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► To cite this version:

Andrés E. Ibañez, William F. Mills, Paco Bustamante, R.A.R. McGill, L.M. Morales, et al.. Variation in blood mercury concentrations in brown skuas (*Stercorarius antarcticus*) is related to trophic ecology but not breeding success or adult body condition. *Marine Pollution Bulletin*, 2022, 181, pp.113919. 10.1016/j.marpolbul.2022.113919 . hal-03771029

HAL Id: hal-03771029

<https://hal.science/hal-03771029>

Submitted on 7 Sep 2022

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Variation in blood mercury concentrations in brown skuas (*Stercorarius antarcticus*) is related to trophic ecology but not breeding success or adult body condition

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Highlights

- Blood THg concentrations in brown skuas measured during early incubation differed between study years, potentially reflecting annual variability in Hg exposure.
- Blood THg concentrations were positively related to $\delta^{15}\text{N}$, reflecting biomagnification.
- Body condition and breeding success were unrelated to blood THg concentrations.

Abstract

Mercury is a pervasive environmental contaminant that can negatively impact seabirds. Here, we measure total mercury (THg) concentrations in red blood cells (RBCs) from breeding brown skuas (*Stercorarius antarcticus*) (n = 49) at Esperanza/Hope Bay, Antarctic Peninsula. The aims of this study were to: (i) analyse RBCs THg concentrations in relation to sex, year and stable isotope values of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$); and (ii) examine correlations between THg, body condition and breeding success. RBC THg concentrations were positively correlated with $\delta^{15}\text{N}$, which is a proxy of trophic position, and hence likely reflects the biomagnification process. Levels of Hg contamination differed between our study years, which is likely related to changes in diet and distribution. RBC THg concentrations were not related to body condition or breeding success, suggesting that Hg contamination is currently not a major conservation concern for this population.

Keywords: pollution, top predator, seabirds, stable isotopes, reproduction, Antarctica

1. Introduction

Mercury (Hg) is a pervasive environmental contaminant that can have deleterious consequences for free-living organisms (Corsolini 2009; Tan et al. 2009). The gaseous, elemental form of Hg (Hg^0) can spread through atmospheric transport from emission sources to remote regions, including the Antarctic (Calle et al. 2015). After its deposition in the marine environment, the inorganic form of Hg (Hg^{II}) is methylated by microorganisms to the more toxic form, methyl-Hg (MeHg, $[\text{CH}_3\text{Hg}]^+$). Once assimilated, MeHg bioaccumulates within marine organisms and biomagnifies through marine food webs (Bargagli 2008; Driscoll et al. 2013). Long-lived, upper trophic level predators, such as many Antarctic seabirds, are therefore potentially exposed to high Hg concentrations through their prey (Bearhop et al. 2000; Bargagli 2008; Tavares et al. 2013; Mills et al. 2020; McKenzie et al. 2021).

Benthic and mesopelagic fish and cephalopods tend to exhibit elevated Hg concentrations, attributed to the increased MeHg production in sediments and at depths associated with low availability of oxygen (Goutte et al. 2015; Polito et al. 2016; Renedo et al. 2020). Some of these species are consumed by seabirds either directly, if they are vertical migrants, or as discards from fisheries, and seabirds can therefore be effective bioindicators of Hg below the photic zone (Carravieri et al. 2021; McKenzie et al. 2021). Hg contamination of seabirds can show high inter- and intraspecific variation, which is often related to factors including age, sex, latitude and trophic ecology (Monteiro et al. 1998; Bearhop et al. 2000a, b; Anderson et al. 2009; Leat et al. 2013; Polito et al. 2016; Carravieri et al. 2021, Mills et al. 2020, 2022). Seabirds are primarily exposed to Hg through their diet, and blood Hg concentrations reflect trophic ecology and differences in exposure over the short-term (Bearhop et al. 2000; Monteiro and Furness 2001). Stable isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$, expressed as $\delta^{13}\text{C}$) and

nitrogen ($^{15}\text{N}/^{14}\text{N}$, expressed as $\delta^{15}\text{N}$) in seabird tissues reflect those of their prey in a predictable way (Peterson and Fry 1987; Hobson and Clark 1992; Bearhop et al. 2002). $\delta^{15}\text{N}$ values exhibit a stepwise increase of 3–5 ‰ at each trophic level, whereas $\delta^{13}\text{C}$ values increase to a lesser extent with trophic level (~0.5–1 ‰), but can be used to infer foraging habitat (Peterson and Fry 1987; Hobson and Clark 1992; Bearhop et al. 2002; Cherel and Hobson 2007; Jaeger et al. 2010). In the marine environment, this includes the relative reliance on an inshore vs. offshore, benthic vs. pelagic diet, and latitude or water mass where a gradient exists, such as in the Southern Ocean (Cherel and Hobson 2007; Phillips et al. 2009; Jaeger et al. 2010). Blood stable isotopes ratios are appropriate for addressing the relationship between Hg contamination and trophic ecology in seabirds, as the timeframe for integration into the blood system is similar (Bond 2010; Albert et al. 2019).

During reproduction, Antarctic seabirds are exposed to multiple environmental and physiological stressors, and the synergistic effects of pollutants can have major repercussions for physiology and life history (Bustnes et al. 2007; Goutte et al. 2014a, 2014b, 2015, 2018). For instance, Hg can have a deleterious effect on body condition (Ackerman et al. 2016; Chételat et al. 2020), the nervous, and reproductive and immune systems (Tan et al. 2009). Ultimately, Hg may have impact reproduction in the short- or long-term (Bustnes et al. 2007; Roos et al. 2012; Dietz et al. 2019; Chételat et al. 2020; Mills et al. 2020). Although intraspecific variation in Hg contamination has been studied in several Southern Ocean seabirds, relationships with fitness parameters have only been investigated for the wandering albatross (*Diomedea exulans*) (Tavares et al. 2013; Carravieri et al. 2014b, 2014c; Goutte et al. 2014a; Bustamante et al. 2016), brown skua (*Stercorarius antarcticus*) and south polar skua (*S. maccormicki*) (Goutte et al. 2014b), Antarctic petrel (*Thalassoica antarctica*) (Carravieri et al. 2018, 2021) and grey-headed albatross (*Thalassarche chrysostoma*) (Mills

et al. 2020). In general, little is known about the drivers and consequences of Hg exposure in Antarctic seabirds.

In the present study, we focus on Hg contamination of brown skuas, which breed in the Antarctic and on sub-Antarctic islands. Brown skuas are opportunistic predators and scavengers in both terrestrial and marine environments, feeding on a wide variety of prey (Reinhardt et al. 2000; Phillips et al. 2004; Ritz et al. 2008; Carneiro et al. 2015; Graña Grilli and Montalti 2015; Borghello et al. 2019). At our study site on the Antarctic Peninsula (Esperanza/Hope Bay), brown skuas breed near numerous Adélie penguin (*Pygoscelis adeliae*) and gentoo penguin (*Pygoscelis papua*) colonies (>100,000 breeding pairs) (Santos et al. 2018), and are heavily reliant on consuming carcasses, eggs and chicks, but also feed on various marine prey (fish and invertebrates) (Borghello et al. 2019; Ibañez et al. 2022). The nearby research station is occupied year-round, and anthropogenic pressure increases during the skua breeding season, with increasing ship calls and tourist visits, and associated release of heavy metals and organic pollutants from waste-disposal sites, construction materials and compounds used for treating effluent (Acero et al. 1999). For instance, a recent study found macro-plastics in skua diet samples (Ibañez et al. 2020). Detrimental consequences of Hg have been reported for Brown skuas elsewhere in the Southern Ocean (Goutte et al. 2014b). Our aims were to compare Hg contamination in red blood cells (RBCs) of brown skuas at Esperanza/Hope Bay between breeding seasons and sexes, and, using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as proxies, in relation to trophic ecology, and to test for relationships with body condition and breeding success.

2. Materials and methods

2.1. Study area and sample collection

Fieldwork was conducted in Bahía Esperanza/Hope Bay, Antarctic Peninsula (63°24'S, 57°01'W) (Fig. 1), during the 2018/19 and 2019/20 breeding seasons from November to January. Skuas were captured and sampled during the early incubation stage (5–10 days after clutch completion). Only active breeders were sampled, as nonbreeders and failed breeders are very difficult to capture. Blood samples (2 ml) were obtained from the brachial vein (using a 25-G needle) from 49 brown skuas ($n_{2018/19} = 24$; $n_{2019/20} = 25$). These were both partners from 16 nests and one adult from 17 nests. Ten individuals - including four from the same two pairs - were sampled in both seasons. Samples were returned to the laboratory within 2–6 hours after extraction. Red blood cells (RBCs) were then isolated by centrifugation and removal of the supernatant, and were stored frozen (-20°C) prior to laboratory analyses. The sex of birds was initially assigned morphologically and was later confirmed by DNA analysis (Fridolfsson and Ellegren 1999; Phillips et al. 2002).

2.2. Body condition and breeding success

Birds were weighed and the tarsus, wing cord and culmen lengths were recorded. As brown skuas display reversed sexual size dimorphism, with females being significantly larger and heavier than males, a body condition index was estimated following Phillips et al. (2002). Briefly, a principal component analysis (PCA) was carried out for each sex incorporating morphometric variables (wing cord, tarsus and culmen length), to obtain PC1 scores accounting for body size (47.5% and 59.8% of the variance explained for males and females, respectively). Bird body mass was regressed against PC1 scores for each sex, and standardized residuals were computed (mean = 0, variance = 1) and used as a measure of

body condition. A measurement (culmen length or flattened wing chord) was missing for seven out of 48 males, and a missing value was therefore imputed (Nakagawa and Freckleton 2008) using a regularized iterative PCA implemented in the R package missMDA (Josse and Husson 2016). Nests were visited every five days to record chick survival; chicks that reached ~ 35 days old were considered to have fledged.

2.3. Total Hg analysis

Previous studies demonstrated that Hg in blood is associated predominantly with the cellular fraction (i.e., RBCs) rather than plasma (Bond and Robertson 2015; Renedo et al. 2018). The vast majority (>90 %) of total Hg (THg) in seabird RBCs is MeHg (Renedo et al. 2018; Albert et al. 2019), and the estimated half-life of Hg in the blood of great skuas (*Stercorarius skua*) is 30–60 days (Bearhop et al. 2000c). Prior to analysis, RBC samples were freeze-dried and homogenized. RBC THg concentrations were measured using an Advanced Mercury Analyser spectrophotometer (Altec AMA 254) at the laboratory Littoral Environnement et Sociétés (LIENSs, France). For each sample, a minimum of two aliquots (range: 1.02–1.86 mg dry weight [dw]) were analyzed, and the means and relative standard deviation among measurements were calculated (all samples RSD <10%). THg concentrations are presented in $\mu\text{g g}^{-1}$ dw. Accuracy was tested using certified reference material (dogfish liver DOLT-5, NRC, Canada; certified Hg concentration: $0.44 \pm 0.18 \mu\text{g g}^{-1}$ dw) every 10 samples. The measured values were $0.43 \pm 0.01 \mu\text{g g}^{-1}$ dw ($n = 15$); thus the recovery was $97.8 \pm 1.7\%$. Blanks were analyzed at the beginning of each set of samples and the detection limit of the method was $0.005 \mu\text{g g}^{-1}$ dw.

2.4. Stable isotope analysis

Stable isotopes were also measured in the RBCs samples. In great skuas, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in RBCs have half-lives of 15.7 and 14.4 days, respectively (i.e., of similar magnitude to the integration period to Hg, see above) (Bearhop et al. 2002). Stable isotope analyses were conducted at the Natural Environment Research Council (NERC) Life Sciences Mass Spectrometry Facility in East Kilbride. Stable isotope ratios of carbon and nitrogen were determined by a continuous-flow mass spectrometer (Delta Plus XP; Thermo Scientific, Bremen, Germany) coupled to an elemental analyzer (vario PYRO cube; Elementar, Langenselbold, Germany). To correct for instrument drift, three internal laboratory standards were analyzed for every 10 samples. Stable isotope ratios are reported as δ -values and expressed as ‰ according to the equation: $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where X is ^{13}C or ^{15}N , R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, and R_{standard} is the ratio of international references Vienna PeeDee Belemnite for carbon and atmospheric N_2 (AIR) for nitrogen. Measurement precision (standard deviation associated with replicate runs of USGS40) was <0.2 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

2.5. Statistical analysis

All data were analysed using R (Core Team 2015). In a first step, generalized linear mixed-effects models (GLMMs; gamma distribution and inverse link function) were used to assess variation in RBC THg concentrations using the ‘lme4’ package in R (Bates et al. 2015). Predictor variables were $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, sex (males, $n = 25$; females, $n = 24$) and season (2018/19 and 2019/20). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were not highly correlated and hence were included in the same models. There were no significant differences in the mean laying date of the first egg ($t = 1.826$ $p = 0.146$) or sampling date between the two seasons ($t = 1.038$ $p = 0.257$). The day of the season that each breeding pair completed their clutch (incubation day) was included as a

covariate and individual identity was included as a random effect, as a subset of birds were sampled in both seasons. Candidate models were fitted based on *a priori* knowledge (Burnham et al. 2011), and two first-order interactions were considered ($\text{sex} \times \delta^{13}\text{C}$ and $\text{sex} \times \delta^{15}\text{N}$). Predictor variables were standardized (mean = 0, variance = 1) to facilitate coefficient comparison and model convergence. Models were ranked using the Akaike Information Criterion adjusted for small sample sizes (AIC_c), and models within two AIC_c units of the top model (i.e., $\Delta\text{AIC}_c \leq 2$) were considered equally plausible (Burnham and Anderson 2002) (Table 2 and Supplementary Table 1). Akaike weights (ω_i) were included as a measure of model support, which quantify the probability that a particular model is the best model among the candidate set (Burnham et al. 2011).

In a second step, we tested for relationships between RBC THg concentrations and body condition using a GLMM (with a Gaussian distribution and identity link function), with the same set of variables as above ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and season) included as covariates and individual identity was included as a random effect. Relationships between blood THg and breeding success (0: failed nest ($n_{\text{nests}} = 8$), 1: one ($n_{\text{nests}} = 13$) or two ($n_{\text{nests}} = 12$) fledged chicks) were tested using GLM, with adult body condition, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ included as covariates. Males and females were analysed together to improve model fit.

3. Results

3.1. Drivers of variation in blood THg

Quantifiable THg concentrations were detected in all RBC samples from brown skuas at Esperanza/Hope Bay in the 2018/19 (mean \pm SD, $0.73 \pm 0.22 \mu\text{g g}^{-1}$ dw; range: 0.41–1.21 $\mu\text{g g}^{-1}$ dw) and 2019/20 ($0.91 \pm 0.44 \mu\text{g g}^{-1}$ dw, 0.44–2.33 $\mu\text{g g}^{-1}$ dw) breeding seasons (Table 1). The most parsimonious GLMM (i.e., $\Delta\text{AIC}_c = 0$) explaining variation in RBC THg

concentrations included $\delta^{15}\text{N}$ (est = -0.25 p = 0.001) and breeding season (est = 0.41 p < 0.0001) as predictor variables (Table 2), indicating a positive correlation with $\delta^{15}\text{N}$ and annual variation in Hg contamination (Fig. 2A). A competitive GLMM (i.e., $\Delta\text{AIC}_c < 2$), albeit with a much reduced ω_i , also included $\delta^{13}\text{C}$ (est = 0.08 p = 0.23) as a predictor variable (Table 2), reflecting a negative relationship between THg concentrations and $\delta^{13}\text{C}$ (Fig. 2B). Sex was not included as predictor in the best-fitting models, indicating that the RBC THg concentrations of males and females did not differ significantly (Table 1). Moreover, males and females did not differ in the isotopic signature (Table 1).

3.2. Relationships between blood THg, body condition and breeding success

Adult body condition was not related to RBC THg concentrations (est = 0.06 p = 0.36) nor to stable isotope ratios ($\delta^{13}\text{C}$: est = -0.26 p = 0.25, $\delta^{15}\text{N}$: est = -0.40 p = 0.10). Similarly, breeding success was not related to individual RBC THg concentrations (est = -0.052 p = 0.98), body condition (est = 2.36 p = 0.35) nor stable isotopes values ($\delta^{13}\text{C}$: est = 1.95 p = 0.54, $\delta^{15}\text{N}$: est = -0.95 p = 0.79).

4. Discussion

In the present study, we examined intraspecific variation in RBC THg concentrations of brown skuas breeding at Esperanza/Hope Bay, Antarctic Peninsula. We also tested whether body condition and breeding success were related to levels of Hg contamination. Although the mean RBC THg concentrations of brown skuas were lower than reported at more northerly breeding colonies in the Southern Ocean (Goutte et al. 2014; Mills et al. 2022), our data are comparable to adults of other Arctic and Antarctic seabirds, including little auks

(*Alle alle*) (Albert et al. 2019; Fort et al. 2014), snow petrels (*Pagodroma nivea*) (Tartu et al. 2015), and Antarctic petrels (*Thalassoica antarctica*) (Carravieri et al. 2021).

4.1. Annual variation in Hg contamination

Breeding season was included as a predictor variable in the most parsimonious GLMM, reflecting the higher RBC THg concentrations of brown skuas in 2019/20 compared to 2018/19 (by $\sim 0.2 \mu\text{g g}^{-1}$ dw). This annual variation contrasts with some other studies of Antarctic seabirds, which found low annual variability in Hg exposure over the short-term at oceanic sites far from point sources (Brasso et al. 2014; Carravieri et al. 2016; Polito et al. 2016; Carravieri et al. 2021). Differences in stable isotope values between the two seasons (i.e., lower $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ by 1 ‰ and 1.4 ‰ in 2019/20 than 2018/19, respectively), indicate that annual variation in contamination could be due to changes in diet and distribution (Table 1). This would indicate a shift in diet to a higher trophic level, and apparently more contaminated prey based on the increase in THg concentrations (Braune et al. 2014). Another non-mutually exclusive explanation relates to annual fluctuations in environmental factors, including oceanographic conditions, light irradiance, timing or extent of sea-ice, or atmospheric deposition, all of which influence Hg transport, MeHg production, and bioavailability to marine predators and their prey in the region (Cossa et al. 2011; Driscoll et al. 2013; Renedo et al. 2020).

4.2. Trophic ecology as a driver of blood THg

Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are effective trophic proxies of foraging habitat and trophic position, respectively, in seabirds, which can contribute to variation in Hg concentrations (Bearhop et al. 2000). The most parsimonious GLMM

explaining RBC THg concentrations included $\delta^{15}\text{N}$ as a predictor variable (Table 2). RBC THg concentrations were positively correlated with $\delta^{15}\text{N}$ values, likely reflecting the biomagnification of MeHg through marine food webs, such that birds foraging at higher trophic levels were exposed to higher levels of Hg. Hg bioaccumulation in the prey (mostly penguins, krill and fish) of brown skuas, and biomagnification in general in Southern Ocean food webs is well documented (Bargagli et al. 1998; Brasso et al. 2014; Calle et al. 2015; Goutte et al. 2015; Polito et al. 2016; Sontag et al. 2019; Seco et al. 2020). An alternative explanation for this, is that skua diet changes according to the phenology and peaks in availability of their prey, and opportunistic changes in consumption of penguin eggs and chicks across the season according to their availability occurs (Graña Grilli et al. 2014; Ibañez et al. 2022). The increase in $\delta^{15}\text{N}$ values would indicate that with the advance of the season, skuas shift from feeding on penguin eggs to chicks, as the latter display higher levels of Hg (McKenzie et al. 2021).

RBC $\delta^{13}\text{C}$ values indicated that in both seasons brown skuas had fed on Antarctic prey during the early incubation and pre-laying period. Although $\delta^{13}\text{C}$ was not included in the top GLMM, it was included as a predictor variable in a competitive model (Table 2), though the ω_i of this model was much lower. Overall, the effect of $\delta^{13}\text{C}$ was weaker than that of $\delta^{15}\text{N}$. In the Southern Ocean, most of the MeHg bioaccumulated by seabirds has a mesopelagic origin (Renedo et al. 2018; 2020), and mesopelagic organisms have elevated Hg concentrations due to increased methylation rates beneath the thermocline (Monteiro et al. 1996; Choy et al. 2009; Blum et al. 2013). RBC THg concentrations showed a weak negative relationship with $\delta^{13}\text{C}$ values, indicating that higher Hg burdens in some skuas might reflect a diet with a higher proportion of pelagic prey (i.e., with lower $\delta^{13}\text{C}$ values). At Esperanza Bay, the contribution of marine resources, including fish (particularly Antarctic Silverfish

Pleuragramma antarcticum) and molluscs, to the diet of breeding skuas is considerable (Borghello et al. 2019; Ibañez et al. 2022). Southern Ocean fish species show extensive variation in Hg concentrations related to preferred habitat (sea-ice, epipelagic, mesopelagic or benthic; Goutte et al. 2015; Polito et al. 2016). Both Antarctic silverfish and Adélie penguins feed in mesopelagic water and exhibit high Hg burdens (Ainley et al. 1992; Brasso et al. 2014; Goutte et al. 2015; Polito et al. 2016), so the reliance of skuas on these prey may contribute to Hg contamination.

4.3. *Sex differences in Hg contamination*

Although sex was not included in the most parsimonious GLMMs (Table 2), THg concentrations were slightly higher in males than females in both seasons (Table 1, Mills et al. 2022). One possible explanation is that egg production provides a route through which females are able to eliminate Hg (Robinson et al. 2012; Ackerman et al. 2020). An alternative explanation relates to the slight sex differences in RBC $\delta^{15}\text{N}$ values (Table 1), which could reflect more lower trophic level prey consumed by females in a pre-laying exodus (known to be undertaken by brown skuas at South Georgia; Carneiro et al. 2016), or other sex-specific variation in diet prior to sampling. However, this would need to be confirmed in future studies.

4.4. *Relationships with body condition and breeding success*

Hg contamination of seabirds may induce changes in physiology, such as immune function (Finkelstein et al. 2007) and energy metabolism, which is involved in detoxification (Lucia et al. 2012; Ackerman et al. 2016). Effects on body condition may ultimately affect fitness-related traits, including breeding success and survival (Labocha and Hayes 2012), and high

blood THg concentrations may cause reproductive impairment or failure (Evers et al. 2008; Ackerman et al. 2016). Here, RBC THg concentrations of brown skuas ranged from 0.41 to 2.33 $\mu\text{g g}^{-1}$ dw (0.10 $\mu\text{g g}^{-1}$ wet weight [ww] to 0.58 $\mu\text{g g}^{-1}$ ww, respectively), suggesting a low risk of MeHg toxicity (Ackerman et al. 2016). Indeed, in our study body condition and breeding success of brown skuas were unrelated to RBC THg concentrations, which is consistent with the recent review of Carravieri et al. (2022). Our data suggest that Hg contamination is not currently a concern for brown skuas at Esperanza/Hope Bay. The threshold of Hg toxicity in seabirds appears to differ in Antarctic compared with subantarctic or subtropical environments (Goutte et al. 2014b; Carravieri et al. 2021). However, previous studies testing for such effects have reported variable results (Kalisinska et al. 2010; Fort et al. 2015; Tartu et al. 2015; Ackerman et al. 2019; Carravieri et al. 2021), suggesting that relationships are complex, and could be modulated by other factors related to individual physiological status or environmental quality. Goutte et al. (2014b) reported short-term effects on breeding success in brown skuas and south solar skuas in relation to Hg contamination, though Hg concentrations were ~ 10.25 and ~ 2.7 fold higher compared to our data, respectively. Long-term effects were more pronounced in south polar skuas from Adélie Land than in brown skuas from Kerguelen Islands, despite the differences in Hg contamination. Selenium (Se) offers a protective effect against Hg toxicity when Se is in molar excess (Manceau et al. 2021). In the case of skuas, blood Se:Hg molar ratio was three fold in brown skuas from Kerguelen Islands than the south polar skuas from Adélie Land (Carravieri et al. 2017). In addition to low RBC THg concentrations of brown skuas in our study, the lack of relationships between with body condition and breeding success in our study may also be explained by the presence of Se. It is possible that background

concentrations of Se in Antarctic latitudes are higher than in more northerly regions and may contribute to our results; however, this hypothesis requires evaluation.

Author contribution

AEI: Conceptualization, Methodology, Investigation, Resources, Formal Analysis, Visualization, Writing, Original Draft. WFM: Conceptualization, Methodology, Investigation, Resources, Writing, Review & Editing. PB: Investigation, Writing, Review & Editing. RARM: Investigation, Writing, Review & Editing. LM: Investigation, Review & Editing. FXP: Review & Editing. DST: Investigation & Review. NSH: Investigation, Review & Editing. RM-J: Investigation, Writing, Review & Editing. RAP: Conceptualization, Methodology, Investigation, Resources, Supervision, Review & Editing. DM: Conceptualization, Investigation, Resources, Supervision, Review & Editing.

Conflict of 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.

Ethical approval

All applicable international, national, and institutional guidelines for sampling, care and experimental use of animals for the study were followed as established by the Article III, Annex II of the Madrid Protocol, Law 24.216 (Taking, Harmful Intrusion and Introduction of Species) within the framework of the projects evaluated and approved by the Environment Office of the IAA and Dirección Nacional del Antártico (DNA).

Acknowledgements

The authors are grateful to the Dirección Nacional del Antártico (DNA) and Instituto Antártico Argentino (IAA) for the support of the field-work activities and logistics in the Antarctic. Thanks to CONICET and Agencia Nacional de Promoción Científica y

Tecnológica (ANPCyT) for the financial support of this project (PIP- CONICET no. 0158 to DM; PICT-2014–3323 to AEI). Stable isotope analyses were funded by the Natural Environment Isotope Facility (grant no. 2325.0920). The authors are grateful to Maud Brault-Favrou for her assistance with Hg analyses. Thanks are also due to the CPER (Contrat de Projet Etat-Région) and the FEDER (Fonds Européen de Développement Régional) for funding the AMA of LIENSs laboratory. The Institut Universitaire de France (IUF) is acknowledged for its support to P. Bustamante as a Senior Member.

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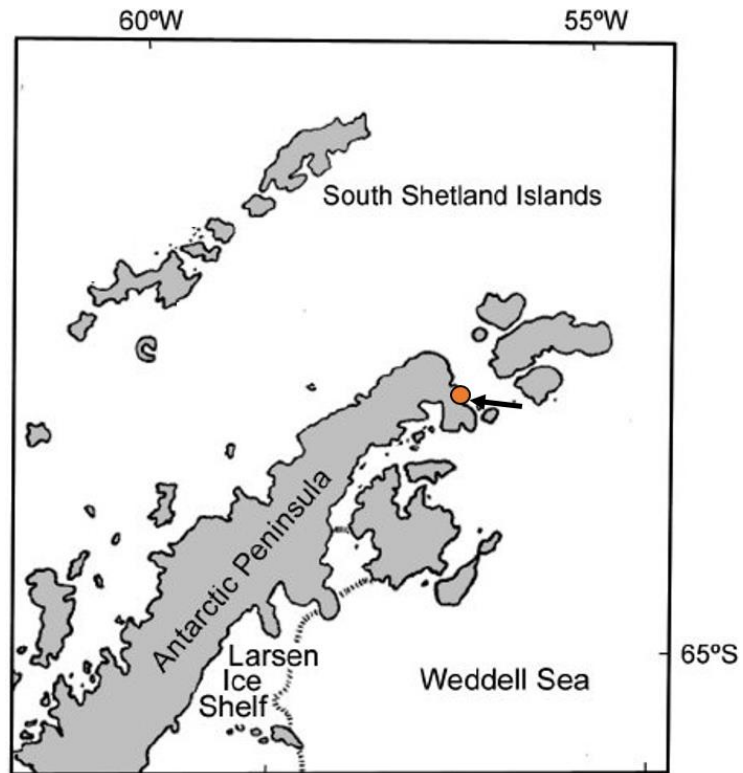


Figure 1. Location of the study site (orange circle), Esperanza/Hope Bay, Antarctic Peninsula ($63^{\circ}24'S$, $57^{\circ}01'W$).

Table 1. Total mercury (THg) concentrations ($\mu\text{g g}^{-1}$ dw), and stable isotope values of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in red blood cells of adult male and female brown skuas (*Stercorarius antarcticus*) at Esperanza/Hope Bay, Antarctic Peninsula ($63^{\circ}24'\text{S}$, $57^{\circ}01'\text{W}$), in the 2018/19 and 2019/20 seasons. Data are means \pm SDs.

| Year | <i>N</i> | Sex | THg ($\mu\text{g g}^{-1}$ dw) | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) |
|-----------|----------|------|--------------------------------|---------------------------|---------------------------|
| 2018-2019 | 11 | F | 0.66 ± 0.25 | -22.09 ± 0.61 | 9.49 ± 0.98 |
| | 13 | M | 0.79 ± 0.18 | -22.59 ± 0.84 | 10.19 ± 1.07 |
| | 24 | Both | 0.73 ± 0.22 | -22.35 ± 0.76 | 9.85 ± 1.07 |
| 2019-2020 | 13 | F | 0.82 ± 0.39 | -23.17 ± 0.47 | 10.91 ± 0.97 |
| | 12 | M | 1.02 ± 0.50 | -23.26 ± 0.26 | 11.61 ± 0.48 |
| | 25 | Both | 0.91 ± 0.44 | -23.22 ± 0.37 | 11.24 ± 0.84 |

Table 2. Model selection for total mercury (THg) concentrations ($\mu\text{g g}^{-1}$ dw) in red blood cells of adult brown skuas (*Stercorarius antarcticus*) from Esperanza/Hope Bay, Antarctic Peninsula. The top five models are shown and all are GLMMs (gamma distribution and inverse link function) ranked according to ΔAICc (i.e., decreasing model fit). AICc: Akaike's Information Criteria adjusted for small sample-sizes; ω_i : Akaike's weights.

| Models (gamma distribution) | AICc | ΔAICc | ω_i |
|--|--------|---------------------|------------|
| Maximal model: $\text{THg} \sim \text{Sex} + \delta^{15}\text{N} + \delta^{13}\text{C} + \text{Season} + \text{Incub day} + \text{Sex}:\delta^{13}\text{C} + \text{Sex}:\delta^{15}\text{N}$ | | | |
| $\text{THg} \sim \delta^{15}\text{N} + \text{Season}$ | -18.87 | 0.00 | 0.309 |
| $\text{THg} \sim \delta^{13}\text{C} + \delta^{15}\text{N} + \text{Season}$ | -17.59 | 1.28 | 0.163 |
| $\text{THg} \sim \delta^{15}\text{N} + \text{Season} + \text{Incub day}$ | -17.12 | 2.12 | 0.146 |
| $\text{THg} \sim \text{Sex} + \delta^{15}\text{N} + \text{Season}$ | -16.31 | 2.56 | 0.085 |
| $\text{THg} \sim \delta^{15}\text{N} + \text{Incub day}$ | -15.59 | 3.28 | 0.059 |

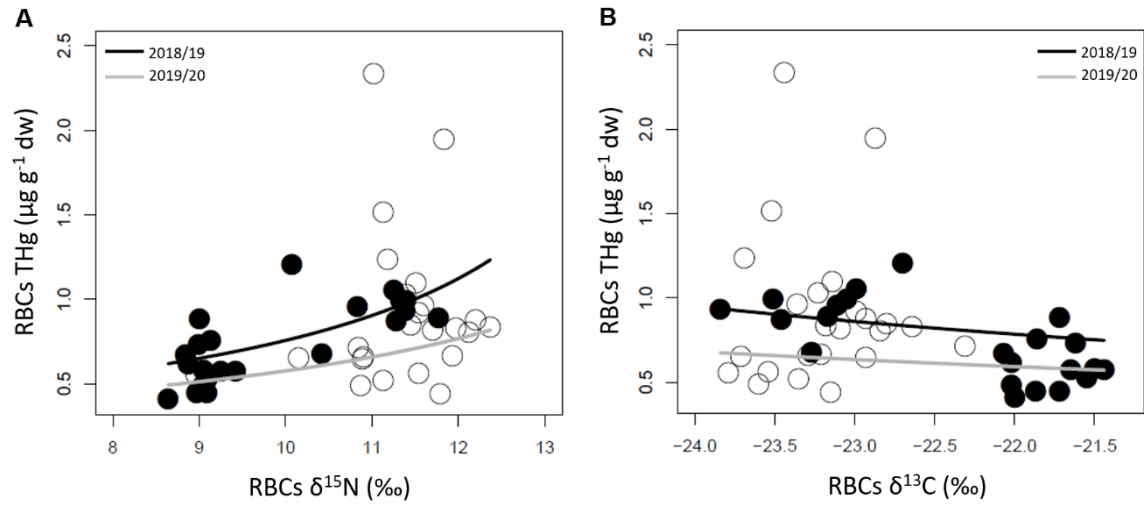


Figure 2. Relationships between total mercury (THg) concentrations ($\mu\text{g g}^{-1} \text{ dw}$) and stable isotope values of (A) nitrogen ($\delta^{15}\text{N}$), and (B) carbon ($\delta^{13}\text{C}$) in red blood cells (RBCs) sampled from adult brown skuas (*Stercorarius antarcticus*) during the early to mid-incubation stage in breeding seasons 2018/19 (black circles) and 2019/20 (white circles).

Supplementary Table 1. (Continuation of Table 2) Model selection for total mercury (THg) concentrations in red blood cells of adult brown skuas (*Stercorarius antarcticus*) from Esperanza/Hope Bay, Antarctic Peninsula. The top five models are shown and all are GLMMs (gamma distribution and inverse link function) ranked according to ΔAICc (i.e., decreasing model fit). AICc: Akaike's Information Criteria adjusted for small sample-sizes; ω_i : Akaike's weights.

| | | | |
|--|--------|------|-------|
| THg ~ $\delta^{13}\text{C} + \delta^{15}\text{N} + \text{Season} + \text{Incub day}$ | -15.38 | 3.49 | 0.053 |
| THg ~ Sex + $\delta^{15}\text{N} + \delta^{13}\text{C} + \text{Season}$ | -14.85 | 4.31 | 0.041 |
| THg ~ Sex + $\delta^{15}\text{N} + \text{Season} + \text{Incub day}$ | -14.55 | 4.31 | 0.035 |
| THg ~ $\delta^{13}\text{C} + \delta^{15}\text{N} + \text{Incub day}$ | -13.19 | 5.68 | 0.018 |
| THg ~ Sex + $\delta^{15}\text{N} + \text{Incub day}$ | -13.17 | 5.70 | 0.017 |
| THg ~ Sex + $\delta^{15}\text{N} + \delta^{13}\text{C} + \text{Season} + \text{Incub day}$ | -12.39 | 6.48 | 0.012 |
| THg ~ $\delta^{15}\text{N}$ | -12.14 | 6.73 | 0.010 |
| THg ~ $\delta^{13}\text{C} + \text{Incub day}$ | -11.44 | 7.43 | 0.007 |
| THg ~ Sex + $\delta^{13}\text{C} + \text{Incub day}$ | -10.60 | 8.27 | 0.005 |
| THg ~ $\delta^{13}\text{C}$ | -10.40 | 8.47 | 0.004 |
| THg ~ $\delta^{13}\text{C} + \text{Season}$ | -10.26 | 8.61 | 0.004 |
| THg ~ $\delta^{15}\text{N} + \delta^{13}\text{C}$ | -10.15 | 8.72 | 0.003 |
| THg ~ Sex + $\delta^{15}\text{N}$ | -10.03 | 8.84 | 0.003 |
| THg ~ $\delta^{13}\text{C} + \text{Season} + \text{Incub day}$ | -9.22 | 9.65 | 0.002 |
| THg ~ Incub day | -9.15 | 9.72 | 0.002 |
| THg ~ Sex + $\delta^{13}\text{C} + \text{Incub day}$ | -9.14 | 9.73 | 0.002 |

| | | | |
|--|-------|-------|--------|
| Null | -8.76 | 10.11 | 0.001 |
| THg ~ Sex + $\delta^{13}\text{C}$ | -8.33 | 10.54 | 0.001 |
| THg ~ Sex + $\delta^{13}\text{C}$ + Season | -7.92 | 10.95 | 0.001 |
| THg ~ Sex + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ | -7.86 | 11.01 | 0.001 |
| THg ~ Sex | -7.30 | 11.57 | 0.0008 |
| THg ~ Sex + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + Season + Incub day + Sex: $\delta^{13}\text{C}$ + Sex: $\delta^{15}\text{N}$ | -7.15 | 11.72 | 0.0006 |
| THg ~ Sex + $\delta^{13}\text{C}$ + Season + Incub day | -6.67 | 12.20 | 0.0005 |
| THg ~ Sex + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + Sex: $\delta^{13}\text{C}$ | -6.38 | 12.49 | 0.0005 |
| THg ~ Season | -6.37 | 12.50 | 0.0004 |
| THg ~ Sex + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + Sex: $\delta^{15}\text{N}$ | -5.65 | 13.22 | 0.0004 |