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Mercury, cadmium, copper, arsenic, and selenium measurements in the feathers of adult eastern brown pelicans (*Pelecanus occidentalis carolinensis*) and chicks in multiple breeding grounds in the northern Gulf of Mexico

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Abstract Several trace metals and metalloids have been introduced into aquatic ecosystems due to anthropogenic activities. Some of these elements like mercury (in the form of methylmercury) are easily transferred from one trophic level to another and can accumulate to toxic quantities in organisms at the top of aquatic food webs. For this reason, seabirds like the eastern brown pelican (*Pelecanus occidentalis carolinensis*) are susceptible to heavy metal and metalloid toxicity and may warrant periodic monitoring. Mercury, cadmium, copper, arsenic, and selenium were measured in the feathers of adult brown pelicans and chicks in several breeding colonies (Shamrock Island, Chester Island, Marker 52 Island, North Deer Island, Raccoon Island, Felicity Island, Gaillard Island, Audubon Island, and Ten Palms Island) in the northern Gulf of Mexico. Overall, most chicks and adults examined had mercury levels in feathers that were below the concentration range in which birds show symptoms of mercury toxicity. However, chicks in the Audubon Island and Ten Palms Island colonies displayed mercury levels that were 3 times higher than values observed in 5 other colonies. In addition, several adults and chicks displayed selenium concentrations that are above what is considered safe for birds. Cadmium quantities in feathers were below levels that trigger toxicity in birds. Similarly, arsenic measurements were at quantities below the average of what has been reported for birds living in contaminated sites. Finally, we identify pelican breeding colonies that may warrant monitoring due to elevated levels of contaminants.

Keywords Heavy metals · Toxicity · Bioaccumulation · Seabird

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Introduction

The eastern brown pelican (*Pelecanus occidentalis carolinensis*) is a year-round resident in the Gulf of

Mexico, with breeding sites in each state within the northern Gulf. They were previously listed as an endangered species in the 1970s due to reproductive failure caused by the organochlorine pesticide DDT and its metabolite DDE (Holm et al. 2003; K.A. King et al. 1977). However, better regulation of the use of pesticides in the states surrounding the Gulf of Mexico has led to a recovery in the pelican population which now exceeds 1970 levels (Holm et al. 2003). Nevertheless, the species is still considered to be one of conservation concern (Jodice et al. 2019) due in part to environmental stressors such as the Deepwater Horizon oil spill event (Haney et al. 2014) and the introduction of other contaminants to their environment such as heavy metals like mercury (Hg).

All forms of Hg are toxic to birds. Hg is released into the atmosphere predominantly through artisanal and small-scale gold mining (Esdaile and Chalker 2018) and power generation via coal combustion (Streets et al. 2018), as well as other industrial and natural processes (Varekamp and Buseck 1986). Once in the atmosphere, it is transported to aquatic ecosystems and is oxidized to inorganic Hg which is often readily available for methylation to methylmercury (MeHg) by microbes containing the *hgcAB* gene, commonly found in anoxic pockets of the water column and sediments (Parks et al. 2013). MeHg is an organic form of Hg and particularly worrisome as it is more toxic than its inorganic counterparts and is easily transferred from one aquatic trophic level to another. With each transfer, MeHg becomes more concentrated in fish tissue (biomagnification) and often results in toxic levels in biota at the top of aquatic food webs such as seabirds. There is recent reporting that MeHg concentrations in Atlantic bluefin tuna have increased from levels present in 1969 to current levels (Schartup et al. 2019). Since pelican diet in the Gulf consists mainly of smaller fish (Lamb et al. 2017a), there is some concern that Hg levels in pelicans might exceed quantities in which Hg becomes toxic to these birds. To date, there are very few studies on the Hg content of adult pelicans and even less on chicks in the Gulf of Mexico (King et al. 1985).

Other elements of environmental concern due to harmful effects on biota are the metals cadmium (Cd) and copper (Cu) and the metalloids selenium (Se) and arsenic (As). Elevated levels of Cd in birds can lead to renal tubular necrosis and testicular atrophy (Scheuhammer 1987; White and Finley 1978). High concentrations of Cu, Se, and As in birds can result in liver damage, deformed embryos and reduced hatchability, reduced egg production, and increased thinning of eggshells (Spallholz and Hoffman 2002; Szymczykv and Zalewski 2003; Chiou et al. 1997; Sanchez-Virosta et al. 2015). Cd, Se, and As are released into the atmosphere through waste incineration, industrial use of coal for power generation, and smelting, respectively (Mason et al. 2000; Yan et al. 2001; Martin et al. 2014). These elements culminate in the watersheds where they persist and bioaccumulate (Mason et al. 2000). Cu is released into watersheds due to the use of antifouling agents and algicides (Henderson and Winterfeld 1975; Franson et al. 2012; Matthiessen et al. 1999; Young et al. 1979). To date, there are very few or no studies investigating the levels of these elements in brown pelicans in the Gulf of Mexico (King et al. 1985).

Thus, the objective of this study was to evaluate the Hg content in adult brown pelicans and chicks in several breeding colonies located in the northern Gulf of Mexico by measuring total Hg levels in feathers. In addition, levels of other environmentally relevant heavy metals (Cd, Cu) and nonmetals (As, Se) were assessed. The data provided here will enable site managers to identify colonies that might be at risk of Hg, Cd, As, and Se toxicity and will contribute to the development of goals for long-term monitoring plans for the species in the region (Ottinger et al. 2019; Jodice et al. 2019).

Materials and methods

Sampling sites and methods

Locations and protocols of feather collection are given in a previous study (Lamb et al. 2017b) and summarized here. Collection permits were obtained from several agencies and include the Florida Fish and Wildlife Conservation Commission (LSSC-13-00005), Louisiana Department of Wildlife and Fisheries (LNHP-13-058 and LNHP-14-045), Texas Parks and Wildlife (SPR-0113-005), Audubon Texas, The Texas Nature Conservancy, and the US Geological Survey Bird Banding Laboratory (22408). Several breeding sites (Fig. 1) across 4 states (Texas, Louisiana, Alabama, Florida) in the northern Gulf of Mexico were visited in the months of May to July 2013–2016. Contour feathers were taken from adults only in colonies at Felicity Island and

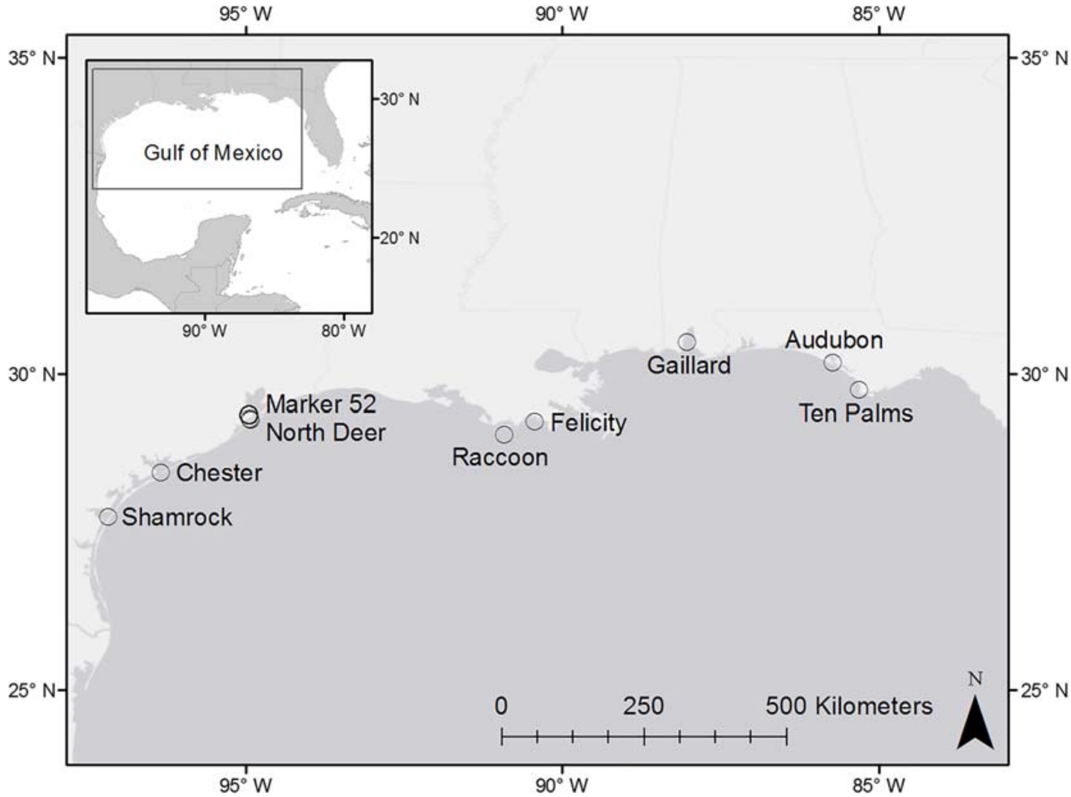

Fig. 1 Locations of brown pelican breeding colonies in the Gulf of Mexico where feather samples were collected. Plot was redrawn from a previous publication (Lamb et al. 2017b)

Raccoon Island; chicks only at colonies at Audubon Island, Marker 52 Island, Ten Palms Island, and North Deer Island; and both adult pelicans and chicks in Shamrock Island, Gaillard Island, and Chester Island. Longitudes and latitudes of these locations are provided in Table S1. While breast feathers are preferred for trace metal and metalloid analysis, the use of contour feathers can provide a minimum estimate of metal and metalloid exposure. These feathers were placed in labeled mini-zip lock bags, and these bags were collectively stored in envelopes.

Chemical analysis

Analysis of trace metals was modified from methods described in the literature (Burger et al. 2015; Mohammed et al. 2017). Feathers were washed with HPLC Grade Acetone and high purity water (MilliQ Millipore, > 18.2 MΩ-cm). Then samples were air dried overnight and additionally left at 60 °C for 1 h to ensure complete dryness (Newtoff and Emslie 2017). Samples were then weighed and digested in 5 mL trace metal grade nitric acid. All digests were accompanied by blanks and the certified reference material (TORT-3, lobster, and SRM 1566 b – Oyster Tissue). The feather/acid digest mixture was heated at 75 °C for 90 min in an Environmental Express block HotBlock®. Heating at this temperature for this duration enabled complete dissolution of feathers and the certified reference material in acid.

Total Hg content in feathers was analyzed with a Brooks Rand MERX-T (Automated Total Mercury System for US EPA Method 1631) after oxidation of an aliquot of acid digest with bromine chloride solution, amendment with hydroxylamine, reduction to elemental Hg with stannous chloride, cold vapor generation, and amalgamation (Ndu et al. 2018). Other metals (Cu and Cd) and nonmetals (As, Se) were determined with an Agilent 7900 ICPMS. The recoveries of Se, As, Cu, Cd, and Hg from the certified reference material ranged from 85 to 106%. More details are provided in Table S2. The detection limits for Hg, Cd, Cu, Se, and As are 0.20 ng g^{-1}, 0.02 ng g^{-1}, 0.09 ng g^{-1}, 0.47 ng g^{-1}, and 0.33 ng g^{-1}, respectively.

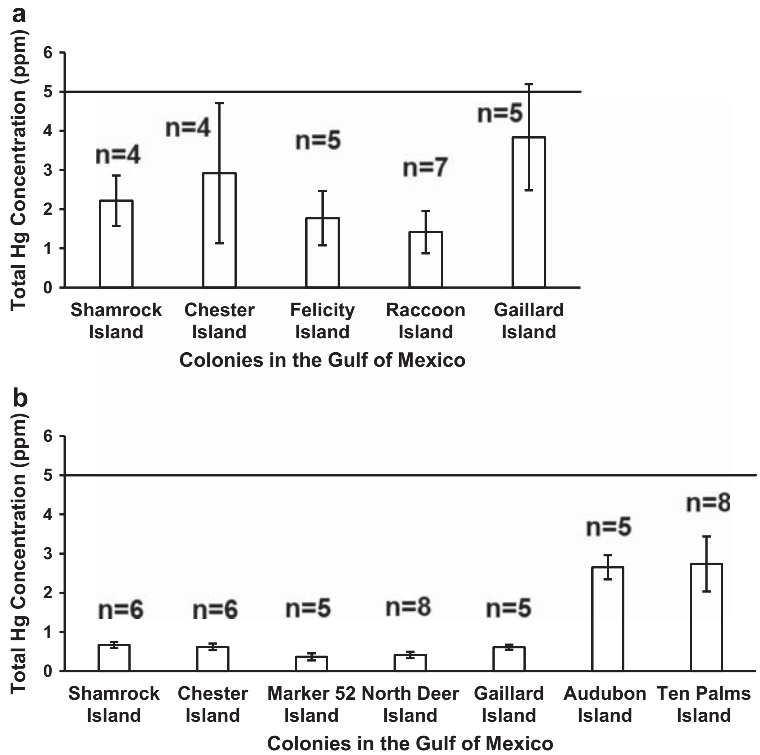

Fig. 2 Total mercury (Hg) measurements in feathers of brown pelicans in **a** adults and **b** chicks. Measurements are in parts per million dry weight and represent the average (± standard error) of about 4 to 8 individuals. Horizontal black line represents the minimum total mercury concentration in feathers where symptoms of mercury toxicity begin to manifest

Results and discussion

Mercury exposure within pelican colonies

The average measurements of total Hg in feathers in adult pelicans and chicks from five colonies are given in Fig. 2 a and b . Total Hg measurements typically ranged from 0.39–8.28 ppm and 0.13–6.68 ppm dry weight in adults and chicks, respectively (Table S3 and Table S4). There was a wider range of total Hg concentrations in adults than with chicks, and adult values were generally higher than values seen in chicks with the exception of the colonies at Audubon Island and Ten Palms Island in Florida (2.65 and 2.74 ppm, respectively). All total Hg measurements in pelicans were below the levels where symptoms of MeHg are observed in birds (5–40 ppm) (Burger and Gochfeld 1997) with exception of four individuals. Two of the birds that exhibited elevated total Hg concentrations were breeding at Gaillard Island, Alabama, while the third was breeding at Chester Island, Texas. The fourth was a chick found in Ten Palms Island, Florida. While there are few measurements of total Hg in muscle tissue in brown pelicans available from other colonies such as in California (Ruelas-Inzunza et al. 2009), to the best of our knowledge, there has been no measurements in feathers in adult individuals in the Gulf of Mexico. Nevertheless, concentrations of total Hg measured in this study are comparable with measurements in American white pelicans in the Gulf of Mexico (1.56–4.21 ppm) and in Lake Chapala, Mexico (0.81–9.57 ppm) (Torres et al. 2014).

The use of feathers to monitor Hg toxicity is relatively common as it is noninvasive and nonlethal. The MeHg content in feathers is typically greater than 95% of the total Hg present in feathers (Evers 2018; Renedo et al. 2017; Thompson and Furness 1989). There is a limit to the amount of information that can be derived from Hg content in feathers. While other bird tissue like blood can provide more recent exposure information (days to weeks), total Hg in feathers reflects exposure to Hg from days to years (Evers 2018; Carvalho et al. 2013). Nevertheless, feathers can provide valuable information as quantities of total Hg in feathers in various species that can cause toxicity are known from laboratory exposure studies. Symptoms of Hg toxicity can range from reduced fertility, abnormal behavior (e.g., altered parent and chick behavior, increased same sex relations), to absolute infertility (Frederick and Jayasena

2011; Burger and Gochfeld 1997). Absolute infertility is a possible extreme consequence of substantially high total Hg measurements in feathers as observed in sparrow hawks (Solonen and Lodenius 1984; Burger and Gochfeld 1997).

For adult brown pelicans, Hg in feathers represents muscle tissue content and dietary exposure at the time of feather molt (Burger 1993; Evers 2018), which occurs before the start of breeding and at locations that may or may not be at the breeding sites due to migratory patterns. Some pelicans migrate after the breeding season, while some remain within the vicinity of breeding grounds. A study found that 50% of brown pelicans that were tagged with GPS monitors in Louisiana remained within 72 km of breeding sites after the breeding season was concluded, while remaining birds moved to other Gulf states (Alabama, Mississippi, and Texas) and the Yucatan Peninsula (King et al. 2013). In a different study where several individual pelicans from several colonies in the northern Gulf of Mexico were tagged with GPS monitoring devises, it was found that individuals from large colonies were more likely to migrate during winter and that the proportion of migrants per colony ranged from 50 to 75% in colonies in the Western and Central Gulf of Mexico (Lamb et al. 2017b). However, one colony in the eastern part of the Gulf had a lower proportion of migrants (Lamb et al. 2017b). Nevertheless, the vast majority (94%) of brown pelicans remained in the Gulf of Mexico, while a few birds crossed the Panama Canal and wintered along the Pacific Coast (Lamb et al. 2018). Understanding migration patterns is essential to deciphering the Hg content in adult feathers.

In contrast to adults, pelican chicks always grow their feathers at the breeding site, meaning that Hg levels in chick feathers represent concentrations at or near the breeding colony (Burger and Gochfeld 1997). Measurements of total Hg in chicks in five out of the seven colonies were below 1 ppm on average, but measurements in the colonies at Audubon Island and Ten Palms Island (Florida) were significantly (one-way ANOVA, $p < 0.05$) higher than values observed in chicks from other colonies. This result was surprising as it was expected that chicks in the Chester Island colony (Texas) would have the highest concentrations of total Hg given the proximity of this colony to a contaminated site with high concentrations of Hg (Apeti et al. 2012). Chicks from Audubon and Ten Palms Islands displayed values consistent with adults of other colonies. Currently, the cause of elevated Hg concentrations in chicks from the Audubon and Ten Palms Islands is unknown but may be related to a specific local food source and warrants further examination. Mercury levels in chicks found in the Gulf of Mexico are comparable with chicks in North Carolina where values of 1.13 ± 0.02 ppm were observed (Newtoff and Emslie 2017). We compared the total Hg content in colonies where feathers were taken from both adults and chicks. Adults in Gaillard Island (Alabama) and Shamrock Island (Texas) colonies had higher total Hg contents in their feathers than chicks (one-way ANOVA, $p < 0.05$), while no difference was found in the Chester Island colony. Differences in total Hg content in feathers between adults and chicks could result from differences in Hg exposure between molting grounds where adults grow feathers and breeding grounds where chicks develop feathers (Burger et al. 1994). In free living chicks, it has not been demonstrated that MeHg toxicity has any effect on survival rates (Ackerman et al. 2008). However, several laboratory studies have shown deleterious effects of MeHg toxicity on chicks. Captive egret nestlings that were fed MeHg in diet experienced reduction in growth and health (Bouton et al. 1999). In a controlled laboratory-based exposure experiment, it was found that MeHg caused weakness of legs and paralysis in egret chicks (Hoffman et al. 2005). Thus, it is essential to monitor these birds to ensure that Hg content does not exceed levels that results in toxicity.

Metal and metalloid concentrations in pelicans

When the metalloid Se was examined in the same subset of pelicans, it was shown that some adults in the Gaillard colony and chicks in Shamrock, North Deer, and Ten Palms colonies had concentrations that exceeded the value of 1.8 ppm that causes toxicity (Burger et al. 2015). A few birds of other colonies had values that were close to 1.8 ppm. Se-Hg ratios can provide some information on the protective ability of Se against Hg toxicity. When the ratio is greater than 1, Se may assist in mitigating the harmful effects of Hg (Whanger 1985; Peterson et al. 2009; Ganther and Sunde 1974). The protective effect of Se in diet has been demonstrated in numerous studies (Parízek and Ostádalová 1960; Ganther and Sunde 1974). Thus, in order to assess the risk of Hg toxicity, both the values of the quantity of Hg in a feather and the molar ratio of Se-Hg in the organism are needed. In all the colonies of chicks examined, the

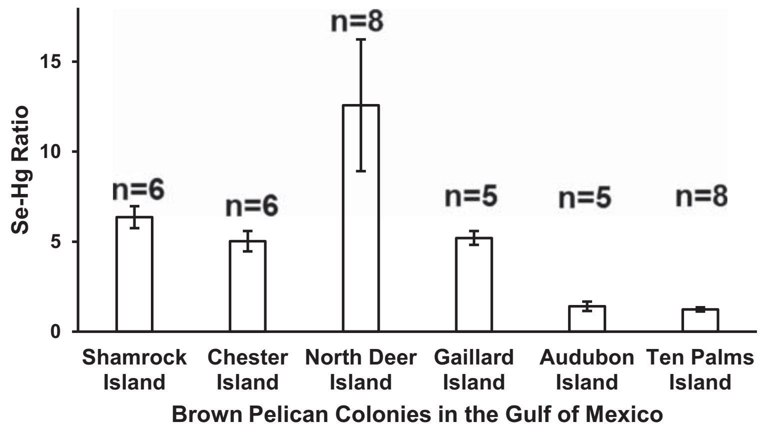

Fig. 3 A plot showing selenium (Se)-mercury (Hg) molar ratios in chicks per colony

molar ratio of Se-Hg was greater than 1 indicating that Se could provide some protection against Hg toxicity.

Furthermore, Se-Hg molar ratios of chicks per colony displayed very little variation (Fig. 3), which might be a characteristic of their local food sources. The Se-Hg ratio of chicks in the Audubon Island and Ten Palms Island colonies showed a distinct difference from other colonies (Fig. 3). The reasons for this difference are not known. Of all colonies included in this study, Audubon Island is the most subject to anthropogenic activity, given its location in the highly urbanized St. Andrew Bay, which is an area that has urban stormwater runoff, industrial point source discharges, and recreational boats and marina repair operations (Huge et al. 2011; Oliver et al. 2001). A more detailed analysis of the geochemistry of Hg at these colonies obtained from ratios of stable Hg isotopes is beyond the scope of this study and is the subject of an upcoming report.

An analysis of other metals and metalloids showed that Cd levels in both adults and chicks, with the exception of 1 chick, were in the range of 0.005–0.02 ppm (Tables S3 & S4), which is less than the range (0.1–2 ppm) where Cd content results in toxicity (Burger et al. 2015). Cu was also measured in feathers (Tables S3 & S4). However, the toxicity threshold of Cu is not known in birds. We simply report these Cu values in feathers as baseline measurements that will allow future researchers to compare datasets in monitoring studies. Just like Cu, the level of As in bird feathers that cause toxicity is not known. Nevertheless, we can compare our measurements of As to measurements made by other researchers in both contaminated and uncontaminated sites. As values measured in adult feathers in this study were below 1 ppm, which is the average value reported for As in feathers obtained from birds in uncontaminated sites (Sanchez-Virosta et al. 2015). With the exception of 4 chicks (1 in Shamrock Island,1 in Chester Island, and 2 in Ten Palms Island), all measurements were also below 1 ppm. All values of As in either adult or chick feathers were well below 10 ppm, the average value reported for As in feathers in contaminated sites (Sanchez-Virosta et al. 2015).

Conclusion

We suggest that adult brown pelicans and chicks in colonies at Gaillard Island, Audubon Island, Ten Palms Island, and Shamrock Island may warrant additional monitoring due to elevated levels of Hg and Se observed in some birds at these colonies. The data obtained in this study will also assist in establishing a baseline and will contribute to the development of endpoints for long-term monitoring plans for the brown pelican in the region. Additional research would elucidate relevant paths of trophic transfer of these elements to these birds and environmental controls of these processes.

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Data availability The dataset for this work can be accessed through the Gulf of Mexico Research Initiative Information and Data Cooperative (GRIIDC) (GRIIDC DOI: https://doi.org/10.7266/aza38gzz).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Field research was conducted with permission from the Clemson University Animal Care and Use Committee (2017-008). Permitting for field data collection was provided by the US Geological Survey Bird Banding Lab (22408) and South Carolina Department of Natural Resources (BB-18-22).

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