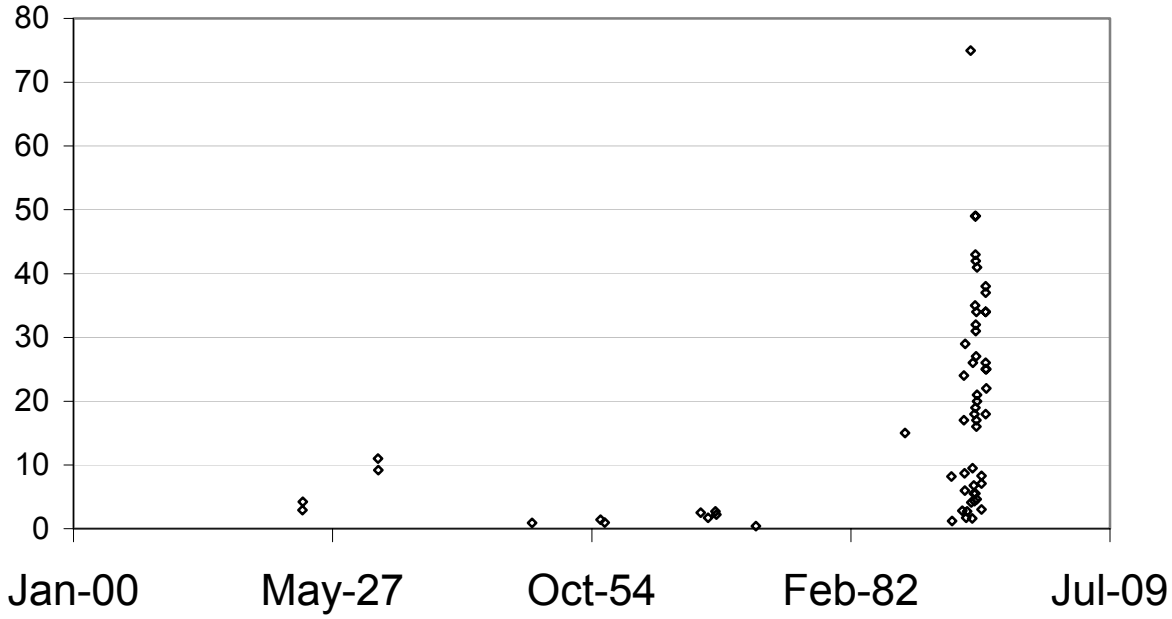


Figure 4. Concentrations of total mercury (mg/kg dry weight) in feathers of individual Great Blue Herons from freshwater areas of the Everglades.



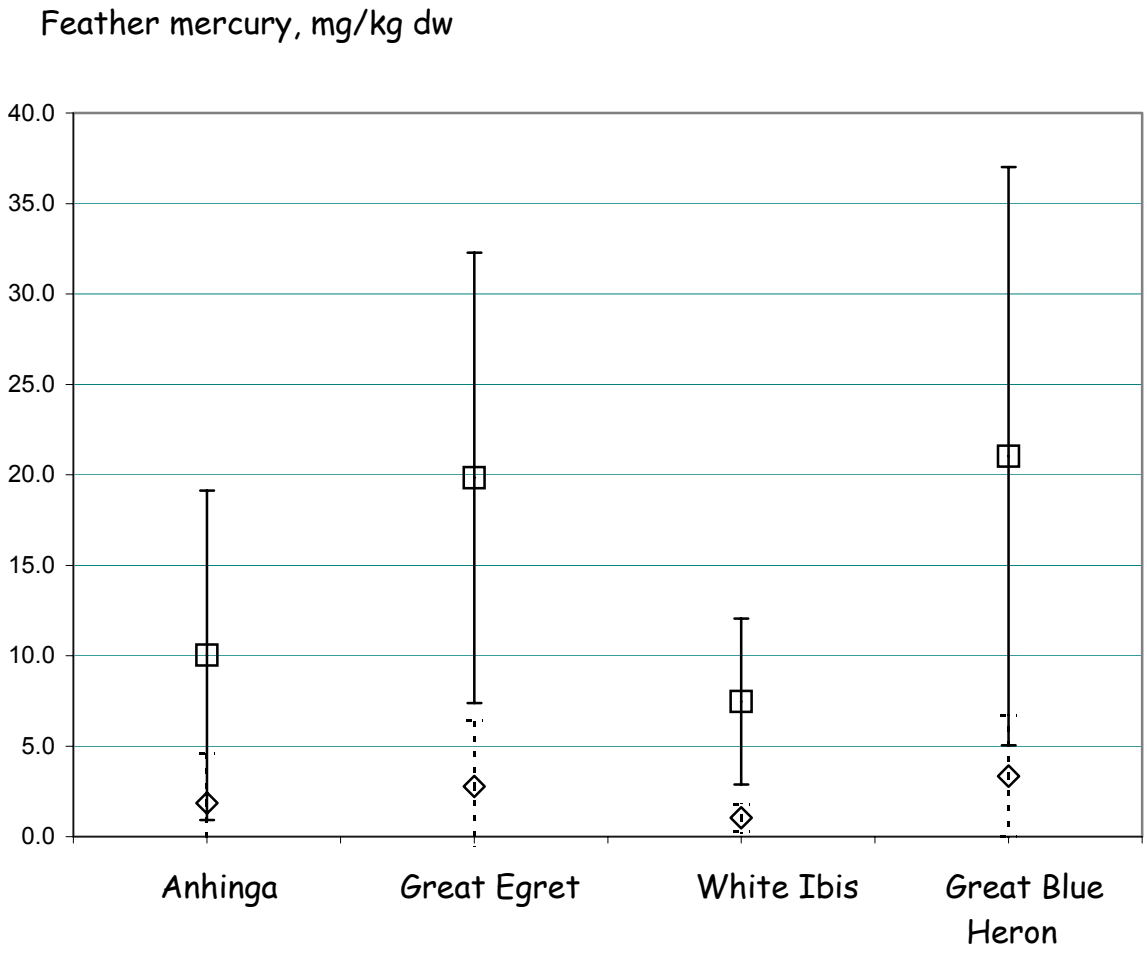
Since there appeared to be no trend during the pre-1980 period, and a very large increase by comparison in the post-1990 samples, we compared the mean concentrations of feathers by species in these two periods. Mean concentrations were significantly higher in the post-1990 samples for all four species (see Figure 5, Table 4), and for most, the standard deviations of the means had almost no overlap between the periods.

Table 4. Mercury concentrations (ppm) in feather samples of piscivorous birds from the Everglades, comparing the same species during two eras: pre-1980's and post-1990.

		Anhinga	Great Egret	White Ibis	Great Blue Herons
Pre-1980's	mean	1.86	2.77	1.04	3.34
	s.d.	2.72	3.64	0.77	3.34
	median	0.87	1.50	0.86	2.35
	n	21	7	33	12
post- 1990	mean	10.03	19.84	7.47	21.03
	s.d.	9.11	12.45	4.58	15.98
	median	6.50	18.00	7.10	19.00
	n	7	37	98	49
Z*		3.475	3.834	7.508	4.264
p		0.00007	0.00001	0.00001	0.00001

* Mann-Whitney U test

Figure 5. Comparison of mean mercury values pre-1980 (diamonds) with mean mercury concentrations of feathers post-1990 (squares). Standard deviations are shown as vertical bars.



Discussion and Conclusions

One of the most important results of this study has been to demonstrate that feather samples taken from museum specimens are not regularly contaminated with exogenous mercury acquired during the preservation or storage process. We found trace amounts of mercury in the acid rinse from museum feathers, which represented far less than one tenth of a percent of the total mercury in the feathers. The strength of this conclusion lies in the assumption that an acid rinse would have removed all mercury that was bound to the surface of the feather. This assumption seems appropriate because unbound inorganic mercury is quite likely to be dissolved in an acid rinse.

Our use of methylmercury as a proxy measurement for total mercury in the museum samples rests on the assumption that the vast majority of mercury in feathers is present in the methylated form. This assumption seems appropriate and parsimonious as several studies have shown that methylmercury constitutes over 95% of feather mercury.

We have also assumed that all or very nearly all of the mercury that was present in the feathers at the time the bird died was also present at the time that we collected feathers. This assumption also seems appropriate since methylmercury is known to be bound covalently with keratinized tissue, and is therefore stable for long periods of time. Thus it seems very unlikely that methylmercury would have been lost from feathers stored in museums, even over a period of many decades.

In comparing recent samples with museum samples, we have clearly compared feathers coming from different parts of the body. We feel that this comparison is warranted only because we have examined the effect of location of feather on mercury concentration in the same individuals. Although our analysis was done with a relatively

small sample size, we found no evidence to support the hypothesis that there is any consistent difference in mercury concentration depending on body location. Probably the most important point, however, is that the magnitude of differences suggested (means of 1.8 – 2.6 ppm) did not seem anywhere near large enough to explain the differences we observed between pre-1980 and post-1990 samples (range of differences in means 6.4 – 17.5 ppm, depending on species).

The lack of any obvious trend in the pre-1980 mercury concentrations suggests that there were no major differences in mercury availability in food to these wetland birds during this period, or at least not of the magnitude of difference seen by comparison with post-1990. Since food is the main source of mercury for the birds (Frederick et al. 1998), and since the relationship between dietary intake and feather mercury concentration is quite well established (Spalding et al. 1999), our findings in this report suggest that mercury contamination in the freshwater Everglades food web was relatively constant throughout the period 1900 through the mid-1970's.

It is not clear whether this constancy is representative of some pre-industrial or pre-settlement condition in south Florida. Of particular interest are the samples of ibises from as early as 1903, and continuing through 1930. It is known that south Florida was largely unsettled during the early part of the 20th century, and that major urban building in the Miami area did not occur until late in the 1920's. Further, during this time, mercury was not a common contaminant from any source, and soil mercury levels during this time were quite low by comparison with late in the century (Rood et al. 1995). This information suggests that the levels of mercury from the early part of the 20th century in the Everglades may have been representative of a pre-settlement condition, and were

certainly representative of a pre-urbanized south Florida. To this extent, the feather concentrations from this time can be used to indicate a NOAEL for these birds.

Since specimens became extremely rare after the mid-1970's, it is impossible to say whether the relatively constant, low-mercury contamination trend continued into the 1980's. At the least, the pattern suggests that the source of contamination that was so evident during the 1990's became functional at some point between the mid-1970's and the early 1990's, a period of approximately 15 years. This may have been a time of greatly increased mercury emissions from the south Florida urban area as suggested by analysis of mercury sources and atmospheric emissions (Husar and Husar 2002).

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