



Mercury bioaccumulation in three colonial seabird species in the Gulf of Maine

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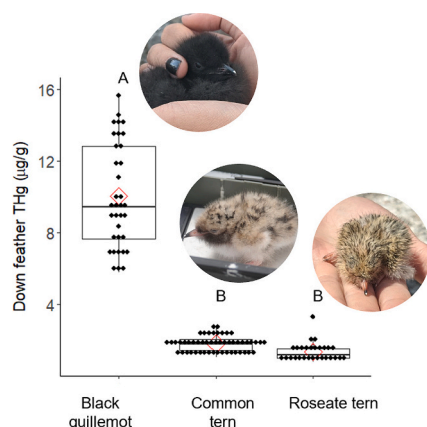
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HIGHLIGHTS

- We measured total mercury (THg) in down and contour feathers from chicks of three seabird species.
- Black guillemots had higher down feather THg levels than either tern species.
- Trophic position partially explains the differences in THg between species.

GRAPHICAL ABSTRACT



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ABSTRACT

The methylated form of mercury, MeHg, is a neurotoxin that bioaccumulates and biomagnifies through aquatic food webs, reaching high concentrations in top trophic species. Many seabird species are wide-ranging and feed on forage fish, so they can be used as sentinel species to assess the level of mercury in pelagic or coastal food webs because they integrate the signal from large areas and from lower trophic levels. The Gulf of Maine provides habitat for many seabirds, including endangered roseate terns (*Sterna dougalii*), common terns (*Sterna hirundo*), and the southernmost breeding population of black guillemots (*Cepphus grylle*). Hg levels were assessed in down of newly hatched chicks of three seabird species to determine pre-hatching Hg exposure. Stable isotopes ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) in down and chick contour feathers grown after hatching were used as indicators of adult female diet in the period before laying the egg (down) and pre-fledging chick diet (contour feathers). Black guillemot down THg concentrations were $10.07 \pm 2.88 \mu\text{g/g}$ (mean \pm 1SD), $5.5\times$ higher than common tern down ($1.82 \pm 0.436 \mu\text{g/g}$), and $7.4\times$ higher than roseate tern down ($1.37 \pm 0.518 \mu\text{g/g}$). Black guillemots also had higher down feather $\delta^{15}\text{N}$ values ($15.1 \pm 0.52 \text{‰}$) compared to common ($13.0 \pm 0.72 \text{‰}$) or roseate terns ($12.8 \pm 0.25 \text{‰}$), and in

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black guillemot down feathers, higher Hg concentrations were correlated with $\delta^{15}\text{N}$, an indicator of trophic level. Repeated testing of the same tissue types across multiple years is needed to monitor THg exposure for seabirds in the Gulf of Maine; additionally, monitoring species composition and Hg presence in prey species of the black guillemot population would help to determine the source of high THg concentrations in this species.

1. Introduction

1.1. Mercury in the food web

Methylmercury, the bioavailable and toxic form of elemental mercury, is a neurotoxin that bioaccumulates in marine biota and is a contaminant of concern for human and wildlife health (Braune et al., 2015; Jackson, 1997; Kirk et al., 2012). Inorganic mercury (Hg^{2+}) enters marine environments through wet and dry deposition and river inputs, where it is transformed into methylmercury (MeHg) through methylation by microbes (Hall et al., 1997). The main source of atmospheric mercury is anthropogenic emissions from artisanal small-scale gold mining and coal combustion, and long-range atmospheric transport means that mercury can enter aquatic environments far from the source of the contaminant (Jackson, 1997). Additionally, nearshore marine environments are influenced by runoff from terrestrial sources of mercury, some of which may be point sources of industrial waste (Buckman et al., 2015; Sunderland et al., 2012).

Although global Hg emissions have decreased due to controls on anthropogenic sources, Hg is retained in sediment and in food webs (Rudd et al., 2018). MeHg bioaccumulates and biomagnifies through the food web, so top predator fish often have elevated tissue MeHg which exceeds criteria calculated to protect humans and wildlife (Chen et al., 2009; Goodale et al., 2008; Harding et al., 2018). Seabirds are frequent subjects of MeHg studies because they can be used as sentinel species to indicate MeHg levels in the marine food web (Furness and Camphuysen, 1997; Goodale et al., 2008; Stenhouse et al., 2018; Thompson et al., 1998).

MeHg affects neurological and immune system function (including increased susceptibility to avian influenza), egg hatchability, and population dynamics in seabirds (Dietz et al., 2019; Heinz et al., 2009; Teitelbaum et al., 2022). Sensitivity to injected MeHg appears to vary with taxonomic group, with Falconiformes (Osprey) and Acciptriformes (American kestrel) having high sensitivities, some species within Charadriiformes (e.g., common tern, herring gull) having medium sensitivities, and Anseriformes (e.g., mallard, Canada goose) having the lowest sensitivities (Heinz et al., 2009). One review found that concentrations of mercury above 1 $\mu\text{g}/\text{g}$ in whole fresh eggs were associated with decreased hatchability and higher embryonic mortality in piscivorous birds (Scheuhammer et al., 2007), which is equivalent to 13.0–13.7 $\mu\text{g}/\text{g}$ in down feathers (Ackerman and Eagles-Smith, 2009). A more comprehensive review identified a series of MeHg toxicity benchmarks for adult birds based on blood THg levels, ranging from low risk (0.2–1 $\mu\text{g}/\text{g}$), moderate risk (1.0–3.0 $\mu\text{g}/\text{g}$), high risk (3.0–4.0 $\mu\text{g}/\text{g}$), and severe risk (>4.0 $\mu\text{g}/\text{g}$) (Ackerman et al., 2016a). There is little currently known about effects of lower concentrations of mercury on chicks for specific species. Juvenile survival, or survival between fledging and adulthood, can be difficult to track, but has been associated with a number of more easily-measured variables, including bodyweight and wing length in Nazca boobies (*Sula granti*; Maness and Anderson, 2013) and bodyweight alone in Atlantic puffins (*Fratercula arctica*; Kress et al., 2017).

The tissue concentration of MeHg within an individual bird is the product of a balance between intake and excretion. MeHg intake is determined by diet, which can change with location, prey availability, energy requirements, and life stage (Albert et al., 2021; Bracey et al., 2021; Scopel et al., 2019). It is difficult to monitor the diet of wild seabirds, particularly outside the breeding season, so indicators such as stable isotope ratios have been used to indicate trophic position and

foraging habitat in comparison with mercury concentrations (Anderson et al., 2009; Peterson et al., 2017; Santos et al., 2017). Higher values of $\delta^{15}\text{N}$ indicate a diet of higher trophic position prey; in the marine environment, organisms foraging offshore or higher in the water column tend to have lower $\delta^{13}\text{C}$ values, while organisms foraging nearshore or in benthic environments have higher $\delta^{13}\text{C}$ values (Graham et al., 2010; Hobson, 1990; Hobson et al., 1994). Organisms foraging in freshwater environments tend also to exhibit lower $\delta^{13}\text{C}$ values.

MeHg is stored in muscle and circulates in blood (Bearhop et al., 2000; Szumilo-Pilarska et al., 2017). In birds, the main form of excretion is the deposition of MeHg into new tissues such as feathers and eggs. During feather formation MeHg is deposited into feather keratin and is separated from the rest of the body when blood supply to the feather is cut off at the end of the growth period, allowing feathers to act as a snapshot of blood mercury concentrations at the time of feather growth (Ackerman et al., 2016a, 2016b; Ackerman and Eagles-Smith, 2009). Most mercury in feathers is MeHg, allowing THg to be measured as a proxy for MeHg (Ackerman et al., 2013). Breeding females deposit MeHg into growing eggs. While still in the egg, chicks grow down feathers, where they in turn deposit MeHg (Ackerman and Eagles-Smith, 2009; Becker and Sperveslage, 1989; Santos et al., 2017). After hatching, as chicks feed and grow, their initial MeHg burden is eventually outweighed by dietary MeHg (Karasov et al., 2007). Blood THg concentrations in chicks are highest at hatching, then decrease, likely due to growth dilution and deposition into feathers, before increasing at fledging (Ackerman et al., 2011). Because blood MeHg concentrations are changing quickly while new feathers are growing, THg in chick contour feathers is variable and does not correlate well to blood concentrations in pre-fledged chicks and is therefore not recommended for monitoring (Ackerman et al., 2016a). However, there is evidence that THg in newly grown chick feathers is correlated with THg in blood of recently fledged gull chicks ($R^2 > 0.8$, Binkowski et al., 2021) and may provide information about species differences (Carravieri et al., 2014). Stable isotope signatures do not accumulate in body tissues in the same way as Hg, so newly grown feathers from fledging chicks can be used for monitoring diet in the period the feathers are growing after hatching (Cherel et al., 2000; Craig et al., 2015; Weiser and Powell, 2011).

1.2. Study system

The Gulf of Maine is one of the fastest-warming bodies of water in the world due to its position by the Gulf Stream (Lotze et al., 2022; Whitney et al., 2022) and is important to both wildlife and humans (Pershing et al., 2015). It contains several large human population centers, supports multiple fisheries both offshore and nearshore, and provides habitat for breeding seabirds. The main source of aquatic mercury in the Gulf of Maine as a whole is oceanographic circulation, with atmospheric deposition, industrial sources, and several major rivers also contributing Hg to the system (Sunderland et al., 2012). Species foraging close to shore or in estuaries may be more affected by point sources such as the Penobscot estuary, the site of a chlor-alkali plant that was active 1967–2000 and has been linked to elevated THg concentrations in bald eagles (*Haliaeetus leucocephalus*; DeSorbo et al., 2018) and American black ducks (*Anas rubripes*; Rudd et al., 2018; Sullivan and Kopec, 2018). Seabirds have previously been used as an indicator of mercury in the Gulf of Maine food web (Bond and Diamond, 2009; Goodale et al., 2008; Stenhouse et al., 2018); between 2001–2006 and 2013, mercury levels in Leach's storm petrels (*Hydrobates leucohora*), which are highly mobile pelagic foragers, remained stable while levels in three inshore species

(black guillemot, common eider, and double-crested cormorant) increased, suggesting that MeHg availability may have increased inshore while remaining stable offshore (Stenhouse et al., 2018). The three species included in this study, common terns (*Sterna hirundo*), roseate terns (*Sterna dougalii*), and black guillemots (*Cepphus grylle*), are of interest because they occupy distinct trophic niches and therefore each provide different information about THg in the surrounding areas; black guillemots have also been recommended as a subject for continued mercury monitoring in the Gulf of Maine (Stenhouse et al., 2018). In all three species, adults provision growing chicks with whole fish; consequently, their diet is limited by gape size and is distinct from that of adults (Arnold et al., 2020; Butler et al., 2020; Gochfeld and Burger, 2020). Both common and roseate terns are classified as Species of Greatest Conservation Need, with common terns classified as threatened in New Hampshire and Maine and roseate terns classified as endangered at both the state and federal level (Arnold et al., 2020; Gochfeld and Burger, 2020).

Knowledge of mercury levels is the first step towards identifying species and populations that may be more vulnerable to mercury exposure. Within this study, we aim to determine which of the three studied species may be the most at risk for mercury exposure by (1) assessing the level of mercury present in these species in the context of currently known toxicity thresholds for seabirds; (2) comparing pre-hatching mercury burden to chick condition after hatching, and (3) comparing trophic position and dietary breadth with mercury burden.

2. Methods

2.1. Sample collection

2.1.1. Collection methods

The Isles of Shoals is a group of seven islands located 9.7 km off the

coast of Maine (Fig. 1). In the 2022 breeding season a total of 48 black guillemot nests were observed on Appledore and Smuttynose I., and an estimated 3066 common tern nests and 124 roseate tern nests were observed on White and Seavey I. White and Seavey I. are the site of the Isles of Shoals Tern Conservation Program and contain a mixed-species colony of common and roseate terns, which was restored to the island in 1997. As part of an ongoing study on seabird ecology on the Isles of Shoals, chicks of all three species were banded, weighed, and measured, and a feather sample was taken. Down feathers were taken within the first week after hatching, and newly grown feather samples of five breast and flank contour feathers from the same feather tracts were taken once these feathers had finished growing. Chicks of all three species that died of natural causes were opportunistically collected throughout the breeding season. Feathers were collected from the same feather tracts using the same methods as live chicks, although more feathers were collected from each chick to allow for repeated measures from the same individual. All feathers collected were placed in paper envelopes and stored in a sealed plastic bag until laboratory analysis.

2.1.2. Sample sizes and characteristics

Feather samples were collected from 33 live black guillemot chicks. Of these, 16 chicks only had down feathers collected, 1 had a contour feather but no down, and 16 had both down and contour feathers. Across all black guillemots sampled, 16 had a known hatch date (Table S-1). Both down and contour feather samples were collected from a total of 28 common tern chicks and 27 roseate tern chicks, all with known hatch dates and hatch orders. Down feathers from an additional 24 common terns, 5 roseate terns, and 1 black guillemot were salvaged postmortem after chicks died of natural causes, most of which were from unmonitored nests. There were no significant differences in THg, $\delta^{15}\text{N}$, or $\delta^{13}\text{C}$ between feathers collected from dead and live chicks (Table S-2).

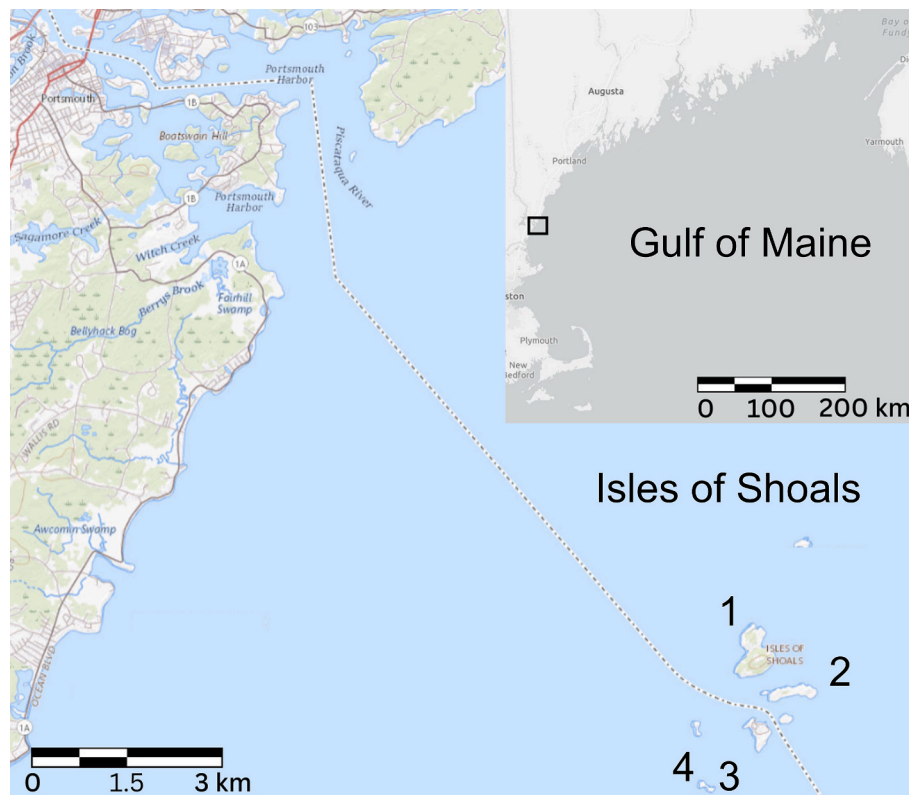


Fig. 1. Location of the Isles of Shoals relative to Portsmouth Harbour and the coast of New Hampshire and Maine. Inset: location of the sample sites within the Gulf of Maine, marked with a black box. All samples were collected from Appledore (1), Smuttynose (2), White (3), and Seavey (4) I. in the Isles of Shoals during summer 2022.

2.2. Sample preparation

After collection, all handling of the feathers was done in a clean room. All tools used to handle feathers were washed and soaked for 24 h in a Citranox bath, rinsed in E-pure water, soaked for 24 h in 10 % HCl, and dried overnight in a fume hood. To remove dirt and debris all feathers were washed in dilute Citranox detergent (Peterson et al., 2019), rinsed in E-pure water and dried for 24–48 h at 40 °C in clean glass vials. The order of vials in each drying batch was randomized.

Peterson et al. (2019) recommend a minimum of 0.006 g down for newly hatched chicks for a representative Hg sample from one individual. Preliminary testing of mercury levels in black guillemot down indicated that Hg levels in 0.006 g down exceeded the calibration curve of the instrument, so a smaller sample of 0.002 g was used. Five low-mass samples (0.0016 ± 0.00055 g) of black guillemot down had a coefficient of variation (CV); the standard deviation divided by the mean, expressed as a percentage) of 10.02 %, higher than the CV of 7.33 % for five high-mass samples (0.0062 ± 0.0018 g) from the same individual (Table S-3). Uncertainty in THg values for black guillemot down feathers will therefore be higher than that for common or roseate terns, for which all THg analysis was done with the recommended 0.006 g down.

For fully grown feathers, Peterson et al. (2019) recommend 0.010 g contour feathers (~5 feathers) for adult birds. Although Hg levels in feathers from growing chicks are more variable than those in adult birds, we limited collection of chick contour feathers to 5 feathers per individual when chicks were too small to take an adequate sample. To find variation in Hg within one individual, we measured Hg in 5 contour feathers per chick for each of five common tern chicks. CV values were <10 % for all but one individual, which had a CV of 12.75 % (Table S-3). Finally, five whole contour feathers could not fit into a single weigh boat, so contour feathers were homogenized with ceramic scissors to allow an entire sample (five feathers from the same individual) to be run at once.

2.3. Mercury analysis

2.3.1. Quality control

We analyzed down feather samples for THg using a DMA-80 Direct Mercury Analyzer (Milestone). Liquid standards (1, 5, or 10 ng Hg), solid standards (DORM-4 or TORT-3), and an air blank were run every 10 samples, with recoveries of 100.27 % \pm 3.57 % for TORT-3 ($n = 22$, mean \pm 1SD), 98.20 % \pm 3.07 % for DORM-4 ($n = 22$), 90.64 % \pm 5.52 % ($n = 21$) for 1 ng Hg, 90.82 % \pm 3.18 % for 5 ng Hg ($n = 17$), and 98.19 % \pm 2.43 % ($n = 5$) for 10 ng Hg (Table S-4). For each species a single matrix spike of 5 ng liquid Hg standard was added to a down feather sample and the amount of Hg recovered was compared with the mean of at least 4 unspiked replicates from the same individual (Table S-5). A matrix spike was also used to compare recovery from whole and homogenized common tern contour feathers. All sample types had matrix spike recovery rates >95 % (Table S-5).

2.4. Stable isotope analysis

For each species, down feathers from 20 newly hatched chicks and contour feathers from 20 fledging chicks were selected for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analysis at the Cornell University Stable Isotope Laboratory (Ithaca, New York; <https://cobsil.cornell.edu/>). From each individual, 1 mg (either subsampled from a homogenate of 5 contour feathers, or enough down feathers to reach 1 mg) was weighed (Mettler Toledo New Classic MS) and packed into a 5 \times 9 mm tin capsule (Costech 041077) using forceps. All materials used to handle the feathers were washed between samples in 90 % isopropyl alcohol.

Samples were analyzed using a Thermo Delta V isotope ratio mass spectrometer interfaced to a NC2500 elemental analyzer. Delta values (δ) for stable carbon and nitrogen isotopes were measured using Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) and atmospheric N_2 ($\delta^{15}\text{N}$) as the primary

reference scales. After every 10 samples an in-house animal standard (white-tailed deer, *Odocoileus virginianus*) was analyzed, with an overall standard deviation of 0.03 ‰ for $\delta^{15}\text{N}$ and 0.10 ‰ for $\delta^{13}\text{C}$. The accuracy of the instrument across a gradient of sample intensities (150–9000 mV for $\delta^{15}\text{N}$, 100–6000 mV for $\delta^{13}\text{C}$) was quantified using a chemical Methionine standard; error associated with linearity was 0.48 ‰ for $\delta^{15}\text{N}$ and 0.20 ‰ for $\delta^{13}\text{C}$. Finally, isotope values were corrected using a two-point linear normalization with two additional in-house standards, corn (*Zea mays*) and brown trout (*Salmo trutta*).

2.5. Data analysis

All analyses were performed using R Statistical Software (v4.2.3; R Core Team, 2023) using RStudio 2023.03.0 + 386 and the packages SIBER (Jackson et al., 2011; Jackson and Parnell, 2023), tidyverse (Wickham et al., 2019), dplyr (Wickham et al., 2023), ggplot2 (Wickham, 2016), and ggpubr (Kassambara, 2023). We used THg, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ measured in down and contour feathers from black guillemots, common terns, and roseate terns, along with body measurements, for the following analyses. THg measured in contour feathers was used to compare chicks in the same life stage across species only and was not compared with either contour feather stable isotope values or down feather THg.

- (1) Dead vs. live chicks: For species where >1 sample was collected postmortem, we compared THg values of dead and live birds using Welch's *t*-tests, chosen for consistency because differences in sample size between dead and live birds made testing for equal variances unreliable in some species and sample types.
- (2) Species comparison: We compared THg levels among the species using a one-way Analysis of Variance (ANOVA) test with post-hoc Tukey HSD tests to identify differences between the species. Assumptions of unimodal distribution and normal distribution of residuals for ANOVA tests were checked using a histogram and normal quantile plot. A down-to-whole egg conversion formula developed by Ackerman and Eagles-Smith (2009) was used to compare the results of this study to other studies measuring THg in whole eggs, and a whole egg to adult female blood conversion formula developed by Ackerman et al. (2016b) was used to compare the results of this study to known toxicity benchmarks identified for adult birds.
- (3) Body condition: We used the residual of the relationship of wing chord length (mm) to weight (g) as a metric of body condition (residual weight). Linear regressions were then used to identify the relationship between residual weight (g) and $\mu\text{g/g}$ THg for each species.
- (4) Trophic position and dietary diversity: Using $\delta^{15}\text{N}$ value as a proxy for trophic position, all three species were compared using ANOVA followed by Tukey HSD tests. Isotopic niche breadth was used as a proxy for dietary diversity. For each species, down and contour feather $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were compared with Welch's *t*-tests, because Levene's tests showed unequal variances between down and contour feather $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for common terns and black guillemots. With the package SIBER (Stable Isotope Bayesian Ellipses in R), the standard ellipse areas corrected for small sample size (SEAc) were calculated using maximum likelihood estimates. Overlap between ellipses was calculated using Bayesian methods with SIBER. R code for analyses with SIBER (Jackson et al., 2011) is available at <https://cran.r-project.org/web/packages/SIBER/>.

Finally, linear regressions were used to identify the relationship between down feather $\delta^{15}\text{N}$ and THg for individuals with both $\delta^{15}\text{N}$ and THg data. The same analyses were repeated for the relationship between $\delta^{13}\text{C}$ and THg.

3. Results

3.1. Mercury concentrations compared to avian toxicity benchmarks

Of 33 black guillemot chicks measured, eight had down THg concentrations >13 µg/g; all common and roseate tern down THg measurements were below this threshold (Table 1). When down feather THg concentrations were converted into female blood equivalent concentrations (Ackerman et al., 2016a, 2016b; Ackerman and Eagles-Smith, 2009), 31 of 33 black guillemots fell within the 1.0–3.0 µg/g moderate risk range identified by Ackerman et al. (2016a, 2016b), and 44 of 51 common terns and 13 of 28 roseate terns were above the 0.2 µg/g known effect level identified by Ackerman et al. (2016a, 2016b) (Table 1).

3.2. Mercury concentrations by species

When comparing species within each feather type, black guillemot down feathers contained significantly higher THg concentrations compared to common or roseate tern down feathers (Fig. 2, one-way ANOVA, $F_{2,111} = 319.5$, $P < 0.001$ with Tukey HSD test). Black guillemot contour feathers also contained significantly higher THg concentrations compared to common or roseate tern contour feathers (one-way ANOVA, $F_{2,67} = 675.9$, $P < 0.001$ with Tukey HSD test). There was no difference in THg concentrations between tern species for down or contour feathers. Overall, black guillemots had down feather THg concentrations of 5.5× that of common terns and 7.4× that of roseate terns (Table 1).

3.3. Body condition and feather mercury

There was no significant correlation between body condition (measured as the residual of wing chord length vs weight), and down feather THg for any species (black guillemots: $R^2 = 0.015$, $t = -0.699$, $df = 32$, $P = 0.489$; common terns: $R^2 = 2.4 \times 10^{-4}$, $t = 0.078$, $df = 25$, $P = 0.938$; roseate terns: $R^2 = 0.046$, $t = -0.490$, $df = 5$, $P = 0.645$).

Table 1

THg ranges by species. THg concentrations (µg/g) in down feathers of three seabird species nesting on the Isles of Shoals. Female blood equivalent concentrations, calculated using the below equations (Ackerman et al., 2016a, 2016b; Ackerman and Eagles-Smith, 2009), are provided in order to compare reported concentrations to known toxicity benchmarks based on adult blood concentrations.

Sample type	Species	N	Min (µg/g)	Max (µg/g)	Mean (µg/g)	SD (µg/g)
Down	Common tern	51	1.08	2.79	1.82	0.436
	Roseate tern	28	0.811	3.31	1.37	0.518
	Black guillemot	34	5.81	15.7	10.07	2.88
Female blood equivalent ^{a,b}	Common tern	51	0.164	0.438	0.282	0.0696
	Roseate tern	28	0.122	0.521	0.210	0.0826
	Black guillemot	34	0.933	2.59	1.64	0.487

$$^a \left[\text{THg}_{\text{g}}^{\text{whole egg}} \right] = e^{-2.517 \pm 0.043 \times [\text{THg}_{\text{g}}^{\text{down}}]^{0.962 \pm 0.020}} \quad (\text{Ackerman and Eagles-Smith, 2009}).$$

$$^b \left[\text{THg}_{\text{g}}^{\text{female blood}} \right] = e^{1.0734 \times \ln(\text{THg}_{\text{g}}^{\text{whole egg}}) + 0.8149} \quad (\text{Ackerman et al., 2016b}).$$

3.4. Stable isotope values by species

There were significant differences among species for $\delta^{15}\text{N}$ in down feathers (Table S-6; one-way ANOVA, $F_{2,44} = 68.44$, $P < 0.001$); there was no difference between common and roseate terns (Tukey HSD test, $P = 0.811$; means \pm 1SD of 13.0 ± 0.72 ‰ and 12.8 ± 0.25 ‰ respectively), but black guillemots had higher down feather $\delta^{15}\text{N}$ values (Tukey HSD test, $P < 0.001$; mean \pm 1SD of 15.1 ± 0.52 ‰). There were also significant differences among species for down feather $\delta^{13}\text{C}$ values (Table S-6; one-way ANOVA, $F_{2,44} = 77.51$, $P < 0.001$). Again, black guillemots were higher (Tukey HSD test, $P < 0.001$; -17.5 ± 0.37 ‰) than both common (-18.8 ± 0.34 ‰) and roseate terns (-18.4 ± 0.14 ‰), and there was no difference between tern species (Tukey HSD test, $P = 0.085$).

The same pattern was observed in contour feathers: there were significant differences among species for both $\delta^{15}\text{N}$ (Table S-6; one-way ANOVA, $F_{2,52} = 77.34$, $P < 0.001$) and $\delta^{13}\text{C}$ (one-way ANOVA, $F_{2,52} = 252.3$, $P < 0.001$). Black guillemots had higher contour feather $\delta^{15}\text{N}$ values (Tukey HSD test, $P < 0.001$; 14.9 ± 0.24 ‰) than both tern species, and common terns (Tukey HSD test, $P < 0.001$; 14.2 ± 0.29 ‰) were higher than roseate terns (Tukey HSD test, $P < 0.001$; 13.9 ± 0.25 ‰). For $\delta^{13}\text{C}$, Black guillemots had higher contour feather values (Tukey HSD test, $P < 0.001$; -16.8 ± 0.18 ‰) than common (-18.0 ± 0.19 ‰) or roseate terns (-17.9 ± 0.17 ‰), but there were no differences between tern species (Tukey HSD test, $P < 0.001$).

3.5. Variation in stable isotope values by developmental stage

There was a significant difference in $\delta^{15}\text{N}$ values between down and contour feathers for common terns ($t = -7.75$, $df = 31.05$, $P < 0.0001$) and roseate terns ($t = -9.47$, $df = 8.46$, $P < 0.0001$), with contour > down feather $\delta^{15}\text{N}$ for both species (Table S-6), but there was no difference in down and contour feather $\delta^{15}\text{N}$ values for black guillemots ($t = 1.86$, $df = 27.35$, $P = 0.968$). There was a significant difference in $\delta^{13}\text{C}$ values between down and contour feathers for all species (common terns: $t = -9.54$, $df = 37.69$, $P < 0.0001$; roseate terns: $t = -6.25$, $df = 9.68$, $P < 0.0001$; black guillemots: $t = -7.28$, $df = 27.95$, $P < 0.0001$), with contour > down feather $\delta^{13}\text{C}$ in all species (Table S-6). Differences between life stages can also be shown through overlap of isotopic niches: in black guillemots, contour feather standard ellipses overlapped down feather SEAc by 80.1 % (Table S-7), while in the tern species contour feather standard ellipses overlapped down feather ellipses by only 47.8 % (common terns, Table S-7) and 3.1 % (roseate terns, Table S-7).

The breadth of the isotopic niche, shown in Fig. 3 as the area of each ellipse, is a proxy for diversity in diet. In black guillemots, down SEAc was higher than contour feather SEAc (0.642 ‰² compared to 0.138 ‰², respectively); the same was true for common terns (SEAc = 0.750 ‰² in down and 0.156 ‰² in contour feathers). In roseate terns there was no difference in isotopic niche diversity between down (SEAc = 0.134 ‰²) and contour feathers (SEAc = 0.133 ‰²).

3.6. Relationship of THg to trophic level and diet

Black guillemot down feathers showed a significant positive relationship with $\delta^{15}\text{N}$ and THg (Fig. S-2, $R^2 = 0.262$, $t = 2.527$, $df = 20$, $P = 0.021$), but there was no significant relationship between $\delta^{15}\text{N}$ and THg in down feathers of common terns (Fig. S-2, $R^2 = 0.008$, $t = 0.423$, $df = 22$, $P = 0.677$). Roseate terns did not have enough down feather samples with both stable isotope and THg measurements for analysis. There was no relationship between $\delta^{13}\text{C}$ and down feather Hg for any species (Table S-8, Fig. S-2). THg and stable isotopes were not compared in contour feathers because of the different accumulation patterns of stable isotopes and THg in feathers.

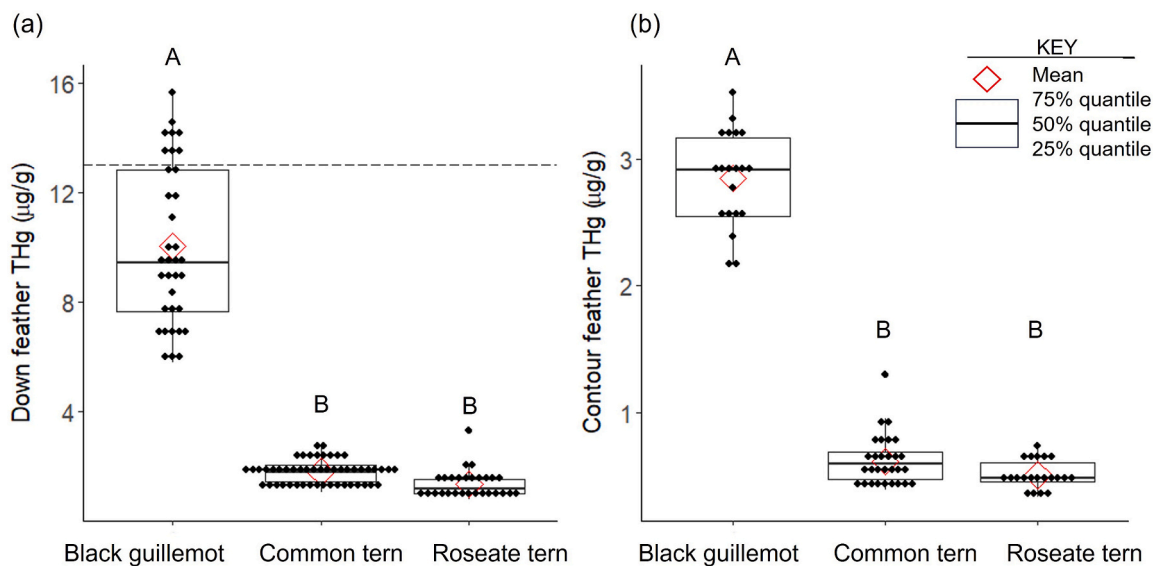


Fig. 2. Feather THg concentrations ($\mu\text{g/g}$; red diamonds indicate means, boxes indicate 75 %, 50 %, and 25 % quantiles) differed among species in both down feathers (a) and contour feathers (b) for black guillemots, common terns, and roseate terns nesting on the Isles of Shoals in summer 2022. The dashed line shows a toxic effect threshold of $13 \mu\text{g/g}$ (down-equivalent, identified in eggs; Ackerman and Eagles-Smith, 2009; Scheuhammer et al., 2007). Letters show results of Tukey HSD test.

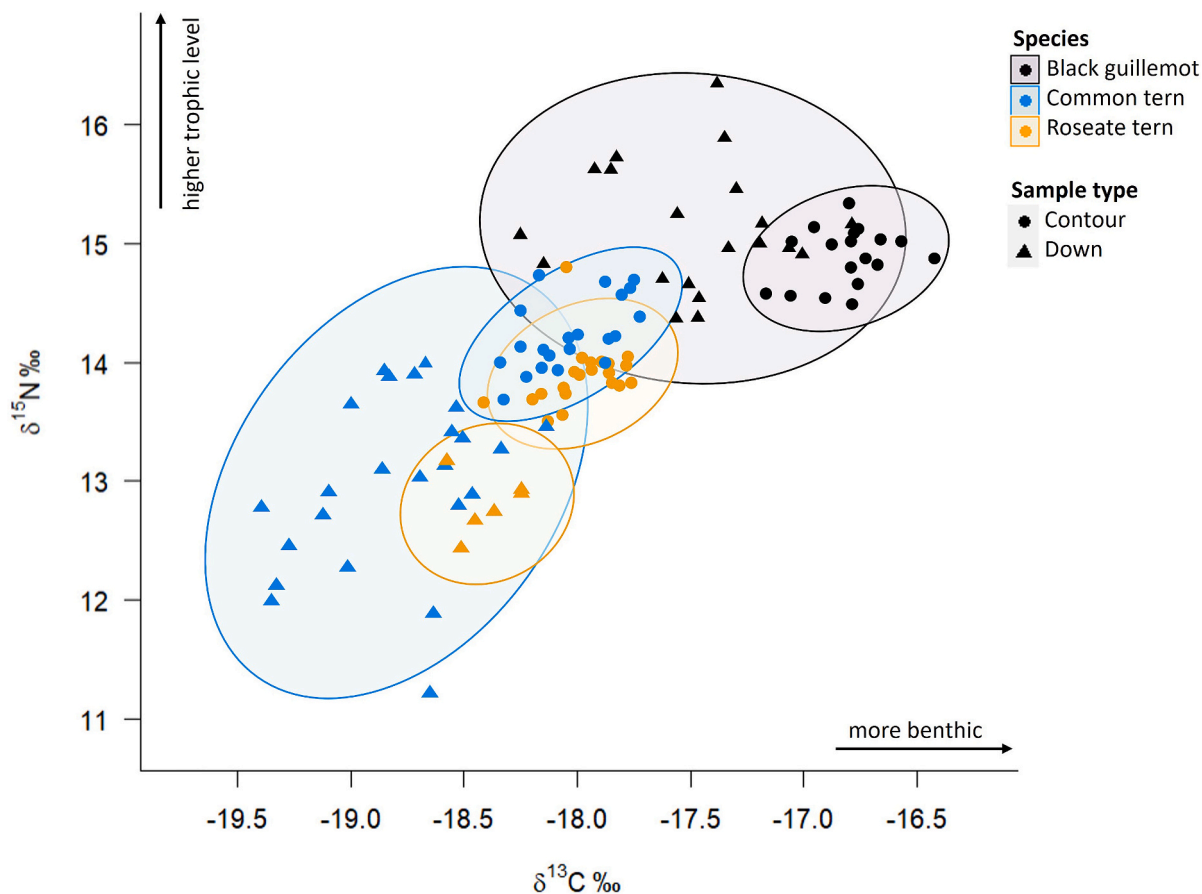


Fig. 3. Isotopic niches of down feathers (triangles) and contour feathers (circles) of black guillemots (purple), common terns (blue), and roseate terns (orange) sampled on the Isles of Shoals in 2022. For each species and life stage one point represents one individual, with some individuals appearing as both down and contour feather samples. Ellipses represent the standard ellipse area, corrected for small sample size (SEAC).

4. Discussion

Our measurements of THg in down feathers of black guillemot, common tern, and roseate tern chicks on the Isles of Shoals in the Gulf of Maine showed potentially biologically significant differences in THg levels between black guillemots and the two tern species (Fig. 2), with higher down and contour feather THg concentrations in black guillemots compared to either tern species and 8 of 33 (24 %) black guillemots having down THg values over 13 µg/g, identified as a toxicity benchmark for impaired hatchability in several bird species (Ackerman and Eagles-Smith, 2009). We suggest differences in diet between the species, established through stable isotope analysis, as a possible explanation for the higher THg levels found in black guillemot down. On a population level, black guillemots in this study had higher mean down and contour feather $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values than either tern species (Fig. 3), and $\delta^{15}\text{N}$ values in black guillemot down feathers showed a positive relationship with THg values (Fig. S-2). Higher $\delta^{15}\text{N}$ values indicate a diet of higher trophic level prey, which has been associated with higher blood mercury levels (Santos et al., 2017). Additionally, higher $\delta^{13}\text{C}$ values have been linked to more benthic food sources (Graham et al., 2010).

4.1. Interspecific differences in down feather THg

Mercury in the egg reflects the blood mercury concentration of the mother (Ackerman et al., 2020, 2016b; Becker and Sperveslage, 1989; Santos et al., 2017). Nearly all down is formed in the egg, before chicks have consumed any external food sources, so down feather mercury concentrations are strongly correlated with egg mercury concentrations (Ackerman and Eagles-Smith, 2009). This initial mercury burden is important for development of the embryo and has been linked to chick success (Ackerman et al., 2011; Eisler, 1987), but most studies of seabird diet take place during the chick provisioning period after the chick has hatched and adults are bringing prey back to the nest (Barrett et al., 2007; Barrett and Anker-Nilssen, 1997; Hof et al., 2018; Stewart et al., 1997). Comparing stable isotope signatures of growing chicks to those in down feathers can be used to identify species for which chick provisioning may not be a good indicator of adult diet, and pre-hatching contaminant exposure of chicks may be different than that of growing chicks.

Black guillemots are benthic feeders, and colonies in the Gulf of Maine have been observed to provision chicks with rock gunnel (*Pholis gunnellus*), making up over 50 % of their diet (Goodale et al., 2008). While both common and roseate terns in the Gulf of Maine feed chicks a diet high in hake (*Merluccius* sp.) and herring (*Clupea* sp.), roseate terns tend to include more sand lance (*Ammodytes* sp.), while common terns are generalists and forage for a wider range of species, including invertebrates (Legett et al., 2023; Yakola et al., 2022). Goodale et al. (2008) suggest that a diet high in long-lived benthic fish species such as rock gunnels could contribute to higher THg concentrations in black guillemots. Additionally, rock gunnels have a long, narrow shape, making larger and older fish (those that have likely accumulated more Hg) edible by chicks that are limited by their gape size (Goodale et al., 2008). While sand lance are a similarly shaped benthic species, they are zooplanktivorous and forage in the water column, meaning their mercury burden and isotopic signature is likely more similar to forage fish such as herring or butterfish than the benthic-foraging rock gunnel (Lindgren et al., 2018; Staudinger et al., 2020).

The mean mercury level in black guillemot down feathers in this study (mean \pm 1SD) was 10.07 ± 2.88 µg/g, with a total range of 5.81–15.70 µg/g ($N = 34$), slightly higher but within one standard deviation of the total down-equivalent mercury levels in 28 black guillemot eggs from 2001 to 2006 (6.93 ± 2.53 µg/g, with a range of 2.17–13.46 µg/g; Goodale et al., 2008; egg THg concentrations were converted to down-equivalent concentrations for direct comparison, but converted values should be interpreted as estimates rather than as precise measurements (Ackerman and Eagles-Smith, 2009)). Without

specific toxicity data for black guillemots, it is difficult to determine whether these mercury levels are affecting the population. We found no evidence for changes in chick condition with higher mercury burden, although 8 of 33 black guillemots measured had down feather THg concentrations above the 13 µg/g threshold for effects on egg hatchability identified by Ackerman and Eagles-Smith (2009) and all but two had adult female blood-equivalent concentrations in the ‘moderate risk’ category (Ackerman et al., 2016b, Fig. 2). Small increases in THg concentrations in the population could therefore put developing black guillemot embryos at higher risk from adverse effects of Hg compared to roseate or common terns.

4.2. Intraspecific differences in down and contour feather stable isotope ratios

Chicks have dietary needs distinct from adult birds, and chick provisioning is affected by factors such as prey proximity (adults cannot leave the nest for too long) and gape size (chicks must consume smaller prey than adults) (Goodale et al., 2008). Energy needs are also important: adults fishing for chicks are more likely to bring back energy-dense, higher trophic level prey that require fewer trips to the nest for the same amount of energy, compared to adults foraging for themselves (Barrett et al., 2007). In this study, dietary diversity, quantified with SEAc, was higher in down feathers compared to contour feathers for common terns and black guillemots (Fig. S-1), indicating that for both species growing chicks had a less diverse diet compared to adult females foraging for themselves prior to laying eggs. In common terns, the higher $\delta^{15}\text{N}$ values in contour feathers compared to down feathers suggest that chicks may be fed on higher trophic level prey compared to adult females, similar to Barrett et al. (2007). The overlap of 47 % between contour and down feathers in this species also indicates a difference between pre-breeding diet of adult females and chicks. In black guillemots, however, there was no difference between contour and down feather $\delta^{15}\text{N}$, and contour feathers fell almost entirely (80 %) within the down feather stable isotope ellipse, suggesting chicks may be fed a limited version of the pre-breeding diet of adult females. Roseate terns showed similar patterns to common terns in trophic level, but had relatively low SEAc in both down and contour feathers (Fig. S-1). This could be the result of the more specialized diet of this species, although sample size was low (6) for roseate tern down feathers.

For common and roseate terns, where fledging chicks are feeding on higher trophic level prey than pre-breeding female birds, measuring Hg in down feathers may not be sufficient to assess Hg exposure of breeding populations. In these species, continued monitoring of chick diet or measurements of Hg in internal tissues of chicks near or after fledging may be necessary to understand exposure to Hg in older chicks.

5. Conclusions

All black guillemot down feathers measured were within a range of THg known to affect reproduction in other species (Ackerman et al., 2016a), and similar to levels measured in the Gulf of Maine in previous years (Goodale et al., 2008; Stenhouse et al., 2018). At the same time, common and roseate terns had down feather concentrations well below known effect levels. Differences in diet are a likely reason for the difference in Hg exposure between the species. Common and roseate tern feeding and breeding ecology has been studied extensively on the Isles of Shoals, although there is no known baseline for important prey species or population growth rates in the black guillemot population. Regular monitoring of Hg levels in biota, including high-level predators such as seabirds, is an effective way to monitor the presence of Hg in a system, and these data are most useful when combined with monitoring of diet and behavior of the indicator species. Continued monitoring of Hg levels in colonies in the Gulf of Maine, alongside currently ongoing research on diet, could therefore be a way to track changes in Hg across the marine food web in the Gulf of Maine.

CRedit authorship contribution statement

Lenny S. Laird: Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Elizabeth C. Craig:** Writing – review & editing, Resources, Investigation, Conceptualization. **Gemma Clucas:** Writing – review & editing, Resources. **Viven F. Taylor:** Writing – review & editing, Resources. **Celia Y. Chen:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.174438>.

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