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Mercury in secondary feathers of depredated versus living Leach's Storm-Petrels (*Hydrobates leucorhous*)

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ABSTRACT

Methyl mercury is a neurotoxin associated with changes in reproductive behavior and success, hormone production, and survival in a variety of organisms. Pelagic seabirds often have high total mercury and methyl mercury concentrations in their tissues. Feathers can indicate internal mercury burden and reflect mercury burden at the time they were grown. On Bon Portage Island, Canada, Leach's Storm-Petrels (*Hydrobates leucorhous*) are depredated by Great Horned Owls (*Bubo virginianus*), American Herring Gulls (*Larus smithsonianus*), and Great Black-backed Gulls (*L. marinus*), which leave behind severed wings (owls) or regurgitated wings (gulls). This meant we could use flight feathers grown during the nonbreeding season to compare mercury burdens in depredated versus live birds and evaluate if mercury had lasting effects that increased predation risk. We compared concentrations of mercury in fourth secondary (S4) flight feathers of 26 live birds and 35 depredated wing carcasses collected on Bon Portage Island, but found no statistical difference. Our results suggest that neurological effects of mercury depurated during the non-breeding season, at least at the concentrations we observed, do not last long enough to increase petrels' risk of predation in the breeding season.

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PALABRAS CLAVE

Ave marina; *Hydrobates leucorhous*; mercurio; riesgo de depredación; pluma secundaria; Procellariiformes

Mercurio en las plumas secundarias de paños boreales (*Hydrobates leucorhous*) depredados versus vivos

RESUMEN

El metilmercurio es una neurotoxina asociada con cambios en el comportamiento y el éxito reproductivo, la producción hormonal y la supervivencia en una variedad de organismos. Las aves marinas pelágicas suelen tener altas concentraciones totales de mercurio y metilmercurio en sus tejidos. Las plumas pueden indicar la carga interna de mercurio y reflejan la carga de mercurio en el momento en que crecieron. En la isla Bon Portage, Canadá, los paños boreales (*Hydrobates leucorhous*) son depredados por los búhos *Bubo virginianus* y las gaviotas, *Larus smithsonianus* y *L. marinus*, que dejan atrás alas cortadas (búho) o regurgitadas (gaviotas). Esto significó que podíamos utilizar las plumas de vuelo crecidas durante la temporada no reproductiva para comparar las cargas de mercurio en aves

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depredadas y vivas y evaluar si el mercurio tenía efectos duraderos que aumentarían el riesgo de depredación. Comparamos las concentraciones de mercurio en las cuartas plumas secundarias (S4) de 26 aves vivas y 35 cadáveres con alas depredadas recogidos en la isla Bon Portage, pero no encontramos diferencias estadísticas. Nuestros resultados sugieren que los efectos neurológicos del mercurio depurado durante la temporada no reproductiva, al menos en las concentraciones que observamos, no duran lo suficiente como para aumentar el riesgo de depredación de los petreles en la temporada reproductiva.

Trace elements that vary in toxicity can come from natural sources, but many are supplemented anthropogenically. Mercury (Hg) production from coal-fired powerplants, gold-mining, cement and metal production, waste incinerators, wildfires, and electric utilities (Pacyna et al. 2003; Driscoll et al. 2007; Webster et al. 2016; UNEP 2018) have caused Hg pollution worldwide, particularly in western regions of the North Atlantic Ocean (Slemr and Langer 1992; Lamborg et al. 2014; Albert et al. 2024).

In aquatic ecosystems, Hg can be converted via bacteria into organic methyl mercury (MeHg) (Yu et al. 2011; Janssen et al. 2016), a biologically active neurotoxin (Clarkson and Magos 2006; Grandjean et al. 2010). MeHg bioaccumulates in individuals and biomagnifies in food webs, causing high burdens of Hg in marine predators, including seabirds (Atwell et al. 1998; Campbell et al. 2005; Carravieri et al. 2018; Mills et al. 2020). In birds, high Hg can impair reproductive behavior, hormone production, egg production, and hatching success (Nocera and Taylor 1998; Frederick and Jayasena 2010; Tartu et al. 2013; Mills et al. 2020), immune system and muscle function (Seewagen 2010; Whitney MC and Cristol DA 2017), as well as flight (Carlson et al. 2014; Whitney MC and Cristol DA 2017; Ma et al. 2018).

Seabirds are often reported to have high total Hg (THg) burdens (Bond and Diamond 2009; Mallory et al. 2018; Pollet et al. 2022; Quillfeldt et al. 2023; Albert et al. 2024) due to their high trophic positions (Carravieri et al. 2018; Mills et al. 2020) and diets that can include deep-water prey occupying Hg-rich environments (Monteiro et al. 1996; Choy et al. 2009; Pollet et al. 2023b). This makes seabirds useful bioindicators of Hg in marine food webs (Monteiro and Furness 1995; Goodale et al. 2008; Whitney M and Cristol D 2017; Pollet et al. 2023b; Albert et al. 2024). Approximately half of seabird species are also in population decline (Croxall et al. 2012) and high THg burdens may be a contributing factor (Mills et al. 2020; Calvert et al. 2024).

THg is measured in birds by sampling a variety of tissues (Monteiro and Furness 1995; Low et al. 2020; Pollet et al. 2022). THg is sequestered in growing feathers and is depurated from the body during molt, decreasing internal THg (Braune and Gaskin 1987; Condon and Cristol 2009; Whitney M and Cristol D 2017; Bottini et al. 2021). Feather-sampling is nonlethal and only mildly invasive, making it a popular method to quantify THg burdens (Monteiro and Furness 1995; Espín et al. 2016). Feathers can also be sampled from carcasses or museum specimens to obtain historical data (Appelquist et al. 1985; Thompson et al. 1992; Bond et al. 2015; Perkins et al. 2019) and reflect THg burdens (e.g., egg-laying or molting body feathers) since the last molt cycle (Braune and Gaskin 1987; Bearhop S, Ruxton GD, et al. 2000; Bottini et al. 2021; Gatt et al. 2021). Some seabirds molt flight

feathers during the non-breeding season (Ainley et al. 1976; Bridge 2006) and feather choice may depend on the purpose of a study. Flight feathers are easy to sample consistently and can be related to a previous time and location during molt due to well-known and predictable molt patterns.

Leach's Storm-Petrels (*Hydrobates leucorhous*) are a pelagic seabird species in the Order Procellariiformes. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) lists Leach's Storm-Petrels as threatened (COSEWIC 2022) and the International Union for the Conservation of Nature listed Leach's Storm-Petrels as vulnerable in 2018 (Birdlife International 2018). Experts identified high THg burdens as an important at-sea threat that may be contributing to declines, alongside other concerns (COSEWIC 2022; Pollet et al. 2023a). Accordingly, in the North Atlantic, survival rates within colonies were correlated with colony-specific THg burdens (Calvert et al. 2024).

Studies on Leach's Storm-Petrels have focused on relating THg in blood samples from live birds to reproductive success, body condition, and long-term survival (Pollet et al. 2017; Krug et al. 2021; Calvert et al. 2024) but have not evaluated if THg accumulation increases predation risk. Avian predation was identified as the primary onshore threat to Leach's Storm-Petrels (Pollet et al. 2023a) and could be affected by high THg burdens if they influence flight capability or cause neurological issues. Comparing THg accumulation in live and depredated individuals via blood samples or contour feathers would require either collecting large samples and later retrieving information on which individuals were depredated or finding whole carcasses, which are not commonly found. Leach's Storm-Petrels are preyed on by owls and gulls, which capture them and leave their wings behind after feeding, either by separating them from the carcass (owls) or regurgitating them (gulls) (Pollet and Shutler 2019a; Hoeg et al. 2021). These wings are readily available, and feathers can be collected to compare THg between depredated and live birds. Molt patterns of Leach's Storm-Petrel wings are not well-studied because the birds spend the non-breeding season at sea, mostly in the tropics (Pollet et al. 2021); however, Ainley et al. (1976) determined flight feathers were molted during the non-breeding season, likely starting in September and ending in March. Flight feathers, therefore, represent THg accumulated during the previous year, but high THg levels may cause lasting neurological damage. Flight feather THg could also be correlated with breeding season THg if individual Leach's Storm-Petrels consume diets with similar amounts of THg in successive years (e.g., Rebstock et al. 2022).

If high THg accumulated over the previous season impairs flight and evasive maneuvers via neurological effects, we predicted that predation rates of breeding birds would be higher for individuals with higher THg in their flight feathers. We tested if THg burden was associated with predation of Leach's Storm-Petrels by comparing THg in flight feathers from live birds versus depredated wing carcasses.

Methods

All procedures were approved by the Acadia University Animal Care Committee. Field work was conducted in the Bon Portage, Nova Scotia (43.4638°N, - 65.7474°W) colony of Leach's Storm-Petrels from 1–29 June 2024. Bon Portage has approximately 39,000 breeding pairs of Leach's Storm-Petrels (Pollet and Shutler 2019b) and an unknown number of breeding Great Horned Owls (*Bubo virginianus*), American Herring Gulls (*Larus*

smithsonianus), and Great Black-backed Gulls (*L. marinus*) that prey on Leach's Storm-Petrels (Pollet and Shutler 2019a; Hoeg et al. 2021).

Live adult Leach's Storm-Petrels were selected by extracting birds from burrows in areas undisturbed by annual monitoring and ongoing or recent studies (e.g., Pollet and Shutler 2019b). For 26 birds, fourth secondary (S4) flight feathers were pulled from both wings to maintain symmetry needed for flight (Swaddle et al. 1996). S4 feathers were selected to mitigate effects on flight and were pulled rather than cut to encourage regrowth (Bortolotti et al. 2008) to reduce the interval flight would be affected. Wings from 35 depredated Leach's Storm-Petrels were collected within the colony. Gulls and owls leave behind either pellets containing regurgitated wings or severed wings (Hoeg et al. 2021). Other predators on the island, such as corvids or small mammals, prey on nestlings and eggs (Hoeg et al. 2021) and are unlikely to leave behind wings that could be misidentified as owl or gull predation. Depredated individuals could have been active breeders but may also have been prospectors visiting the colony prior to breeding (Bond et al. 2023), information we did not have. Only fully intact or lightly weathered carcass wings were selected to minimize loss of material during testing and to constrain date of depredation to the present year as best as possible. S4 feathers were washed with deionized water and gently scrubbed to remove extraneous material.

To fit flight feathers into weigh boats, S4 feathers from live birds and wing carcasses were cut into small pieces ($<0.5 \times 0.5$ cm) with scissors. We selected this method because although freezing feathers with liquid nitrogen and using a mortar and pestle to homogenize can make samples less variable, from 4% relative standard deviation (RSD) to 10% RSD, it also results in a THg loss of 9.3% (Peterson et al. 2019). A representative sample of each feather (including vane, rachis, and calamus) was weighed to the nearest 0.1 mg. THg for homogenized S4 feathers was measured with an MA-3000 Direct Mercury Analyzer (Nippon Instruments), using thermal decomposition, gold amalgamation, and atomic absorption spectrometry (TDA-AAS) and reported in ng/g dry weight. To ensure accuracy, standards DORM-4 (% recovery = $104.5 \pm 3.2\%$; $n = 9$) and DOLT-5 (% recovery = $94.0 \pm 1.8\%$; $n = 9$) were tested at the beginning of each analysis and the method detection limit was 5.58 ng/g calculated as 3X the standard deviation of repeated blank analyses ($n = 20$). To test how repeatable samples were, triplicates of individual S4 feathers were tested every 10–11 samples. Triplicate S4 feather samples had a %RSD of $15.75\% \pm 16.24\%$ ($n = 4$).

All statistical analyses were conducted in R Version 4.3.2 (R Core Team 2023). We compared THg in wing carcasses and living birds using ANOVA. Although data were not normally distributed, we present only parametric results because both are fairly robust against non-normal data (Blanca Mena 2017; Schmidt and Finan 2018) and non-parametric tests had p values within 0.05 of parametric tests. Attempts to log transform the data had no effect on the distribution.

Results

There was no significant difference between THg in S4 feathers from wing carcasses ($\bar{X} = 12,005.9$ ng/g; $sd = 6,109.9$ ng/g; $n = 35$) versus living ($\bar{X} = 12,821.9$ ng/g;

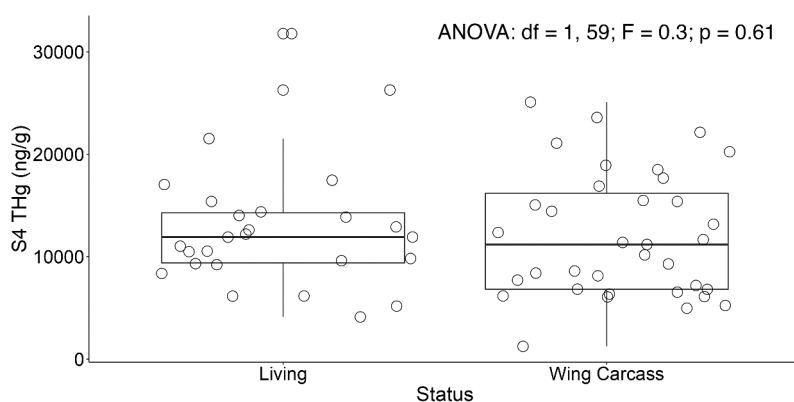


Figure 1. Log transformed mercury in fourth secondary flight feathers from living Leach's Storm-Petrels (*Hydrobates leucorhous*) and wing carcasses left behind from owl and gull predation.

sd = 6,232.3 ng/g; $n = 26$) Leach's Storm-Petrels (analysis of variance [ANOVA]: df = 1, 59; $F = 0.3$; $p = 0.61$; Fig. 1).

Discussion

Living and dead Leach's Storm-Petrels had similar S4 THg concentrations, failing to support our hypothesis that THg from the non-breeding season affects probability of predation. Although we know of no other studies that have tested effects of THg burden on predation in procellariiforms, other studies on Leach's Storm-Petrels have also failed to find correlations between THg burden from blood (Pollet et al. 2017) or liver samples (Krug et al. 2021) and survival, reproductive success, or body condition. Our results do not mean that THg burden has no effect on predation risk, however. Because S4 feathers indicate THg burden prior to the previous molt, the lack of difference between THg in live Leach's Storm-Petrels and wing carcasses may simply show that THg does not have lasting effects after depuration, at least at the levels we observed. Contemporary THg accumulated during the breeding season may still affect predation risk in the breeding colonies, but we were unable to acquire blood or contour feathers from depredated Leach's Storm-Petrels to measure contemporary THg burden.

We were limited to flight feathers from wing carcasses, but to evaluate how well they reflected contemporary THg burdens, we also sampled contour feathers from the same 26 living birds from which we had collected S4 feathers. Contour feathers are molted continuously, including during the breeding season (Ainley et al. 1976) and are likely more closely associated with breeding season THg burden. We found no correlation between S4 feathers and contour feathers from the same live birds ($y = 6,900 - 0.002x$; $R^2 < 0.01$; $p = 0.97$; $n = 26$). Although we cannot make direct comparisons between THg in the breeding season and non-breeding season between different feather types, the difference in seasonal THg across individuals suggests that Leach's Storm-Petrels either change diets or consume prey with divergent THg between fall versus winter diets when these feather types are grown. Leach's Storm-Petrels spend the breeding season in the North Atlantic and North Pacific and the non-breeding season mostly in the tropics (Ainley et al.

1976; Pollet et al. 2021). A petrel consuming a high-THg diet in the breeding season may depurate THg through molting during the non-breeding season and then consume a low-THg diet during the breeding season, resulting in a mismatch between flight feather and internal THg during breeding seasons. Although some studies have found positive correlations between flight feather THg and blood THg during molt (e.g., Bearhop S, Phillips RA, et al. 2000; Bearhop S, Ruxton GD, et al. 2000; Bottini et al. 2021), only a few have found correlations outside the molting period (Evers et al. 1998; Kenow et al. 2007), and others have found no significant correlation (Rimmer et al. 2005; Szumilo-Pilarska et al. 2017).

In summary, THg accumulated during the previous season for Storm-Petrels breeding on Bon Portage Island, at the concentrations we observed, did not have lasting effects on flight or neurology that increased predation risk. These results should not be interpreted as THg having no effect on Storm-Petrel predation risk, however, and THg accumulated during the breeding season may still increase predation risk. Finally, the lack of correlation between contour and S4 feathers from living birds indicates that THg accumulated and depurated during the previous season is not correlated with contemporary THg in the breeding season, suggesting a change in diet or in dietary THg burden.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Generative AI was not used in the production of this manuscript.

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