SG GRANT 200000000646260987: Evaluation of mercury levels in the endangered Black-capped Petrel *Pterodroma hasitata*

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Abstract:
The Diablotin Black-capped Petrel (*Pterodroma hasitata*) is an endangered gadfly petrel in the western North Atlantic, listed as globally Endangered by the International Union for the Conservation of Nature, and currently being reviewed for listing under the U.S. Endangered Species Act. There are an estimated 2,000 pairs of Petrels nesting at five documented sites on the island of Hispaniola, in the Caribbean, although to date only 100 nests have been located. The species' at-sea distribution extends over the western north Atlantic, Caribbean Sea and northern Gulf of Mexico. This expansive marine range exposes it to many conservation threats at sea including, but not limited to, offshore energy development and shipping, and associated marine pollution. In the last years, several eggs in monitored nests have failed to hatch, raising concerns of exposure to contaminants (Rupp and Brown, pers. comm.). In April 2018 and May 2019, we collected samples of breast feathers from adults captured at nesting sites (n = 10) and at sea (n = 10), respectively. We analysed total mercury concentrations in whole feathers via nitric-acid extraction following Hammerschmidt and Fitzgerald (2006). Results showed high concentrations of total Hg, with mean concentrations of 30.3 ppm dry weight (range: 15.2-53.9 ppm dry weight; sd: 10.8, n = 20). These results confirm earlier suggestions of high susceptibility of mercury by Black-capped Petrels but the mechanisms of bioaccumulations remain unclear.

Through anthropogenic emissions, heavy metal contaminants such as mercury have become increasingly prevalent in the marine food chain (Lamborg et al. 2014). Mercury has been showed to affect seabird physiology, fitness and development (Evers et al. 2008, Tartu et al. 2013), and have rapid consequences at the population level (Bond et al. 2015). Atmospheric mercury is deposited into open ocean systems, where it represents 90% of all mercury inputs into the surface ocean (Mason et al. 2012). Mercury is naturally present in the environment but human activities have increased natural atmospheric concentrations by ca. 450% since 1450 (Zhang et al. 2014, Outridge et al. 2018). Although occurring in all ocean basins, inputs of anthropogenic mercury are spatially variable and concentrations of mercury available to enter the food chain depend on biophysical oceanic transport and processes (Mason et al. 2012, Zhang et al. 2014). Once into aquatic ecosystems, inorganic mercury may be converted to methylmercury, the more toxic form that is assimilated into the marine food webs and biomagnifies through the food chain (Driscoll et al. 2013). In seabirds, exposure to mercury thus depends on the location of foraging areas (Anderson et al. 2009), and the type, size and ecology of prey (Becker et al. 2016).

The Diablotin Black-capped Petrel *Pterodroma hasitata* (Figure1) is an endangered gadfly petrel endemic to the Caribbean and occurs in waters of the western North Atlantic Ocean, Caribbean Sea, and Gulf of Mexico (Jodice et al. 2021). The species is considered Endangered throughout its range (BirdLife International 2018) and is being considered by the U.S. Fish and Wildlife Service for listing as Threatened under the Endangered Species Act (U.S. Fish and Wildlife Service 2018). Two phenotypes have been described: a smaller dark form and a heavier light form, which are genetically distinct (Manly et al. 2013). Our recent tracking of adults has shown an extensive use of the southern Caribbean Sea by breeding birds, in areas off Colombia and Venezuela where explorative drilling and active extraction of hydrocarbon are ongoing (Satgé et al. 2019). Although such activities do not occur in the western North Atlantic, global mercury models suggest a high prevalence of total mercury in the mixed layer of areas used by petrels in this basin (Zhang et al. 2014, Satgé et al. *In Prep*.) The foraging ecology of the Black-capped Petrel is still under study but its diet is considered to be mainly composed of mesopelagic cephalopods (Haney 1987, Moser and Lee 1992). Since high concentrations of mercury have been detected in pelagic seabirds that feed extensively on cephalopods (e.g. Carravieri et al. 2014), we posit that Black-capped Petrels are exposed to high background concentrations of mercury throughout the annual cycle. The only previous analysis of mercury levels in the Black-capped Petrel was performed by (Whaling et al. 1980, cited in Simons et al. 2013) and suggested a mean Total Hg concentration of 18.0ppm (n = 22) in feathers. However, these results were only summarized in a conference abstract, and the methods were never published. Therefore, the objective of this project was to measure contemporary mercury levels in Black-capped Petrel feathers.

During previous fieldwork, we collected 4-5 breast feathers from adult Black-capped Petrels captured at nesting sites in the Dominican Republic, and at sea offshore Cape Hatteras, North Carolina, USA. We collected breast feathers because they could be easily sampled without causing undue stress to captured birds, and because they are considered to be more representative of body metal levels than flight feathers (Furness et al. 1986). We ringed petrels with individually numbered metal rings (U.S. Geological Survey Bird Banding Laboratory, Maryland, USA). Samples were individually identified and were stored in a cool and dry area. We washed one feather per individual with acetone and high purity water prior to shipment to the USGS Mercury Research Lab. Upon receipt, feather samples were digested in 4.5M HNO_3 at 60°C for 8 hours to extract methylmercury. Extracts were treated with ultraviolet light for 3-5 days to destroy dissolved organic matter and then oxidized with bromine monochloride (BrCl) at 50°C for five days to convert methylmercury to the inorganic form (Hg(II)). Total mercury analysis was performed according to U.S. EPA Method 1631. Briefly, aliquots of oxidized samples were neutralized with hydroxylamine hydrochloride followed by stannous chloride addition to release mercury from the solution as the gaseous Hg(0) form. Mercury was purged onto gold traps using ultra high purity argon, desorbed, and measured by cold vapor atomic fluorescence spectrometry. All laboratory analyses were performed by the USGS Mercury Research Lab, Wisconsin, USA. All samples were collected under authorization by and following guidelines from Clemson University IACUC (AUP2018-005 and AUP2019-033, respectively).

Of 10 adult Black-capped Petrels sampled at nesting sites in the Dominican Republic in April 2018, and 10 other adults sampled at sea offshore Cape Hatteras in May 2019, 15 individuals were of the dark phenotype and 5 of the light phenotype. Mean Total Hg concentration was 30.3 ppm dry weight (min. = 15.2, max. = 53.9, sd = 12.13)(Figure 2). There were no statistically significant differences in Total Hg concentrations in the dark phenotype compared to the light phenotype (t-test: $p = 0.56$).

Due to its high trophic position in the marine food chain of the western North Atlantic, Caribbean Sea and Gulf of Mexico, the Black-capped Petrel is highly susceptible to mercury bioaccumulation (Monteiro et al. 1998, Simons et al. 2013). Our results are in the top tier of Total Hg concentrations measured in breast feathers of other *Pterodroma* species (Figure 2). The moulting process of the Black-capped Petrel is poorly known but it is assumed to follow a similar pattern to that of the Bermuda Petrel *Pterodroma cahow*, in which body feathers are moulted after the breeding season (Simons et al. 2013). We collected breast feathers in the spring during the breeding season, thus reflecting dietary intake related to the last moult (i.e., June-August of the previous year). After the breeding season, Black-capped Petrels appear to leave the Caribbean basin to spend the non-breeding period in the western North Atlantic (Jodice et al. 2015, Satgé et al. *In Prep*.) Therefore, the burdens measured in our study seem to reflect mercury exposure in the western North Atlantic. Two parallel studies by our group provide new insight about mercury exposure as it relates to the trophic ecology of Black-capped Petrel and its exposure to marine threats. Firstly, our recent analysis of prey DNA in Petrel faecal samples suggests that the species has a more diverse diet than previously thought, comprising more fish species, including mesopelagic species and species that perform diel migrations (Satgé et al. *In Prep.*) Secondly, our analysis of macro-scale exposure to marine threats suggests that Black-capped Petrel phenotypes have different distributions at sea and are differently exposed to modelled mercury concentrations in the oceanic mixed layer (Satgé et al. *In Prep*.) Our results do not support this difference but the limited sample sizes prevent any definite conclusion. Therefore, future research should focus on 1) increasing sample size for mercury analyses of breast feathers between phenotypes, 2) assessing exposure to mercury in the Caribbean basin by analyzing blood samples from breeding adults, and 3) assessing impacts on the reproductive success.

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Figure 1.Diablotin Black-capped Petrel *Pterodroma hasitata*. Photo by Kate Sutherland. Used with permission.

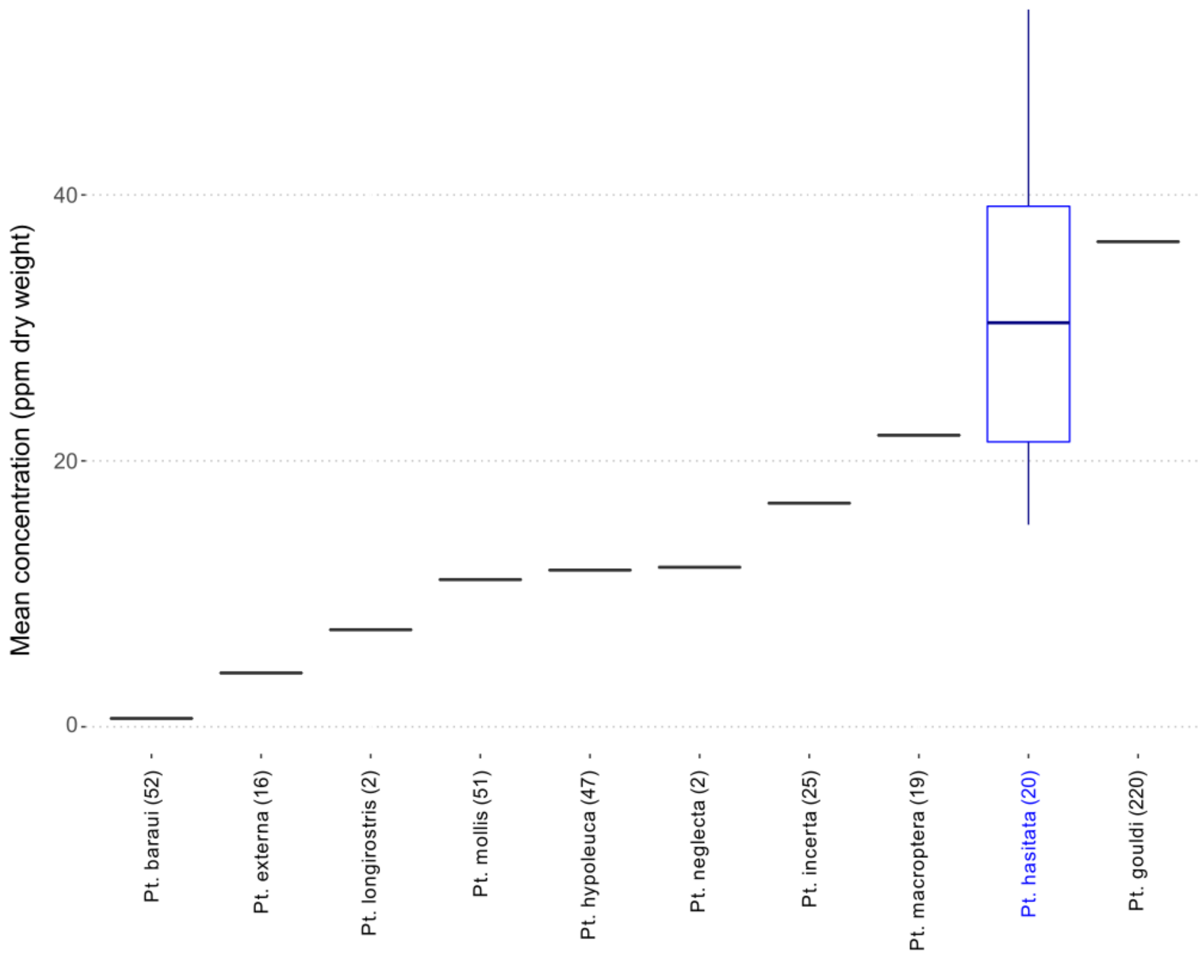

Figure 2. Mean Total Hg concentrations (dry weight) in breast feathers of *Pterodroma* species. Our results are show as a boxplot to provide more details on the distribution of measured concentrations. Numbers in parentheses represent sample sizes. References: P. baraui: Kojadinovic et al 2007; P. externa, longirostris, and neglecta: Ochoa-acuña et al 2002; P. mollis: Thompson et al 1993, Carravieri et al 2004, Becker et al 2016; P. hypoleuca: Burger and Gochfeld 2000; P. incerta: Thompson et al 1993, Becker et al 2016; P. macroptera: Carravieri et al 2004, Becker et al 2016; P. gouldi: Lyver et al. 2017.

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