

Mercury Exposure and Diet in Brown Pelicans (*Pelecanus occidentalis*) in North Carolina, USA

KIERSTEN N. NEWTOFF AND STEVEN D. EMSLIE*

Department of Biology and Marine Biology, 601 South College Road, University of North Carolina Wilmington, Wilmington, North Carolina, 28403, USA

*Corresponding author; E-mail: emslies@uncw.edu

Abstract.—Methylmercury biomagnifies in food chains and can lower reproductive success in many organisms, particularly in top predators such as Brown Pelicans (*Pelecanus occidentalis*). To determine and compare variability in mercury exposure in this species, chick feathers and egg membranes were collected from seven breeding sites located in the remote Pamlico Sound and the more human-impacted Cape Fear River in North Carolina, USA. The average concentration of total mercury in egg membrane was 0.20 ± 0.14 ppm dry weight, a level associated with slightly reduced reproductive success in some birds, while chick feather total mercury concentrations (1.13 ± 0.02 ppm fresh weight) were well below the lowest observable adverse effects level. Mercury exposure did not vary between the Cape Fear River and Pamlico Sound, but did vary significantly among three Cape Fear River colonies. Diet analysis using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ revealed minimal differences in the trophic level and foraging location of prey between islands in close proximity, indicating that differences in mercury availability are not due to differences in diet composition. The source of mercury variation in Brown Pelicans remains unknown and in need of further study. Received 8 June 2016, accepted 11 November 2016.

Key words.—biomagnification, Brown Pelican, carbon, methylmercury, nitrogen, *Pelecanus occidentalis*, stable isotopes.

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Mercury is a widely distributed heavy metal that can negatively impact species and ecosystems (Driscoll *et al.* 2013). Methylation of inorganic mercury into the bioavailable methylmercury (CH_3Hg or MeHg) especially occurs in aquatic systems where it can then biomagnify within the food chain. Exposure to high levels of methylmercury can cause severe damage to the central nervous system and impact development of young, particularly in top predators. Piscivorous birds and mammals are especially vulnerable and may suffer from declines in reproductive success and can develop physical abnormalities (Heinz and Hoffman 2003; Evers *et al.* 2008). Birds are increasingly used as biomonitors to quantify the mercury load in the environment (Furness and Camphuyzen 1997; Goodale *et al.* 2008) with eggs and feathers often sampled since these tissues serve as elimination paths of mercury and can easily be collected and analyzed (Barrett *et al.* 1985; Braune and Gaskin 1987).

Brown Pelicans (*Pelecanus occidentalis*) in Alabama, Florida, Georgia, South Carolina, North Carolina, and points northward along the USA Atlantic coast were removed from the Federal List of Endangered and Threatened Wildlife in 1985 (U.S. Fish and Wild-

life Service 1985) and from wherever found outside those areas in 2009 (U.S. Fish and Wildlife Service 2009) after recovering from major population declines in the mid-20th century, largely due to DDT and other factors (Shields 2002). The Brown Pelican is a piscivorous species. It mainly eats small fish that form schools near the surface such as menhaden (*Brevoortia* sp.), which comprise 95% of their diet in populations along the Atlantic Coast (Shields 2002). Methylmercury assimilates into Brown Pelican tissues through this piscivorous diet, which can be measured using stable isotope analyses of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$). Thus, continued monitoring of this species and its exposure to pollutants can help prevent future population declines (U.S. Fish and Wildlife Service 2009).

Despite their sensitivity to pollutants, mercury in Brown Pelicans has not been examined since 2002 when, of five aquatic birds studied in the Gulf of California, Brown Pelicans had the highest levels of mercury (2.85 ppm) in their muscle tissue (Ruelas-Inzunza *et al.* 2009). Mercury was last measured in Brown Pelicans in North Carolina, USA, in 1994 with whole egg samples from Wainwright Island, Pamlico Sound, and

South Pelican Island in the Cape Fear River (Wickliffe and Bickham 1998). This analysis yielded mean mercury levels of 0.34 ppm from the former site and 0.26 ppm from the latter, opposite of expectations given greater chemical residues the authors measured in the Cape Fear River region. Here, we determined total mercury concentrations in chick feathers and egg membranes at seven breeding sites located in the same two watersheds in coastal North Carolina. We salvaged hatched eggs at these colonies as a non-destructive sampling method that does not impact the reproductive success of the pelicans, but can still be used to quantify chick exposure to mercury prior to hatching. Chick feathers are indicative of the local mercury burden near the colonies where they hatched.

Our objectives were to: 1) quantify mercury concentrations in Brown Pelican tissues and compare them to lowest observable adverse effects levels (LOAEL); 2) compare mercury exposure between the two watersheds, one of which has a heavy human impact (lower Cape Fear River) while the other is larger and relatively pristine (Pamlico Sound); and 3) investigate if any differences in mercury exposure among the colonies are due to diet or other factors.

METHODS

Study Area

In May and June 2013, approximately 20 eggshells and attached membranes were salvaged at each of the seven Brown Pelican breeding colonies in North Carolina, USA: North Pelican (NP), South Pelican (SP), and Ferry Slip (FS) Islands in the lower Cape Fear River, and New Dump (ND), Department of Transportation (DOT), and Beacon (BE) Islands, and Island MN (MN) in Pamlico Sound (Fig. 1). Only one eggshell was collected from each sampled nest. In July and August 2013, 10-15 breast feathers from each of up to 20 chicks with juvenile plumage were collected at each colony during annual banding operations. Samples were stored in clean plastic bags until analysis. Body feathers were selected because they exhibit the least amount of mercury variation compared to flight feathers from the same individual (Furness *et al.* 1986).

Limited samples of regurgitated menhaden were collected at three colonies in the Pamlico Sound; additional sampling of prey could not be conducted without further disturbances to the colonies. Menhaden were

stored in a freezer until analysis. Total length could not be measured due to missing heads and/or tail fins, but most prey were small and less than an estimated 15 cm in total length in life. Four menhaden from each of the three colonies, BE, DOT, and MN, were analyzed for stable isotope and mercury concentrations; an additional six menhaden from SP recovered during Royal Tern banding on 7 July 2013 also were analyzed to provide data on this prey in the Cape Fear River.

Mercury Analyses

Feathers were prepared for mercury analysis by rinsing with acetone and deionized water three times and then air drying to remove any surficial mercury from atmospheric deposition or skin excretions (Monteiro and Furness 2001). The egg membrane was separated from the eggshell by soaking it in deionized water and completing the separation by hand. The membrane was scrubbed using a brush and deionized water to remove any remnants of albumen or yolk that might influence the levels of mercury in the membrane. Membrane and chick feathers were air-dried under a fume hood for 24+ hr. Frozen fish were slightly thawed before white dorsal musculature was removed from each fish. Samples were placed in a drying oven at 60 °C for 6 days and then removed and ground to a fine powder with a mortar and pestle.

Three randomly chosen feathers per individual, analyzed independently, and a 0.01-0.02 mg sub-sample of egg membrane and fish underwent cold vapor atomic absorption spectroscopy for total mercury using a Direct Mercury Analyzer-80 (DMA-80). Total mercury can be used as a proxy of MeHg since almost all of the mercury content in seabird body feathers is in this bioavailable form (Bond and Diamond 2009). The percentage of MeHg in egg membranes has yet to be determined, but in egg yolks and albumin most of the mercury is MeHg (Evers *et al.* 2003; Bond and Diamond 2009). Regardless, most LOAELs are reported in total mercury. Each run on the DMA-80 consisted of 20 samples that were preceded and followed by two machine blanks, a sample blank, and two samples of two standard reference materials (DORM-3 – fish protein, DOLT-4 – dogfish liver, certified reference materials provided by the National Research Council Canada). Mean percent recovery of DORM-3 was $102.4 \pm 2.9\%$ and DOLT-4 was $103.5 \pm 3.5\%$ (values often exceed 100% due to slight variations of mercury in standards and/or machine error). All mercury concentrations are reported in parts per million (ppm; mg/kg) dry weight or fresh weight. The DMA-80 detection limit was 0.005 ng mercury.

Stable Isotope Analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)

Feathers were prepared for stable isotope analysis by soaking in a 2:1 ratio of chloroform and methanol solution for 24 hr. They were then rinsed with the chloroform:methanol solution twice and allowed to air dry for at least 48 hr (Wassenaar 2008). A 0.5-mg sub-sample of prepared egg membranes and fish (same cleaning protocol as with mercury) and feathers were loaded into tin cups. The samples were combusted in a Costech 1040 elemental analyzer interfaced with a

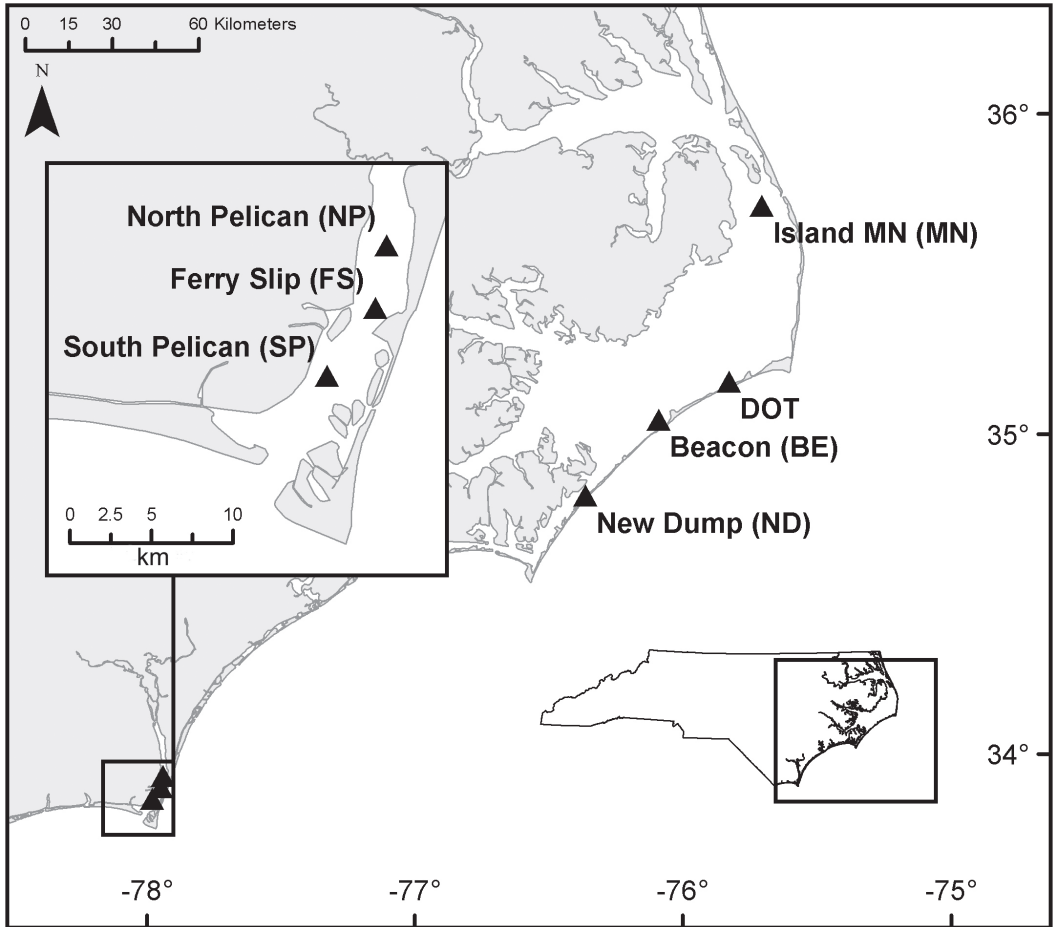


Figure 1. Locations of Brown Pelican breeding colonies in North Carolina, USA, in 2013 and where tissue samples were collected as described in the text. Abbreviated names for the islands are given in parentheses. DOT = Department of Transportation.

Thermo Finnigan Delta V Plus stable isotope mass spectrometer. Each run consisted of 27 samples, three duplicate samples, a machine blank, two sample blanks, and seven U.S. Geological Survey 40 and nine U.S. Geological Survey 41 L-glutamic Acid standard reference materials. Sample precision was based on the standard and sample duplicates and was 0.2‰ for both isotopes. Stable isotope values underwent two-point normalization and linearity corrections (see Paul *et al.* 2007). Fish samples were not lipid-digested, but all carbon isotope values have been lipid corrected according to the equation from Logan *et al.* (2008): $\delta^{13}C = [(a \times C:N + b) / (C:N + c)] + \delta^{13}C$, where a , b , and c are constants set for fish muscle across all species (7.415, -22.732, and 0.746, respectively) and the C:N ratio is unique to each sample, as calculated during mass spectrometry.

Statistical Analyses

Non-parametric statistical tests were used to analyze the mercury and stable isotope data across egg mem-

branes, chick feathers, and fish because sample sizes differed and were often too small to achieve normality (SAS Institute, Inc. 2012). Mann-Whitney tests were applied when comparing two categories, whereas Kruskal-Wallis tests were used with three or more categories. If the Kruskal-Wallis test proved significant ($\alpha < 0.05$), then a Dunn's post hoc comparison test was used to test pairwise comparisons. All averages are written with \pm one standard deviation.

RESULTS

Mercury Levels

Total mercury in Brown Pelican egg membranes ranged between 0.03-0.77 ppm dry weight (average = 0.20 ± 0.14 , $n = 103$) and was not correlated with $\delta^{15}N$ (linear re-

gression, $R^2 < 0.002$) or $\delta^{13}\text{C}$ ($R^2 < 0.0001$) in this tissue. The concentration of mercury in the egg membrane can be extrapolated to indicate approximate levels in the yolk and albumin (see Brasso *et al.* 2012), tissues typically measured to determine the LOEL of mercury. The range of total mercury values we derived for Brown Pelican egg membranes correlate with total mercury values between 3.482-4.587 ppm in penguin egg contents (albumen and yolk; Brasso *et al.* 2012). Variation in mercury within an individual's feathers was low; standard errors ranged from < 0.001 to 0.118, averaging at 0.032. The average feather mercury value across colonies was 1.13 ± 0.75 ppm. Similar to egg membranes, the total mercury in chick feathers was not influenced by $\delta^{15}\text{N}$ (linear regression, $R^2 = 0.018$) or $\delta^{13}\text{C}$ ($R^2 = 0.034$).

Spatial Trends in Mercury and Stable Isotopes

Egg membranes were not significantly different between the pooled Cape Fear ($n = 51$) and Pamlico Sound ($n = 52$) colonies in average mercury, $\delta^{15}\text{N}$, or $\delta^{13}\text{C}$ concentrations (Mann-Whitney, $P = 0.77$, 0.49, and 0.15, respectively). In chick feathers, the mean mercury value in the pooled Cape Fear River colonies (1.21 ± 1.0 ppm, $n = 65$) was slightly higher than the Pamlico Sound colonies (1.05 ± 0.32 ppm, $n = 62$), but this difference was not statistically significant (Mann-Whitney, $P = 0.87$). Feathers from the Cape Fear colonies also were 0.18‰ higher

in $\delta^{15}\text{N}$ (Mann-Whitney, $P = 0.04$) and 1.26‰ higher in $\delta^{13}\text{C}$ ($P < 0.001$). Menhaden collected from SP (0.37 ± 0.22 ppm, $n = 6$) were significantly higher in mercury than pooled colonies in Pamlico Sound (0.088 ± 0.034 , $n = 12$; Mann-Whitney, $P < 0.01$) but similar in $\delta^{15}\text{N}$ ($P = 0.089$) and $\delta^{13}\text{C}$ ($P = 0.21$).

Among individual colonies, mercury in egg membranes varied significantly (Kruskal-Wallis, $df = 6$, $P < 0.005$) and averaged between 0.14 ppm and 0.34 ppm. The differences in egg membranes were exclusively among the Cape Fear breeding colonies (Table 1). Post-hoc comparisons indicated that FS had significantly less mercury than SP (Table 1; Mann-Whitney, $P = 0.003$) despite their close proximity of 5 km (Fig. 2A). However, FS did not differ from SP in $\delta^{13}\text{C}$ (Mann-Whitney, $P = 0.20$) or $\delta^{15}\text{N}$ ($P = 0.68$), suggesting the two populations were feeding on prey occupying the same trophic level and foraging habitat (Fig. 2B).

Mercury in Brown Pelican chick feathers also varied significantly among colonies (Kruskal-Wallis, $df = 5$, $P < 0.01$). Post-hoc comparisons indicated NP had significantly more mercury than MN, ND, FS, and SP (Table 1; Mann-Whitney, $P < 0.05$). BE and FS also contained significantly more mercury than SP (Table 1; Mann-Whitney, $P < 0.05$; Fig. 3A). In the Cape Fear region, there was a decreasing trend of mercury in chick feathers with decreasing latitude. These three islands did not differ in $\delta^{15}\text{N}$, but SP was approximately 0.5‰ lower than NP in $\delta^{13}\text{C}$ (Mann-Whitney, $P < 0.001$; Fig. 3B).

Table 1. Mean \pm SD mercury (Hg), $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ values in Brown Pelican egg membranes and chick feathers collected at the seven breeding islands in North Carolina. MN = Island MN; DOT = Department of Transportation.

Sample Site	Egg Membrane				Chick Feathers			
	<i>n</i>	Hg (ppm)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	<i>n</i>	Hg (ppm)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Pamlico Sound								
MN	20	0.17 ± 0.09	15.7 ± 0.8	-16.9 ± 1.1	21	0.98 ± 0.23	13.6 ± 0.1	-18.0 ± 0.8
DOT	7	0.34 ± 0.21	14.2 ± 1.7	-16.6 ± 1.4	0	–	–	–
Beacon	21	0.16 ± 0.14	15.2 ± 0.8	-17.1 ± 0.8	22	1.19 ± 0.42	14.0 ± 0.4	-17.9 ± 0.7
New Dump	4	0.27 ± 0.08	13.8 ± 0.7	-16.1 ± 1.5	19	0.97 ± 0.19	14.1 ± 0.3	-18.2 ± 0.1
Cape Fear River								
North Pelican	8	0.18 ± 0.13	14.9 ± 0.5	-18.4 ± 2.9	20	1.82 ± 1.66	14.1 ± 0.3	-16.6 ± 0.3
Ferry Slip	24	0.14 ± 0.17	15.0 ± 0.5	-17.1 ± 0.6	20	1.03 ± 0.11	14.1 ± 0.3	-16.7 ± 0.8
South Pelican	19	0.27 ± 0.11	15.0 ± 0.5	-16.9 ± 0.8	25	0.86 ± 0.18	14.0 ± 0.3	-17.0 ± 0.2

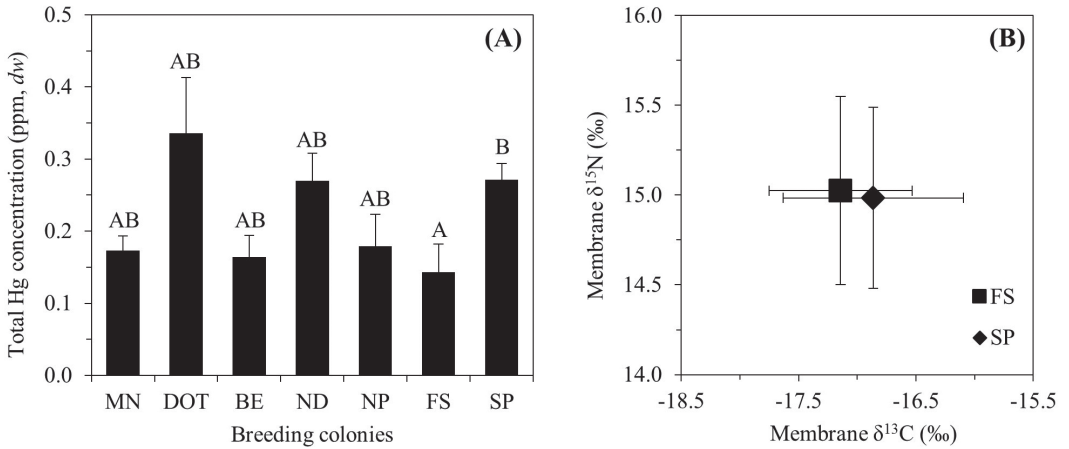


Figure 2. Average total mercury (Hg) concentration in Brown Pelican egg membranes with standard deviation error bars (A). Columns significantly different ($P < 0.05$) from each other do not share a letter. Sample size is approximately 20 for each site, except for New Dump (ND; $n = 4$) and North Pelican (NP; $n = 8$). Biplot of average stable isotope composition with standard deviation error bars in egg membrane at Ferry Slip (FS) and South Pelican (SP), which were significantly different in mercury concentrations (B). *dw* = dry weight.

DISCUSSION

Our data on mercury and stable isotope concentrations in egg membrane and chick feathers for the first time allow comparisons not only between these tissues in Brown Pelicans, but also among breeding colonies in two watersheds in North Carolina. The extrapolated whole egg mercury values from all colonies fall within the 2.82-6.10 ppm

range, which Evers *et al.* (2003) determined as moderate in toxicity in Common Loons (*Gavia immer*) and as having the potential to induce significant reproductive impairment in some individuals in some species. These values also are over an order of magnitude higher than whole egg measurements reported by Wickliffe and Bickham (1998) from the Pamlico and Cape Fear River watersheds, indicating a considerable increase in

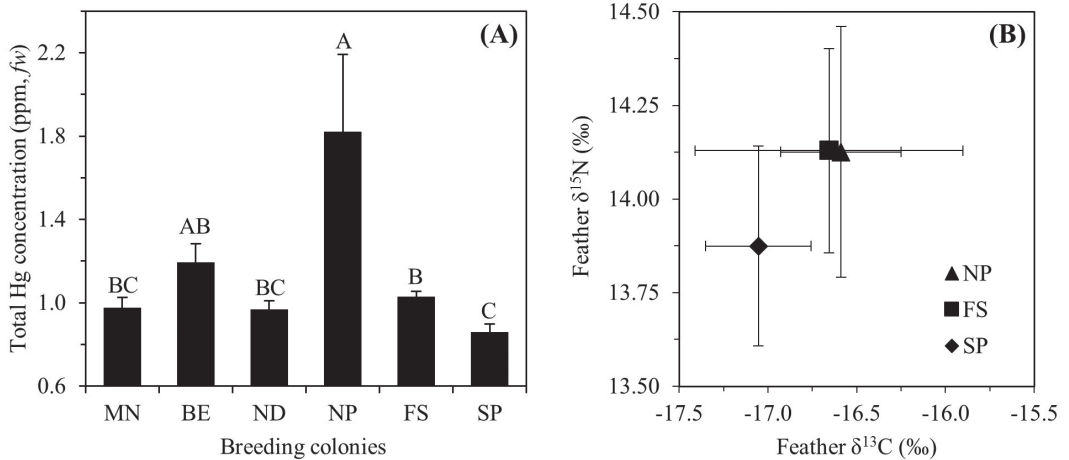


Figure 3. Average total mercury (Hg) concentration in Brown Pelican chick feathers with standard deviation error bars (A). Columns significantly different ($P < 0.05$) from each other do not share a letter. Sample size is approximately 20 for each site. Biplot of average stable isotope composition with standard deviation error bars in chick feathers at North Pelican (NP), Ferry Slip (FS) and South Pelican (SP), which were significantly different in mercury concentrations (B). *fw* = fresh weight.

mercury exposure in Brown Pelicans since 1994, when the studied eggs were collected. We were unable to assess the impact of mercury on Brown Pelican productivity because colonies were not visited regularly enough to accurately quantify reproductive success. Although there is a significant positive correlation between mercury levels in eggshell and associated membrane with whole egg contents, the relation may not be strong enough to use membrane mercury as a predictor of mercury in whole egg content (Morera *et al.* 1997; Kennamer *et al.* 2005). In addition, we sampled only one egg per nest, and Brown Pelicans typically produce clutches of two to four eggs (Shields 2002). Mercury concentrations can vary by egg sequence in marine birds and ducks (Kennamer *et al.* 2005; Akearok *et al.* 2010), but this has not been investigated in Brown Pelicans. If intraclutch variation in mercury levels does occur in Brown Pelicans, it may also explain some of the variation in our results. Thus, while salvaged egg membranes are beneficial to use since they do not impact reproductive success, caution should be taken in extrapolating to whole egg concentrations until more focused studies on mercury distribution in Brown Pelican eggs are conducted.

The LOAEL of mercury in bird feathers is not well established. Bowerman *et al.* (1994) did not find any correlations between mercury in adult Bald Eagle (*Haliaeetus leucocephalus*) feathers (average = 21 ppm) and nesting success or productivity. However, another study on Mallards (*Anas platyrhynchos*) observed lower reproductive success in dosed hens with 9 ppm in their feathers (Heinz 1979). Brown Pelicans likely have similar physiological tolerances to that of Bald Eagles since they are both piscivorous (but phylogenetically distant). Regardless, the average Brown Pelican chick feather mercury concentration in North Carolina (1.13 ppm) was well below that reported for Bald Eagles and Mallards and is likely not of concern.

We expected to see greater concentrations of mercury in Brown Pelicans breeding on islands in the Cape Fear River because they receive the effluent from about 17%

of the State, whereas the Pamlico Sound is largely removed from anthropogenic influence. The small area of the Cape Fear Estuary compared to the Pamlico Sound leads to an increased density of wetlands and salt marshes, which are known to have more bacteria that convert inorganic mercury into methylmercury (Zillioux *et al.* 1993). Despite assumed differences in the anthropogenic influence between the locations, differences in mercury in both egg membranes and feathers were minimal. The only significant difference observed was an approximate 1.27‰ enrichment in $\delta^{13}\text{C}$ in chick feathers in the Cape Fear River colonies.

In the Cape Fear River breeding colonies, egg membrane mercury concentrations were higher at SP than at FS, while the chick feather mercury was highest at NP, then FS, and lowest at SP. A possible explanation for this variation in mercury is a difference in diet composition, since higher trophic levels ($\delta^{15}\text{N}$) and offshore feeding ($\delta^{13}\text{C}$) are correlated with higher mercury loads (Power *et al.* 2002; Kehrig *et al.* 2013). However, the egg membranes did not differ in $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ among these colonies. The chick feathers did not differ in $\delta^{15}\text{N}$, but there were miniscule differences (0.5‰) in $\delta^{13}\text{C}$. Most of the regurgitated fish were menhaden (K. Newtoff, pers. obs.), a species previously identified as their primary food source in this region (Schreiber 1980); therefore, differences in mercury concentration in eggs among colonies do not appear to be a result of consumption of different prey species. The difference in mercury is possibly due to different mercury exposures of their prey. The menhaden samples we were able to collect and analyze had varying levels of mercury while maintaining similar stable isotope concentrations between colonies. However, the menhaden collected in the Cape Fear River were obtained from a Royal and Sandwich tern colony, and these birds may select menhaden differently than Brown Pelicans.

Egg membrane total mercury concentrations in Brown Pelican colonies investigated here extrapolate to whole egg values that are higher than those previously measured by Wickliffe and Bickham (1998) and may be a

cause for concern. Egg membranes are non-destructive and can be gathered and stored easily, but a targeted study examining the relationship between egg membrane and whole egg mercury concentrations needs to be conducted in Brown Pelicans. Although there were no noticeable reproductive consequences in the breeding colonies, nest counts and monitoring are recommended in future years to accurately assess reproductive success and detect possible declines. Mercury concentrations in the chicks were not significantly elevated to cause any neurological distress, but accumulations in their tissues can be passed down by females to eggs, causing lethal consequences for the eggs (Evers *et al.* 2003).

It is projected that mercury concentrations will increase globally as developing nations industrialize, which increases the demand for electricity, likely in the form of coal-fueled power plants (Streets *et al.* 2009). Although the emissions in the United States have declined, the long-range transport of mercury will affect ecosystems around the world. The mercury and stable isotope values presented here characterize recent trends and bridge a gap in the literature on pollutant exposures in this once-endangered species, but also provide impetus for future research that will elucidate the role of prey selection on mercury exposure in this species.

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