



# Accumulation of the Toxic Metal Mercury in Multiple Tissues of Marine-Associated Birds from South Florida

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## Abstract

One of the best studied global “hot spots” for ecological mercury (Hg) contamination is south Florida (USA), where elevated Hg concentrations in environmental media and regional wildlife were first described over thirty years ago. While Hg contamination has lessened in this region, it is still critical to monitor Hg uptake and potential risks in south Florida wildlife, especially in marine-associated birds, which are known to accumulate potentially toxic Hg levels. In this study, total Hg (THg) concentrations were measured in liver, kidney, muscle, and feathers of 101 individuals from seven species of south Florida birds: brown pelican *Pelecanus occidentalis*, double-crested cormorant *Phalacrocorax auratus*, herring gull *Larus argentatus*, laughing gull *Leucophaeus atricilla*, northern gannet *Morus bassanus*, royal tern *Thalasseus maximus*, and osprey *Pandion halietus*. A sizeable proportion of individuals (>40%) were found to contain THg concentrations in internal tissues that exceeded estimated toxicity thresholds for Hg-related effects. Certain species, especially osprey, were found to exhibit a higher rate of threshold exceedances than others and should continue to be monitored for Hg-related effects in future studies. Feather THg concentrations exhibited a lower rate of toxicity threshold exceedances (12%) and were not significantly correlated with those in internal tissues in most cases, suggesting that they may not be well suited for monitoring Hg exposure in these species unless sources of data variation can be better understood. The results of the present study contribute significantly to our understanding of trends in Hg accumulation and Hg-related health risks in south Florida marine-associated birds.

Because of their abundance in coastal areas, high trophic position, and relatively long lifespans, marine-associated birds have the tendency to accumulate significant concentrations of environmental toxicants (Burger and Gochfeld 2004; Egwumah et al. 2017). This pattern has been well demonstrated for the toxic heavy metal mercury (Hg), which has been shown to concentrate in individuals from a sizeable number of bird populations at levels above known toxicity thresholds (reviewed in Ackerman et al. 2016). Several aspects of bird physiology are sensitive to Hg toxicity, including behavior, immune function, endocrinology, nervous system function, reproduction, growth, and general

health and survival (Whitney and Cristol 2017). At particularly high levels of exposure, toxicological responses to Hg have the potential to result in population-level effects in birds. For example, previous studies have demonstrated notable reductions in reproductive output in several avian species exposed to high, but still ecologically relevant Hg levels (Jackson et al. 2011; Varian-Ramos et al. 2014). Because of such effects, significant declines in bird populations have been linked with Hg exposure at several locations throughout the world (e.g., Evers et al. 2008), sites generally referred to as “Hg hot spots” in the scientific literature.

One of the best studied Hg hot spots in the contiguous USA where bird population declines have been reported is south Florida, particularly in sites within the Florida Everglades (Frederick et al. 2004; Rumbold 2019). Elevated Hg concentrations in this region have been proposed to be a function of high levels of regional atmospheric Hg deposition from municipal/medical waste incineration and power generation combined with optimal conditions for bacterial transformation of inorganic Hg to the highly persistent, bioavailable, and toxic organometal, monomethylmercury (MeHg) (Gilmour et al. 1998; Gabriel et al. 2010). Wading

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bird populations in the Everglades had exhibited declines beginning around the 1940s (Ogden et al. 1999), and Hg exposure was first implicated as a possible contributor to these declines when Ogden et al. (1974) reported high levels of Hg in a variety of tissues from Everglades wildlife including eggs of several waterbird species (e.g., great egret *Ardea alba*, 0.13–0.91 mg/kg w.w., double-crested cormorant *Phalacrocorax auratus*, 0.31–0.44 mg/kg w.w., brown pelican *Pelecanus occidentalis*, 0.12–0.65 mg/kg w.w.). Later, elevated Hg levels and possible associations between Hg toxicity and population-level responses were reported in several south Florida avian species, including wood stork *Mycteria americana* (Burger 1993; Beyer et al. 1997), great white heron *Ardea herodias occidentalis* (Spalding et al. 1994; Beyer et al. 1997), great egret (Beyer et al. 1997; Frederick et al. 1999; Sepulveda et al. 1999), double-crested cormorant (Sepulveda et al. 1998), and white ibis *Eudocimus albus* (Heath and Frederick 2005). Mercury levels in some of these species (e.g., great egrets, Rumbold et al. 2001; Frederick et al. 2002, white ibis, Heath and Frederick 2005) and other Everglades wildlife have since experienced significant declines (e.g., American alligator *Alligator mississippiensis*, Rumbold et al. 2002, finfish, Rumbold 2005). This has been partly attributed to significant declines in atmospheric Hg loading since the early 2000s, which has resulted from reduced use of Hg for commercial and industrial uses as well as more stringent regulations on waste incineration and industrial emissions (Atkeson and Parks 2002; Rumbold et al. 2008; Rumbold 2019). However, numerous Hg hot spots still remain within this ecosystem as well as in other locations in south Florida (Rumbold et al. 2008; Julian 2013). This is likely because of features that promote high bacterial methylation rates within the south Florida ecosystem, such as high sulfur-loading (Gabriel et al. 2010). Furthermore, elevated Hg levels have been reported in other south Florida bird populations (e.g., osprey *Pandion haliaetus*, Lounsbury-Billie et al. 2008; Rumbold et al. 2017) in more recent years. Therefore, continued studies on Hg levels in waterbirds in this general region remain necessary for understanding the risks that Hg poses to resident wildlife populations.

The goal of the current study was to obtain updated information on Hg accumulation in a broad range of marine-associated birds from south Florida habitats. To accomplish this, we examined total Hg (THg) concentrations in multiple tissues (i.e., liver, kidney, breast muscle, feather) of individuals from seven marine-associated bird species: double-crested cormorant, brown pelican, osprey, herring gull *Larus argentatus*, laughing gull *Larus atricilla*, royal tern *Sterna maxima*, and northern gannet *Morus bassanus*. We examined differences in THg concentrations associated with tissue type, species, and stage of maturity (when possible), and compared measurements of THg in tissues from these

individuals with toxicity thresholds estimated for all tissues, based on recommendations from earlier studies (Burger and Gochfeld 1997; Ackerman et al. 2016). We also examined correlations between THg concentrations in different tissue types, which could be useful for comparing data between studies that use different approaches for evaluating Hg accumulation in birds.

## Methods

### Sample Collections

Bird carcasses were obtained from four federally licensed wildlife rehabilitation centers located in the southeast Florida region: South Florida Wildlife Center (SFWC, Fort Lauderdale), Pelican Harbor Seabird Station (PHSS, North Miami), Florida Keys Wild Bird Rehabilitation Center (FKWBHC, Tavernier), and Key West Wildlife Center (KWWC, Key West). All specimens were collected in accordance with Florida Fish and Wildlife Conservation Commission permit LSSC-12–00,075 and US Fish and Wildlife Service permit MB82910A-0. No Institutional Animal Care and Use Committee (IACUC) authorization was needed, as all specimens obtained from these centers were already deceased. Specimens were transported to Nova Southeastern University (Fort Lauderdale, FL) frozen. Birds were categorized as sexually mature or immature based on plumage and body size. Sex was recorded when possible but was not determined for all specimens because some individuals were either immature, or mature but not in reproductively active conditions.

Specimens were stored frozen at  $-20\text{ }^{\circ}\text{C}$  or lower until dissections were performed. During dissection, a series of morphometric measurements were collected from thawed individuals: tarsus length, tail length, wing chord, wing span, bill depth, and three measurements of bill length (i.e., from base, feathers, and nostril). Up to 10 recent growth feathers were collected from contour areas closest to the inner wing and body and stored in polyethylene bags at room temperature. Approximately 2–5 g each of pectoralis major muscle, liver, and kidney tissues was sampled, packaged in aluminum foil, and stored at  $-80\text{ }^{\circ}\text{C}$  until shipped frozen to the University of North Florida (Jacksonville, FL) for THg analysis.

### THg Analysis

Liver, kidney, and muscle samples and feathers were weighed and dried at  $60\text{ }^{\circ}\text{C}$  for 48–60 h, or until there was no further change in sample weight. Feathers were rinsed for 1 min in 0.25 M NaOH following by three rinses in deionized water prior to drying to remove possible external

contaminants (Bond and Diamond 2009). After drying, samples were re-weighed to determine percent moisture, then crushed using a mortar and pestle. Feather samples were cut into smaller pieces using scissors and flash-frozen using liquid nitrogen prior to crushing because it facilitated the homogenization process and has been shown to increase the precision of feather THg measurements (Peterson et al. 2019). THg concentrations were measured in homogenized samples via thermal decomposition (combustion), amalgamation, and atomic absorption spectrometry using a calibrated DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, CT), following EPA Method 7473 (U.S. EPA 2007). Approximately 0.05 g of homogenized sample was loaded into the DMA-80 and analyzed for THg using the methods described in Nam et al. (2011). Quality control procedures included analysis of laboratory method blanks, duplicate tissue samples, and certified reference materials for each group of 10 samples analyzed. Dry-weight (d.w.) measurements of THg concentrations in all tissues were used for comparison with estimated toxicity thresholds, which were determined on a dry-weight basis using information from Ackerman et al. (2016) and are described below. Measurements of THg concentrations in d.w. in liver, kidney, and muscle were also converted to wet-weight (w.w.) measurements using moisture data for comparisons with past studies, which are generally reported on a w.w. basis. Feather THg concentrations were only reported in mg/kg d.w., as in past studies.

## Data Analysis

Results were grouped by species and tissue type and analyzed using descriptive statistics to summarize and compare data with toxicity threshold values and results from past studies. Because of the presence of extreme positive outliers in some datasets, both the arithmetic and geometric means were reported.

Toxicity thresholds for all tissues were estimated based on the blood equivalent toxicity benchmarks of <0.2 (background), 0.2–1.0 (lower risk), 1.0–3.0 (moderate risk),

3.0–4.0 (higher risk), and > 4.0 (severe risk) mg/kg w.w., which were recommended by Ackerman et al. (2016) because they represent levels at which a range of effects on avian health and reproduction may occur. Toxicity thresholds based on these benchmarks were estimated for internal tissues and feathers in a d.w. basis using predictive relationships established between blood and these tissues by Eagles-Smith et al. (2008) using > 600 birds from four species exhibiting a broad range of THg concentrations (Ackerman et al. 2016). The resulting estimates for toxicity thresholds used in the present study are presented in Table 1. The percentage of samples that exceeded estimated toxicity thresholds was reported for each tissue type by species.

Kruskal–Wallis ANOVA and Dunn’s multiple comparison test were used to compare THg concentrations in internal tissues within species as group variances were not homogenous and attempts to transform data to achieve homoscedasticity were not successful. When data from both immature and mature individuals from the same species were available (i.e., all species except for osprey and royal tern, of which only adults were obtained), mean levels observed in liver (selected as the best indicator of internal Hg burden) were also compared by stage of maturity using the Student t-test. THg concentrations in all tissues were also compared by site of collection (i.e., the wildlife center at which the sample was obtained) using one-way ANOVA followed by Tukey’s *b* test except for a small number of datasets (i.e., herring gull liver and kidney) in which Kruskal–Wallis ANOVA and Dunn’s multiple comparison test were used. The results of comparisons between maturity stages and site of collection were used to determine whether data were combined or separated before comparisons of THg levels between species were conducted.

THg concentrations in all tissues were compared between species using Kruskal–Wallis ANOVA and Dunn’s multiple comparison test because group variances were not homogenous and attempts to transform data to achieve homoscedasticity were not successful. Graphical depiction of these data was arranged in relation to foraging guild (i.e., piscivore for

**Table 1** THg concentrations (mg/kg d.w.) in liver, kidney, muscle, and feathers used as toxicity thresholds in the present study

Tissue	Blood equivalent toxicity benchmarks				
	Background (<0.2)	Lower risk (0.2–1.0)	Moderate risk (1.0–3.0)	Higher risk (3.0–4.0)	Severe risk (> 4.0)
Liver	< 1.39	> 1.39–7.30	> 7.30–22.67	> 22.67–30.49	> 30.49
Kidney	< 1.49	> 1.49–7.40	> 7.40–22.14	> 22.14–29.48	> 29.48
Muscle	< 0.52	> 0.52–2.58	> 2.58–7.14	> 7.14–9.31	> 9.31
Feathers	< 1.10	> 1.10–12.01	> 12.01–61.45	> 61.45–94.19	> 94.19

Values were determined using predictive relationships established between blood and these tissues by Eagles-Smith et al. (2008), as reported in Ackerman et al. (2016). Values correspond to blood equivalent toxicity benchmarks of <0.2 (background), 0.2–1.0 (lower risk), 1.0–3.0 (moderate risk), 3.0–4.0 (higher risk), and > 4.0 (severe risk) mg/kg w.w

all species except for laughing gull, which is dominantly a crustaceavore, Ackerman et al. 2016) and prior, unpublished estimates of trophic level (TL) for each species derived via stable isotope analysis (laughing gull: 3.90, royal tern: 4.11, herring gull: 4.59, brown pelican: 4.78, northern gannet and osprey: 5.15, Kerstetter et al. 2016). An estimated average TL of 4.50 was used for double-crested cormorant, which exhibited a range of TL from 3.40–5.59 in the prior study (Kerstetter et al. 2016).

Last, correlations between THg concentrations in all tissue types were examined for each species using Spearman's rank-correlation coefficient. When significant correlations were observed between feather THg concentrations and other tissues, regression analysis was used to characterize the strength of the predictive relationship.

All statistical analyses were performed using SPSS software, v. 26.0 (IBM, Armonk, NY, USA). Graphs were prepared using GraphPad Prism, v. 8.0 (GraphPad Software, San Diego, CA, USA).

## Results

THg concentrations were measured in liver, kidney, muscle, and feathers in a total of 101 birds from the seven study species. Dry-weight measurements are provided in Table 2,

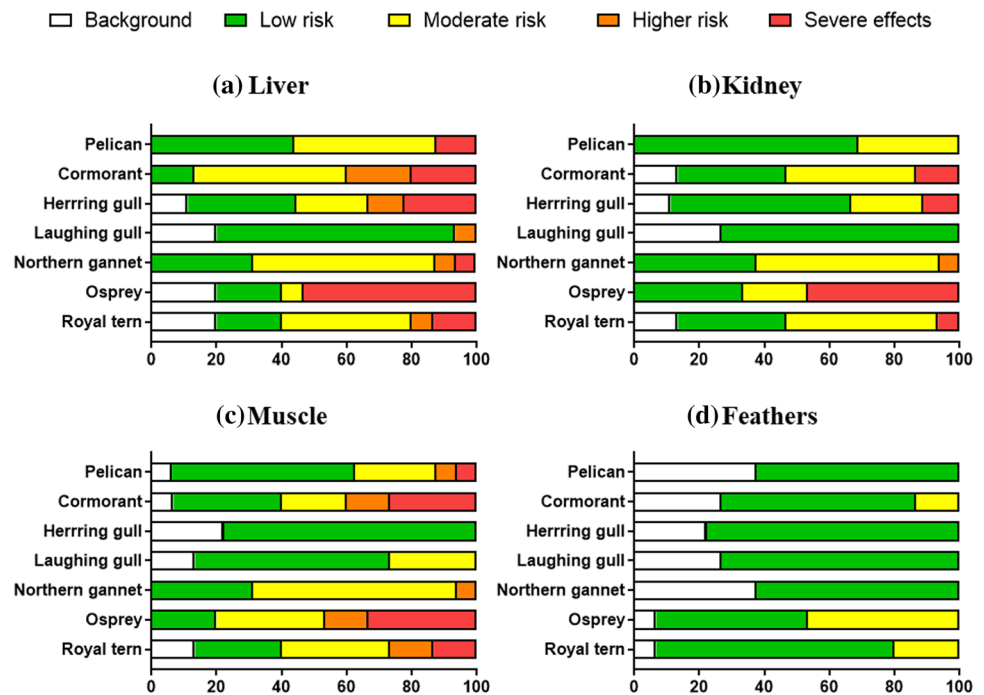
whereas a graphical depiction of toxicity threshold exceedances is presented in Fig. 1. Overall, less than 10% of internal tissue samples examined (9.9%, 8.9%, and 7.9% in liver, kidney, and muscle respectively) were found to exhibit THg concentrations at or below background levels, whereas 24% of feather samples exhibited background levels of Hg exposure. Correspondingly, a sizeable proportion of internal tissue samples (56.4%, 43.6%, and 54.4% in liver, kidney, and muscle, respectively) were found to exceed toxicity thresholds for at least moderate Hg-related effects, while only 12% of feather samples were found to exceed thresholds for moderate toxicity. Toxicity threshold exceedance rates varied by species with the lowest observed in laughing gull (6.6%, 0%, 26.6%, and 0% above thresholds for moderate toxicity in liver, kidney, muscle, and feathers, respectively) and the greatest observed in osprey (60%, 66.6%, 80%, and 46.6% above thresholds for moderate toxicity in liver, kidney, muscle, and feathers, respectively). The greatest number of threshold exceedances for severe toxicity were also observed in osprey, in which 53.3% of liver samples, 46.6% of kidney samples, and 33.3% of muscle samples exhibited THg concentrations above those associated with severe risks. High exceedance rates for moderate or greater risks (> 50%) were also observed in certain tissues in most species other than laughing gull. This was particularly true for the cormorant, in which 40% of liver and muscle samples exhibited THg

**Table 2** Dry-weight measurements of THg (mg/kg d.w.) in liver, kidney, pectoralis major muscle, and contour feathers of seven species of marine-associated birds from south Florida

Species	N	Liver	Kidney	Muscle	Feathers
Brown pelican (2014–2016)	16	13.82 ± 13.93	6.55 ± 4.81	3.23 ± 2.62	2.02 ± 2.0
		9.49 ± 2.40	5.27 ± 1.92	2.47 ± 2.14	1.39 ± 2.44
		2.49–56.03	2.40–17.20	0.52–10.15	0.30–7.61
Double-crested cormorant (2014–2016)	15	52.96 ± 106.6	32.14 ± 86.56	10.80 ± 21.45	5.51 ± 7.64
		21.51 ± 3.58	8.09 ± 4.50	4.05 ± 4.10	2.34 ± 4.50
		1.71–426.4	0.67–343.4	0.30–86.47	0.13–28.53
Herring gull (2014–2016)	9	68.59 ± 172.6	8.76 ± 10.55	4.50 ± 6.52	2.58 ± 2.35
		10.37 ± 6.40	5.34 ± 2.83	2.35 ± 3.70	1.53 ± 3.48
		1.15–527.8	1.36–35.37	0.36–16.78	0.19–7.34
Laughing gull (2015)	15	3.86 ± 6.05	2.06 ± 1.12	1.75 ± 1.27	1.63 ± 1.08
		2.25 ± 2.60	1.70 ± 2.07	1.37 ± 2.16	1.27 ± 2.22
		0.40–25.0	0.25–4.31	0.24–5.40	0.25–3.81
Northern gannet (2015–2016)	16	12.89 ± 8.19	10.28 ± 6.94	3.93 ± 1.97	2.0 ± 1.58
		10.49 ± 2.0	8.13 ± 2.12	3.43 ± 1.77	1.43 ± 2.53
		2.89–32.16	1.92–26.66	0.82–8.91	0.15–5.85
Osprey (2014–2016)	15	78.61 ± 128.4	185.8 ± 378.5	8.19 ± 7.34	10.33 ± 7.85
		16.98 ± 9.23	25.01 ± 9.18	4.79 ± 3.41	6.68 ± 3.06
		0.53–490.4	1.76–1,381.0	0.62–21.14	0.74–24.37
Royal tern (2015–2016)	15	15.80 ± 15.78	10.29 ± 8.70	4.89 ± 4.33	10.22 ± 12.68
		8.35 ± 3.88	6.07 ± 3.56	3.02 ± 3.13	6.37 ± 2.95
		0.78–54.63	0.34–31.47	0.36–15.46	ND–45.08
Total	101				

Values shown include (in order, by row) arithmetic mean ± SD, geometric mean ± geometric SD, and range. Specimen collection years are included for each respective species

**Fig. 1** Percentage of **a** liver, **b** kidney, **c** muscle, and **d** feather samples that exhibited THg concentrations that fell within the five different toxicity threshold categories used in this study. Tissue concentrations that correspond to toxicity threshold categories are presented in Table 1



concentrations above those associated with higher or severe risks for Hg toxicity.

Internal THg levels were typically highest in liver, followed by kidney then muscle. However, significant

differences between w.w. concentrations in internal tissues were only observed in certain species (Kruskal–Wallis ANOVA and Dunn’s post-test, Table 3). No significant differences were observed in liver THg concentrations

**Table 3** Wet-weight measurements of THg (mg/kg w.w.) in liver, kidney, and muscle of seven species of marine-associated birds from south Florida

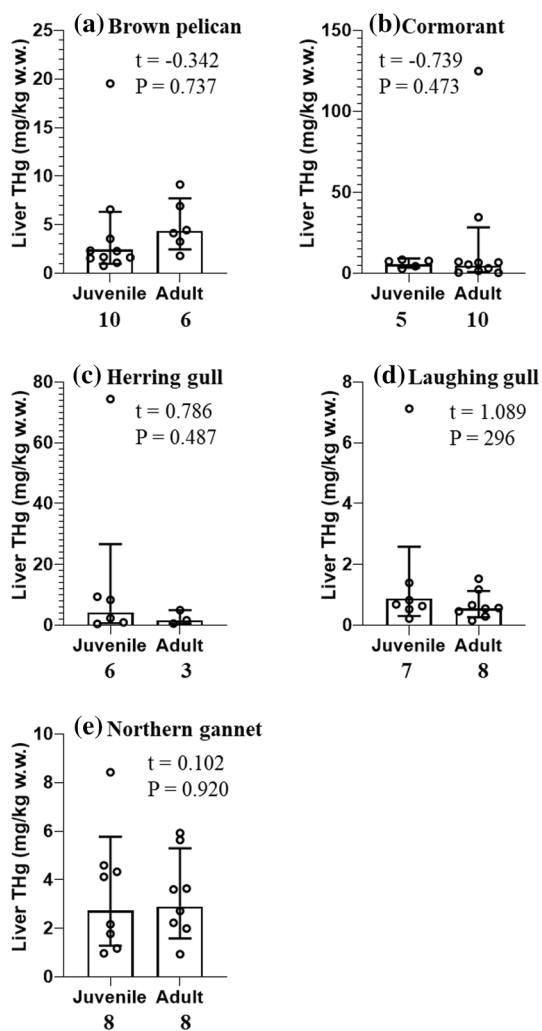
Species	N	Liver	Kidney	Muscle	Results of mean comparisons
Brown pelican	16	4.41 ± 4.67	1.71 ± 1.28	0.89 ± 0.59	H = 20.12
		3.05 ± 2.34	1.39 ± 1.91	0.73 ± 1.94	P < 0.0001*
		0.76–19.53	0.63–5.05	0.15–2.57	L > K > M
Double-crested cormorant	15	14.72 ± 31.54	7.47 ± 18.88	2.96 ± 5.80	H = 8.59
		4.95 ± 4.36	2.05 ± 4.44	1.12 ± 4.21	P = 0.014*
		0.32–124.9	0.16–75.02	0.06–23.43	L > K = M
Herring gull	9	11.44 ± 23.86	3.85 ± 4.57	1.12 ± 1.27	H = 5.10
		3.07 ± 5.11	1.95 ± 3.59	0.64 ± 3.06	P = 0.078
		0.44–74.43	0.39–13.25	0.13–3.31	NS
Laughing gull	15	1.12 ± 1.71	0.55 ± 0.30	0.45 ± 0.29	H = 4.47
		0.68 ± 2.49	0.45 ± 2.14	0.37 ± 2.03	P = 0.107
		0.15–7.12	0.06–1.17	0.07–1.26	NS
Northern gannet	16	3.39 ± 2.07	2.52 ± 1.65	0.96 ± 0.50	H = 18.50
		2.81 ± 1.93	2.02 ± 2.07	0.84 ± 1.79	P < 0.0001*
		0.93–8.43	0.48–6.0	0.18–2.29	L = K > M
Osprey	15	23.64 ± 35.63	50.42 ± 98.44	2.19 ± 2.18	H = 5.56
		5.37 ± 9.15	7.49 ± 8.69	1.24 ± 3.37	P = 0.062
		0.15–132.1	0.48–352.4	0.18–6.98	NS
Royal tern	15	5.30 ± 5.23	3.38 ± 2.95	1.35 ± 1.02	H = 6.98
		2.80 ± 3.88	1.91 ± 3.69	0.90 ± 2.95	P = 0.03*
		0.26–16.90	0.11–9.66	0.11–3.48	L > M, L = K, K = M

NS Not significant

Values shown include (in order, by row) arithmetic mean ± SD, geometric mean ± geometric SD, and range. Final column reports the results of comparison of means tests (Kruskal–Wallis ANOVA and Dunn’s post hoc test). Significant differences are shown with an asterisk (\*)

between juvenile and adult individuals in the species for which samples from both groups were obtained (Student *t*-test, Fig. 2). Furthermore, no significant differences were observed by site of collection for any tissues in any species (one-way ANOVA or Kruskal–Wallis one-way ANOVA, Fig. 3). Therefore, all data were combined by tissue type for comparison between species.

Species-specific differences were observed in THg concentrations in all tissues and generally appeared to correspond well to foraging guild and prior estimates of TL (Fig. 4). Significantly lower concentrations of THg were observed in liver ( $P=0.003$ ) and kidney ( $P=0.0002$ ) of laughing gull compared with those observed in all other species (Kruskal–Wallis ANOVA and Dunn's post-test, Fig. 4).



**Fig. 2** Mean liver THg concentrations (mg/kg w.w.) in juvenile and adult individuals of **a** brown pelican, **b** double-crested cormorant, **c** herring gull, **d** laughing gull, and **e** northern gannet. Open circles represent individual measurements, whereas bars represent geometric means  $\pm$  geometric SD. Results of comparisons of means tests (student *t*-test) are shown. *N* are shown below *x*-axis labels

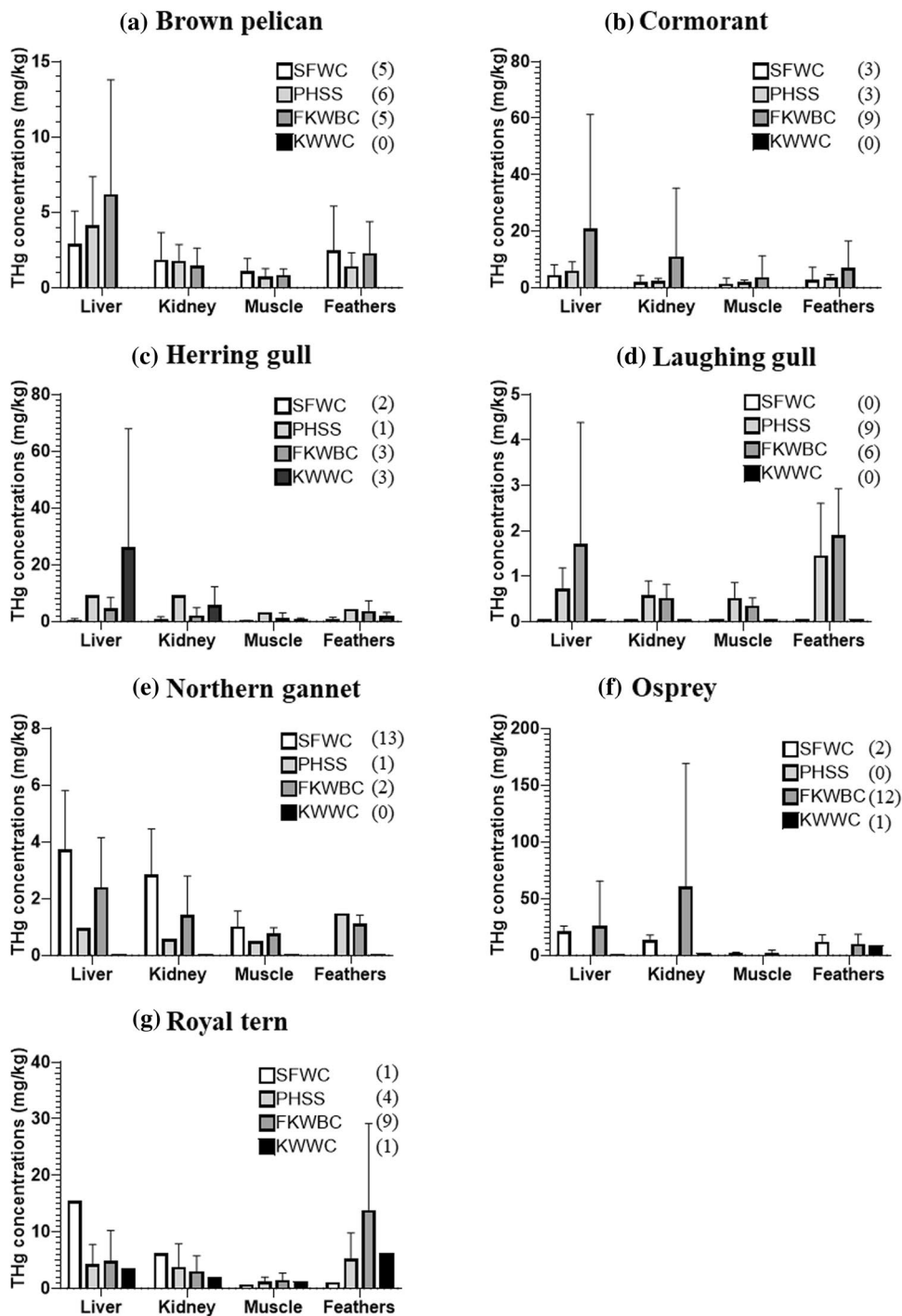
Muscle ( $P=0.019$ ) and feather ( $P<0.0001$ ) THg concentrations also differed significantly by species, but results of statistical tests were more variable (Kruskal–Wallis ANOVA and Dunn's post-test, Fig. 4).

Significant correlations were generally observed between THg concentrations in internal tissues of all species with only few exceptions (Spearman correlation coefficient, Table 4). In contrast, THg concentrations in feathers were not significantly correlated with those in internal tissues in most species, except for double-crested cormorant (liver, kidney, and muscle), laughing gull (muscle only), and osprey (liver and kidney only) (Spearman's correlation coefficient, Table 4). In most instances when feather THg concentrations were correlated with those in internal tissues, little of the variation in feather THg levels was explained by that in internal tissues (Fig. S-1 in Supplementary Information).

## Discussion

The results of this study indicate that several marine-associated bird species in south Florida may accumulate toxicologically relevant levels of Hg. This is based on the high percentage of toxicity threshold exceedances observed in this study, particularly in internal tissues, in which 40–50% of samples exhibited THg concentrations above levels previously shown to be associated with moderate to severe health effects (Ackerman et al. 2016). However, it is noteworthy to point out that high exceedance rates observed for tissues capable of MeHg detoxification, such as liver and kidney (Manceau et al. 2021), may exaggerate toxicity risks in these populations. This is because these tissues have been shown to be capable of demethylating MeHg to form high molecular weight Hg–selenium (Se)–protein compounds, which can then undergo degradation in lysosomes, creating inorganic Hg selenides and/or stable Hg–Se–protein fragments (Ikemoto et al. 2004; Scheuhammer et al. 2007). While these insoluble Hg–Se compounds may accumulate over time, they are largely believed to be toxicologically inert (Scheuhammer et al. 2007; Eagles-Smith et al. 2009) and may comprise a significant proportion of THg in these tissues (e.g., inorganic forms of Hg can make up as much as 40–60% of liver THg at THg concentrations above 8.5 mg/kg d.w., Eagles-Smith et al. 2009). Therefore, since toxicity thresholds such as the ones used herein assume that all of the THg observed in a given tissue is in the biologically available methylated form (Ackerman et al. 2016), this is not likely to be the case in the present study and our liver and perhaps even kidney exceedance rates presumably overestimate toxicity risks. Still, it is important to note that all species except for laughing gull also exhibited moderately high exceedance rates in muscle, which shows little capacity for MeHg demethylation (Scheuhammer et al. 2007) and

**Fig. 3** THg concentrations in liver, kidney, muscle, and feathers of **a** brown pelican, **b** double-crested cormorant, **c** herring gull, **d** laughing gull, **e** northern gannet, **f** osprey, and **g** royal tern in relation to site of collection. Bars represent arithmetic means  $\pm$  SD in mg/kg w.w. for liver, kidney, and muscle or mg/kg d.w. for feathers. *N* are shown for each site in the legend. SFWC: South Florida Wildlife Center, PHSS: Pelican Harbor Seabird Station, FKWBC: Florida Keys Wild Bird Rehabilitation Center, and KWWC: Key West Wildlife Center. No mean values differed significantly by site at  $\alpha=0.05$  (one-way ANOVA for all comparisons except kidney and muscle for herring gull, which were analyzed using Kruskal–Wallis one-way ANOVA)

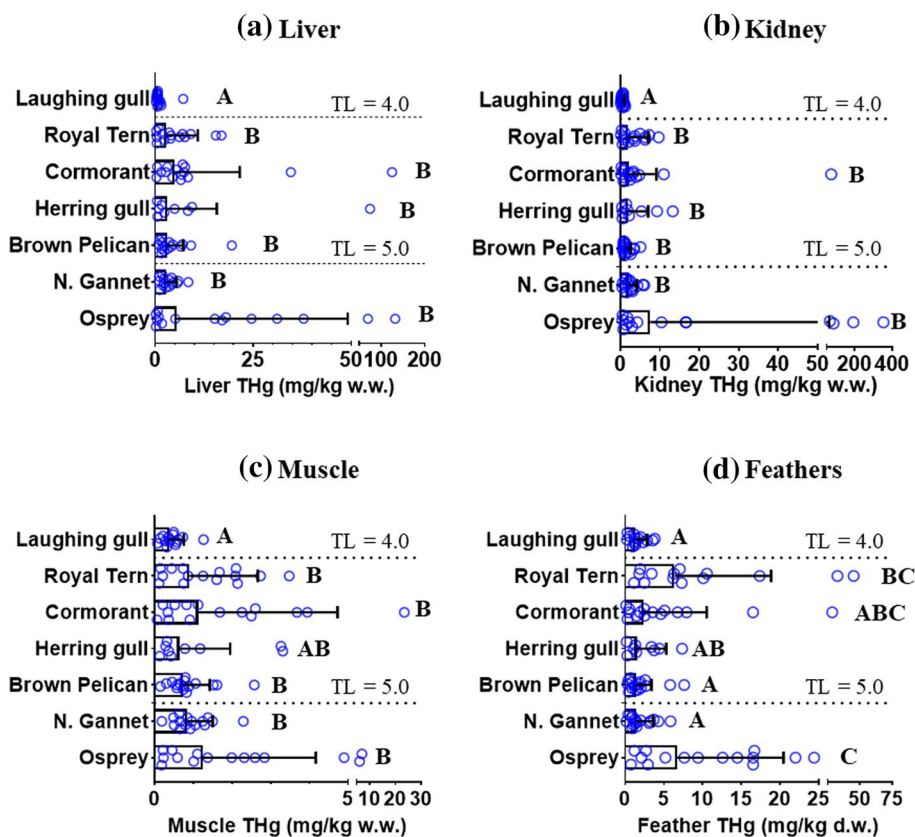


exhibits higher MeHg/THg ratios (e.g., up to > 90%, Des-Granges et al. 1998). Thus, there is still reason to consider that south Florida populations of some species examined in this study may be at risk of Hg-related health effects. This is particularly true for the osprey, in which a sizeable proportion of muscle (46.6%) samples were found to contain THg concentrations that exceeded benchmarks for severe toxicity.

In addition to other probable factors, species-specific differences in THg concentrations and rates of toxicity

threshold exceedances observed in this study are likely to be strongly related to dietary habits. This is best exemplified by the significantly lower THg levels observed in internal tissues of laughing gull compared with those in all other species, as laughing gull was the single crustaceavore examined in the present study as well as the only species to exhibit a single estimated TL < 4.0 in previous work by Kerstetter et al. (2016). The lack of consistent, significant differences between internal THg concentrations in all other

**Fig. 4** THg concentrations in **a** liver, **b** kidney, **c** muscle, and **d** feathers of brown pelican, double-crested cormorant, herring gull, laughing gull, northern gannet, osprey, and royal tern from south Florida. Open circles represent individual measurements, whereas bars represent geometric means  $\pm$  geometric SD in mg/kg w.w. for liver, kidney, and muscle or mg/kg d.w. for feathers. *N* for each group is provided in Tables 2 and 3. Species are arranged from top to bottom based on previous measurements of trophic level (TL) by Kerstetter et al. (2016), as described in the text. Dotted lines represent cutoff values for TLs of 4 and 5. Uppercase letters represent homogenous subgroups (Kruskal–Wallis ANOVA and Dunn’s post hoc test,  $\alpha=0.05$ )



species likely reflects overlap in feeding habits given that all are largely piscivorous. However, the notably higher rate of toxicity threshold exceedances in osprey (as well as the significantly higher THg concentrations in osprey feathers) are believed to reflect its likely status as the top avian predator within this system. Associations between feeding habits and Hg accumulation have been reported in other studies on seabirds (e.g., Gochfeld 1980; Anderson et al. 2009; Blévin et al. 2013; Hosseini et al. 2013), and broad comparisons between avian groups suggest that aquatic piscivores along with terrestrial carnivores generally exhibit the highest THg levels of all bird species (Ackerman et al. 2016). Nonetheless, variations in the strength of relationships between diet and Hg accumulation have been reported in several studies (e.g., Thompson et al. 1993; Bearhop et al. 2000). Therefore, it is also important to not discount the role of other factors in driving species-specific differences in Hg accumulation patterns. This can include factors such as behavior, migratory patterns, foraging habitat, physiological differences, and/or temporal changes in diet or any of these aforementioned factors, all of which have been shown to influence Hg uptake (Anderson et al. 2009).

Notwithstanding evidence for the potential for Hg toxicity in south Florida birds, the results of the present study also support the hypothesis that Hg accumulation may have progressively declined in these populations (Rumbold et al.

2001; Frederick et al. 2002; Heath and Frederick 2005), perhaps even in species that still exhibit moderately high toxicity threshold exceedance rates. This is best supported by comparisons of the present data with those previously reported for south Florida samples from some of the same species examined in this study (Table 5). This includes double-crested cormorant, for which THg concentrations were examined in liver, kidney, and brain of south Florida samples ( $n=84$ ) by Sepulveda et al. (1998). As summarized in Table 5, THg concentrations in both liver and kidney of double-crested cormorant examined in the present study were lower than those reported by Sepulveda et al. (1998); although occasionally high levels were still observed. Reduced levels of THg were also observed in feathers of south Florida osprey examined in the present study in comparison with those reported in samples collected a decade and a half earlier (2000–2001) by Lounsbury-Billie et al. (2008) (Table 5). However, it is important to note that while it improves the overall precision of feather THg measurements, the sample preparation approach used in the present study (i.e., cutting feathers with scissors, followed by liquid nitrogen-facilitated crushing of samples using a mortar and pestle) has been shown to reduce THg measurements by close to 10% of the measurements made using other approaches (i.e., whole-feather analysis or scissor-cut feathers without homogenization) (Peterson et al. 2019).



**Table 4** Results of correlation analysis (Spearman rank-order correlation coefficient, *P*-value) conducted for THg concentrations in tissues of seven species of marine-associated birds from south Florida

Species Tissue	Kidney	Muscle	Feather
<i>Brown pelican</i>			
Liver	0.876, <i>P</i> < 0.0001*	0.515, <i>P</i> = 0.043*	0.241, <i>P</i> = 0.367
Kidney		0.685, <i>P</i> = 0.004*	0.223, <i>P</i> = 0.404
Muscle			0.059, <i>P</i> = 0.831
<i>Double-crested cormorant</i>			
Liver	0.814, <i>P</i> = 0.0004*	0.786, <i>P</i> = 0.0008*	0.529, <i>P</i> = 0.0454*
Kidney		0.689, <i>P</i> = 0.0057*	0.786, <i>P</i> = 0.0008*
Muscle			0.546, <i>P</i> = 0.0377*
<i>Herring gull</i>			
Liver	0.567, <i>P</i> = 0.1206	0.633, <i>P</i> = 0.076	0.133, <i>P</i> = 0.7435
Kidney		0.550, <i>P</i> = 0.1328	0.500, <i>P</i> = 0.1777
Muscle			0.600, <i>P</i> = 0.097
<i>Laughing gull</i>			
Liver	0.746, <i>P</i> = 0.002*	0.525, <i>P</i> = 0.0471*	0.225, <i>P</i> = 0.419
Kidney		0.729, <i>P</i> = 0.0029*	0.396, <i>P</i> = 0.1446
Muscle			0.643, <i>P</i> = 0.0116*
<i>Northern gannet</i>			
Liver	0.929, <i>P</i> < 0.0001*	0.706, <i>P</i> = 0.003*	-0.05, <i>P</i> = 0.856
Kidney		0.791, <i>P</i> = 0.0004*	0.159, <i>P</i> = 0.556
Muscle			-0.073, <i>P</i> = 0.788
<i>Osprey</i>			
Liver	0.761, <i>P</i> = 0.0015*	0.636, <i>P</i> = 0.0128*	0.668, <i>P</i> = 0.008*
Kidney		0.375, <i>P</i> = 0.1692	0.789, <i>P</i> = 0.0008*
Muscle			0.289, <i>P</i> = 0.2949
<i>Royal tern</i>			
Liver	0.836, <i>P</i> = 0.0002*	0.714, <i>P</i> = 0.0037*	0.054, <i>P</i> = 0.8525
Kidney		0.775, <i>P</i> = 0.0011*	-0.021, <i>P</i> = 0.9438
Muscle			0.182, <i>P</i> = 0.5150

Significant correlations are shown with an asterisk (\*)

**Table 5** Comparisons of THg concentrations in tissues of south Florida marine-associated birds examined in the present study with values reported for south Florida individuals from the same species in previously published studies

Species Age class	Tissue	Year(s)	<i>N</i>	THg ± SD (range)	References
<i>Double-crested cormorants</i>					
Juvenile	Liver	1994–1996	40	12.0 ± 18.0 (0.90 – 83.0)	Sepulveda et al. 1996
		2014–2015	5	6.07 ± 2.42 (2.69 – 8.34)	Present study
	Kidney	1994–1996	33	4.80 ± 6.20 (0.25 – 33.0)	Sepulveda et al. 1996
		2014–2015	5	2.47 ± 1.60 (0.75 – 4.74)	Present study
Adult	Liver	1994–1996	33	48.0 ± 67.0 (0.44 – 250.0)	Sepulveda et al. 1996
		2015–2016	10	19.0 ± 38.50 (0.32 – 124.0)	Present study
	Kidney	1994–96	22	12.0 ± 20.0 (0.29 – 77.0)	Sepulveda et al. 1996
		2015–2016	10	9.96 ± 13.08 (0.16 – 75.02)	Present study
<i>Osprey</i> Adult	Feathers	2000–2001	17	16.4 ± 1.51 (NR)	Lounsbury-Billie et al. 2008
		2014	110	17.8 ± 16.10 (0.375–93.65) <sup>a</sup>	Rumbold et al. 2017
		2014–2016	15	10.33 ± 7.85 (0.74 – 24.37)	Present study

<sup>a</sup>Values reported in Rumbold et al. are for multiple locations in Florida, including south Florida sites in Monroe County.

Data are presented in mg/kg w.w. for liver and kidney and mg/kg d.w. for feathers. Values are arithmetic means ± SD, followed by ranges in parentheses. NR: not reported.

Therefore, it is perhaps better to consider that the observations made in the present study may be only slightly lower but still within the range of earlier findings, especially since other somewhat recent (i.e., 2014) measurements of THg concentrations in south Florida osprey feathers prepared without homogenization (Rumbold et al. 2017) were largely comparable to those reported by Lounsbury-Billie et al. (2008) (Table 5).

Possible responses to Hg exposure in marine-associated birds at levels above the toxicity benchmarks used in this study may include alterations in health and reproduction (Ackerman et al. 2016), effects that have the potential to lead to population-level declines in species for which high rates of toxicity threshold exceedances may occur (e.g., osprey). As an example, Heath and Frederick (2005) reported possible associations between above-threshold feather THg levels and reductions in pre-breeding female plasma estradiol concentrations and nesting effort in white ibis. Based on these data, the authors suggested that alterations in hormone-mediated reproductive behaviors due to Hg exposure may have resulted in higher rates of nest abandonment and lowered reproductive success. While such observations are largely correlative, more direct experimental work has supported the hypothesis that exposure to Hg at ecologically realistic levels can affect health and reproduction in south Florida seabirds. Spalding et al. (2000a, 2000b) reported a variety of health effects in captive great egret nestlings that were orally dosed with MeHg at levels comparable with those in some south Florida prey fish species (i.e., 0.5 mg/kg w.w.). These responses included anemia, decreased appetite and weight, reduced activity, behavioral changes, and various immunological responses. In a more recent study, Frederick and Jayasena (2010) reported that chronic dietary exposure to even lower levels of MeHg (0.05–0.3 mg/kg w.w.) could also impair reproduction in adult white ibis by altering male reproductive behavior, resulting in an increase in male-male pairing and a concomitant decline in successful heterosexual pairing and nestling production.

Despite these points, it remains unclear whether Hg exposure is affecting south Florida bird populations, including osprey, in large part because previous studies have reported comparable if not higher levels of Hg accumulation in other osprey populations without any apparent effects on reproduction (Hughes et al. 1997; Cahill et al. 1998; DesGranges et al. 1998; Anderson et al. 2008). For example, Cahill et al. (1998) observed mean ( $\pm$ SD) feather THg concentrations of  $20.0 \pm 11.0$  mg/kg d.w. in adult osprey from a Hg-contaminated freshwater lake in central CA but no evidence of declines in breeding success. In a follow-up study conducted at the same location, Anderson et al. (2008) reported no association between productivity (i.e., number of young produced per active nest) and Hg exposure in osprey over a broad range of feather THg concentrations (2 to > 20 mg/kg

d.w.). Based on this, both Lounsbury-Billie et al. (2008) and Rumbold et al. (2017) concluded that south Florida osprey populations—which exhibit lower feather THg concentrations (Table 5)—are unlikely to be experiencing significant effects from Hg exposure. Still, both expressed the need for continued monitoring of Hg exposure and effects in these populations because of the possibility of effects on younger age classes during more sensitive life stages. As Rumbold et al. (2017) also stressed, the assessment of Hg toxicity in birds using feather THg data only can be complicated due to high intra- and interspecific variation in these values, as well as interspecific differences in sensitivity to Hg.

Concerns about the predictive value of using feathers to assess Hg toxicity in birds are relevant to the present study. This is because, with only few exceptions, we did not observe significant or meaningful associations between THg concentrations in feathers with those measured in internal tissues. Similar inconsistencies in the strength of relationships between feather THg measurements and internal Hg burden have been reported in numerous studies (e.g., Gochfeld et al. 1996; Eagles-Smith et al. 2008), demonstrating the limitations of this approach despite its general attractiveness as a lower-effort and noninvasive method for screening possible Hg-related effects in bird populations (Low et al. 2019; Jaspers et al. 2019; Peterson et al. 2019). Such inconsistencies are likely to be partly due to the high variability in feather THg measurements, both between species or individuals from the same locations as well as between feathers or even feather subcomponents from the same individual (Peterson et al. 2019). These variations are in turn likely associated with species-specific and/or individual differences in age, movement patterns, foraging behavior, and molting periodicity, as feather THg concentrations are generally believed to reflect blood THg levels at the time of feather growth. Therefore, any variations in feather replacement strategy and/or Hg exposure or remobilization from internal stores during molting can lead to sizeable differences in feather Hg uptake. Additional factors that have also been shown to influence feather THg levels include sample condition (e.g., fraying) and sample preparation (Peterson et al. 2019). Despite these points, it is important to note that THg levels in downy feathers from young individuals have been shown to exhibit much lower variability than that observed in fully grown feathers from older birds and also correlate reasonably well with Hg levels in eggs or internal tissues (Ackerman and Eagles-Smith 2009; Peterson et al. 2019). Thus, feather THg measurements still hold some value for Hg monitoring in at least some bird taxa and/or age classes, provided that potential sources of data uncertainty can be controlled for or addressed (Ackerman et al. 2016; Low et al. 2019).

As a final point, it is important to acknowledge that the use of rehabilitation centers as a source for samples in the

present study may have provided us with a somewhat skewed characterization of Hg contamination in south Florida seabirds, as pollutant levels in health-compromised individuals may not necessarily be typical of those in an overall population. This has been previously demonstrated for Hg in past studies. For example, Frank et al. (1983) observed significantly greater brain THg concentrations in emaciated common loons *Gavia immer* compared with those in healthy individuals. Therefore, future studies that obtain samples from healthy, free-flying individuals may be needed to obtain data more representative of overall south Florida bird populations. Still, the data reported in this study provide a good comparison with those from earlier research on these populations, as several studies have also used debilitated, moribund, or dead individuals as study animals (e.g., Spalding et al. 1994; Sundlof et al. 1994; Beyer et al. 1997; Sepulveda et al. 1998).

## Conclusions

In conclusion, the present study has contributed valuable recent data on Hg accumulation in several marine-associated bird species from south Florida, suggesting potential for risks of Hg toxicity in some of these species. We echo earlier recommendations for further evaluation of Hg risks to species for which higher rates of toxicity threshold exceedances were observed, such as osprey, especially since these observations were made in a broad range of tissue types.

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**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interests** The authors have no relevant financial or non-financial interests to disclose.

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