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Feather mercury concentrations and physiological condition of great egret and white ibis nestlings in the Florida Everglades

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ABSTRACT

Mercury contamination in the Florida Everglades has reportedly played a role in the recent decline of wading birds, although no studies have identified a mechanism leading to population-level effects. We assessed feather mercury levels in great egret (*Ardea alba*; $n=91$) and white ibis (*Eudocimus albus*; $n=46$) nestlings at breeding colonies in the Florida Everglades during a year (2006) with excellent breeding conditions (characterized by hydrology leading to concentrated prey) and a year with below average breeding conditions (2007). We also assessed the physiological condition of those nestlings based on levels of plasma and fecal corticosterone metabolites, and stress proteins 60 and 70. Mercury levels were higher in both species during the good breeding condition year (great egret = 6.25 μg/g ± 0.81 SE, white ibis = 1.47 μg/g ± 0.41 SE) and lower in the below average breeding year (great egret = 1.60 μg/g ± 0.11 SE, white ibis = 0.20 μg/g ± 0.03 SE). Nestlings were in better physiological condition in 2006, the year with higher feather mercury levels. These results support the hypothesis that nestlings are protected from the harmful effects of mercury through deposition of mercury in growing feathers. We found evidence to suggest shifts in diets of the two species, as a function of prey availability, thus altering their exposure profiles. However, we found no evidence to suggest they respond differently to mercury exposure.

1. Introduction

Mercury contamination in wetland ecosystems may have negative consequences for resident species, particularly apex predators. Under certain conditions inorganic mercury is microbially transformed to methylmercury (MeHg) within wetlands (Zillioux et al., 1993; St. Louis et al., 1994; Rumbold and Fink, 2006). It is the MeHg that biomagnifies as it progressively moves up the food chain (Nriagu, 1989); sometimes resulting in a suite of negative responses among birds that feed within aquatic environments (see Burger et al., 1992; Burger and Gochfield, 1997; Frederick et al., 2002). Consequently, one hypothesis for the recent decline of wading birds in the Florida Everglades is the bioaccumulation of mercury (Frederick and Spalding, 1998; Spalding et al., 1994; Sundlof et al., 1994; Frederick et al., 2002; Heath and Frederick 2005).

Most research in the Everglades on mercury accumulation in wading birds has focused on great egrets (*Ardea alba*; see Frederick and Spalding, 1998; Spalding et al., 1994; Sundlof et al., 1994; Rumbold et al., 2001; Frederick et al., 2002) because great egrets are long-lived and are an upper trophic level piscivore (Rumbold 2005). Early research focused on potential

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harmful effects of mercury in exposed nestlings (Bouton et al., 1999; Frederick et al., 1999; Sepulveda et al., 1999). However, free ranging great egret chicks that were dosed with mercury were found to have similar health and survival rates as control egrets within the Everglades (Sepulveda et al., 1999). Moreover, controlled dosing of chicks in pens produced equivocal results (Bouton et al., 1999). This study and others (Spalding et al., 2000a,b; Kenow et al., 2003) revealed that nestlings are to a degree, buffered against the effects of mercury during early growth periods because mercury is sequestered in growing feather tissues. However, after completion of feather growth and cessation of this elimination pathway, growth, health, and feeding behaviors may be impacted via continued mercury ingestion (Spalding et al., 2000a,b).

It is also possible that mercury exposure could adversely affect adult wading birds. Heath and Frederick (2005) suggested that subacute effects of mercury exposure could be a contributing factor in the recent decline of white ibis (*Eudocimus albus*) in the Everglades as a result of fewer birds nesting, or increased nest failure. This hypothesis was based on a negative correlation between feather mercury levels in great egret nestlings (as a surrogate measure of adult ibis exposure) and total numbers of white ibis nests initiated across the Everglades during a seven-year period (1994–2001). Although Heath and Frederick (2005) did not report the relationship between great egret feather mercury levels and great egret nest numbers during the same period, we found no correlation ($r^2=0.26$, $P=0.14$, 6 *df*) between the great egret feather mercury levels reported in Heath and Frederick (2005) and numbers of great egret nests during this period in the Everglades (Everglades regions included Water Conservation Areas 1–3; Crozier and Gawlik, 2003). The number of great egret nests in the Everglades has been increasing in the Everglades for the past 20 years, even during periods of high mercury levels. White ibis nest numbers have only recently begun increasing (Crozier and Gawlik, 2003; Cook and Herring, 2007). Therefore, if the mercury hypothesis is valid for the white ibis, this species must respond differently to mercury exposure than does the great egret. We expected that in years when mercury concentration are higher in white ibis that they would be in poorer physiological condition.

We conducted this study to test for a differential response to mercury exposure for great egret and white ibis nestlings, taking into consideration interannual differences in mercury levels and habitat conditions. To be consistent with previous studies, we measured mercury concentrations in feathers taken from nestlings over two nesting seasons. As noted above, sequestration of mercury in growing feathers may, to a degree, buffer nestlings from its harmful effects. However, for most species it is unclear how fast mercury moves to the feather (i.e., half-life of the available mercury) or if there is a threshold dose that could overwhelm this elimination pathway. In either case it is reasonable to expect that there are at least subtle physiological responses associated with dietary mercury exposure either prior to elimination, or if the elimination pathway is overwhelmed.

We assessed chick physiological condition during two years with contrasting hydrological patterns by measuring hormone (corticosterone) and stress protein levels to determine the condition of nestlings prior to fledging given potentially different mercury exposure levels associated with landscape level variability. Corticosterone serves as a physiological signal to a bird to modify its behavior and metabolism in response to potentially adverse change. Corticosterone is released into the blood stream via the adrenocortical tissue when birds become stressed (e.g., low food availability) inducing a response, and allowing them to overcome the short-term deficiency (Astheimer et al., 1992; Wingfield et al., 1992; Silverin, 1998). In nestlings, the release of corticosterone into the blood stream can facilitate begging and allow them to restore depleted energy reserves by increasing parental provisioning (Kitaysky et al., 2001). Increased corticosterone levels in birds have been correlated with food shortages (Kitaysky et al., 1999; 2001, Herring, 2008) and ingestion of methylmercury (Thaxton et al., 1981, Burgess et al., 2005, Pollock and Machin, 2008). Corticosterone can be measured either directly from the blood stream or excretia, although fecal corticosterone metabolite levels are lower than circulating levels (Wasser et al., 2000) because of rapid and extensive metabolization before excretion results in lower overall levels than circulating levels.

Stress proteins are a group of highly conserved intracellular polypeptides found in all organisms, from bacteria to humans, indicating a crucial role in cellular survival (Sørensen et al., 2003). They function as molecular chaperones for proteins within cells and present a major molecular barrier to alterations in cellular homeostasis (Sørensen et al., 2003; Tomás et al., 2004). Under normal cellular conditions, stress proteins are involved in routine cellular protection. However, their molecular chaperone role is amplified during periods of increased stress in order to regulate cell protein damage (Sørensen et al., 2003). In many species and taxa, stress proteins are induced in response to a variety of stressors including heavy metals (Martínez et al., 2001; Werner and Nagel, 1997), nutritional stress (Merino et al., 2002), high density food limitation, and others (see Herring and Gawlik, 2007). Stress proteins have recently been experimentally demonstrated to increase in response to decreasing levels of food availability for birds (Herring, 2008).

Within the Everglades ecosystem, prey densities are a critical component of habitat quality and are directly related to hydrology. Variability in prey communities may influence prey selection of adult wading birds and the diet of nestlings (Kushlan, 1979; Smith, 1997). It is possible that dietary differences may be associated with interannual differences in nestling feather mercury concentrations and thereby influence the physiological responses of those nestlings to mercury exposure (Hoffman and Curnow, 1979; Doi et al., 1984; Braune, 1987; Goutner and Furness, 1997). Mercury exposure (ingestion) could also interact with the availability of and/or quality of prey, producing possible synergistic, antagonistic, or ameliorative effects.

2. Materials and methods

2.1. *Selection of nests*

During the dry season (Dec–May) in the Florida Everglades of both 2005/2006 and 2006/2007 (hereafter 2006 and 2007), we

collected data on feather mercury and physiological markers in great egret and white ibis nestlings at the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Lox), Palm Beach County, and in Water Conservation Area (WCA) 2 and 3, Broward County, Florida (Fig. 1). Within colonies, we randomly selected nests for monitoring and subsequent feather mercury sampling. All nests were marked with flagging tape during the egg laying period, and revisited every 6–7 days to monitor nest survival and to determine when eggs hatched.

2.2. *Sampling of nestlings*

We measured mercury concentrations in scapular feathers of nestlings because all prior studies of wading bird nestling mercury levels in the Everglades sampled these feathers and at the same approximate age (Frederick et al., 1999; 2002; Rumbold et al., 2001). Scapular feathers are also grown at similar times in great egret and white ibis nestlings (Kushlan and Bildstein, 1992; McCrimmon et al., 2001), and thus allow for comparisons between species. Nearly 100% of the mercury in feathers is in the form of methyl-mercury (Thompson and Furness, 1989) and represent body burdens at the time of feather growth (Scheuhammer, 1987). Feather mercury concentrations are also positively correlated with mercury exposure, concentrations in other tissues (Ohlendorf and Harrison, 1986, Spalding et al., 2000b, Ackerman et al., 2007, Tsao et al., 2009), and hormone levels in adults (Heath and Frederick, 2005).

We collected blood, fecal material, and feathers when the first hatched nestling within a nest was at least 14 days old. At this stage, the proportion of adult culmen length that egret and ibis nestling culmens represent are similar, represent 46% and 39% respectively. Blood samples were collected (up to 1 ml) using a 27.5-gauge needle to puncture the brachial or jugular vein and then stored in a heparinized storage tube. Fecal samples were extracted directly from the cloaca of birds

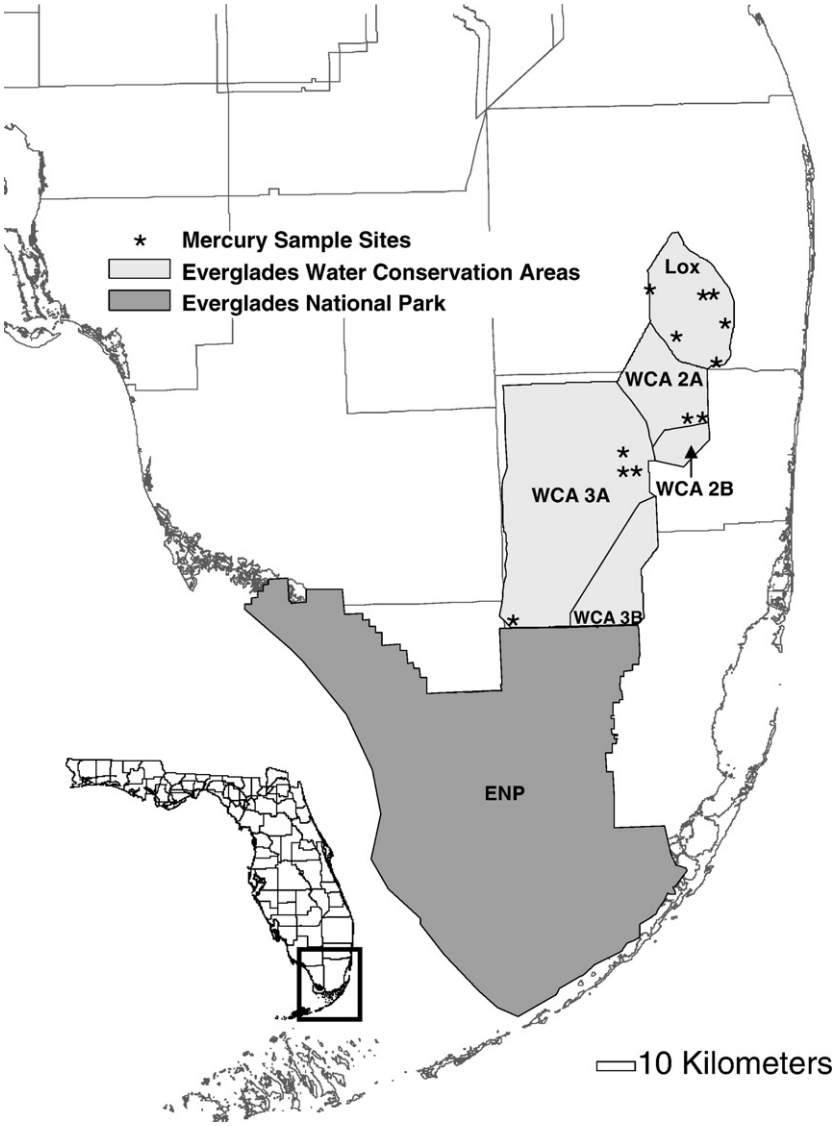

Fig. 1 – Mercury sampling sites of great egret and white ibis nestlings during the 2006 and 2007 breeding seasons at the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Lox) and Water Conservation Areas (WCA) 2A and 3A in the Florida Everglades.

with a micro pipette and stored in a vacuutainer. Blood and fecal samples were stored on ice until transport to the laboratory. We also took standard measurements (mass, culmen, bill depth, wing flat, tarsus) to the nearest gram or mm. We then collected 8–10 scapular feathers. Feathers were placed in individual, labeled bags and stored dry until shipment to the analytical lab. In the lab, blood samples were centrifuged (10,000 g, 15 min); plasma and red blood cells were separated and frozen at −20 °C for later analysis. Fecal samples were also frozen at −20 °C for later analysis.

2.3. *Physiological condition*

To assess physiological condition of great egret and white ibis nestlings, we measured plasma and fecal corticosterone metabolites, and red blood cell stress protein 60 and 70 levels. Plasma corticosterone samples were homogenized and then mixed with methanol and vortexed for 30 min. After centrifugation (15 min, 2500 ×g) we transferred the plasma corticosterone supernatant to a new vial, which was then evaporated under a stream of nitrogen gas. Fecal samples were homogenized and dried using a Labconco CentriVap Concentro (Labconco, Kansas City, MO). Dried samples (0.5 g) were then mixed with 5 ml of 95% ethanol and vortexed for 30 min. After centrifugation (15 min, 2500 ×g) we transferred the supernatant to a new vial, which was then evaporated under a stream of nitrogen gas. Corticosterone metabolites were then resuspended in diluted extraction buffer and measured using the Correlate-EIA™ Corticosterone Enzyme Immunoassay Kit (ELISA; Rothschild et al., 2008) following the manufacturer's instructions (Assay Design, Inc., Ann Arbor, MI).

Cross reactivity was as follows: deoxycorticosterone (21.3%), desoxycorticosterone (21.0%), progesterone (0.46%), testosterone (0.31%), tetrahydrocorticosterone (0.28%), aldosterone (0.18%), cortisol (0.04%), pregnenolone (0.03%), β-estradiol (0.03%), cortisone (0.03%), and 11=dehydrocorticosterone acetate (0.03%; Assay Design, Inc., Ann Arbor, MI). The minimum detectable level of corticosterone metabolites was 0.05 ng/g (Assay Design, Inc., Ann Arbor, MI). We conducted a standard assay validation for each bird species, which included an assessment of parallelism, recovery of exogenous corticosterone, and intra and inter-assay precision to confirm that the ELISAs accurately measured corticosterone metabolites in both great egret and white ibis samples as per Astalis et al., 2004, Goymann, 2005, Herring, 2008, Rothschild et al., 2008. We also confirmed that fecal corticosterone metabolite levels did not change after freezing, as is the case in mammals (Khan et al., 2003), by freezing and measuring fecal corticosterone metabolite levels monthly for 6 months (Herring and Gawlik, in press).

For the stress proteins, red blood cell supernatant was mixed with 1×extraction reagent and a protease inhibitor cocktail (Sigma), vortexed for 5 min, and then sonicated for 1 min. Samples were again centrifuged (15 min, 2500 g) and the supernatant removed. We measured SP60 (HSPD1) and SP70 (SP72/ HSPA1A) in the supernatant using ELISA kits specific to just those stress proteins and not all other SP60 and SP70 family members (Assay Designs, Inc., Ann Arbor, MI). All samples were run in one ELISA kit for each assay. Inter-assay coefficients of variation for SP60 and SP70 internal standards were 5% and 7% respectively. All samples were processed in duplicate, and means of duplicates were used in all analyses. All ELISA kits were validated using serial dilutions and spike tests to determine percent recovery (Herring, 2008).

2.4. *Mercury analysis*

Mercury concentrations in feathers were determined by the Florida Department of Environmental Protection Chemistry Laboratory in Tallahassee, Florida using methods previously described (Rumbold et al., 2001; Frederick et al., 2002). All feathers collected from each individual chick were weighed and then cut into small pieces and homogenized using a mixture of concentrated acids (5:2 ratio of trace metal-grade sulfuric acid to trace metal-grade nitric acid). A subsample was then digested and analyzed using a modified version of EPA Method 245.6 (US EPA, 2001). Mercury in feather samples was first oxidized to Hg^{2+}, using potassium permanganate and potassium persulfide, with the addition of hydroxylamine hydrochloride to reduce excess oxidizing agents. Mercuric ions in solution were then reduced to atomic mercury stannous chloride and purged into atomic absorption spectrophotometer (Varian SpectraAA 400 with SPS5 autosampler, Mulgrove, Victoria, Australia) using UHP-grade nitrogen. Mercury concentrations are reported as mg/kg dry weight.

Quality control samples consisted of matrix spikes (MS), matrix spike duplicates (MSD), and laboratory control samples (LCS) and duplicates. Insufficient amounts of samples were available for other laboratory duplicates, and in the 2007 samples to allow matrix spikes. Recoveries in the 2006 samples for MS and MSD were 93.3% and 94.8%, respectively, for a relative percent difference (RPD) of 102%. Recoveries in LCS ranged from 95.4% to 108% (n=14, mean=99.2%) with RPD between LCSs ranging from 0.21% to 7.25% (n=7 pairs, mean=2.8%). In 2007, weights of 20 feather samples (12 egret and 8 ibis) were lost prior to calculating concentration; instead the average digestate weight of other samples were used in the calculations.

2.5. *Statistical analysis*

2.5.1. *Physiological condition*

We constructed general linear models to examine the effects of year, region, species, mercury levels and all possible interactions on chick physiological condition metrics. Response variables were fecal and plasma corticosterone metabolites, and stress proteins 60 and 70. All response variables were log transformed to meet assumptions of normality and homoscedascity. We removed all interaction terms except species×mercury from the models when they were not significant. We retained the species×mercury in the model because this interaction term was explicitly specified *a priori* to test our main hypothesis that egret and ibis nestlings physiologically respond differently to mercury ingestion.

2.5.2. *Mercury analysis*

We ran a 3-way analysis of covariance (ANCOVA) with terms for species, year, region, and their interactions (JMP, 2003). We included an index of age of nestlings as a covariate in the model to determine if it significantly influenced the main

Table 1 – General linear model results for great egret and white ibis nestling physiological condition by year, region, species, and mercury levels during 2006 and 2007 in the Florida Everglades

Effect	df	F	p	Parameter estimate	SE
Stress protein 60 (n=113)					
Model	6	1.18	0.32		
Error	106				
Year		3.72	0.05	3.87	2.00
Region		0.02	0.87	0.02	0.15
Species		0.59	0.44	0.11	0.15
Mercury		0.18	0.66	−0.04	0.11
Species × mercury		1.63	0.20	0.10	0.08
Stress protein 70 (n=87)					
Model	6	0.44	0.84		
Error	80				
Year		0.39	0.52	−0.11	0.18
Region		1.47	0.22	0.18	0.14
Species		1.25	0.25	−0.16	0.14
Mercury		0.84	0.35	0.09	0.10
Species × mercury		0.06	0.80	0.01	0.07
Fecal corticosterone (n=119)					
Model	6	6.41	<0.01		
Error	112				
Year		3.70	0.05	1.96	2.0
Region		0.03	0.73	−0.52	0.18
Species		0.95	0.32	−0.18	0.18
Mercury		0.09	0.76	−0.04	0.15
Species × mercury		0.11	0.73	−0.03	0.10
Plasma corticosterone (n=112)					
Model	6	0.39	0.88		
Error	105				
Year		0.01	0.89	−0.05	0.37
Region		0.33	0.56	−0.21	0.37
Species		0.51	0.47	0.26	0.36
Mercury		0.13	0.71	−0.10	0.27
Species × mercury		0.04	0.82	0.04	0.20

effect. The age index consisted of the log of culmen lengths of great egret and white ibis nestlings (Custer and Peterson, 1991). We observed a significant species × year interaction and subsequently ran separate ANCOVA models to examine the effects of year and region on the mercury levels for each species. Mercury data met assumptions of homoscedascity (Levene's test; JMP, 2003), residuals were normal after log transformation, and all ANCOVA tests met the requirement of parallelism. We then used linear correlation to examine the relationship between log mercury levels and log culmen length. We standardized least-squares mean of mercury to a culmen length of 8 cm for great egret and 7 cm for white ibis nestlings to allow direct comparisons to previous studies.

3. Results

We collected feather samples from 137 nestlings, 53 during 2006 (26 white ibis and 27 great egret) and 84 during 2007 (20 white ibis and 64 great egret). Mean age of nestlings sampled was 18±0.5 SE (range=10–35 days) and 20±1.0 SE (range=12–30 days) for egret and ibis respectively.

Table 2 – Least square means±SE values of physiological metrics measured in great egret and white ibis nestlings by species and year during 2006 and 2007 in the Florida Everglades

Physiological parameter	Great egret 2006	Great egret 2007	White ibis 2006	White ibis 2007
Fecal corticosterone[a]	0.75±0.31	2.02±0.23	8.34±0.52	15.46±0.38
Plasma corticosterone[a]	29.87±5.55	32.47±4.01	24.21±20.21	20.26±14.08
Stress protein 60[b]	16.54±1.18	23.01±1.26	14.05±1.41	26.91±1.27
Stress protein 70[b]	1.28±7.94	17.69±4.17	8.37±9.70	8.70±4.55

[a] Presented as ng/g.
[b] Presented as ng/ml.

3.1. Physiological condition

None of the physiological condition metrics had a significant species × feather mercury interaction (Table 1). We observed a significant effect of year on SP60 and fecal corticosterone metabolite levels while no other main effects were significant (Table 1). SP60 and fecal corticosterone levels were lower during 2006 than in 2007 (Table 2). Concentrations of SP70 and plasma corticosterone were similar between years for both species (Table 2).

3.2. Feather mercury

We observed a significant species × year interaction, with Tukey tests revealing that white ibis mercury concentrations were lower than egrets in 2006 and 2007 ($P<0.001$; Fig. 2). In 2006, mercury levels in ibis feathers were, however, similar to levels observed in egrets in 2007 ($P=0.96$). We found mercury concentrations in both great egret and white ibis nestlings differed between years (Table 3). For both species, feather

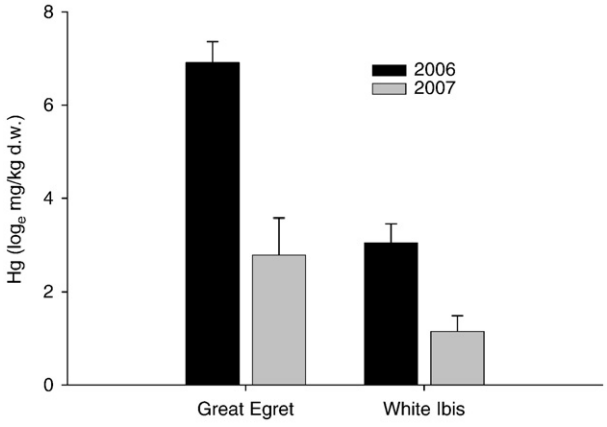

Fig. 2 – Mercury concentrations in great egret and white ibis nestling feathers during the 2006 (black bars) and 2007 (white bars) breeding season in the Florida Everglades. Means are presented as least-squares mean±SE.

Table 3 – Analysis of covariance model results for great egret ($n=91$) and white ibis ($n=46$) nestling mercury levels by year and location during 2006 and 2007 in the Florida Everglades

	Great egret			White ibis		
	df	*F*	*p*	*df*	*F*	*P*
Model	5	18.75	<0.01	2	25.52	<0.01
Error	80	30.26		41	16.55	

Effect	*df*	*F*	*p*	Estimate	SE	*df*	*F*	*p*	Estimate	SE
Year	1	28.80	<0.01	0.50	0.11	1	34.34	<0.01	1.22	0.23
Location	2	0.79	0.45	0.16	0.14	1	26.10	<0.01	−0.94	0.17
Culmen	1	6.62	0.01	0.02	0.01	1	1.06	0.31	−0.01	0.01

Log culmen length was used to control for age differences.

mercury levels were higher in 2006 than 2007 (Fig. 2). Mercury levels in white ibis nestlings differed by region, with higher levels in WCA 3A than Lox, whereas great egret chick mercury levels did not differ by region (Table 3). In both years, <20% of the variation in mercury concentrations were associated with culmen length, however mercury levels were not correlated with culmen length in white ibis, but did increase with culmen length in great egrets (Fig. 3).

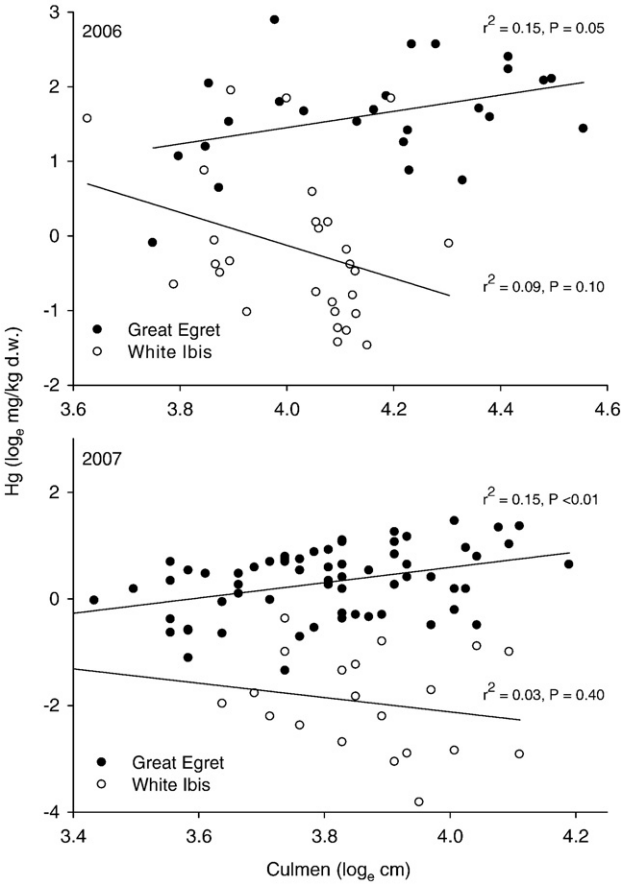

Fig. 3 – Correlation between great egret and white ibis nestling log culmen length and log mercury levels during 2006 and 2007 based on analysis of covariance. Culmen length is used as a surrogate for age in this analysis.

4. Discussion

4.1. *Nestling mercury and physiological condition*

We observed no interaction between species and feather mercury levels for any of our physiological metrics, suggesting that the physiological condition of these two species did not differ due to mercury in the their diet. Collectively these results indicate that white ibis nestlings did not respond differently to mercury exposure during the nestling stage than did great egrets in years with different mercury levels.

We did detect between-year differences in SP60 and fecal corticosterone metabolites, which were higher in 2007 than 2006. Increased levels of SP60 and fecal corticosterone metabolites during 2007 as compared to 2006, likely reflected differences in habitat conditions. During 2007, the biomass of prey across the landscape was 83% lower (8 g/m^2 ± 1 SE) than in 2006 (48 g/m^2 ± 12 SE; Gawlik and Botson, 2008), which could have led to a decrease in food being delivered to nestlings and a subsequent increase in chick stress levels. In nestlings, the release of corticosterone into the blood stream can facilitate begging and allows them to restore depleted energy reserves by increasing parental provisioning (Kitaysky et al., 2001). Further, during prolonged periods of nutritional stress, increased upregulation of stress proteins can occur, possibly as a mechanism to maintain homeostasis within cells and prevent cellular damage (Sørensen et al., 2003; Merino et al., 2002).

4.2. *Diet and mercury concentrations*

This study and Frederick et al. (2002) both showed that great egret nestlings have higher mercury concentrations relative to white ibis nestlings. Diet studies suggested that great egrets can be generalized as an upper trophic level piscivore (Frederick et al., 1999; McCrimmon et al., 2001), whereas white ibis tend to be more of a lower trophic level generalist (Kushlan and Bildstein, 1992). On average great egret adults consume more fish, which generally have increased levels of mercury relative to the lower trophic level invertebrates that are often consumed by white ibis (Cleckner et al., 1998). This difference in prey selection is the simplest explanation for the observed interspecific differences in mercury levels.

An obvious explanation for the small between-year difference in feather mercury might be a reduction in bioavailable MeHg in 2007; however, fish [e.g., Eastern mosquitofish (*Gambusia holbrooki*), sunfish (Lepomis spp.), and largemouth bass (*Micropterus salmoides*)], collected as part of the regional monitoring program, were reported to have higher mercury levels in 2007 as compared to 2006 (Axelrad et al., 2009; Gabriel et al., in review). An alternate explanation for the between-year differences in feather mercury concentration might be a between-year shift in the diets of these birds.

White ibis switch from a diet of predominantly invertebrates to fish when fish density exceeds a threshold (Kushlan, 1979). The landscape clearly produced higher densities and biomass of fish in 2006 than in 2007 (Gawlik and Botson, 2008) and bolus samples collected from white ibis nestlings in 2006 had a higher proportion of fish in their diet than was expected based on the literature (Dorn et al., 2008). This evidence suggests that ibis switched from their typical diet of invertebrates to fish in 2006, and correspondingly incurred higher feather mercury levels in their nestlings. It is also possible that great egrets took a higher proportion of invertebrates in 2007 than 2006. Previous studies that have shown extensive piscivory in prey items fed to great egret nestlings (Smith, 1997; Strong et al., 1997, McCrimmon et al., 2001), except during periods of drought or low fish density years, such as we observed in 2007, when great egrets might shift their diets and consume proportionally more invertebrates (Smith, 1997).

4.3. *Everglades regional mercury differences*

Feather mercury concentrations differed between regions in the white ibis but not in the great egret. The regional variation in ibis mercury (lower in northern colonies and higher in southern colonies) was consistent with previous reports of mercury gradients in the Everglades, with higher concentrations in the WCA-3 than Lox (Cleckner et al., 1998; Gilmour et al., 1998; Hurley et al., 1998, Axelrad et al., 2009). The fact that great egrets did not have higher mercury levels in southern colonies is curious, and the patterns observed in white ibis do not provide an obvious answer for why we did not see the same pattern between species.

4.4. *Nestling age and mercury concentrations*

Our analysis indicated that great egret feather mercury concentrations increased with increasing culmen length; we interpreted this to indicate that mercury levels increased as great egret nestlings got older. Rumbold et al. (2001) reported a similar pattern for great egrets at one Everglades colony but not at another. Although, Rumbold et al. (2001) could not rule out the small sample size or narrow range in ages of nestlings sampled as contributing factors as to why the regression of feather mercury on culmen length was not significant at one colony, they suggested that exposure level was simply lower there. Other studies have observed that nestling feather mercury levels may be independent (Thompson et al., 1991; Goutner and Furness, 1997; Goutner et al., 2001) or even negatively correlated with nestling age (Goutner ad Furness, 1997). Monteiro and Furness (1995) maintain that contradictions in age-related differences can be reconciled simply based on level of exposure. Increases in mercury concentration with age of nestlings require that the natural "growth dilution" be overcome by a certain level of mercury exposure found in heavily contaminated environment.

White ibis nestlings did not show a correlation between feather mercury levels and culmen length, suggesting that white ibis nestlings were fed prey items low in mercury continually (i.e., any possible increase in mercury concentration did not overcome natural growth dilution; Monteiro and Furness, 1995). Sample size and range in ages of nestlings sampled were not a problem in the present study; white ibis nestlings were between 12 to 30 days old and were collected from 46 nestlings. Samples were collected from eight separate colonies and therefore represent a broad level of colony exposure associated with landscape differences in mercury levels. Frederick et al. (2002) also observed no relationship between white ibis mercury concentrations and culmen length (chick age), suggesting the pattern may be more consistent in white ibis than great egrets.

The only estimate of mercury concentrations in white ibis nestlings in the Everglades is from 1998 (Frederick et al., 2002). A comparison between studies shows that mercury concentrations in white ibis nestlings for 2007 were similar to those in 1998 whereas estimates for 2006 were approximately two times higher than those in 1998. Our results from two years also suggest that interannual variation in white ibis mercury concentrations may be high, complicating comparisons between only several years, and making it difficult to determine if there was a trend over the last 9 years. Annual monitoring of mercury levels in white ibis seems warranted to understand long-term patterns. Further, measuring levels of mercury in other taxa besides fishes in the Everglades may be warranted to understand potential ingestion levels for species that are not strict piscivores.

4.5. *Conclusions*

Results from this study demonstrated that mercury levels in wading bird nestlings can vary greatly between years across the Florida Everglades. The overall substantive decreases in mercury levels in wading bird nestlings from those levels in the early 1990s appear to have diminished the potential for short-term detrimental effects during the nestling period. Indeed we observed no patterns of physiological response in either species to suggest that they were negatively affected by mercury concentrated in either year. Current patterns of physiological condition in both species' nestlings were likely influenced more by landscape habitat condition patterns (e.g., prey densities) than any other factors, such as wading bird prey mercury concentrations. Further, the fact that during the year both species had higher mercury concentrations they were in better physiological condition, which may mitigate any issues in the short term, but long term effects are still poorly understood.

Our findings do not address the possibility that mercury affects birds at an older stage, perhaps when they are juveniles and developing cognitively and acquiring foraging skills. This might account for lower nest numbers, if fewer juvenile ibis reach breeding age because of poor cognitive development (Adams and Frederick, 2008). However, juvenile white ibis

dosed with mercury do not show any cognitive leaning impairment, and in some instances increase their cognitive abilities (Adams and Frederick, 2008). Although not definitive, we believe the collective weight of evidence favors the hypothesis that the current levels of mercury in the Everglades ecosystem do not have a large negative effect on wading bird nestlings or their development.

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