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Relationships between blood mercury levels, reproduction, and return rate in a small seabird

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Abstract Mercury (Hg) is a ubiquitous heavy metal that occurs naturally in the environment, but its levels have been supplemented for decades by a variety of human activities. Mercury can have serious deleterious effects on a variety of organisms, with top predators being particularly susceptible because methylmercury bioaccumulates and biomagnifies in food webs. Among birds, seabirds can have especially high levels of Hg contamination and Leach's storm-petrels (*Oceanodroma leucorhoa*), in particular, have amongst the highest known levels. Several populations of Leach's storm-petrels have declined recently in the Northwest Atlantic. The causes of these declines remain uncertain, but the toxic effects of Hg could be a potential factor in this decline. Here, we tested for relationships between adult blood total Hg (THg) concentration and several offspring development parameters, and adult return rate of Leach's storm-petrels breeding on Bon Portage Island (43° 28′ N, 65° 44′ W), Nova Scotia, Canada, between 2011 and 2015 (blood samples $n = 20$, 36, 6, 15, and 13 for each year, respectively). Overall, THg levels were elevated (0.78 ± 0.43 µg/g wet wt.) compared to other species of seabirds in this region, and varied significantly among years. However, we found no associations between THg levels and reproductive parameters or adult return rate. Our results indicate that levels of mercury observed in Leach's storm-petrel blood, although elevated, appear not to adversely affect their offspring development or adult return rate on Bon Portage Island.

Keywords Leach's storm-petrel · Mercury · *Oceanodroma leucorhoa* · Reproduction

Introduction

Mercury (Hg) is a naturally occurring trace element that may leach from geological deposits into aquatic ecosystems, or be volatilized by volcanoes, hot springs, and forest fires (Mason and Sheu 2002; Fitzgerald et al. 2007; Wiedinmyer and Friedli 2007). Mercury is also a by-product of human activities where the main sources are small-scale gold mining and burning of fossil fuels (Pacyna et al. 2006; UNEP 2013). Volatilized Hg can be transported through the atmosphere to reach locations far from sources (Fitzgerald et al. 2007; Cossa et al. 2011), making Hg pollution a global issue. Although natural contributions of Hg to the atmosphere have stayed relatively stable over the last 150 years, anthropogenic contributions have increased dramatically, resulting in increased Hg loading, particularly in aquatic ecosystems (Thompson et al. 1992; Asmund and Nielsen 2002).

Hg is most toxic when it is transformed into methylmercury (MeHg), mostly by bacteria in marine and freshwater ecosystems. Methylmercury bioaccumulates in tissues of organisms and biomagnifies from one trophic level to the next, resulting in top predators with high concentrations of MeHg in their bodies. This phenomenon is most pronounced in predators in aquatic food webs (Atwell et al.

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1998; Campbell et al. 2005; Spencer et al. 2011), where MeHg can have negative neurological, immunological, and reproductive effects (Wolfe et al. 1998).

Seabirds have been used as bioindicators of various contaminants in marine environments (Furness and Camphuysen 1997; Burger and Gochfeld 2004) including Hg (Kahle and Becker 1999; Goodale et al. 2008; Burgess et al. 2013). In seabirds, levels of Hg are often higher than human thresholds for toxic effects (Thompson 1990). In any case, high concentrations of Hg in seabird tissues have been associated with a variety of negative consequences, including direct effects such as decreased fitness through endocrine disruption and egg infertility, and indirect effects such as increased numbers of parasitic helminths (Wayland et al. 2001; Dietz et al. 2013; Tartu et al. 2015). Seabirds are capable of demethylating MeHg, especially in the liver, and storing it as less toxic inorganic Hg (Kim et al. 1996). They can also depurate MeHg through molting, and to a lesser extent through excreting guano and laying eggs (Monteiro and Furness 1995). Presumably these mechanisms partly buffer the deleterious effects of Hg, but other threats, such as global climate change could interact with Hg (Pinkney et al. 2015). Currently, there are insufficient data to evaluate these assumptions of cumulative effects for many seabird populations.

Leach's storm-petrel (*Oceanodroma leucorhoa*) is a small, abundant seabird of the northern hemisphere that feeds off the continental shelf during the breeding season (Pollet et al. 2014a) and over much of the Atlantic Ocean during the non-breeding season (Pollet et al. 2014b). Populations of this species in the North Atlantic have declined over the past 50 years (Robertson et al. 2006; Newson et al. 2008; Hedd et al. 2015). Several factors may be responsible for these declines including introductions of mammalian predators to breeding islands (Bicknell et al. 2009), increased gull populations (Stenhouse and Montevecchi 1999; Sanz-Aguilar et al. 2009), and attraction to offshore structures with bright lights that lead to disorientation, and collisions with structures or incineration by gas flares (Wiese et al. 2001). Leach's storm-petrels also have some of the highest reported concentrations of Hg amongst seabirds in the Gulf of Maine (Goodale et al. 2008; Bond and Diamond 2009). It is not clear, however, whether these concentrations are contributing to population declines by impairing reproduction or reducing adult survival.

The main goal of this study was to assess the effects of Hg on breeding success and apparent survival in Leach's storm-petrels. Specifically, we related blood Hg levels to egg-laying date, egg volume, nestling growth, hatching rate (defined as the percent of eggs that hatched), fledging rate (defined as the percent of chicks that fledged), and adult return rate to the breeding colony (defined as the percent of previously banded adults seen in any subsequent season).

Materials and methods

Study site and threats

This study was conducted during summers of 2011 through 2015 on Bon Portage Island (also called Outer Island; 43° 28′ N, 65° 44′ W) off the southwest coast of Nova Scotia, Canada. The island is ~3.0 × 0.5 km and has an estimated 50,000 breeding pairs of Leach's storm-petrels (Oxley 1999). Apparent survival has been low at this site since 2009 (Fife et al. 2015). The main threat to storm-petrels at this colony appears to be predation, mainly by great black-backed gulls *(Larus marinus*), herring gulls (*L. argentus*), and great horned owls (*Bubo virginianus*). However, threats from oil and gas infrastructure are likely relatively low at this site because foraging ranges of Leach's storm-petrels at this colony do not overlap with locations of offshore oil and gas platforms (Pollet et al. 2014a).

Field data collection

We monitored and banded adults in ~250 uniquely numbered burrows, distributed among twelve 12 × 12-m plots. As part of a different study, we deployed global location sensors (GLS) on randomly selected Leach's storm-petrels in each of the study plots and those birds were included in the present study. We visited burrows up to two times during incubation to fit adults with uniquely numbered Canadian Wildlife Service metal bands, and to determine return rates of adults from previous years. Leach's storm-petrels have high breeding site fidelity (Morse and Kress 1984, Huntington et al. 1996) so that return rate is a reliable proxy for overwinter survival. We limited visits during this time to reduce chances of nest desertion (Blackmer et al. 2003). If eggs were present in burrows, we measured their length and maximum width to the nearest 0.1 mm, and determined approximate laying and hatching date by candling following Weller's 1956 criteria. Egg volume (V, in mm^3) was estimated using Hoyt's 1979 formula: $V = 0.51 \times LW^2$, where L is length and W is width. We included these measures because early egg-laying dates tend to increase reproductive success (Wanless and Harris 1988, Sydeman et al. 1991), and egg volume can relate to breeding success in some seabird species (Thomas 1983, Croxall et al. 1992). We checked each burrow two days after estimated hatching date to record hatching success. Once chicks hatched, we weighed them weekly to monitor their growth. For each nestling, we calculated growth rate as mass gain per day (g d^{-1}) during the linear growth phase (between 5 and 30 d post-hatch; Huntington et al. 1996). We considered fledging to have occurred if, at 60 d post-hatching, a burrow was empty and during the previous visit the nestling had weighed ≥60 g and had a wing chord ≥120 mm.

To quantify Hg levels, we collected blood samples during the incubation period from 126 randomly selected adults. Only one member from each breeding pair was sampled. Skin over the left brachial vein was wiped with ethanol and the vein was punctured with a 26-gauge needle. In 2011–2013, blood samples (maximum of 150 µL) were collected via hematocrit capillary tubes and transferred to centrifuge tubes that were then sealed. Samples were kept on ice in the field for no more than 2 h. Samples were then frozen until processing at Acadia University. For 2014 and 2015, blood samples were collected in hematocrit capillary tubes, which were then sealed at both ends. In total, we collected blood samples from 126 adult Leach's storm-petrels between 2011 and 2015 ($n = 20$, 36, 42, 15, and 13 for each year, respectively). However, some samples ($n = 36$) for 2013 were not sealed properly and could not be used for the analysis, leaving $n = 6$ for that year. Samples were kept refrigerated for no more than a week until shipped on ice to the Environment Canada laboratory in Ottawa, at which point samples were frozen at −20 °C until processing. We did not sex individuals for this study. The Acadia University Animal Care Committee approved all animal handling procedures (Protocol # 06–09).

Mercury speciation

We quantified MeHg and divalent mercury for the 2011–2013 samples using digestion in basic methanol (25 % potassium hydroxide KOH) of dried and homogenized samples, followed by an ethylation purge and trap preconcentration and analysis using gas chromatography-atomic fluorescence spectrometry (Bloom et al. 1988; Edmonds et al. 2010, 2012). Certified reference materials, including dogfish liver tissue (DOLT-4), and lobster hepatopancreas tissue (TORT-2), indicated good recovery of mercury with an overall mean recovery of 108 ± 2.4 % ($n = 26$). No samples were below the mean detection limit (three times the standard deviation of blank concentrations) of 1.96 ng/g (based on a sample mass of 5 mg dry weight). For the 2011–2013 samples, total mercury (THg) was expressed as the sum of MeHg and divalent mercury species in digests. For 2014 and 2015 samples, THg was quantified using a Direct Mercury Analyser-80 (Milestone Scientific). Samples were thermally and chemically decomposed under a continuous flow of ultra-pure oxygen. A gold amalgamator trapped mercury and realised mercury vapour under heat. Mercury was quantified with a single wavelength atomic absorption spectrometer TORT-3 and DOLT-4 samples were used to evaluate accuracy. Recovery was 111.1 ± 5.4 % ($n = 26$). The similar recovery rate between both methods, allow us to compare data across years with confidence.

Statistical analyses

All data were tested for normality and homogeneity of variance; data that did not conform to these assumptions (blood THg concentrations and egg volume) were $\log_{10}$-transformed. We used analysis of variance (ANOVA) to test for differences in blood THg concentration (expressed in µg/g wet wt) across years, after ensuring that assumptions of normality and homogeneity of variance were met, followed by Tukey's multiple comparisons test for post hoc comparisons between pairs. We used generalized linear regressions to test for associations between blood THg concentration and laying date (day of the year) estimated from candling, egg volume, and nestling growth rate, with year as a covariate. Finally, we used a logistic regression to test for associations between blood THg concentration and hatching success, fledging success, and adult return rate. Data were analyzed in R version 3.0.2 (R Development Core Team 2012).

Results

Adult THg ranged from 0.24 to 2.33 µg/g wet wt. (mean ± SD = 0.82 ± 0.45 µg/g wet wt.) and differed significantly

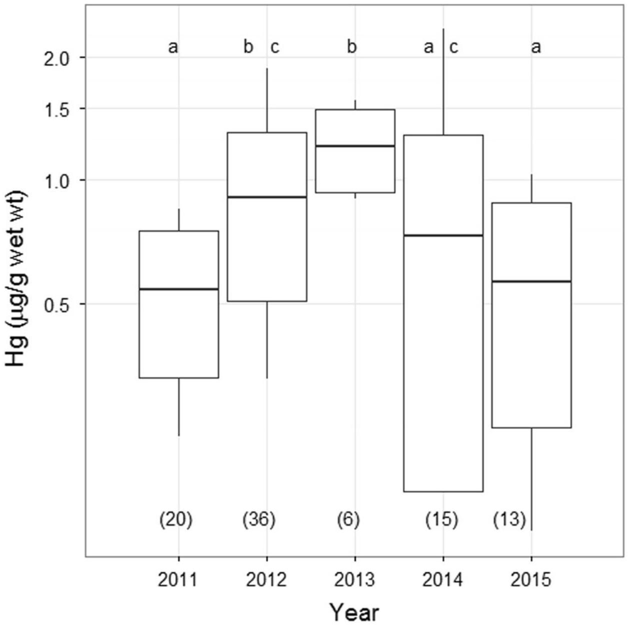

Fig. 1 Adult blood total mercury (THg) in blood of Leach's storm-petrels (log scale) by year. Sample sizes are given in *parentheses*. *Boxes* sharing the same letter are not significantly different ($P > 0.05$). *Middle line* represents mean, *box outlines* ± 1 standard deviation, and *whiskers* are minimum and maximum values

among years ($F_{4,\ 85} = 6.94$, $P < 0.001$). Post hoc Tukey tests indicated that values for 2011 were significantly lower than for 2012 and 2013, and values for 2014 and 2015 were significantly lower than values for 2013 (Fig. 1). Variation in THg was not associated with variation in lay date ($F_{1,\ 69} = 0.007$, $P = 0.9$) and lay date did vary among years ($F_{1,\ 69} = 0.56$, $P = 0.64$, Fig. 2a, Table 1). Variation in THg was not associated with variation in egg volume ($F_{1,\ 69} = 1.34$, $P = 0.26$) and egg volume did not vary among years ($F_{1,69} = 0.33$, $P = 0.57$, Fig. 2b, Table 1). Variation in THg was also not associated with variation in chick growth ($F_{1,\ 38} = 0.01$, $P = 0.9$) but chick growth did vary among years ($F_{1,\ 38} = 3.89$, $P = 0.01$, Fig. 2c, Table1). Finally, there was no significant relationship between adult THg and hatching success, fledgling success, or return rate (Table 2).

Discussion

Adult Leach's storm-petrels at our study site had blood concentrations of THg that are considered elevated, even for seabirds that often have higher blood Hg concentrations

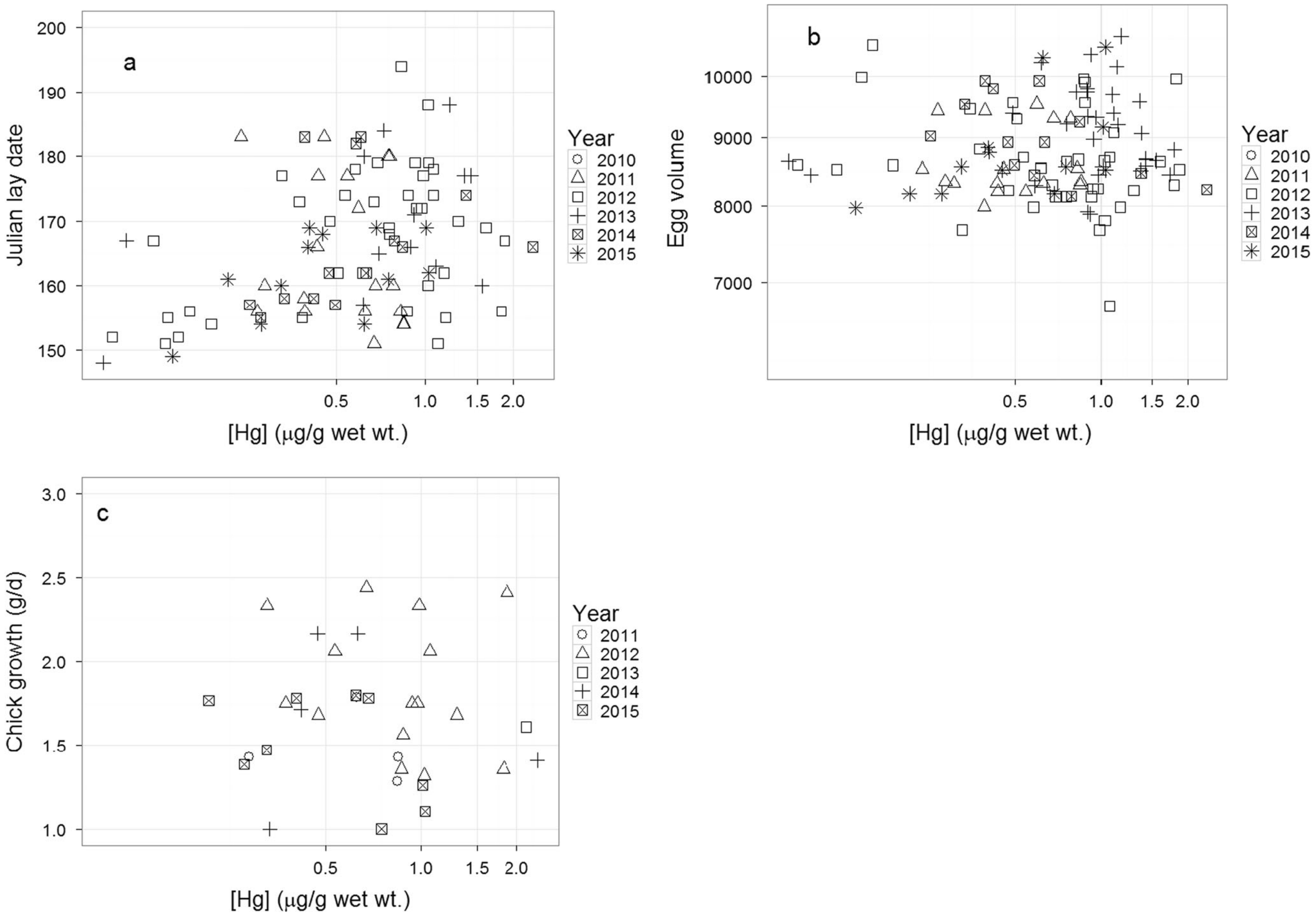

Fig. 2 Relationships between adult blood total mercury (THg) concentration (log scale) and **a** egg-laying date, **b** egg volume (mm^3, log scale), and **c** chick growth in Leach's storm-petrels from Bon Portage Island between 2011 and 2015

Table 1 Mean (±SD) adult blood total mercury (THg) and reproductive parameters in leach's storm-petrels on Bon Portage for each year of this study

Year	Blood THg (μg/g wet wt.)	Lay date (day of year)	Egg volume (mm^3)	Chick growth (g.day^{-1})
2011	0.54 ± 0.21	164.25 ± 10.54	8662.43 ± 537.34	1.08 ± 0.64
2012	0.91 ± 0.40	171.00 ± 8.31	8525.88 ± 641.61	1.86 ± 0.39
2013	1.51 ± 0.51	172.75 ± 5.31	9137.40 ± 1081.30	1.17 ± 0.63
2014	0.76 ± 0.57	166.00 ± 9.20	8937.87 ± 596.70	1.70 ± 0.51
2015	0.56 ± 0.31	161.85 ± 6.46	8791.63 ± 798.97	1.25 ± 0.52

than other birds (Thompson et al. 1993). In addition, adult Leach's storm-petrels from Bon Portage had higher average blood concentrations of THg than levels reported for Leach's storm-petrels from other islands in the Gulf of Maine (Table 3), but lower concentrations of THg than Leach's storm-petrels breeding further north (Burgess, pers. obs.). During our 5-year study, we saw an increase in adult blood THg level concentrations for the first three years, followed by a decrease in the last two years. Whereas annual fluctuations in Hg are not atypical (Bond et al. 2015), in our case, higher values for 2013 could be explained by handling issue. Sample storage issues in 2013 resulted in desiccation of a large number of samples and remaining samples ($n = 6$) might have suffered from partial desiccation; this could result in inflation of THg values. However values from 2013 were not different than values from 2012.

Despite relatively high levels of adult blood THg, we found no negative associations between THg levels and egg-laying date, egg volume, nestling growth rate, hatching success, fledging success, or return rate. In other seabirds, there is similarly no evidence of changes in reproductive success and survival in relation to Hg levels (Thompson et al. 1991; Mitro et al. 2008; Wayland et al. 2008; Goutte et al. 2014a, b). In common loons (*Gavia immer*), for instance, Hg concentrations must reach 3 µg/g wet wt. to negatively affect reproductive outcomes (Burgess and Meyer 2008; Evers et al. 2008). In our study, despite high levels of Hg detected compared to other seabirds of the area, Leach's storm-petrels levels were well below this, so they may not reach a threshold where effects can be detected. In many species, including storm-petrels, high concentrations of Hg may be buffered in several ways. For instance, liver and to a lesser extent kidney and brain tissues have the capacity to demethylate and store Hg (Kim et al. 1996; Wolfe et al. 1998; Henny et al 2002). In addition, although part of the Hg burden stays in bird tissue, a large portion of MeHg is depurated through feathers during molt (Braune and Gaskin 1987; Monteiro and Furness 1995), and a smaller fraction is excreted through guano or deposited in eggs (Burgess et al. 2013).

Table 2 Results of logistic regressions of adult blood mercury (THg) versus hatching success, fledging success, and return rates of Leach's storm-petrels on Bon Portage Island; *n* corresponds, in sequence, to the number of eggs monitored for hatching, the number of chicks monitored for fledgling, and the number of adults monitored for return rate

Response variable	*n*	Estimate	SE	*z* ratio	*P*
Hatching	90	0.11	0.89	0.13	0.90
Fledging	56	0.13	1.55	0.08	0.94
Return rate	77	1.24	1.11	1.12	0.26

Global Hg emissions and atmospheric deposition have increased in the last century, and are predicted to continue to increase in the future (Streets et al. 2009; Pacyna et al. 2010; UNEP 2013). However, Hg concentrations have increased in some seabirds (Braune 2007; Bond et al. 2015) but not others (Burgess et al. 2013). Although we found high levels of THg in blood samples of Leach's storm-petrels in comparison to other seabirds from the Gulf of Maine (Goodale et al. 2008), we detected no relationship between THg and Leach's storm-petrel reproduction or survival. Results of our study suggest that Hg on its own is unlikely to explain low survival of Leach's storm-petrels recently observed on Bon Portage Island (Fife et al. 2015). It remains to be seen whether increases in Hg will lead to detectable detriments in survival or immunologic response via additive effects (Hawley et al. 2009). It is also possible that Hg had disruptive reproductive effects before laying (Evers et al. 2008), which we did not assess in this study.

Females have the potential to excrete mercury into an egg, but past studies have failed to determine that seabird females necessarily have significantly lower levels of mercury than males. For example, in breeding northern (*Macronectes halli*) and southern giant petrels (*M. giganteus*), females had higher levels of Hg than males, whereas in Forster's terns (*Sterna forsteri*) and gentoo penguins (*Pygoscelis papua*), males had higher levels of Hg than females (Becker et al. 2002; Ackerman et al. 2016). These differences could arise from variation in niche use, such as sex-biased diets, or a number of other factors beyond the scope of this study. The small sample size of this study may reduce the likelihood of detecting a significant effect of Hg, and although this study presents an initial baseline for the effects of Hg on some offspring development, a follow-up

Table 3 Mean (+SD) adult blood total mercury (THg) concentrations (µg/g wet weight) for adult Leach's storm-petrels breeding on several islands in the Gulf of Maine, Machias Seal Island (New Brunswick), and Bon Portage Island (Nova Scotia)

Location	Years	THg (µg/g)	*n*	Analysis method	Source
Gulf of Maine	2001–2006	0.54 ± 0.37	28	Atomic absorption spectroscopy	Goodale et al. (2008)
Machias Seal Island	2005–2006	0.40 ± 0.48	16	Atomic absorption spectrophotometry	Bond and Diamond (2009)
Bon Portage Island	2011–2014	0.78 ± 0.44	90	Atomic fluorescence spectrometry	This study

study on the influence of mercury should include the sexing of the birds.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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