The Auk 122(1):255–267, 2005

RELATIONSHIPS AMONG MERCURY CONCENTRATIONS, HORMONES, AND NESTING EFFORT OF WHITE IBISES (*EUDOCIMUS ALBUS*) IN THE FLORIDA EVERGLADES

Julie A. Heath[1] and Peter C. Frederick

Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, Florida 32611, USA

Abstract.—Mercury, a common wetland pollutant, can affect wildlife populations through acute toxicity or through physiological effects that modify behavior and negatively influence reproductive success. We compared body-feather mercury concentrations of free-living male and female adult White Ibises (*Eudocimus albus*) during three breeding seasons in the Florida Everglades and examined the relationships among mercury, hormone concentrations, and body-condition scores. Female White Ibises consistently had lower mercury concentrations than males. Prebreeding females' estradiol concentrations were negatively correlated with mercury concentrations. However, we found no relationship between mercury and female testosterone, progesterone, and corticosterone concentrations. Incubating male White Ibises showed a significant positive relationship between testosterone and mercury concentrations, but no other significant hormonal correlations with mercury concentrations. We used a seven-year standardized data set of Great Egret (*Ardea alba*) chick-feather mercury concentrations as a measure of temporal changes in mercury bioavailability in the Everglades and related that measure to annual numbers of White Ibis nests. White Ibis nesting was negatively correlated with the mercury exposure index. Low numbers of nesting White Ibises may have been the result of fewer birds nesting or high abandonment rates. Our results suggest that mercury exposure may cause fewer birds to nest or more birds to abandon nests because of subacute effects on hormone systems. However, the results are correlative; they call for further investigation in free-living populations and in the laboratory. *Received 23 May 2003, accepted 23 September 2004.*

Key words: *Ardea alba*, endocrine, *Eudocimus albus*, Great Egret, methylmercury, toxicology, wetlands, White Ibis.

Relaciones entre las Concentraciones de Mercurio, Hormonas y el Esfuerzo de Nidificación de *Eudocimus albus* en los Everglades, Florida

Resumen.—El mercurio, un contaminante común de los humedales, puede afectar las poblaciones silvestres por medio de su toxicidad aguda o a través de efectos fisiológicos que modifican el comportamiento e influencian negativamente el éxito reproductivo. Comparamos las concentraciones de mercurio en las plumas del cuerpo de machos y hembras silvestres de *Eudocimus albus* durante tres estaciones reproductivas en los Everglades de Florida y examinamos las relaciones entre el mercurio, las concentraciones de hormonas e índices de condición corporal. Las hembras consistentemente presentaron concentraciones de mercurio menores que los machos. Las concentraciones de estradiol en hembras pre-reproductivas

[1]Present address: Department of Biology, 130 Gittleson Hall, Hofstra University, Hempstead, New York 11549, USA. E-mail: biojah@hofstra.edu

estuvieron correlacionadas negativamente con las concentraciones de mercurio. Sin embargo, no encontramos una relación entre el mercurio y las concentraciones de testosterona, progesterona y corticosterona en las hembras. Los machos que estaban incubando exhibieron una relación positiva entre las concentraciones de testosterona y mercurio, pero no existieron otras correlaciones entre las concentraciones de hormonas y de mercurio. Empleamos un conjunto de datos de las concentraciones de mercurio en las plumas de pichones de *Ardea alba* estandarizados a través de siete años como una medida de los cambios temporales de la biodisponibilidad de mercurio en los Everglades y relacionamos esta medida con los números anuales de nidos de *E. albus*. La nidificación de *E. albus* estuvo correlacionada negativamente con el índice de exposición al mercurio. Los números bajos de *E. albus* nidificantes podrían haber sido el resultado de una cantidad menor de aves nidificando o de tasas altas de abandono. Nuestros resultados sugieren que la exposición al mercurio puede llevar a que menos aves nidifiquen o a que un número mayor de ellas abandonen sus nidos debido a efectos menos agudos sobre sus sistemas hormonales. Sin embargo, los resultados son correlativos y sugieren la necesidad de realizar más investigaciones en poblaciones silvestres y en condiciones de laboratorio.

BIOACCUMULATION OF MERCURY in wetlands has been identified as a potential threat to wildlife, especially to predatory animals such as piscivorous birds (Scheuhammer 1987, Zillioux et al. 1993, Thompson 1996, Gariboldi et al. 1998, Wolfe et al. 1998, Snodgrass et al. 2000). Wetland soils sequester mercury, and as physical conditions change (i.e. decreased water levels, anoxia), inorganic mercury (Hg) can be converted to the more toxic methylmercury (MeHg; Zillioux et al. 1993, Snodgrass et al. 2000). In shallow-depression wetlands, such as the Florida Everglades, that conversion process can be thorough and rapid (Gilmour et al. 1998). As mercury moves through aquatic ecosystems from water to periphyton to invertebrates and small fish, mercury concentrations increase (Cleckner et al. 1998). Therefore, long-legged wading birds that feed on small fish and invertebrates are exposed to high concentrations of methylated mercury (Jurczyk 1993).

Although mercury is known to affect reproductive success of birds through teratogenesis and poor egg viability (Scheuhammer 1987), reproduction may also be affected by altered behavior of adults or young (Heinz 1979, Nocera and Taylor 1998). For example, mercury exposure affected Mallard (*Anas platyrhynchos*) breeding behavior (Heinz 1979) and was associated with decreased nesting attempts and nest attentiveness in Common Loons (*Gavia immer*; Barr 1986). At high doses, mercury is a neurotoxin that affects behavior primarily via nerve demyelination and reduction in nerve function (Clarkson 1993). However, the mechanisms by which mercury may affect avian reproductive behavior at sublethal doses are poorly understood. Meyer et al. (1998) suggested that endocrine disruption via mercury contamination might explain changes in Common Loon behavior (Nocera and Taylor 1998) and reproductive success.

Mercury is associated with reduced steroidogenesis in mammals (Burton and Meikle 1980, Friedmann et al. 1998) and fish (Hontela et al. 1992, 1995; Leblond and Hontela 1999); however, few studies have examined the effects of mercury on the endocrine system of birds (but see French et al. 2001). Given *in vitro* at high doses, mercury decreased oviductal progesterone and testosterone receptor affinities in domestic fowl (*Gallus gallus*; Lundholm 1991). Hens given oral mercury doses had decreased prostaglandin synthesis, which affected calcium absorption in the eggshell gland mucosa, resulting in thinner eggshells and decreased hatchability (Lundholm 1995). Thus, it is likely that mercury can affect avian endocrine systems associated with behavior and reproductive physiology.

The extent to which mercury may affect an individual's physiology or behavior depends on exposure and excretion. Birds of different sexes or reproductive status can have different metabolic pathways that affect mercury mobilization and excretion. In particular, adult female birds may have lower circulating mercury concentrations than males, because mercury can be excreted into eggs (Braune and Gaskin 1987).

For example, adult female Herring Gulls (*Larus argentatus*) were estimated to excrete 20% more mercury than males, and females' liver mercury concentrations showed a positive correlation with egg mercury concentrations (Lewis et al. 1993).

Here, we describe relationships among mercury exposure, reproductive physiology, and nesting effort in adult free-living White Ibises (*Eudocimus albus*) breeding in the Florida Everglades. We examined feather mercury concentrations among free-living adult male and female White Ibises over three years. To determine whether relationships existed among mercury and hormone concentrations, we compared feather mercury concentrations with testosterone, estradiol, progesterone, and corticosterone concentrations, and with body-condition scores, of male and female White Ibises in different reproductive stages. We also compared within- and among-year differences in mercury concentrations in nestling Great Egrets (*Ardea alba*) and adult White Ibises. Although those samples were from different species that may not eat the same prey items, and different age classes that may have temporally distinct mercury exposure, we predicted that mercury concentrations in Great Egrets would show annual changes similar to those in White Ibises. We also predicted that White Ibis nesting effort would be related to annual mercury availability, and compared numbers of White Ibis nesting attempts in the Everglades over a seven-year period with mercury concentrations in Great Egret chick feathers, a standardized measure of avian mercury bioavailability.

Methods

Sampling Adult White Ibises

From January through June of 1999, 2000, and 2001, we captured 118 adult White Ibises away from their nests in Everglades Water Conservation Areas (WCAs) 1, 2, 3A, and 3B (Dade and Broward counties) using mist nets and rocket nets (Heath and Frederick 2003). In 1998, we captured 15 adult White Ibises outside the Everglades ecosystem, at colonies in central Florida (Lake, Polk, and Orange counties). Those 15 birds were included in models of mercury effects on hormone concentrations, but not in analyses of Everglades mercury trends. All birds were trapped between sunrise and 4 h past sunrise to avoid heat stress to the birds and control for diel variation in hormone concentrations.

Once birds were captured, we immediately collected a 3-mL blood sample from the jugular vein with a 22-gauge needle and 5-mL syringe (mean time from bird capture to completion of blood collection: 10.4 ± 0.7 min). Blood was transferred to a 5-mL heparinized Vacutainer (BD, Franklin Lakes, New Jersey) and stored on ice until arrival at the laboratory (3–5 h after collection). We centrifuged the blood for 10 min at 2,000 rpm and then separated the plasma from the cellular fraction. Plasma was stored at –20°C until analysis.

To measure mercury exposure, we collected three mature feathers from the bird's scapular region. Many White Ibises were molting ≥5% of their body feathers during the breeding season (Heath et al. 2003). If birds were molting or showed signs of new feather growth (some feathers whiter than others), we chose the whitest (newest) feathers for collection to ensure that feathers represented the most current mercury exposure. Feathers were stored in paper envelopes until analyzed.

We marked each bird with a federal band placed on the leg, above the tibio-tarsal joint. Male White Ibises are larger than females (Kushlan and Bildstein 1992, Heath 2002), and we estimated the sex of a bird from its bill and body size. We later confirmed that subjective assessment using discriminant-function analysis based on body measurements of birds of known sex (Heath 2002, Heath et al. 2003). We measured body mass to the nearest gram, and straight and curved bill length, bill depth, wing chord, and tarsus length to the nearest millimeter. We scored bill and leg colors by holding a paint swatch (Wal-Mart [Bentonville, Arkansas] brand, numbers 0071–1111) up to the body part and recording the color that most closely resembled the bill or leg (Heath 2002). We developed a discriminant-function model based on color scores to classify reproductive stage (i.e. prebreeding, display, egg production, incubation, chick rearing). That model correctly classified known-stage birds 96.4% of the time (Heath 2002).

Sampling Great Egret Chicks

Mercury binds with growing feather tissue, and feather mercury values are positively

correlated with mercury exposure in captive Great Egret chicks (Spalding et al. 2000). Chick feathers provide a contamination history of the time during which the feather was grown (Thompson and Furness 1989, Burger et al. 1993, Burger and Gochfeld 2000), are easy to collect, and represent a specific spatial and temporal sample (Frederick et al. 2001b, Gariboldi et al. 2001).

For years 1994–2001, mercury exposure of Everglades wading birds (i.e. Ciconiiformes) was monitored by collection of feathers from Great Egret chicks. Great Egret feather samples were collected in April and May of 1994–2001 (except for 1996). Up to 1 g of growing feathers were collected from the scapular area of chicks captured on the nest at approximately 20–30 days of age. Culmen length (millimeters) and mass (grams) were also recorded. Feathers were stored in paper envelopes until analyzed (Frederick et al. 2001b). The data set is described in detail in Frederick et al. (2001b).

White Ibis Nesting Effort

We used annual estimates of White Ibis nesting attempts to examine the relationship between White Ibis nesting (Frederick et al. 1996, 1997, 2001a) and annual mercury bioavailability. Between 1994 and 2001, monthly systematic aerial surveys—a series of east–west transects spaced 2.5 km apart at 245 m altitude—of WCAs 2, 3A, and 3B were conducted from February through June to estimate number of breeding pairs. Once colonies were detected, their location was circled, and two observers repeatedly counted the number of White Ibises present at the colony. Number of breeding pairs was estimated as the highest number of birds counted between February and June.

Analyses

Mercury concentrations.—Feather samples were analyzed for total mercury concentrations by the Florida Department of Environmental Protection, Chemistry Section, Tallahassee, Florida. 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, Palo Alto, California; for details, see Frederick et al. 2001b).

Hormone concentrations.—All plasma steroid concentrations were analyzed with a radioimmunoassay procedure described in detail in Heath et al. (2003). Minimum detectable concentrations were 24 pg mL^{-1} for testosterone, 30 pg mL^{-1} for estradiol, 42 pg mL^{-1} for progesterone, and 44 pg mL^{-1} for corticosterone. Many vertebrates respond to capture and handling stress by increasing circulating hormone concentrations (Harvey et al. 1984, Macniven et al. 1992). We performed a regression to see whether hand-ling time had a significant effect on corticosterone or progesterone concentrations. We found a significant relationship between handling time and corticosterone concentrations ($y = 517 + 1.64x$, $r_s = 0.18$, $P = 0.0001$), and between progesterone concentrations and handling time ($y = 518 + 1.50x$, $r_s = 0.12$, $P = 0.0002$). We used those equations to correct for the effects of handling time and used the corrected results for further analyses (Heath 1997).

Body condition.—We calculated a condition index from mass regressed (least squares) against a principal component score that corrected for size variation in females and males separately (Green 2001). Principal component scores were created from a model based on tarsus length, bill depth, and wing length (Heath 2002). The relationship between size score and body mass was linear (female $r_s = 0.45$, male $r_s = 0.45$). Individual residuals were considered a body-condition "score." A positive score represented a greater mass:size ratio than expected (good condition), and a negative score represented a lower mass:size ratio than expected (poor condition).

Statistical analysis.—Prior to analyses, we examined data to test basic assumptions (e.g. normality). If parametric requirements could not be met, the appropriate nonparametric test was used (Hollander and Wolfe 1999). To examine effects of reproductive stage, trap location, year, and sex on mercury concentrations in adult White Ibises, we performed a four-way ANOVA. If interaction terms were not significant, they were removed from the model. We compared mercury concentrations in male and female White Ibises with those in Great Egret chicks separately in two two-way ANOVAs with species and year as main factors. Adult White Ibis hormone concentrations differed between males and females; within each sex, hormones changed among reproductive

stages (Heath et al. 2003). To examine mercury effects on physiological parameters, we conducted multiple Spearman rank correlations between mercury and hormone concentrations and body-condition scores for each sex in each reproductive stage.

To estimate annual mean mercury concentrations for Great Egrets, we used the adjusted means from a linear model that accounted for variation caused by age and colony (Spalding et al. 2000, Frederick et al. 2001b). Culmen length (age) and colony were a (significant) covariate and block, respectively. Adjusted means from that model were used for further comparisons of Great Egret mercury concentrations (mercury exposure measure) with White Ibis nesting effort via a Spearman rank-correlation test. All pairwise means comparisons were made with least-significant-difference tests.

We report descriptive statistics as mean ± SE. Statistical analyses were done on SAS software, versions 6.12 and 8 (SAS Institue, Cary, North Carolina).

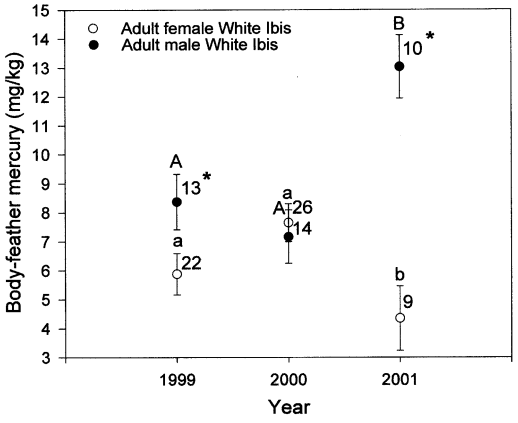

FIG. 1. Effect of year and sex on mercury concentrations in body feathers of adult White Ibises in the Everglades. In 1999 and 2001, males had significantly higher mercury concentrations than females (*), but in 2000 they were not significantly different. Mercury concentrations in females were lower in 2001 than in 2000, but concentrations in males were higher in 2001 than in 2000. Within-sex differences among years are represented by letters: males in capitals, females in lower case; means with different letters were significantly different. Sample sizes are represented at upper right of symbol.

RESULTS

MERCURY CONCENTRATIONS IN ADULT WHITE IBISES

We collected 94 feather samples ($n = 57$ female, $n = 37$ male) from adult White Ibises. Mercury concentration ranges were 0.33–17 mg kg^{-1} in feathers of female White Ibises ($\bar{x} = 6.44 \pm 0.51$ mg kg^{-1}) and 0.69–20 mg kg^{-1} in those of male White Ibises ($\bar{x} = 9.17 \pm 0.84$ mg kg^{-1}). The interaction of year and sex had a significant effect on White Ibis mercury concentrations ($F = 4.68$, df = 2 and 82, $P = 0.012$; Fig. 1 and Table 1). Reproductive stage and trap location did not affect mercury concentrations ($F = 0.18$, df = 4 and 82, $P = 0.94$ and $F = 1.80$, df = 2 and 82, $P = 0.17$, respectively; Table 1).

We collected 12 feather samples ($n = 9$ female, $n = 3$ male; range = 1.5–22 mg kg^{-1}; $\bar{x} = 6.02 \pm$

TABLE 1. ANOVA to compare effects of year, sex, reproductive stage, and trap location on White Ibis feather mercury concentrations.

Source	Degrees of freedom	Sum of squares	*F*-value	*P*-value
Model	11	543.1156	2.84	0.0034
Error	82	1426.0780		

Source	Degrees of freedom	Type III sum of squares	*F*-value	*P*-value
Year	2	9.2142	0.26	0.7679
Sex	1	194.4444	11.18	0.0012
Reproductive stage	4	12.7151	0.18	0.9467
Location	2	62.5671	1.8	0.1720
Year*sex	2	162.9214	4.68	0.0119

1.87 mg kg^{-1}) from White Ibises breeding outside of the Everglades in 1998 and 3 feather samples (n = 2 female, n = 1 male; range = 3.9–12.0 mg kg^{-1}) from White Ibises breeding in WCA 1 in 2001. Those samples were included in analyses of mercury relationships to reproductive physiology.

Mercury and White Ibis Reproductive Physiology

Mercury exposure negatively correlated with female estradiol concentrations during the prebreeding stage (P = 0.03; Table 2), and that negative relationship showed a nonsignificant tendency into the display stage as well (P = 0.12; Table 2). Mercury concentrations showed a nonsignificant positive trend with female progesterone concentrations during incubation (P = 0.17; Table 2). There were no significant correlations among female mercury exposure and testosterone or corticosterone concentrations (Table 2). Finally, mercury exposure showed a nearly significant positive correlation with female body-condition score during egg production (P = 0.08; Table 2).

Mercury concentrations positively correlated with male plasma testosterone concentrations during incubation (P = 0.04; Table 3). Like female White Ibises, males showed a nonsignificant positive correlation between mercury concentrations and plasma progesterone during incubation (Table 3). There were no significant relationships among mercury concentrations and male estradiol or corticosterone concentrations (Table 3). However, mercury concentrations showed a significant positive correlation with body-condition scores during the prebreeding stage (Table 3).

Mercury Concentrations in Feathers of Great Egret Chicks

Feather samples were collected from 563 Great Egret chicks at nine colonies in the Everglades between 1994 and 2001. For the three years in which we were able to compare mercury in both Great Egret chicks and adult White Ibises, mercury concentration patterns in adult female White Ibises were significantly different from those in Great Egret chicks (year*species: F = 14.22, df = 2 and 275, P < 0.0001; Fig. 2), but there were no significant differences in annual mercury patterns between adult male White

Table 2. Spearman rank-correlation statistics for the relationships among female White Ibis reproductive stage, hormone concentrations, and mercury exposure from 1998 to 2001 in Everglades and central Florida populations. Numbers in bold indicate significance at $P \leq 0.05$; numbers in parentheses represent nonsignificant trends.

	Testosterone			Estradiol			Progesterone			Corticosterone			Condition Score		
Stage	r_s	P	n	r_s	P	n	r_s	P	n	r_s	P	n	r_s	P	n
Prebreeding	0.50	0.25	7	**–0.78**	**0.03**	**7**	–0.08	0.83	9	0.40	0.31	8	0.25	0.45	11
Display	0.39	0.22	11	(–0.44)	(0.12)	(13)	–0.02	0.94	11	–0.21	0.53	11	–0.14	0.58	16
Egg laying	–0.29	0.39	11	0.37	0.19	14	0.05	0.85	12	–0.26	0.46	10	(0.40)	(0.08)	(19)
Incubation	0.24	0.48	10	–0.04	0.90	10	(0.63)	(0.17)	(6)	–0.11	0.75	10	–0.18	0.61	10
Chick rearing	–0.06	0.85	10	–0.33	0.33	11	–0.42	0.26	9	–0.35	0.31	10	0.30	0.36	11

TABLE 3. Spearman rank-correlation statistics for the relationships among male White Ibis reproductive stage, hormone concentrations, and mercury exposure from 1998 to 2001 in Everglades and central Florida populations. Numbers in bold indicate significance at $P \leq 0.05$; numbers in parentheses represent nonsignificant trends. No statistics were performed on groups with $n < 5$.

	Testosterone			Estradiol			Progesterone			Corticosterone			Condition Score		
Stage	r_s	P	n	r_s	P	n	r_s	P	n	r_s	P	n	r_s	P	n
Prebreeding	–0.15	0.63	12	0.30	0.42	9	–0.26	0.46	10	0.11	0.74	11	**0.69**	**0.009**	**9**
Display	–	–	4	–	–	3	0.01	0.90	5	0.60	0.28	5	0.20	0.74	5
Egg laying	–	–	3	–	–	2	–	–	3	–	–	3	–0.50	0.67	5
Incubation	**0.64**	**0.04**	**10**	–0.07	0.86	8	(0.62)	(0.10)	(8)	–0.20	0.60	9	0.33	0.38	9
Chick rearing	0.19	0.64	8	0.02	0.95	8	0.28	0.49	8	0.50	0.20	8	–0.33	0.37	9

Ibises and Great Egret chicks (year*species: F = 0.88, df = 2 and 255, P = 0.42; Fig. 2). However, mercury concentrations in adult male White Ibises were significantly higher than those in Great Egret chicks in all years (F = 21.65, df = 1 and 255, $P < 0.0001$; Fig. 2).

We used measures of mercury concentrations in Great Egret chicks in 1994–2001 (no data from 1996) to examine how annual changes in wading birds' mercury exposure in WCAs 2 and 3 may have been related to White Ibis nesting efforts. Mercury concentrations in Great Egret chicks from 1998 to 2001 were significantly lower than in earlier years (1994–1995, 1997; overall F = 83.49, df = 6 and 547, $P < 0.0001$; Fig. 3; Frederick et al. 2001b). Furthermore, annual mercury concentrations in Great Egret chicks between 1994 and

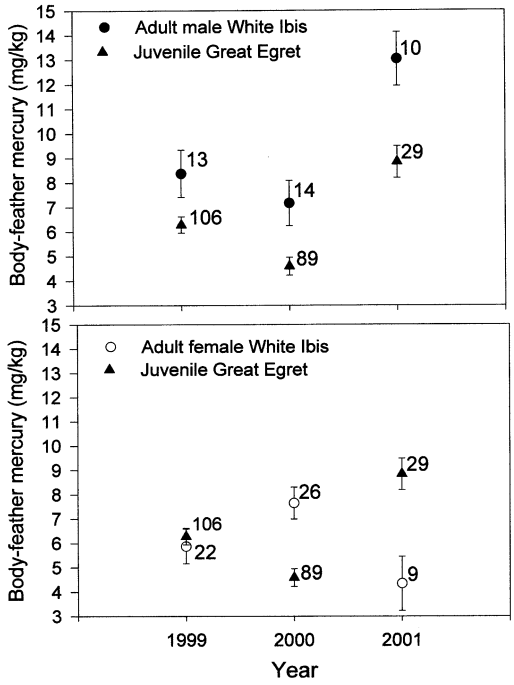

FIG. 2. Comparison of mercury concentrations in Great Egret chicks and adult White Ibis males (top) and females (bottom) in the Everglades. Male White Ibises and Great Egrets showed similar temporal patterns in mercury concentrations, but mercury concentrations were consistently higher in male White Ibises than in Great Egrets. Mercury concentrations in Great Egrets and in adult female White Ibises showed significantly different temporal patterns. Sample sizes are represented at upper right of symbol.

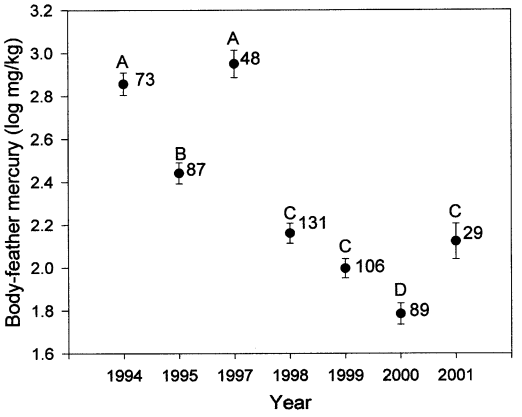

FIG. 3. Annual difference in mercury concentrations in Great Egret chicks in the Everglades (least-squares means ± SE). Means are adjusted for culmen length (age) and location. Means with different letters were significantly different. Sample sizes are represented at upper right of symbol.

2001 were negatively correlated with the number of White Ibis nesting attempts in WCAs 2 and 3 (Spearman-rank $r_s = -0.77$, $P = 0.04$; Table 4).

TABLE 4. Nesting effort of White Ibises and body-feather mercury (Hg) concentrations in Great Egret chicks. The number of White Ibis nests negatively correlated with Great Egret feather mercury concentrations (Spearman-rank $r_s = -0.77$, $P = 0.04$).

Year	Maximum number of White Ibis nests	Mean Great Egret feather Hg
1994	5182	17.40
1995	8177	11.48
1997	5989	19.10
1998	4971	8.67
1999	14014	7.36
2000	32204	5.96
2001	13144	8.35

DISCUSSION

MERCURY CONCENTRATIONS IN FEATHERS OF ADULT WHITE IBISES

In two of three years, adult male White Ibises had higher feather mercury concentrations than adult female White Ibises. Male and female White Ibises forage together in mixed flocks, and differences in mercury load are probably not related to differences in diet or trophic-level exposure. However, male White Ibises are larger than females (present study: males 800–1,160 g, females 610–900 g) and likely consume more prey than female birds. For that reason, male White Ibises might be expected to accumulate more mercury than females. Indeed, during periods of increased food consumption (prebreeding for males, egg production for females; Kushlan and Bildstein 1992, Heath et al. 2003), both sexes tended to show a positive relationship between body-condition scores and feather mercury concentrations, which suggests a relationship between mass (or recent food intake) and mercury concentration. If sexual size-dimorphism completely explained sex differences, mercury concentrations in males would be consistently higher than those in females. However, males and females had different temporal patterns in mercury concentrations across years and, in 2000, male and female White Ibises had similar feather mercury concentrations.

Mercury excretion via egg production is another explanation for lower mercury concentrations in female White Ibises as compared with males, but (like body size) it does not explain the difference in temporal patterns between the sexes, because the proportion of mercury deposition in eggs is dose-independent (Monteiro and Furness 2001a). In addition, there was no evidence that females excreted more mercury with increasing clutch sizes (J. A. Heath unpubl. data). A dosing study of Cory's Shearwaters (*Calonectris diomedea*) also found sex-related differences in mercury excretion not caused by egg formation (Monteiro and Furness 2001a). Sex-specific physiological factors other than (or in addition to) egg formation are likely influencing sex-biased mercury concentrations (Monteiro and Furness 2001a) and may explain the different temporal patterns observed in the present study.

MERCURY CONCENTRATIONS IN ADULT WHITE IBISES COMPARED WITH THOSE IN GREAT EGRET CHICKS

Mercury concentrations in feathers of adult male White Ibises showed a temporal pattern similar to that in Great Egret chick feathers. In the Everglades, adult White Ibises molted their body feathers during the breeding season,

which was within 1–2 months of Great Egret sampling. Mercury is rapidly transferred from blood to feathers in growing (Bearhop et al. 2000, Monteiro and Furness 2001b, Fournier et al. 2002) and molting birds (Monteiro and Furness 2001a), and the plumage:blood ratio is dose-independent in chicks (Monteiro and Furness 2001b). Therefore, among-year comparisons of chick-feather mercury concentrations probably accurately reflected temporal changes in mercury availability during the nesting season of wading birds. Adult males and chicks also share similar excretion pathways (excrement and plumage), which may explain why male White Ibises showed temporal patterns similar to those of Great Egret chicks but adult female White Ibises did not. These results may be applicable to future designs for contaminant monitoring programs. Specifically, body-feather mercury concentrations in adult males may better reflect temporal or spatial variation in exposure than those in female feathers, because males' mercury-excretion pathways are more limited than females'.

Mercury and White Ibis Reproductive Physiology

Multiple correlative relationships existed between feather mercury and White Ibis hormone concentrations. Female White Ibises showed a significant negative relationship between mercury exposure and estradiol concentrations during the prebreeding period and a nonsignificant positive trend between progesterone and mercury concentrations during incubation. Late in the prebreeding stage, female White Ibises were beginning to undergo ovarian follicular development and associated changes in tissues and body condition (Heath et al. 2003). During that stage, mercury may affect estradiol concentrations (and, consequently, ovarian development) by directly affecting estradiol synthesis or estradiol control mechanisms. Studies in fishes and mammals have shown significant interactions among mercury, estradiol, and progesterone (Mondal et al. 1997, Davis et al. 2001). For example, inorganic mercury stimulated fish oocytes to increase progesterone synthesis via increased transcription of the enzyme 3-beta-hydroxysteroid dehydrogenase (3β-HSD), which is responsible for converting pregnenolone to progesterone (Mondal et al. 1997). Initial increased progesterone production by fish oocytes was also associated with decreased progesterone conversion to estradiol (Mondal et al. 1997). In the present study, there was no concomitant increase in progesterone and decrease in estradiol in prebreeding White Ibises (as was reported in fish oocytes). Future research that examines the mechanisms by which mercury may affect avian hormones will require controlled experiments that examine all endocrine pathways, including hormone synthesis and secretion and metabolism control.

Incubating male White Ibises showed a significant positive relationship between testosterone and mercury concentrations. Those results were inconsistent with a mammalian study that demonstrated decreased testosterone production in male rats exposed to 80 $\mu g\ kg^{-1}$ of methylmercury twice a day (Friedmann et al. 1998). The mechanism by which mercury affects testosterone concentrations is unclear. As described above, mercury could stimulate production of the testosterone precursor progesterone, which may subsequently convert to testosterone.

This is the first avian field study to report descriptive relationships (and lack thereof) among mercury and steroid hormone concentrations. Our objective was to explore mercury's potential role as an endocrine disruptor during periods when avian reproductive physiology may be vulnerable to mercury's effects. Although we found few relationships between mercury and hormone concentrations, we found two intriguing correlations during stages where specific hormones play a particular role in reproduction. For example, during the prebreeding stage, estradiol is integral to ovarian development, and that is the period when birds with high mercury concentrations had low estrogen concentrations. Male birds that incubate typically have low circulating testosterone concentrations, which is thought to aid in nest attendance (Ketterson and Nolan 1994). In the present study, males with high mercury had high testosterone during incubation. In both cases, if mercury is acting as an endocrine disruptor, mercury exposure would result in poor reproductive success. During our field study, we could not determine whether mechanistic relationships existed between mercury and hormone concentrations. Although the relationships we report are correlative, they may help future field or laboratory investigations to

focus more narrowly on hormones important to specific aspects of reproductive physiology and behavior, such as gonad development or nest attendance.

MERCURY AND WHITE IBIS NESTING EFFORT

The annual number of White Ibis nests in the Everglades was negatively correlated with feather mercury concentrations in Great Egret chicks, a measure of annual mercury bioavailability. Decreased nest numbers could be the result of decreased nesting attempts or high rates of abandonment. If White Ibises attempted to breed and then failed between monthly surveys, annual nesting estimates would be low. Thus, if a functional (or causative) relationship exists, mercury could adversely affect nesting effort by decreasing nesting attempts or increasing abandonment, or by a combination of those responses.

Nest abandonment is the most common cause of nest failure for White Ibises in the Everglades (Frederick and Collopy 1989, Frederick and Spalding 1994). Abandonment usually occurs after rain causes water levels to rise, prey become less concentrated (Frederick and Collopy 1989, Gawlik 2002), and food availability decreases (Gawlik 2002). Many of the environmental factors that influence food availability, such as water level (Kahl 1964), surface water drying rate (Kushlan 1979, Frederick and Collopy 1989), and rainfall (Ogden et al. 1980) can also affect the conversion rate of mercury to methylmercury (e.g. changes in hydrology; Snodgrass et al. 2000). Therefore, a correlative relationship between mercury exposure and nest number may be indicative of functional relationships between environmental conditions and nesting behavior.

The variability within the Everglades ecosystem and the ability of White Ibises to forage many kilometers away from a colony make it almost impossible to relate the environmental parameters to individual reproductive behavior. However, it is possible to compare White Ibis nesting effort in years that had similar environmental variables but different mercury bioavailability (i.e. significantly higher feather mercury concentrations in Great Egret chicks). During the 1999–2001 breeding seasons, White Ibises attempted to breed and formed multiple colonies in the WCAs. Colonies initiated around the same time each year and were large (>7,000 pairs). In all three of those years, large April storms caused a reversal of water drying-trends during the breeding season, when many birds were incubating and feeding young. Only in 2001, when feather mercury levels were highest, did >50% of White Ibises (including whole colonies) abandon their nests after the water reversal.

Most likely, White Ibis nesting effort and abandonment were affected by a combination of environmental conditions and mercury exposure. For example, White Ibis nest attendance and incubation behavior may change because of decreased physiological promotion of nest attendance, increasing the chance for abandonment under challenging environmental conditions, such as increased water levels and decreased prey availability. In a laboratory study, Zebra Finches (*Taeniopygia guttata*) exposed to methylmercury spent less time incubating and had lower hatching success, and males had significantly lower progesterone concentrations than Zebra Finches not exposed to methylmercury (Heath 2002). Nest attendance in birds during incubation and chick brooding has been correlated with increased progesterone concentrations (Silver and Cooper 1983, Hector et al. 1985, Fivizzani and Oring 1986, Davis et al. 1995) and decreased testosterone concentrations in males (reviewed in Ketterson and Nolan 1994). Disruption of the endocrine system by mercury could adversely affect the physiological promotion of parental care; birds exposed to mercury may therefore be more likely to abandon their nests.

In sum, the evidence provided here suggests that White Ibis endocrinology and nesting behavior may be affected by mercury exposure at tissue contamination concentrations that are below those that have been suggested to cause acute effects (Scheuhammer 1987, Thompson 1996). However, evidence of the interactions among hormones, mercury, and behavior reported here is correlative. Sexual differences in adult White Ibises' mercury exposure and excretion may result in effects that are specific to each sex. Furthermore, mercury exposure, reproductive stage, and other environmental conditions probably mediate the effect of mercury on breeding. Continued research in a controlled setting with captive individuals may produce applicable toxicological models

of the relationships among mercury exposure, hormone concentrations, and parental care that could be applied to White Ibises in the Everglades. In particular, mercury exposure may interfere with the hormonal mechanisms associated with reproductive behavior (e.g. nest attendance). At low exposure levels, we hypothesize that the interactions between mercury and steroid hormones may adversely affect nesting success by causing abandonment during periods of short-term environmental changes (e.g. decreased food availability).

Acknowledgments

We thank P. Epanchin, E. Fenichel, and S. Wright for assistance in capturing White Ibises. We also thank a number of assistants that conducted colony surveys and collected Great Egret feathers. We appreciate the help of R. Dusek and M. Spalding for demonstrating bleeding and laparoscopy techniques. We are grateful for the laboratory space provided by L. J. Guillette, Jr., for hormone analyses. This paper benefited from the comments of K. Sieving, D. Gawlik, C. A. Lott, and two anonymous reviewers. A grant from the U.S. Army Corps of Engineers supported this research. This is publication number R-10053 of the Journal Series, Florida Agricultural Experiment Station.

Literature Cited

Barr, J. F. 1986. Population dynamics of the Common Loon (*Gavia immer*) associated with mercury-contaminated waters in northwestern Ontario. Canadian Wildlife Service, Occasional Paper, no. 56. Ottawa, Ontario.

Bearhop, S., G. D. Ruxton, and R. W. Furness. 2000. Dynamics of mercury in blood and feathers of Great Skuas. Environmental Toxicology and Chemistry 19:1638–1643.

Braune, B. M., and D. E. Gaskin. 1987. Mercury levels in Bonaparte's Gulls (*Larus philadelphia*) during autumn moult in the Quody Region, New Brunswick, Canada. Archives of Environmental Contamination and Toxicology 16:539–549.

Burger, J., and M. Gochfeld. 2000. Metal levels in feathers of 12 species of seabirds from Midway Atoll in the northern Pacific Ocean. Science of the Total Environment 257:37–52.

Burger, J., J. A. Rodgers, and M. Gochfeld. 1993. Heavy metal and selenium levels in endangered Wood Storks (*Mycteria americana*) from nesting colonies in Florida and Costa Rica. Archives of Environmental Contamination and Toxicology 24:417–420.

Burton, G. V., and A. W. Meikle. 1980. Acute and chronic methylmercury poisoning impairs rat adrenal and testicular function. Journal of Toxicology and Environmental Health 6:597–606.

Clarkson, T. W. 1993. Mercury: Major issues in environmental health. Environmental Health Perspectives 100:31–38.

Cleckner, L. B., P. J. Garrison, J. P. Hurley, M. L. Olson, and D. P. Krabbenhoft. 1998. Trophic transfer of methylmercury in the northern Florida Everglades. Biogeochemistry 40:347–361.

Davis, B. J., H. C. Price, R. W. O'Connor, R. Fernando, A. S. Rowland, and D. L. Morgan. 2001. Mercury vapor and female reproductive toxicity. Toxicological Sciences 59:291–296.

Davis, L. S., J. F. Cockrem, G. D. Miller, and G. S. Court. 1995. An incubation timer for seabirds: Progesterone and its relationship to hatching in Adélie Penguins. Emu 95:245–251.

Fivizzani, A. J., and L. W. Oring. 1986. Plasma steroid hormones in relation to behavioral sex role reversal in the Spotted Sandpiper, *Actitis macularia*. Biology of Reproduction 35:1195–1201.

Fournier, F., W. H. Karasov, K. P. Kenow, M. W. Meyer, and R. K. Hines. 2002. The oral bioavailability and toxicokinetics of mercury in Common Loon (*Gavia immer*) chicks. Comparative Biochemistry and Physiology, Part A 133:703–714.

Frederick, P. C., and M. W. Collopy. 1989. Nesting success of five ciconiiform species in relation to water conditions in the Florida Everglades. Auk 106:625–634.

Frederick, P. C., J. A. Heath, R. Hylton, J. D. Semones, G. Babbitt, and M. Spalding. 2001a. Factors affecting breeding status of wading birds in the Everglades. Final report, U.S. Army Corps of Engineers, Jacksonville, Florida.

Frederick, P. C., J. Salatas, and J. Surdick. 1996. Monitoring and research on wading birds in the Water Conservation Areas of the Everglades: The 1996 nesting season. Final report, U.S. Army Corps of Engineers, Jacksonville, Florida.

FREDERICK, P. C., J. SALATAS, J. SURDICK, AND S. BOUTON. 1997. Monitoring and research on wading birds in the Water Conservation Areas of the Everglades: 1996 and 1997. Final report, U.S. Army Corps of Engineers, Jacksonville, Florida.

FREDERICK, P. C., AND M. G. SPALDING. 1994. Factors affecting reproductive success of wading birds (Ciconiiformes) in the Everglades ecosystem. Pages 659–692 *in* Everglades: The Ecosystem and Its Restoration (S. Davis and J. C. Ogden, Eds.). St. Lucie Press, Delray Beach, Florida.

FREDERICK, P. C., M. G. SPALDING, AND R. DUSEK. 2001b. Wading birds as bioindicators of mercury contamination in Florida: Annual and geographic variation. Environmental Toxicology and Chemistry 21:262–264.

FRENCH, J. B., I. C. T. NISBET, AND H. SCHWABL. 2001. Maternal steroids and contaminants in Common Tern eggs: A mechanism of endocrine disruption? Comparative Biochemistry and Physiology C–Toxicology and Pharmacology 128:91–98.

FRIEDMANN, A. S., H. L. CHEN, L. D. RABUCK, AND B. R. ZIRKIN. 1998. Accumulation of dietary methylmercury in the testes of the adult brown Norway rat: Impaired testicular and epididymal function. Environmental Toxicology and Chemistry 17:867–871.

GARIBOLDI, J. C., A. L. BRYAN, JR., AND C. H. JAGOE. 2001. Annual and regional variation in mercury concentrations in Wood Stork nestlings. Environmental Toxicology and Chemistry 20:1551–1556.

GARIBOLDI, J. C., C. H. JAGOE, AND A. L. BRYAN, JR. 1998. Dietary exposure to mercury in nestling Wood Storks (*Mycteria americana*) in Georgia. Archives of Environmental Contamination and Toxicology 34:398–405.

GAWLIK, D. E. 2002. The effects of prey availability on the numerical response of wading birds. Ecological Monographs 72:329–346.

GILMOUR, C. C., G. S. RIEDEL, M. C. EDERINGTON, J. T. DELL, J. M. BENOIT, G. A. GILL, AND M. C. STORDAL. 1998. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. Biogeochemistry 40:327–345.

GREEN, A. J. 2001. Mass/length residuals: Measures of body condition or generators of spurious results? Ecology 82:1473–1483.

HARVEY, S., J. G. PHILLIP, A. REES, AND T. R. HALL. 1984. Stress and adrenal function. Journal of Experimental Zoology 232:633–645.

HEATH, J. A. 1997. Corticosterone levels during nest departure of juvenile American Kestrels. Condor 99:806–811.

HEATH, J. A. 2002. White Ibis (*Eudocimus albus*) reproductive physiology. Ph.D. dissertation, University of Florida, Gainesville.

HEATH, J. A., AND P. C. FREDERICK. 2003. Trapping White Ibises with rocket nets and mist nets in the Florida Everglades. Journal of Field Ornithology 74:187–192.

HEATH J. A., P. C. FREDERICK, T. M. EDWARDS, AND L. J. GUILLETTE, JR. 2003. Reproductive physiology of free-living White Ibises (*Eudocimus albus*) in the Florida Everglades. General and Comparative Physiology 133:118–131.

HECTOR, J. A. L., J. P. CROXALL, AND B. K. FOLLETT. 1985. Reproductive endocrinology of the Wandering Albatross *Diomedea exulans* in relation to biennial breeding and deferred sexual maturity. Ibis 128:9–22.

HEINZ, G. H. 1979. Methylmercury: Reproductive and behavioral effects on three generations of Mallard ducks. Journal of Wildlife Management 43:394–401.

HOLLANDER, M., AND D. A. WOLFE. 1999. Nonparametric Statistical Methods, 2nd ed. John Wiley and Sons, New York.

HONTELA, A., P. DUMONT, D. DUCLOS, AND R. FORTIN. 1995. Endocrine and metabolic dysfunction in Yellow Perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence River. Environmental Toxicology and Chemistry 14:725–731.

HONTELA, A., J. B. RASMUSSEN, C. AUDET, AND G. CHEVALIER. 1992. Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. Archives of Environmental Contamination and Toxicology 22:278–283.

JURCZYK, N. U. 1993. An ecological risk assessment of the impacts of mercury contamination in the Florida Everglades. M.S. thesis, University of Florida, Gainesville.

KAHL, M. P. 1964. Food ecology of the Wood Stork (*Mycteria americana*). Ecological Monographs 34:97–117.

KETTERSON, E. D., AND V. NOLAN, JR. 1994. Male parental behavior in birds. Annual Review of Ecology and Systematics 25:601–628.

KUSHLAN, J. A. 1979. Feeding ecology and prey

selection in the White Ibis. Condor 81: 376–389.

Kushlan, J. A., and K. L. Bildstein. 1992. White Ibis (*Eudocimus albus*). *In* The Birds of North America, no. 9 (A. Poole, P. Stettenheim, and F. Gill, Eds.). Academy of Natural Sciences, Philadelphia and American Ornithologists' Union, Washington, D.C.

Leblond, V. S., and A. Hontela. 1999. Effects of *in vitro* exposures to cadmium, mercury, zinc, and 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane on steroidogenesis by dispersed interrenal cells of rainbow trout (*Oncorhynchus mykiss*). Toxicology and Applied Pharmacology 157:16–22.

Lewis, S. A., P. H. Becker, and R. W. Furness. 1993. Mercury levels in eggs, tissues, and feathers of Herring Gulls *Larus argentatus* from the German Wadden Sea Coast. Environmental Pollution 80:293–299.

Lundholm, C. E. 1991. Influence of chlorinated hydrocarbons, Hg2+ and methyl-Hg+ on steroid hormone receptors from eggshell gland mucosa of domestic fowls and ducks. Archives of Toxicology 65:220–227.

Lundholm, C. E. 1995. Effects of methyl mercury at different dose regimes on eggshell formation and some biochemical characteristics of the eggshell gland mucosa of the domestic fowl. Comparative Biochemistry and Physiology, Part C 110:23–28.

Macniven, E., D. Decatanzaro, and E. V. Younglai. 1992. Chronic stress increases estrogen and other steroids in inseminated rats. Physiology and Behavior 52:159–162.

Meyer, M. W., D. C. Evers, J. J. Hartigan, and P. S. Rasmussen. 1998. Patterns of Common Loon (*Gavia immer*) mercury exposure, reproduction, and survival in Wisconsin, U.S.A. Environmental Toxicology and Chemistry 17:184–190.

Mondal, S., B. Mukhopadhyay, and S. Bhattacharya. 1997. Inorganic mercury binding to fish oocyte plasma membrane induces steroidogenesis and translatable messenger RNA synthesis. Biometals 10: 285–290.

Monteiro, L. R., and R. W. Furness. 2001a. Kinetics, dose-response, and excretion of methylmercury in free-living adult Cory's Shearwaters. Environmental Science and Technology 35:739–746.

Monteiro, L. R., and R. W. Furness. 2001b. Kinetics, dose-response, excretion and toxicity of methylmercury in free-living Cory's Shearwater chicks. Environmental Toxicology and Chemistry 20:1816–1823.

Nocera J. J., and P. D. Taylor. 1998. *In situ* behavioral response of Common Loons associated with elevated mercury (Hg) exposure. [Online.] Conservation Ecology, vol. 2. Available at www.ecologyandsociety.org/vol2/iss2/art10/index.html.

Ogden, J. C., H. W. Kale II, and S. A. Nesbitt. 1980. The influence of annual variation in rainfall and water levels on nesting by Florida populations of wading birds. Transactions of the Linnaean Society of New York 9:115–126.

Scheuhammer, A. M. 1987. The chronic toxicity of aluminum, cadmium, mercury, and lead in birds: A review. Environmental Pollution 46:263–295.

Silver, R., and M. Cooper. 1983. Avian behavioral endocrinology. BioScience 33:567-572.

Snodgrass, J. W., C. H. Jargoe, A. L. Bryan Jr., H. A. Brant, and J. Burger. 2000. Effects of trophic status and wetland morphology, hydroperiod, and water chemistry on mercury concentrations in fish. Canadian Journal of Fish and Aquatic Sciences 57:171–180.

Spalding, M. G., P. C. Frederick, H. C. McGill, S. N. Bouton, and L. R. McDowell. 2000. Methylmercury accumulation in tissues and its effects on growth and appetite in captive Great Egrets. Journal of Wildlife Diseases 36:411–422.

Thompson, D. R. 1996. Mercury in birds and terrestrial mammals. Pages 341–356 *in* Interpreting Environmental Contaminants in Animal Tissues (W. N. Beyer, G. H. Heinz, and A. Redmon, Eds.). Lewis Publishers, Boca Raton, Florida.

Thompson, D. R., and R. W. Furness. 1989. Comparison of the levels of total and organic mercury in seabird feathers. Marine Pollution Bulletin 20:577–579.

Wolfe, M. F., S. Schwarzbach, and R. A. Sulaiman. 1998. Effects of mercury on wildlife: A comprehensive review. Environmental Toxicology and Chemistry 17:146–160.

Zillioux, E. J., D. B. Porcella, and J. M Benoit. 1993. Mercury cycling and effects in freshwater wetland ecosystems. Environmental Toxicology and Chemistry 12:2245–2264.

Associate Editor: W. H. Karasov