# *Deleterious effects of mercury contamination on immunocompetence, liver function and egg volume in an antarctic seabird*

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# Deleterious effects of mercury contamination on immunocompetence, liver function and egg volume in an Antarctic seabird

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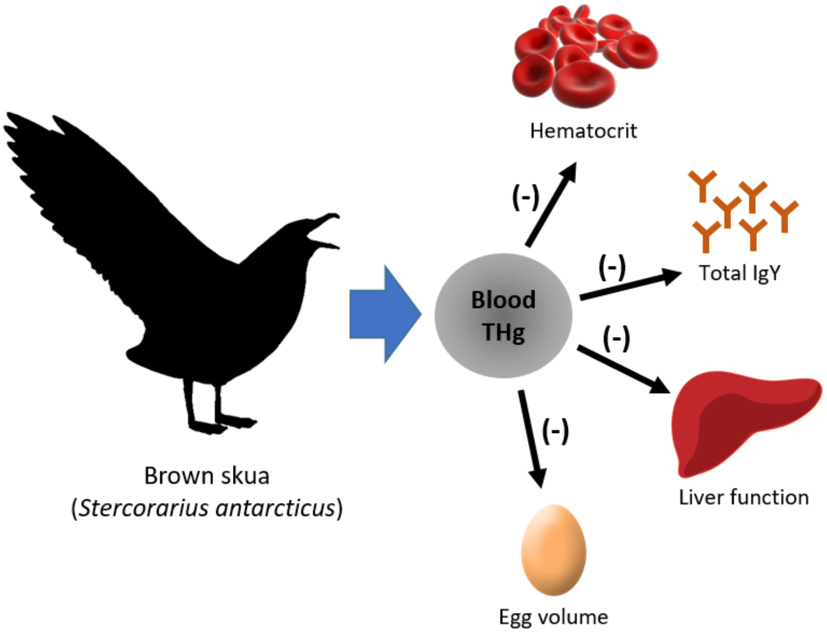
# GRAPHICAL ABSTRACT



# HIGHLIGHTS

- Blood THg concentrations were measured in brown skuas on the Antarctic Peninsula.
- Higher blood THg concentrations had deleterious effects on physiology.
- Higher blood THg concentrations disrupted immune and liver function.
- Higher blood THg concentrations were associated with lower egg volume.

# ABSTRACT

Mercury (Hg) is a globally important pollutant that can negatively impact metabolic, endocrine and immune systems of marine biota. Seabirds are long-lived marine top predators and hence are at risk of bioaccumulating high Hg concentrations from their prey. Here, we measured blood total mercury (THg) concentrations and relationships with physiology and breeding parameters of breeding brown skuas (*Stercorarius antarcticus*) (*n* = 49 individuals) at Esperanza/Hope Bay, Antarctic Peninsula. Mean blood THg concentrations were similar in males and females despite the differences in body size and breeding roles, but differed between study years. Immune markers (hematocrit, Immunoglobulin Y [IgY] and albumin) were negatively correlated with blood THg concentrations, which likely indicates a disruptive effect of Hg on immunity. Alanine aminotransferase (GPT) activity, reflecting liver dysfunction, was positively associated with blood THg. Additionally, triacylglycerol and albumin differed between our study years, but did not correlate with Hg levels, and so were more likely to reflect changes in diet and nutritional status rather than Hg contamination. Egg volume correlated negatively with blood THg concentrations. Our study provides new insights into the sublethal effects of Hg contamination on immunity, liver function and breeding parameters in seabirds. In this Antarctic species, exposure to sublethal Hg concentrations reflects the short-term risks which could make individuals more susceptible to environmental stressors, including ongoing climatic changes.

**Keywords:** pollution; mercury; seabirds; physiology; reproduction; Antarctica

## 1. INTRODUCTION

Mercury (Hg) is a global environmental pollutant that can have deleterious consequences for humans and wildlife (Corsolini 2009; Tan et al., 2009; Driscoll et al., 2013). Although Hg occurs naturally, its availability has increased in marine and terrestrial environments in different organic and inorganic forms (MeHg, inorganic Hg, etc) (Aronson et al., 2011). The gaseous, elemental form of Hg ($Hg^0$) can spread through atmospheric transport from emission sources to distant regions (Calle et al., 2015). Once deposited in marine environments, the inorganic form of Hg ($Hg^{II}$) is methylated by microorganisms to the more toxic form, methyl-Hg (MeHg, $[CH_3Hg]^+$). MeHg bioaccumulates within the tissues of marine organisms and biomagnifies through marine food webs (Bargagli 2008; Driscoll et al., 2013; Ibañez et al., 2022a; Matias et al., 2022). Seabirds, which are typically long-lived and feed at high trophic positions, are potentially at risk of accumulating high Hg levels in their tissues from dietary exposure (Bearhop et al., 2000; Bargagli 2008; Tavares et al., 2013; McKenzie et al., 2021; Mills et al., 2020; 2022; Ibañez et al., 2022a).

Hg in Antarctic ecosystems derives from natural and human sources, and the Antarctic continent constitutes a sink for Hg, which condensates in colder regions after evaporation and long-range transportation from lower latitudes (Angot et al., 2016). Artisanal and small-scale gold mining, which releases large quantities of Hg into the environment, is mostly concentrated in the Southern Hemisphere (Keane et al., 2023). There are also local sources of Hg, including volcanic activity and the release of Hg stored in sea ice which increase its bioavailability for microbial methylation (Cossa 2013; de Ferro et al., 2014; Gionfriddo et al., 2016). Human activity that is associated with nearby research stations on the Antarctic Peninsula increases during spring and summer, which results in the release of pollutants (heavy metals and organic compounds) from waste-disposal sites, construction materials and compounds used for treating effluent (Acero et al., 1999).

Hg is a neurological, immune and endocrine disruptor (Tartu et al., 2013; Whitney and Cristol 2017), and, ultimately, can Hg contamination can have short- or long-term fitness consequences for seabirds (Bustnes et al., 2007; Roos et al., 2012; Dietz et al., 2019; Chételat et al., 2020; Mills et al., 2020; Goutte et al., 2014a, 2014b). Hg contamination may also negatively impact body condition (Tan et al., 2009; Ackerman et al., 2016; Chételat et al., 2020), although, in the wild, body condition indices are unreliable indicators of Hg sublethal effects (Carravieri et al., 2022). Although many studies have examined inter- and intraspecific variation in Hg contamination of seabirds in the Southern Ocean (Carravieri et al., 2014a; 2016; 2017; Mills et al., 2020; 2022; Quillfledt et al., 2023), few have tested for relationships with fitness parameters. Indeed, these 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), Antarctic petrel (*Thalassoica antarctica*) (Carravieri et al., 2018; 2021), grey-headed albatross (*Thalassarche chrysostoma*) (Mills et al., 2020), and brown skua (*Stercorarius antarcticus*) (Ibañez et al., 2022a) and south polar skua (*S. maccormicki*) (Goutte et al., 2014b).

Brown skuas breed on the Antarctic continent and sub-Antarctic islands and are opportunistic predators that feed on a wide diversity of prey in both terrestrial and marine environments (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; Ibañez et al., 2022b). As the brown skua is a migratory seabird, birds are exposed to pollutants not just during the breeding season but also in the nonbreeding season when they visit regions with higher anthropogenic pressure (Albert et al., 2022). At Bahía Esperanza/Hope Bay–located on the Antarctic Peninsula–brown skuas breed close to large colonies of Adélie penguins (*Pygoscelis adeliae*) and gentoo penguins (*Pygoscelis papua*). They feed mainly on penguins and to a lesser extent on marine prey (e.g., fishes and invertebrates) (Borghello et al., 2019; Ibañez et al., 2022b). Macro-plastics have been found recently in skua diet samples collected during the breeding season (Ibañez et al., 2020), which may represent a possible route for chemical pollutants such as Hg (Hamilton et al., 2023). Overall, there has been little research concerning the physiological effects of Hg contamination on Antarctic seabirds (Carravieri et al., 2021; Goutte et al., 2014), and more studies are needed to understand the potential sublethal effects. In the present study, we focus on the sublethal effects of Hg contamination of brown skuas. Total Hg (the sum of inorganic and organic Hg) concentration was measured in red blood cells of brown skuas at Esperanza/Hope Bay. Our aims were to: (i) compare blood THg concentrations between two breeding seasons and sexes; and (ii) relate blood THg concentrations to markers of energy metabolism, immunocompetence and liver function, and to egg volume.

## 2. MATERIALS AND METHODS

### 2.1. *Fieldwork and sample collection*

Fieldwork for this study was conducted at Bahía Esperanza/Hope Bay, Antarctic Peninsula (63°24′S, 57°01′W) (Fig. 1), from November to January during the 2018/19 and 2019/20 breeding seasons. Brown skuas were sampled during the early incubation stage (5–10 days after clutch completion). Blood samples (~2 ml) were obtained from the brachial vein using a 25-G needles ($n$ = 49, $n$ = 24 and $n$ = 25 in 2018/19 and 2019/20, respectively). Sampled birds included both individuals from 16 nests and one adult from 17 nests. Once in the laboratory (within 2–6 hours after extraction) serum and red blood cells were separated by centrifugation (2000 rpm for 10 min), placed in sterile plastic eppendorf tubes and were stored frozen (–20°C) prior to laboratory analyses.

Eggs were measured (length and breadth) using digital calipers and volumes ($mm^3$) calculated as 0.00048 x length (mm) x breadth $(mm)^2$ (Phillips et al., 2004). We calculated the total clutch volume as the average of the volume of both eggs. The sex of birds was initially assigned morphologically based on body size, and later confirmed by DNA analysis (Fridolfsson and Ellegren 1999; Phillips et al., 2002).

### 2.2. *Total Hg analysis*

Studies have demonstrated that Hg in blood is associated predominantly with the cellular fraction (i.e., red blood cells) rather than plasma (Bond and Robertson 2015; Renedo et al., 2018). THg in seabird RBCs is mostly (>90%) MeHg (Renedo et al., 2018; Albert et al., 2019). Prior to analysis, red blood cells were freeze-dried and homogenized. Blood THg concentrations were measured using an Advanced Mercury Analyser spectrophotometer (Altec AMA 254) (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 (RSD) among measurements were calculated (all samples RSD <10%). THg concentrations are presented in $\mu g\ g^{-1}$ dw. Accuracy was tested using certified reference material (CRM; dogfish liver DOLT-5, NRC, Canada; certified Hg concentration: 0.44 ± 0.18 $\mu g\ g^{-1}$ dw) every 10 samples. Recovery of the CRM was 97.8 ± 1.7%. Blanks were analyzed at the beginning of each set of samples. The limit of quantification of the AMA was 0.1 ng and the detection limit of the method was 0.005 $\mu g\ g^{-1}$ dw.

### 2.3. *Hematological determinations*

Serum concentrations of five energy metabolism markers (including total proteins, uric acid, triacylglycerol, cholesterol and glucose), albumin, aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) enzymes were measured in each bird using colorimetric commercial kits (Wiener Lab). All assays were conducted using an automatic analyzer (Ibañez et al., 2015).

Serum circulating levels of Immunoglobulin Y (IgY) were determined by direct ELISA using peroxidase conjugated anti-chicken IgY antibodies (Sigma, St Louis, MO, USA, A-9046) (Martínez et al., 2003; Ibañez et al., 2018). For this, 96-well microtiter plates (Nunc PolySorp; Nunc, Roskilde, Denmark) were coated during 1 h at 37 °C with serum samples diluted (1/30,000) in 0.1 M carbonate-bicarbonate buffer (pH = 9.6). Then the plates were washed three times with PBS- 0.05% Tween 20 and incubated with 1% non-fat milk (Nestlé coffee-mate) in PBS-Tween-20 during 1 h at 37 °C to block the free binding sites. After new washing, the wells were incubated with peroxidase- conjugated anti-chicken IgY. Finally, the wells were washed and ABTS [2,2-azino-di (3-ethylbenzthiazoline sulfonate)] was added as substrate. After incubating for 30 min (at room temperature) color development was stopped with oxalic acid 2% and then read as optical density (OD) at 405 nm.

To determine the hematocrit value a heparinized capillary was filled in the laboratory with 100 µl of blood from the Eppendorf tube that contained heparinized blood obtained in the field. The capillary was then centrifuged at 5000 rpm for 15 min (Ibañez et al., 2015), and a digital caliper used to measure the length (mm) of the red blood cell fraction and the total blood volume. Hematocrit values are presented as a percentage of total volume.

### 2.4. *Statistical analysis*

Data were analysed using R (R Core Team 2015). Blood THg concentrations, physiological parameters and egg volumes were checked for normality and homogeneity of variances using Shapiro-Wilk and Levene’s tests, respectively. Differences in blood THg between sexes and seasons were tested using general linear mixed models (GLMMs; Gaussian distribution and identity link function), with the individual identity and the breeding pair included as random effects to account for partners potentially being more similar than non-partners in blood THg concentrations. Relationships between physiological markers (total proteins, uric acid, triacylglycerol, cholesterol, hematocrit, albumin, IgY, GOT and GPT), egg volume and blood THg concentrations were also tested using GLMMs, with sex (except in the case of egg volume) and season included as covariates, and individual identity included as a random effect. All GLMMs were fitted using the “nlme” package in R (Pinheiro et al. 2017).

## 3. RESULTS

### 3.1. *Sex and annual variation in Hg contamination*

Detectable blood THg concentrations were found in all samples from brown skuas at Bahía Esperanza/Hope Bay in 2018/19 (mean ± SD, 0.73 ± 0.22 μg $g^{-1}$ dw) and 2019/20 (0.91 ± 0.44 μg $g^{-1}$ dw) (Table 1). There were no significant differences in blood THg concentrations (log-transformed) between sexes (est = 0.23, p = 0.48) or seasons (est = 0.19, p = 0.56) (Table 1).

### 3.2. *Physiological markers and egg volume*

Physiological parameters, in particular immune and hepatic functions, did not differ between sexes (all $p > 0.05$), but were negatively associated with blood THg concentrations. Significant negative relationships were found between albumin (est = -0.31, $p < 0.0001$), hematocrit (%) (est = -9.27, $p < 0.0001$) and IgY (est = -0.06, $p < 0.05$) and blood THg concentrations (Fig. 2; Table 2). A significant positive relationship was found between GPT activity and blood THg concentrations (est = 13.10, $p < 0.01$) (Fig. 2; Table 2). Mean albumin levels differed significantly between seasons (est = 0.31, $p < 0.0001$). Triacylglycerol levels also varied annually (est = 55.89, $p < 0.001$) and were unrelated to blood THg concentrations (est = -11.04, p = 0.55) (Table 2). Uric acid, total proteins, cholesterol, glucose and GOT show no relationship with sex, season or blood THg (Table 2). Egg volume ($mm^3$) showed a significant negative relationship with blood THg concentrations (est = -16.40, $p < 0.05$) (Fig. 3), and did not differ significantly between seasons (est = 4.25, p = 0.19).

## 4. DISCUSSION

In our study, blood THg concentrations of brown skuas ranged from 0.41 to 2.33 μg $g^{-1}$ dw (equivalent to 0.10 to 0.58 μg $g^{-1}$ wet weight [ww], assuming a 79% moisture content;

Eagles-Smith et al., 2008; Ackerman et al., 2016). These concentrations are comparable to those found in adults of other Antarctic seabirds, including snow petrels (*Pagodroma nivea*) (Tartu et al., 2015), and Antarctic petrels (*Thalassoica antarctica*) (Carravieri et al., 2021). However, they were lower than those reported in skuas at lower latitude breeding colonies in the southwest Atlantic Ocean sector of the Southern Ocean (Mills et al., 2022), but were comparable to those associated with reduced breeding success in south polar skuas at Adélie Land (Goutte et al., 2014b). Despite the low values of blood THg measured here, we found significant negative effects on immunity and hepatic enzymes, and on breeding parameters.

### 4.1. *Impacts of Hg contamination on immunocompetence*

Hg is often associated with immunosuppressive effects at sublethal levels, but mostly in captive studies (Fallacara et al., 2011; Kenow et al., 2007; Lewis et al., 2013a). Despite the relatively low concentrations (initially suggesting a low risk of MeHg toxicity; Ackerman et al., 2016), IgY and hematocrit were negatively related to blood THg concentrations in our study (Fig. 2). These results indicate a negative impact of Hg contamination on the immune status of brown skuas. Our results agree with previous studies on captive zebra finches (*Taeniopygia guttata*) that have shown negative effects of Hg exposure on B-cell proliferation (Lewis 2012; Lewis et al., 2013). Also, impaired macrophage phagocytosis was related to high Hg levels in black-footed albatrosses (*Phoebastria nigripes*) in the North Pacific (Finkelstein et al., 2007). Hematocrit values decreased with blood THg concentrations, which agrees with the hemolytic and anemia-inducing effects of Hg (Zolla et al., 1997). Erythrocytes are an important target of Hg and the majority of Hg in blood is found in the cellular fraction (~90%) (Bond and Robertson 2015). *In vitro* studies have demonstrated that the exposure of erythrocytes to low concentrations of Hg induce structural changes in the external surface of the membrane. These changes are mediated by the translocation of phosphatidylserine to the external surface of the erythrocyte cell membrane as a signal that may prompt cellular apoptosis (Eisele et al., 2006; Lim et al., 2010). Another possible explanation behind the decrease in hematocrit may be an association with lowered renal function, also linked to hemolytic processes (Rivarob et al., 1983; Chitra et al., 2013).

Serum IgY and albumin are useful indicators of health in birds (Lumeij 1987; Ibañez et al., 2018). Fitness traits are mediated by hormones including luteinizing hormone, which is a pituitary hormone involved in the onset of breeding (Dawson et al., 2001); corticosterone, an adrenal hormone in the stress response (Wingfield and Sapolsky 2003); and prolactin, a pituitary hormone involved in the expression of parental care (Angelier and Chastel 2009). Trace metal pollution may have different effects on hormones of the hypothalamic–pituitary–adrenal (HPA) axis, such as corticosterone (Tan et al., 2009; Tartu et al., 2013). Serum IgY and albumin concentrations were negatively related to blood THg concentrations of brown skuas in our study (Fig. 2). One possible explanation for this may be increasing immunosuppressive effects and catabolic activity associated with endocrine disruption of the HPA axis (Coutinho and Chapman 2011), as Hg accumulates in the pituitary gland and thyroid in vertebrates (Colborn et al., 1993; Tan et al., 2009; Meyer et al., 2014; Tartu et al., 2013). In this scenario, the levels of Hg found in brown skuas at Esperanza/Hope Bay may induce protein catabolism and immunosuppression (IgY and albumin decline) because corticosterone release is disrupted (Ibañez et al., 2018). Another possible explanation is impaired protein synthesis in the liver. However, these hypotheses would need to be confirmed in future studies on skuas, particularly as a Hg-induced deterioration in immunocompetence is likely to affect disease risk and, ultimately, population dynamics.

### 4.2. *Impacts of Hg contamination on liver function*

GPT was positively related to blood THg concentrations of brown skuas. This suggests that Hg contamination impacted the liver function of brown skuas. The liver is the major organ involved in the biotransformation, metabolism, protein synthesis and detoxification processes, which also reflect its susceptibility to pollutants, leading to tissue damage. GOT and GPT enzymes are considered to be biochemical markers of impaired liver function. Liver-cell damage, such as degeneration and necrosis, may increase GOT and GPT levels (Gowda et al., 2009; Mari et al., 2010; Ibañez et al., 2015; Yang et al., 2015; Choi et al., 2017). The biological mechanism of association between Hg exposure and liver dysfunction is mainly explained by oxidative stress, cell death, and impaired metabolism (Malhi et al., 2010). For instance, in male rodents exposed to Hg, levels of GOT, GPT, and gamma glutamyl-transferase activities were elevated, and tissue damage or necrotic changes observed in most livers (Waddam 2009). Also, histological analyses described degenerative changes and lysed areas in liver parenchyma in Hg-exposed zebrafish (Maricella et al., 2016). Hg exposure in zebrafish induced deregulation of oxidative stress, intrinsic apoptotic pathways, and resulting hepatotoxicity through cell death, mitochondrial dysfunction, endocrine disruption, and metabolic disorders (Ung et al., 2010; Maricella et al., 2016). In the spectacled caiman (*Caiman crocodilus*), negative associations between Hg and alkaline phosphatase activity (a liver cytoplasmic enzyme involved in the hepatocytic functions) were observed (Lemaire et al., 2018). Therefore, our results suggest that variation in GPT activity in response to elevated blood Hg levels, may indicate hepatotoxicity even at the low levels of contamination found in Antarctica.

### 4.3. *Annual variations in Hg contamination and association with physiology*

In a previous study of brown skuas at Hope Bay, we found annual variation in blood THg concentrations was related to trophic ecology, but had no impact on adult body condition (Ibañez et al., 2022a). Blood THg concentrations were higher in 2019/20 than 2018/19 (by ~0.2 μg $g^{-1}$ dw) (Ibañez et al., 2022a). This was potentially due to changes in diet composition, and the consumption of more contaminated prey (Braune et al., 2014). Also, annual fluctuations in environmental conditions may influence Hg transport, MeHg production, and bioavailability to marine predators and their prey (Cossa et al., 2011; Driscoll et al., 2013; Renedo et al., 2020). In the current study, mean albumin and triacylglycerol levels differed between years, but were not linked directly to blood Hg contamination. Albumin and triacylglycerol are markers of nutritional status (Ibañez et al., 2018). In this scenario, annual dietary differences may affect nutritional status. This may in turn moderate the effects of Hg on metabolism, with potentially limited food resources (thus lower Hg levels) associated with lower albumins and triacylglycerols.

### 4.4. *Impacts of Hg contamination on egg volume*

Hg contamination of seabirds may induce changes on body condition that ultimately affect breeding fitness and survival (Evers et al., 2008; Labocha and Hayes 2012; Ackerman et al., 2016). Effects of Hg on reproduction can be reflected at different levels including egg neglect (Tartu et al., 2015), lower breeding success (Tartu et al., 2016), reproductive failure (Mills et al., 2020), or population dynamics (Goutte et al., 2014b, Goutte et al., 2015). However, the effects of Hg contamination during the non-breeding season on subsequent breeding success are poorly known. In little auks (*Alle alle*), Fort et al. (2014) suggested a carry-over effect, in that individuals with the highest Hg concentrations laid smaller eggs. In the present study, egg volume was negatively associated with female blood THg concentrations (Fig. 3). Although not significant, THg concentrations were slightly higher in males than females in both seasons (Table 1; Mills et al., 2022; Ibañez et al., 2022a), possibly because egg production provides a route through which females are able to eliminate Hg (Robinson et al. 2012; Ackerman et al. 2020). An explanation for the association of smaller eggs laid with higher blood THg concentrations of brown skuas relates to the trophic ecology and Hg contamination during female pre-laying exodus or differences in prey consumption prior to sampling. Therefore, our results support a previous study which also suggested carry-over effects of Hg on the reproduction of great skuas (*Stercorarius skua*) with a specific influence of female winter distribution and Hg contamination on egg volume (Albert et al., 2022). However, this would need to be confirmed in future studies by measuring THg in feathers grown during the nonbreeding season to infer the degree of Hg contamination since the last moult (Fort et al., 2014).

The threshold of Hg toxicity in seabirds appears to be related to the latitude of the study site. Toxicity appears to differ in Antarctic compared with lower latitudes such as subantarctic or subtropical environments (Goutte et al., 2014b; Carravieri et al., 2021). Blood THg concentrations of brown skuas in our study would initially suggest a low risk of MeHg toxicity (Ackerman et al., 2016). These sublethal THg concentrations had deleterious effects on physiology and egg volume, but there was no relationship with breeding success (Ibañez et al., 2022a). Goutte et al. (2014b) reported short-term effects of Hg on breeding success in brown skuas and south polar skuas, though concentrations were higher than in brown skuas at our study site. Selenium (Se)-Hg interactions are often observed in the blood and internal tissues of marine predators, and Se has a protective effect against Hg toxicity when Se is in molar excess (Carravieri et al., 2017; 2020; Manceau et al., 2021). Our results may also be explained by the presence of Se, as was reported for brown skua chicks from the Southern Ocean (Carravieri et al., 2017), south polar skuas (Goutte et al., 2014b) and the spectacled caiman (Lemaire et al., 2018). If so, higher concentrations of Se at Antarctic latitudes may reduce the negative effects of Hg on physiology and reproduction; however, this hypothesis requires further investigation.

## 5. CONCLUSIONS

Our study demonstrated negative effects of Hg contamination on physiology and breeding parameters in brown skuas on the Antarctic Peninsula. Despite low blood THg concentrations, which are below or similar to those of other Antarctic seabirds, the association between Hg and the physiological and breeding parameters are of concern. The detrimental impact on egg volume highlights the importance of investigating the relationships between blood THg and the hormones that play a role in stress responses and reproductive decisions, as well as, Hg concentration in chicks during development. This is particularly as skuas and other predators in the Antarctic may become more susceptible to pollutants and other environmental stressors, given the evidence for rapid, ongoing climatic changes in the region.

## CReditT authorship contribution statement

**Andrés E Ibañez:** Conceptualization, Methodology, Investigation, Resources, Formal Analysis, Visualization, Writing – Original Draft. **William F Mills:** Methodology, Investigation, Resources, Writing – Review & Editing. **Paco Bustamante**: Investigation, Writing – Review & Editing. **Lara M Morales:** Investigation, Writing – Review & Editing. **Diego S Torres:** Investigation, Writing – Review & Editing. **Beatriz D´Astek:** Investigation, Resources. **Rocío Mariano-Jelicich:** Investigation, Writing – Review & Editing. **Richard A Phillips:** Conceptualization, Methodology, Investigation, Resources, Supervision, Writing – Review & Editing. **Diego Montalti:** Investigation, Resources, Supervision, Writing – 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) (permits number 2018-FEAMB-CT-GA-70 and 2019-FEAMB-CT-GA-66). In addition, all the protocols conducted on this project were evaluated and approved by the Institutional Commitee for the Care and Use of Study Animals (CICUAE) of Natural Science and Museum Faculty (FCNyM-UNLP).

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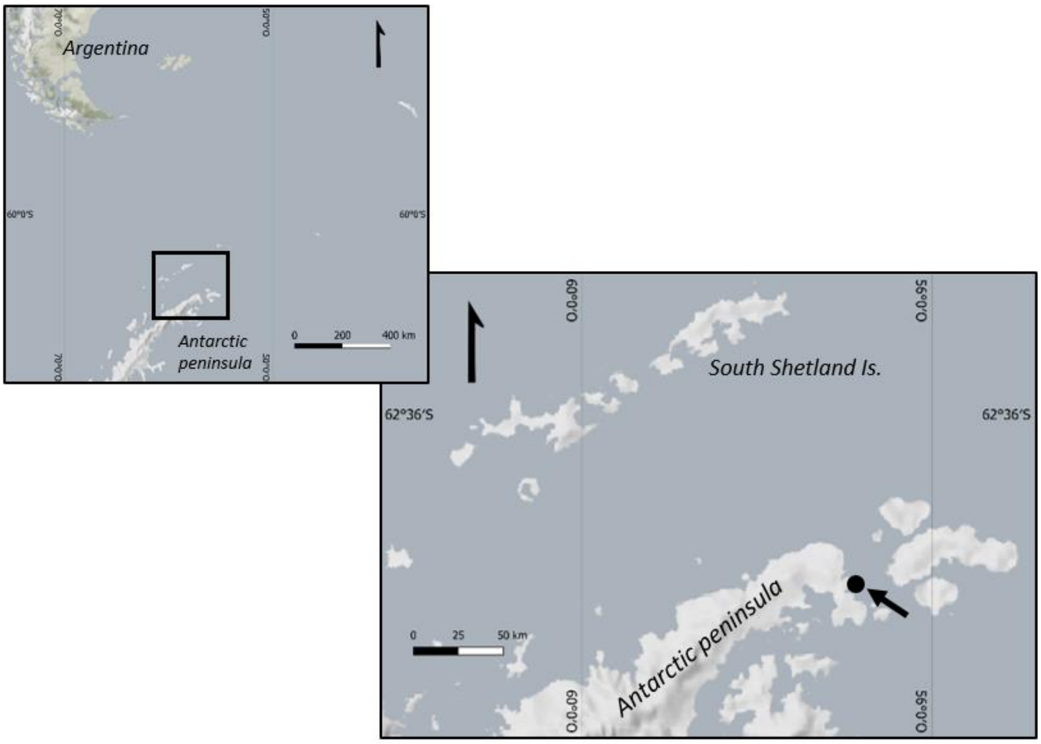

**Figure 1.** Location of the study site (black circle), Esperanza/Hope Bay, Antarctic Peninsula (63◦24′ S, 57◦01′ W).

**Table 1.** Total mercury (THg) concentrations (µg $g^{-1}$ dw) in red blood cells of adult male and female brown skuas (*Stercorarius antarcticus*) at Esperanza/Hope Bay, Antarctic Peninsula (63°24′S, 57°01′W), in the 2018/19 and 2019/20 breeding seasons. Data are means ± SDs.

| Year | *N* | Sex | THg (µg $g^{-1}$ dw) | Range (µg $g^{-1}$ dw) |
|---|---|---|---|---|
| 2018-2019 | 11 | F | 0.66 ± 0.25 | 0.41 - 1.21 |
| | 13 | M | 0.79 ± 0.18 | 0.48 - 1.06 |
| | 24 | Both | 0.73 ± 0.22 | 0.41 - 1.21 |
| 2019-2020 | 13 | F | 0.82 ± 0.39 | 0.49 - 1.94 |
| | 12 | M | 1.02 ± 0.50 | 0.44 - 2.33 |
| | 25 | Both | 0.91 ± 0.44 | 0.44 - 2.33 |

**Table 2.** Serum hematological markers of adult brown skuas (*Stercorarius antarcticus*) at Esperanza/Hope Bay, Antarctic Peninsula (63°24′S, 57°01′W), in the 2018/19 and 2019/20 breeding seasons. Data are means ± SDs. Uric acid (mg/dl), triacylglycerol (mg/dl), total proteins (d/dl), cholesterol (mg/dl), glucose (mg/dl), albumin (g/dl), GOT (U/l) and GPT (U/l). Serum hematological markers that showed an association with season are indicated with [a] ($p < 0.05$).

| | Season 2018-2019 | | Season 2019-2020 | |
|---|---|---|---|---|
| Parameter | Mean ± SD | Min-Max | Mean ± SD | Min-Max |
| Uric acid | 8.126 ± 2.776 | 3.87 - 14.37 | 6.234 ± 2.069 | 2.250 - 9.56 |
| Triacylglicerols | 63.41 ± 18.40 | 31 - 99 | 118.1 ± 49.76 | 53 - 286 |
| Total proteins | 2.809 ± 0.392 | 2.1 - 3.7 | 2.790 ± 0.497 | 1.9 - 3.5 |
| Cholesterol | 245 ± 58.06 | 103.6 - 354.9 | 244.8 ± 65.63 | 113.3 - 351.5 |
| Glucose | 340.6 ± 43.41 | 269 - 420 | 330.6 ± 49.95 | 170  379 |
| Albumin | 1.020 ± 0.135 | 0.73 - 1.260 | 1.282 ± 0.233 | 0.87 - 1.63 |
| GOT | 75.95 ± 27.20 | 25 - 160 | 105.4 ± 20.79 | 69 - 153 |
| GPT | 27.70 ± 12.84 | 9.1 - 63 | 27.27 ± 7.192 | 15 - 39 |
| Hematocrit (%) | 45.60 ± 4.967 | 35.6 - 53.5 | 41.33 ± 6.204 | 30.10 - 30.90 |
| IgY (OD 405nm) | 0.215 ± 0.069 | 0.11 - 0.38 | 0.237 ± 0.075 | 0.104 - 0.430 |

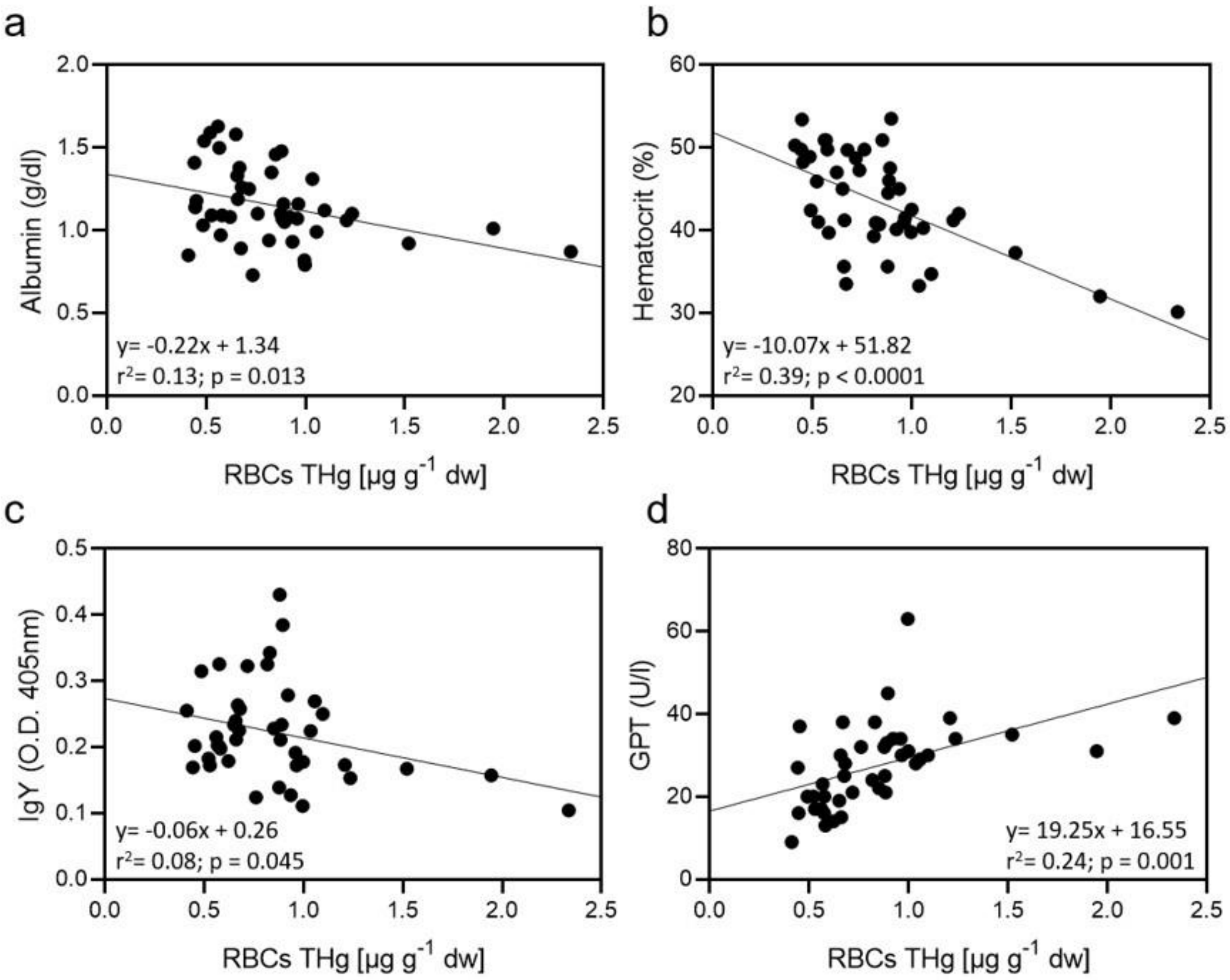

**Figure 2.** Relationships between red blood cells THg concentrations (µg $g^{-1}$ dw) and **(a)** albumin (g/dl), **(b)** hematocrit (%), **(c)** IgY (OD 405nm) and **(d)** GPT (U/l) in the blood of adult brown skuas (*Stercorarius antarcticus*) at Esperanza/Hope Bay, Antarctic Peninsula (63°24′S, 57°01′W) in the 2018/19 and 2019/20 breeding seasons.

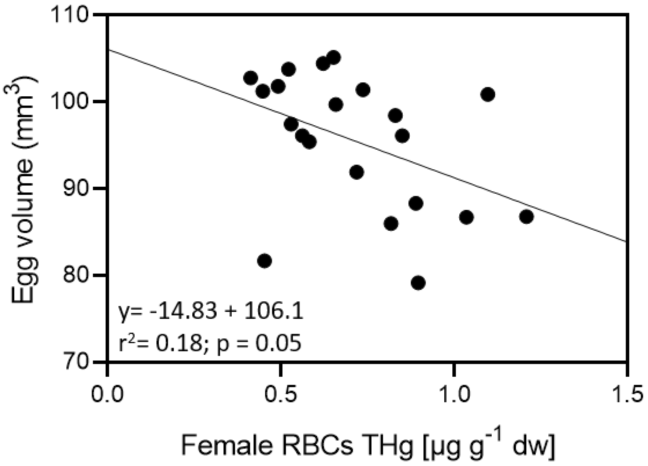

**Figure 3.** Relationships between red blood cells THg concentrations (µg $g^{-1}$ dw) and egg volume ($mm^3$) of female brown skuas (*Stercorarius antarcticus*) at Esperanza/Hope Bay, Antarctic Peninsula (63°24′S, 57°01′W) in the 2018/19 and 2019/20 breeding seasons.