

THE USE OF NOVEL BIOMARKERS TO DETERMINE DIETARY MERCURY ACCUMULATION IN NESTLING WATERBIRDS

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Abstract—Mercury (Hg) depuration into growing feathers is a well-studied phenomenon in waterbirds. Although the kinetics of Hg excretion in relation to molt and diet has been studied extensively, the relationship between the individual nutritional condition of nestlings and dietary Hg accumulation has not been investigated. In the present study, a body-condition index (BCI) and nutritional condition index (NCI) for nestlings of two waterbird species occupying different trophic positions on the aquatic food web were determined and used to predict Hg accumulation through diet. Candidate models consisting of these indices and nestling age were compared using Akaike's information criterion corrected for small sample sizes. For both species, the top-performing model contained the sole parameter of nutritional condition index (NCI). The relationship between Hg and NCI was stronger in the species foraging higher on the trophic web, which experienced higher rates of Hg depuration into feathers. Models containing BCI could not be discounted (AICc < 2) for one of the species and the utility of this index is discussed. *Environ. Toxicol. Chem.* 2012;31:1143–1148. © 2012 SETAC

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INTRODUCTION

Monitoring mercury (Hg) accumulation in waterbirds is a commonly used conservation tool [1–4], and it has been established that feathers from nestlings are particularly useful for indicating Hg content of a local foraging habitat [5–9]. This is due largely to the limited movement of adults around a central breeding location [10]. Mercury accumulated through diet during the nestling phase is eliminated from the body into growing feathers and should represent the Hg content of the prey items in the habitat surrounding the nest. Because of the ephemeral nature of prey items in estuarine systems and individual foraging habits of adults, however, a study investigating the interaction between the nutritional condition of developing waterbird nestlings and Hg accumulated through diet would strengthen our use of feathers as biomarkers. A dietary study would also strengthen our understanding of the dynamics of terrestrial consumers foraging in aquatic food webs. One might expect nestlings fed prey items that are higher on the trophic web would maintain a better nutritional condition at the expense of accumulating more Hg than a nestling fed a diet of low-trophic level prey items. Such a tradeoff has been observed in piscivorous waterbirds, but the relationship has not been investigated in species foraging primarily on invertebrate prey [11].

Ptilochronology is an accurate, inexpensive technique that uses growth bars, or alternating patterns in deposition of pigment over a 24-h period into growing feathers to determine the nutritional condition of an individual [12,13]. Limited dietary nutrition will result in slower feather growth and thus in narrower growth bars [14]. Empirical studies using ptilochronology have established the utility of growth bar width as a biomarker to determine habitat and diet quality [15–18]. When coupled with Hg analysis, ptilochronology can further our

understanding of the relationship between nutritional condition and Hg accumulation in waterbird nestlings.

The goal of the present study was to apply novel biomarkers in waterbird species to predict Hg accumulation through diet originating from a local foraging habitat. Specifically, our aims were to demonstrate whether a measure of individual nutritional or body condition can predict Hg accumulation in two waterbird species occupying different ends of a foraging habitat-use spectrum and to demonstrate the utility of these biomarkers at two locations along the East Coast of the United States experiencing different regimes with respect to disturbance.

MATERIALS AND METHODS

Study species

The diet of the glossy ibis (*Plegadis falcinellus*) typically consists of invertebrates, mollusks, and gastropods, although fish, reptiles, and amphibians are taken occasionally as well (<http://bna.birds.cornell.edu/bna/species/545>; [19]). A habitat generalist foraging strategy allows double-crested cormorants (*Phalacrocorax auritus*) to be opportunistic and exploit multiple prey, with various fish species comprising the bulk of diet (<http://bna.birds.cornell.edu/bna/species/441>). Because they forage on higher trophic-level fish (~250 fish species have been documented in the diet) and at more foraging locations within a landscape (found commonly in urban and rural regions), double-crested cormorants are considered effective sentinels of ecosystem contaminant load [5].

Study areas

Virginia, USA. Chimney Pole and Chincoteague Causeway heronries are located on relatively pristine natural lagoonal marshes in Hog Island and Chincoteague Bays on the eastern shore of Virginia within the Virginia Coast Reserve (<http://www1.vcrllter.virginia.edu/home1/index.php>) (37°28'N, 75°43'W and 37°56'N, 75°25'W respectively) [20,21]. Breeding populations of wading and shore birds are within close

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proximity to undeveloped shoreline and marsh foraging habitat and serve as effective indicators of the health of the Virginia barrier island ecosystem [22].

New York, USA. Hoffman Island, Canarsie Pol, and Swinburne Island are in the urbanized New York metropolitan region and served as comparison sites with Virginia. Hoffman Island is a 4.0-ha island located 1.5 km offshore from Staten Island, New York (40°35'N, 74°4'W) [23]. Swinburne Island is located next to Hoffman Island in the lower New York Harbor (40°35'N, 74°4'W) and is a small (1 ha) island that supports a breeding colony of 264 cormorant pairs (New York City Audubon's Harbor Herons Project: 2007 Nesting Survey; www.harborestuary.org/reports/Nesting_Survey.pdf). Canarsie Pol is a large (96 ha) dredged-material island in Jamaica Bay, Brooklyn, New York (40°36'N, 73°50'W) [24].

Field work

During the 2009 (April 10–July 7) and 2010 (May 5–July 17) breeding seasons, colonies were visited weekly until egg laying had occurred. At this point, randomly selected nests were marked with numbered wooden stakes. Nestlings were marked with U.S. Fish and Wildlife Service aluminum and plastic colored leg bands, and nests were monitored during the incubation, nestling, and fledging phases as part of an ongoing study (C.E. Clarkson, University of Virginia, Charlottesville, VA, USA, unpublished data). Once nestlings possessed fully formed feathers, individuals of both species were caught and mass (g), wing chord (mm), tarsometatarsus length (tarsus) (mm), and culmen length (mm) were measured. A primary feather (Primary 1, P1) was collected from each individual and stored in a paper envelope for later analysis. Regurgitations were collected opportunistically from handled chicks and preserved immediately with 70% ethanol in glass collection jars. Feathers and regurgitant were collected under the appropriate federal permit (U.S. Geological Survey Master Banding Permit 23573) and with permission from the Animal Care and Use Committee (ACUC 3702, University of Virginia).

Laboratory work

Feather analysis. The first 10 d of feather growth were used to determine nutritional condition, because this distal portion of the feather was fully formed and had no blood supply. Previous studies have demonstrated that the Hg contained in a portion of feather with no blood supply resists change [25]. Individual feathers were placed on an index card and a size 0 insect pin was inserted through the distalmost growth bar. Another insect pin was inserted into the tenth growth bar from the distal end of the feather. The feather was removed from the index card and the distance between the two insect pins was measured with a Tresna Instruments digital caliper, accurate to ± 0.01 mm. The distance between these two growth bars indicated the total feather length produced in the first 10 d of feather production and was divided by 10 to determine a nutritional condition index (NCI) (mm/d) for each individual. In some individuals, fewer than 10 growth bars were clearly visible, and the total length of the feather was divided by the number of growth bars measured (no fewer than seven growth bars). To determine measurement repeatability, feather measurements were taken once for each individual and then a second measurement was taken approximately one month later. The same researcher (C.E. Clarkson) performed all measurements. The two measurements were compared using intraclass correlation.

Hg Analysis. All feathers were cut at the tenth growth bar prior to Hg analysis. Samples were analyzed for total Hg (THg)

concentrations using a Tekran[®] cold-vapor atomic fluorescence spectrophotometer according to U.S. Environmental Protection Agency (U.S. EPA) method 1631, Revision E (2002) under a Class 100 clean bench at the University of Virginia. Although the majority of mercury contained in feathers is primarily in the methylated form [MeHg] [26], results will be reported as Hg to avoid confusion. The minimum detection limit for THg was determined to be 0.19 ng L^{-1} from the standard deviation (0.06) of seven aliquots of a 0.65 ng L^{-1} solution (U.S. EPA). Digestion procedures followed a modified protocol of the Series 2600 Total Mercury Analysis of Human Hair (Tekran, Toronto, ON, Canada). Feathers were heated in an oven at 60°C overnight to determine dry weight and then placed in borosilicate Erlenmeyer flasks covered with Teflon spheres. The feathers were digested with 5 ml of concentrated HNO₃ overnight at room temperature, then slowly heated to boiling over 1.5 h and refluxed for another 3 h. Two nitric acid method blanks without feathers were included in each batch of feathers (16–18 in a batch). After cooling overnight, all samples were diluted to approximately 50 ml using 0.5% bromium monochloride (BrCl) solution. An additional two 0.5% BrCl method blanks without feathers or nitric acid were included in each batch of feathers for quality assurance purposes. All samples and blanks were capped and double bagged until subsequent analysis. Overall Hg laboratory accuracy was determined by participating in an interlaboratory proficiency test administered by Environment Canada in January 2010. All quality control metrics are within U.S. EPA guidelines.

Diet analysis. Wet weight (g) and size (mm) of diet samples were determined and prey items were identified to species-level when possible using dichotomous keys. Frequency and percent biomass (PB) of each dietary item were determined for both species.

Statistical analysis. Nestling body condition is often used as a biomarker for habitat condition and a novel body-condition index (BCI) was created for each individual based on the structural size of a chick at a given age. First, a principal components analysis (PCA) was performed using tarsus and wing chord measurements for each chick. The first principal components axis described a positive relationship for the two variables (0.70 for both species) and accounted for 83% of the total variance in nestling structural size for glossy ibis and 62% for double-crested cormorants. Each individual's principal component 1 (PC1) score served as a measurement of its structural size. The BCI was obtained by using the residuals from a linear regression of culmen on the PC1 scores. Culmen length is invariant in many waterbirds of a similar age and is commonly used as a proxy for age [27–29,9]. All chicks in the present study were of similar age (25–30 d). Individuals with a negative BCI were considered to be structurally smaller at a given age and therefore more stunted in growth than those with a positive score.

Nutritional condition index, Hg analysis, and BCI were computed for 48 glossy ibis nestlings during the two breeding seasons (Table 1). Mercury content and NCI was measured for 30 cormorants during the two breeding seasons; however, full morphometric measurements were not taken on all individuals, and BCI was calculated for 10 individuals ($n = 5$ for both 2009 and 2010).

The capacity of the BCI, NCI, and culmen (proxy for age) to predict Hg content was examined using an information-theoretic approach [30]. Mercury content was the dependent variable, and BCI, culmen, and NCI were the predictor variables. Akaike's Information Criterion adjusted for small sample sizes

Table 1. Descriptive statistics by site for glossy ibis (*Plegadis falcinellus*, GLIB) and double-crested cormorant (*Phalacrocorax auritus*, DCCO) nestlings^a

State	Site	n	THg	n	NCI	n	BCI
GLIB							
New York	Hoffman Island	18	3.22 ± 0.52	18	2.28 ± 0.11	18	0.27 ± 0.22
	Canarsie Pol	8	2.96 ± 1.10	8	1.90 ± 0.07	8	0.03 ± 0.29
Virginia	Chincoteague	11	3.21 ± 0.83	11	2.48 ± 0.17	11	-0.08 ± 0.33
	Chimney Pole	11	4.41 ± 0.62	11	2.41 ± 0.15	11	-0.38 ± 0.22
DCCO							
New York	Swinburne Island	19	12.60 ± 1.40	19	2.71 ± 0.12	0	N/A
Virginia	Chimney Pole	11	10.88 ± 1.89	11	2.90 ± 0.19	10	0.00 ± 0.

^a Averages ± standard error are presented. No difference existed in parameters by site based on a general linear model ANOVA (culmen length was used as a covariate to control for nestling age). THg = feather mercury content (μg/g) dry weight, NCI = Nutritional Condition Index (mm/d); BCI = body-condition index.

(AIC_c) was computed for each candidate model using an all subsets approach [30]. The most parsimonious model (lowest AIC_c) was selected as the best-fit model to predict Hg content. Akaike weights (ω_i), parameter likelihoods, and evidence ratios (ER) were calculated for all competing models [31].

All statistical analyses were performed using R statistical package (Ver 2.13.1). All data were checked for normality using Kolmogorov-Smirnov tests and visual inspection of histograms and transformed to LOG(y + 1) if necessary. Means are reported ± standard error (SE) and results were considered significant if $p \leq 0.05$.

RESULTS

No difference existed in any of the measurements for either species by state based on a general linear model ANOVA (glossy ibis: NCI: $F = 2.05$, $p = 0.12$; BCI: $F = 1.38$, $p = 0.26$; Hg: $F = 1.59$, $p = 0.21$; double-crested cormorant: NCI: $F = 0.87$, $p = 0.36$; Hg: $F = 0.00$, $p = 0.96$; culmen was used as a covariate to control for nestling age). There was high repeatability in feather measurements based on intraclass correlation ($r = 0.92$).

Cormorant feathers collected from New York were taken from the scapular region, whereas feathers collected in Virginia were the same as those collected from glossy ibis (P1). While Hg deposition into growing feathers is variable by feather tract,

mercury analysis did not reveal a difference in Hg ($t = -0.73$, $p = 0.47$, $df = 19$) nor NCI ($t = 0.90$, $p = 0.38$, $df = 16$) between scapulars and primary remiges of the same bird [25].

Nutritional and body condition as indicators of Hg

Glossy ibis. Three candidate models were chosen to explain variation in log_e Hg content (model goodness-of-fit = 0.10) (Table 2). The most parsimonious model (ω_i = 0.25) contained the single parameter NCI, whereas the second best-performing model (ω_i = 0.23) included the parameters NCI and BCI. The last model considered (ω_i = 0.21) contained the single parameter BCI. The relative importance of NCI at predicting variation in feather log_e Hg content was greater than BCI (parameter likelihood values: 0.48, 0.44, respectively, evidence ratio value = 1.19). There was a weak negative relationship between average NCI and BCI and log_e Hg (Fig. 1).

Double-crested cormorants. Only one model was chosen to explain variation in log_e Hg content (model goodness-of-fit: 0.46). As with glossy ibis, the most parsimonious model (ω_i = 0.51) contained the single parameter NCI (Table 2). Nutritional condition index declined with increasing Hg (Fig. 2).

Diet

For glossy ibis nestlings, the majority of prey items in the regurgitant were from the orders Coleoptera (Dysticidae), Diptera (Tabanidae and Muscidae) and Xiphosurida (Limuli-

Table 2. Top-ranked models predicting mercury content of feathers using AIC analysis adjusted for small sample sizes (AIC_c)^a

Species	Model	K	AIC _c	ΔAIC _c	ω _i	ER
GLIB						
	NCI ^b	2	-129.12	0	0.25	
	NCI + BCI ^b	3	-129.32	0.09	0.23	1.05
	BCI ^b	2	-128.78	0.34	0.21	1.19
	NCI + log _e culmen	3	-127.12	2.29	0.08	3.15
	BCI + log _e culmen	3	-127.11	2.30	0.08	3.16
	GLOBAL	4	-127.32	2.50	0.07	3.48
	NULL	1	-125.56	3.37	0.05	5.40
	Log-culmen	2	-125.56	3.56	0.04	5.93
DCCO						
	NCI ^b	2	34.32	0	0.51	
	NCI + culmen	3	36.93	2.61	0.14	3.68
	NCI + BCI	3	37.04	2.72	0.13	3.89
	NULL	1	37.36	3.04	0.11	4.56
	Culmen	2	38.99	4.67	0.05	10.33
	BCI	2	39.16	4.84	0.05	11.25
	GLOBAL	4	42.73	8.41	0.01	66.88
	BCI + culmen	3	43.15	8.83	0.01	82.50

^a The most parsimonious model (ΔAIC_c = 0) is presented first.

^b These models can be considered the best at predicting total mercury content in chicks (ΔAIC_c < 2).

GLIB = glossy ibis (*Plegadis falcinellus*), DCCO = double-crested cormorant (*Phalacrocorax auritus*), K = number of parameters estimated in model, ω_i = Akaike model weights, ER = evidence ratio; see text for descriptions of model parameters.

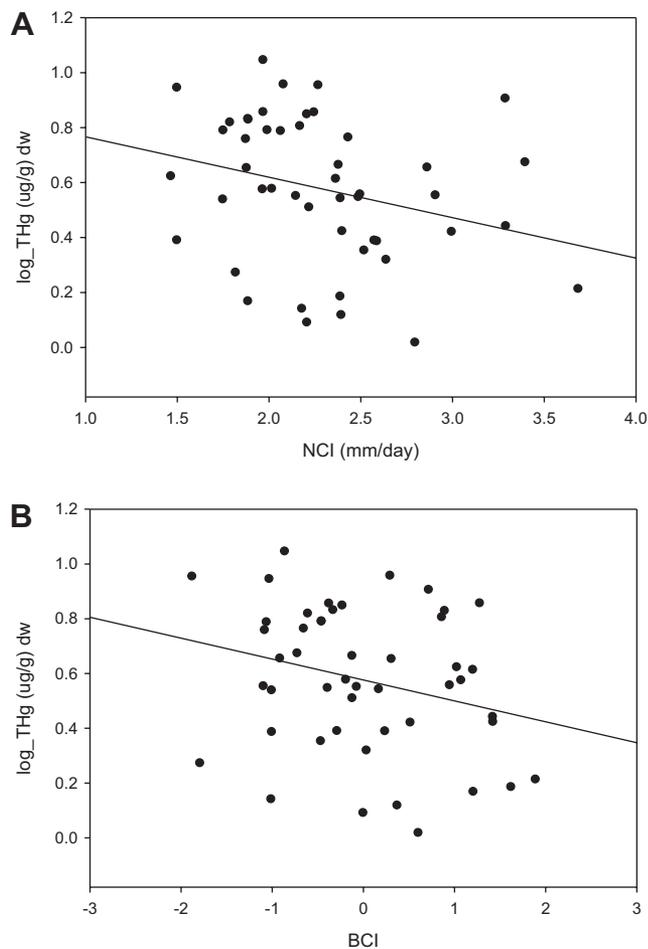


Fig. 1. Relationships of log₁₀ THg content and (A) nutritional condition index (NCI, mm/day) of nestling glossy ibis (*Plegadis falcinellus*) feathers and (B) body-condition index (BCI). Nestlings in better nutritional and body condition tended to have less mercury (Hg) burden.

dae). From diet samples, it was clear that some adults foraged in freshwater systems and selected aquatic macroinvertebrates and amphibians, whereas others foraged primarily in saltwater systems, choosing to forage on eggs of fish and horseshoe crabs. Double-crested cormorant nestlings were fed all marine-derived prey items and predominantly fish species

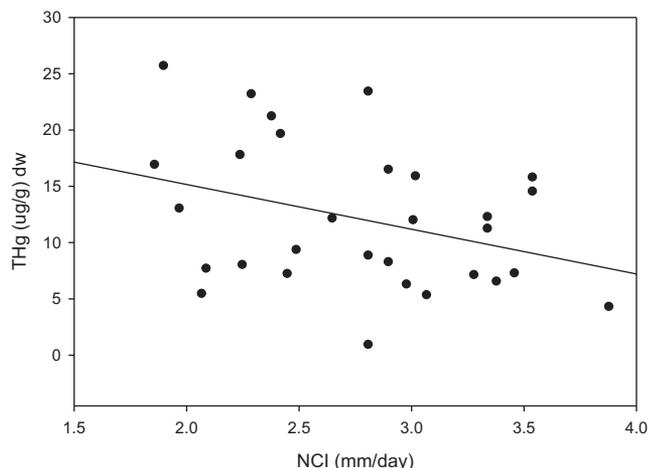


Fig. 2. Relationship between total mercury (THg) content and nutritional condition index (NCI, mm/day) of nestling double-crested cormorants (*Phalacrocorax auritus*).

(Fundulidae, Batrachoididae, and Atherinopsidae) and grass shrimp (*Palaemonetes* spp.) (Table 3).

DISCUSSION

The fact that Hg content of feathers did not differ significantly between colonies for the same species suggests that bioavailable Hg may not differ greatly, even though the New York colony resides in a more Hg-contaminated environment. A sufficient dietary overlap may also exist in waterbird foraging, regardless of geographic locale, to make dietary Hg content as much a function of trophic level of prey as it is of geographic variability. Although the total amount of Hg in the New York metro region may be higher, the bioavailable portion may be equally high in the state of Virginia, where large shallow estuaries present ideal locations for the conversion of Hg into a bioavailable form [32].

Many studies have investigated the depuration of Hg into rapidly growing nestling waterbird feathers [33,34]; however, no studies have combined this analysis with ptilochronology to determine the interaction between individual nutritional condition and Hg accumulation. Although the results of the present study suggest that NCI serves as an accurate predictor of the Hg load that nestling waterbirds experience through diet, the fact that individuals in better nutritional condition tend to have lower Hg burdens seems counterintuitive for piscivorous species and requires further investigation. Individual nestlings with a higher Hg burden yet lower NCI may be consuming high

Table 3. Frequency and percent biomass (PB) of prey items fed to glossy ibis (GLIB, $n = 20$) and double-crested cormorant (DCCO, $n = 10$) nestlings^a

Prey species	GLIB		DCCO	
	Frequency	PB	Frequency	PB
Fish				
Unidentified eggs	22	4.21	0	0
Fundulidae	0	0	42	60.87
<i>Opsanus tao</i>	0	0	4	5.80
<i>Menidia menidia</i>	0	0	2	2.90
Arthropoda				
Limulidae	200	38.24	0	0
<i>Palaemonetes</i> spp.	0	0	21	30.43
<i>Uca pugnax</i>	1	0.19	0	0
Mollusca				
<i>Gemma gemma</i>	3	0.57	0	0
Amphibia				
Anura	2	0.38	0	0
Gastropoda				
<i>Littorina irrorata</i>	6	1.14	0	0
Arachnida				
Araneae	1	0.19	0	0
Annelida				
Oligochaeta	6	1.14	0	0
Insects				
Hemiptera				
<i>Belostoma</i> spp.	2	0.38	0	0
Odonata				
<i>Aeshna</i> spp.	6	1.14	0	0
Coleoptera				
Dysticidae	11	2.10	0	0
Lepidoptera	3	0.57	0	0
Diptera				
Tabanidae	225	43.02	0	0
Muscidae	31	5.93	0	0
Chironomidae	3	0.57	0	0
Tipulidae	1	0.19	0	0

^a Due to small sample size, regurgitant samples for New York and Virginia were combined for the analysis.

trophic level prey items that do not supply adequate energy content to promote feather growth. A nestling glossy ibis that is fed a larger percentage of predatory insects (Odonata, Araneidae) will likely accumulate more Hg than an individual that is fed prey items that forage low on the aquatic food web yet supply high levels of energy. This is illustrated in the present study by individuals that preyed on horseshoe crab eggs, which are high-energy diet items but are lower on the trophic web than predacious insects. The phenomenon of higher rates of mercury accumulation in birds foraging on insects rather than fish has been documented by Critol et al. [35]. A study investigating the relationship between energy content, trophic position, and Hg content of commonly consumed prey items would strengthen the present study.

Shortly after fledging, blood and tissue concentrations of Hg increase as feather growth ceases, and this excretory pathway no longer exists [34,36,37]. The depuration of Hg into growing feathers therefore represents a highly efficient method of reducing body load of Hg during critical periods of development. In the present study, the first 10 d of feather growth were analyzed, but diet samples were obtained during the period of feather collection, when nestlings were between 25 and 30 d old. Therefore, the diet sample collected may not accurately reflect the diet nestlings were fed during the first 10 d of feather growth. A comprehensive study of dietary changes over the course of the nestling phase is needed to account for this source of potential variation. However, because adult foraging behavior dictates nestling diet, it seems safe to assume that a nestling fed a more nutritious diet during the first 10 d of feather growth would be maintained on this diet for the duration of the nestling phase.

Models incorporating the BCI of the individual could not be discounted for glossy ibis in the present study and suggest that the structural size of developing nestlings may be a useful predictor of Hg content for this species. Immature rats dosed with mercury demonstrated inhibited growth in proximal tibia [38]. When fed ad libitum diets containing selenium and methylmercury, adult mallards (*Anas platyrhynchos*) produced embryos with small or malformed wings and legs [39] and stunted growth has been documented in the embryos of waterbird species exposed to methylmercury [40]. Traditional body condition indices are calculated as the residuals of body mass on a structural skeletal component (tarsus, wing cord) or as mass gain over the nestling period [41]. These indices could be misleading in the present study for two reasons: First, nestlings were weighed once during the study and at different times of day. Because nestling mass varies with time of day and feeding schedule, this single mass measurement would lend itself poorly to comparison [42]. Second, metrics of developmental health, such as nestling growth-rate are useful for detecting certain contaminants; however, because nestlings depurate Hg into actively growing feathers, catabolism of body tissue may not take place until after this excretory pathway ceases [34]. Because feather growth does not cease until after fledging, use of traditional mass-based growth-rate of nestlings may not be an adequate biomonitoring tool for detecting high levels of Hg in local food sources. Further, a single feather collection is less invasive than multiple trips into a breeding colony to obtain data for growth-rate calculations and represents a more efficient biomarker that causes fewer disturbances. In glossy ibis, the parameter BCI was the third best-performing model and parameter likelihood values suggest that BCI was only strengthened as a predictive tool when combined with NCI. Because the goal of the present study was to identify a biomarker capable of effectively predicting Hg content in nestlings as a proxy for

local habitat conditions, body condition indices, whether based on mass gain or bone growth, may be insufficient predictors of Hg in pre-fledged chicks. This is supported by the fact that BCI was not found to be a predictive tool in double-crested cormorants, which is of particular interest considering the much higher Hg accumulation in this species. Despite these shortcomings, the novel BCI used in the present study may be a useful biomarker for predicting contaminants that are known to stunt growth yet for which no known efficient excretory pathway exists, such as cadmium [43,44].

While ptilochronology has not been applied as a biomarker in waterbird studies, its use could greatly enhance our understanding of local habitat quality and the interactions between dietary items and individual nutritional condition. In addition, the capacity to reduce observer-caused disturbance in species prone to nest abandonment could increase the attractiveness of the technique to conservation and management agencies. A single feather can serve as a dietary record for the entirety of the nestling period without the need to enter breeding colonies repeatedly. The present study demonstrates that the nutritional condition of the individual is not only tied to energy content of diet, but also can be used to gain insight into the dietary contaminant loads derived from diet. When applied appropriately, there is a promising future of ptilochronology as a biomarker in waterbirds.

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