

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

Article

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

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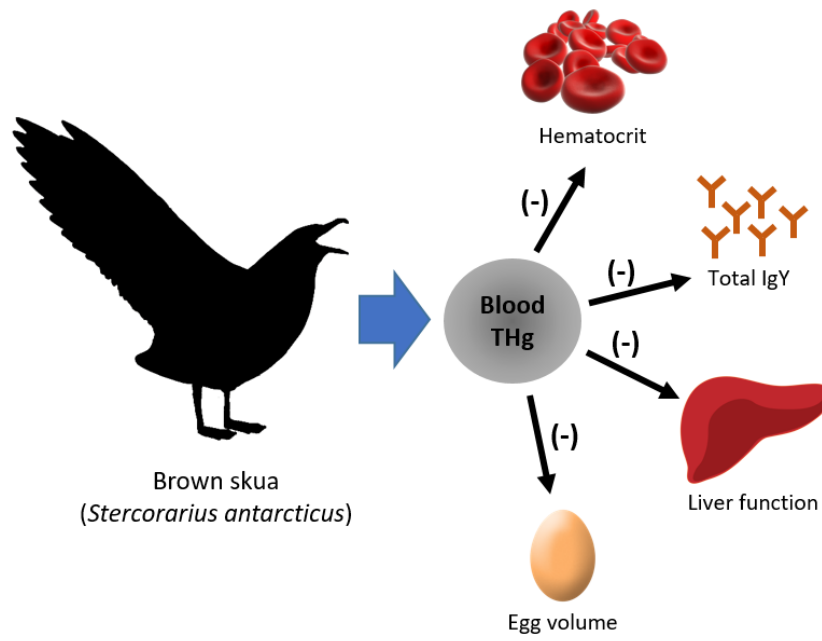
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GRAPHICAL ABSTRACT



25

HIGHLIGHTS

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

30 **ABSTRACT**

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

50
51 **Keywords:** pollution; mercury; seabirds; physiology; reproduction; Antarctica

1. INTRODUCTION

52
53 Mercury (Hg) is a global environmental pollutant that can have deleterious consequences
54 for humans and wildlife (Corsolini 2009; Tan et al., 2009; Driscoll et al., 2013). Although
55 Hg occurs naturally, its availability has increased in marine and terrestrial environments
56 in different organic and inorganic forms (MeHg, inorganic Hg, etc) (Aronson et al., 2011).
57 The gaseous, elemental form of Hg (Hg^0) can spread through atmospheric transport from
58 emission sources to distant regions (Calle et al., 2015). Once deposited in marine
59 environments, the inorganic form of Hg (Hg^{II}) is methylated by microorganisms to the
60 more toxic form, methyl-Hg (MeHg, $[\text{CH}_3\text{Hg}]^+$). MeHg bioaccumulates within the tissues
61 of marine organisms and biomagnifies through marine food webs (Bargagli 2008;
62 Driscoll et al., 2013; Ibañez et al., 2022a; Matias et al., 2022). Seabirds, which are
63 typically long-lived and feed at high trophic positions, are potentially at risk of
64 accumulating high Hg levels in their tissues from dietary exposure (Bearhop et al., 2000;
65 Bargagli 2008; Tavares et al., 2013; McKenzie et al., 2021; Mills et al., 2020; 2022;
66 Ibañez et al., 2022a).

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

78 Hg is a neurological, immune and endocrine disruptor (Tartu et al., 2013; Whitney and
79 Cristol 2017), and, ultimately, can Hg contamination can have short- or long-term fitness
80 consequences for seabirds (Bustnes et al., 2007; Roos et al., 2012; Dietz et al., 2019;
81 Chételat et al., 2020; Mills et al., 2020; Goutte et al., 2014a, 2014b). Hg contamination
82 may also negatively impact body condition (Tan et al., 2009; Ackerman et al., 2016;
83 Chételat et al., 2020), although, in the wild, body condition indices are unreliable

84 indicators of Hg sublethal effects (Carravieri et al., 2022). Although many studies have
85 examined inter- and intraspecific variation in Hg contamination of seabirds in the
86 Southern Ocean (Carravieri et al., 2014a; 2016; 2017; Mills et al., 2020; 2022; Quillfeldt
87 et al., 2023), few have tested for relationships with fitness parameters. Indeed, these have
88 only been investigated for the wandering albatross (*Diomedea exulans*) (Tavares et al.,
89 2013; Carravieri et al., 2014b; 2014c; Goutte et al., 2014a; Bustamante et al., 2016),
90 Antarctic petrel (*Thalassoica antarctica*) (Carravieri et al., 2018; 2021), grey-headed
91 albatross (*Thalassarche chrysostoma*) (Mills et al., 2020), and brown skua (*Stercorarius*
92 *antarcticus*) (Ibañez et al., 2022a) and south polar skua (*S. maccormicki*) (Goutte et al.,
93 2014b).

94 Brown skuas breed on the Antarctic continent and sub-Antarctic islands and are
95 opportunistic predators that feed on a wide diversity of prey in both terrestrial and marine
96 environments (Reinhardt et al., 2000; Phillips et al., 2004; Ritz et al., 2008; Carneiro et
97 al., 2015; Graña Grilli and Montalti 2015; Borghello et al., 2019; Ibañez et al., 2022b).
98 As the brown skua is a migratory seabird, birds are exposed to pollutants not just during
99 the breeding season but also in the nonbreeding season when they visit regions with
100 higher anthropogenic pressure (Albert et al., 2022). At Bahía Esperanza/Hope Bay–
101 located on the Antarctic Peninsula–brown skuas breed close to large colonies of Adélie
102 penguins (*Pygoscelis adeliae*) and gentoo penguins (*Pygoscelis papua*). They feed mainly
103 on penguins and to a lesser extent on marine prey (e.g., fishes and invertebrates)
104 (Borghello et al., 2019; Ibañez et al., 2022b). Macro-plastics have been found recently in
105 skua diet samples collected during the breeding season (Ibañez et al., 2020), which may
106 represent a possible route for chemical pollutants such as Hg (Hamilton et al., 2023).
107 Overall, there has been little research concerning the physiological effects of Hg
108 contamination on Antarctic seabirds (Carravieri et al., 2021; Goutte et al., 2014), and
109 more studies are needed to understand the potential sublethal effects. In the present study,
110 we focus on the sublethal effects of Hg contamination of brown skuas. Total Hg (the sum
111 of inorganic and organic Hg) concentration was measured in red blood cells of brown
112 skuas at Esperanza/Hope Bay. Our aims were to: (i) compare blood THg concentrations
113 between two breeding seasons and sexes; and (ii) relate blood THg concentrations to
114 markers of energy metabolism, immunocompetence and liver function, and to egg
115 volume.

116

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 $0.00048 \times \text{length (mm)} \times \text{breadth (mm)}^2$ (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 $\mu\text{g g}^{-1}$ dw. Accuracy was tested using certified reference material (CRM; dogfish liver DOLT-5, NRC, Canada; certified Hg concentration: $0.44 \pm 0.18 \mu\text{g g}^{-1}$ dw) every 10 samples. Recovery of the CRM was $97.8 \pm 1.7\%$. 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 $0.005 \mu\text{g g}^{-1}$ dw.

149

150

2.3. Hematological determinations

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

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

168 To determine the hematocrit value a heparinized capillary was filled in the laboratory
169 with 100 µl of blood from the Eppendorf tube that contained heparinized blood obtained
170 in the field. The capillary was then centrifuged at 5000 rpm for 15 min (Ibañez et al.,
171 2015), and a digital caliper used to measure the length (mm) of the red blood cell fraction
172 and the total blood volume. Hematocrit values are presented as a percentage of total
173 volume.

174

175

2.4. Statistical analysis

176 Data were analysed using R (R Core Team 2015). Blood THg concentrations,
177 physiological parameters and egg volumes were checked for normality and homogeneity
178 of variances using Shapiro-Wilk and Levene's tests, respectively. Differences in blood
179 THg between sexes and seasons were tested using general linear mixed models (GLMMs;
180 Gaussian distribution and identity link function), with the individual identity and the

181 breeding pair included as random effects to account for partners potentially being more
182 similar than non-partners in blood THg concentrations. Relationships between
183 physiological markers (total proteins, uric acid, triacylglycerol, cholesterol, hematocrit,
184 albumin, IgY, GOT and GPT), egg volume and blood THg concentrations were also
185 tested using GLMMs, with sex (except in the case of egg volume) and season included as
186 covariates, and individual identity included as a random effect. All GLMMs were fitted
187 using the “nlme” package in R (Pinheiro et al. 2017).

188

189

3. RESULTS

190

3.1. Sex and annual variation in Hg contamination

191 Detectable blood THg concentrations were found in all samples from brown skuas at
192 Bahía Esperanza/Hope Bay in 2018/19 (mean \pm SD, $0.73 \pm 0.22 \mu\text{g g}^{-1}$ dw) and 2019/20
193 ($0.91 \pm 0.44 \mu\text{g g}^{-1}$ dw) (Table 1). There were no significant differences in blood THg
194 concentrations (log-transformed) between sexes (est = 0.23, p = 0.48) or seasons (est =
195 0.19, p = 0.56) (Table 1).

196

197

3.2. Physiological markers and egg volume

198 Physiological parameters, in particular immune and hepatic functions, did not differ
199 between sexes (all p > 0.05), but were negatively associated with blood THg
200 concentrations. Significant negative relationships were found between albumin (est = -
201 0.31, p < 0.0001), hematocrit (%) (est = -9.27, p < 0.0001) and IgY (est = -0.06, p < 0.05)
202 and blood THg concentrations (Fig. 2; Table 2). A significant positive relationship was
203 found between GPT activity and blood THg concentrations (est = 13.10, p < 0.01) (Fig.
204 2; Table 2). Mean albumin levels differed significantly between seasons (est = 0.31, p <
205 0.0001). Triacylglycerol levels also varied annually (est = 55.89, p < 0.001) and were
206 unrelated to blood THg concentrations (est = -11.04, p = 0.55) (Table 2). Uric acid, total
207 proteins, cholesterol, glucose and GOT show no relationship with sex, season or blood
208 THg (Table 2). Egg volume (mm^3) showed a significant negative relationship with blood
209 THg concentrations (est = -16.40, p < 0.05) (Fig. 3), and did not differ significantly
210 between seasons (est = 4.25, p = 0.19).

211

212

4. DISCUSSION

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

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

224

225 4.1. Impacts of Hg contamination on immunocompetence

226 Hg is often associated with immunosuppressive effects at sublethal levels, but mostly in
227 captive studies (Fallacara et al., 2011; Kenow et al., 2007; Lewis et al., 2013a). Despite
228 the relatively low concentrations (initially suggesting a low risk of MeHg toxicity;
229 Ackerman et al., 2016), IgY and hematocrit were negatively related to blood THg
230 concentrations in our study (Fig. 2). These results indicate a negative impact of Hg
231 contamination on the immune status of brown skuas. Our results agree with previous
232 studies on captive zebra finches (*Taeniopygia guttata*) that have shown negative effects
233 of Hg exposure on B-cell proliferation (Lewis 2012; Lewis et al., 2013). Also, impaired
234 macrophage phagocytosis was related to high Hg levels in black-footed albatrosses
235 (*Phoebastria nigripes*) in the North Pacific (Finkelstein et al., 2007). Hematocrit values
236 decreased with blood THg concentrations, which agrees with the hemolytic and anemia-
237 inducing effects of Hg (Zolla et al., 1997). Erythrocytes are an important target of Hg and
238 the majority of Hg in blood is found in the cellular fraction (~90%) (Bond and Robertson
239 2015). *In vitro* studies have demonstrated that the exposure of erythrocytes to low
240 concentrations of Hg induce structural changes in the external surface of the membrane.
241 These changes are mediated by the translocation of phosphatidylserine to the external
242 surface of the erythrocyte cell membrane as a signal that may prompt cellular apoptosis
243 (Eisele et al., 2006; Lim et al., 2010). Another possible explanation behind the decrease
244 in hematocrit may be an association with lowered renal function, also linked to hemolytic
245 processes (Rivarob et al., 1983; Chitra et al., 2013).

246 Serum IgY and albumin are useful indicators of health in birds (Lumeij 1987; Ibañez et
247 al., 2018). Fitness traits are mediated by hormones including luteinizing hormone, which

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

265

266 *4.2. Impacts of Hg contamination on liver function*

267 GPT was positively related to blood THg concentrations of brown skuas. This suggests
268 that Hg contamination impacted the liver function of brown skuas. The liver is the major
269 organ involved in the biotransformation, metabolism, protein synthesis and detoxification
270 processes, which also reflect its susceptibility to pollutants, leading to tissue damage.
271 GOT and GPT enzymes are considered to be biochemical markers of impaired liver
272 function. Liver-cell damage, such as degeneration and necrosis, may increase GOT and
273 GPT levels (Gowda et al., 2009; Mari et al., 2010; Ibañez et al., 2015; Yang et al., 2015;
274 Choi et al., 2017). The biological mechanism of association between Hg exposure and
275 liver dysfunction is mainly explained by oxidative stress, cell death, and impaired
276 metabolism (Malhi et al., 2010). For instance, in male rodents exposed to Hg, levels of
277 GOT, GPT, and gamma glutamyl-transferase activities were elevated, and tissue damage
278 or necrotic changes observed in most livers (Waddam 2009). Also, histological analyses
279 described degenerative changes and lysed areas in liver parenchyma in Hg-exposed
280 zebrafish (Maricella et al., 2016). Hg exposure in zebrafish induced deregulation of

281 oxidative stress, intrinsic apoptotic pathways, and resulting hepatotoxicity through cell
282 death, mitochondrial dysfunction, endocrine disruption, and metabolic disorders (Ung et
283 al., 2010; Maricella et al., 2016). In the spectacled caiman (*Caiman crocodilus*), negative
284 associations between Hg and alkaline phosphatase activity (a liver cytoplasmic enzyme
285 involved in the hepatocytic functions) were observed (Lemaire et al., 2018). Therefore,
286 our results suggest that variation in GPT activity in response to elevated blood Hg levels,
287 may indicate hepatotoxicity even at the low levels of contamination found in Antarctica.

288

289 4.3. Annual variations in Hg contamination and association with physiology

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

303

304 4.4. Impacts of Hg contamination on egg volume

305 Hg contamination of seabirds may induce changes on body condition that ultimately
306 affect breeding fitness and survival (Evers et al., 2008; Labocha and Hayes 2012;
307 Ackerman et al., 2016). Effects of Hg on reproduction can be reflected at different levels
308 including egg neglect (Tartu et al., 2015), lower breeding success (Tartu et al., 2016),
309 reproductive failure (Mills et al., 2020), or population dynamics (Goutte et al., 2014b,
310 Goutte et al., 2015). However, the effects of Hg contamination during the non-breeding
311 season on subsequent breeding success are poorly known. In little auks (*Alle alle*), Fort
312 et al. (2014) suggested a carry-over effect, in that individuals with the highest Hg
313 concentrations laid smaller eggs. In the present study, egg volume was negatively
314 associated with female blood THg concentrations (Fig. 3). Although not significant, THg

315 concentrations were slightly higher in males than females in both seasons (Table 1; Mills
316 et al., 2022; Ibañez et al., 2022a), possibly because egg production provides a route
317 through which females are able to eliminate Hg (Robinson et al. 2012; Ackerman et al.
318 2020). An explanation for the association of smaller eggs laid with higher blood THg
319 concentrations of brown skuas relates to the trophic ecology and Hg contamination during
320 female pre-laying exodus or differences in prey consumption prior to sampling.
321 Therefore, our results support a previous study which also suggested carry-over effects
322 of Hg on the reproduction of great skuas (*Stercorarius skua*) with a specific influence of
323 female winter distribution and Hg contamination on egg volume (Albert et al., 2022).
324 However, this would need to be confirmed in future studies by measuring THg in feathers
325 grown during the nonbreeding season to infer the degree of Hg contamination since the
326 last moult (Fort et al., 2014).

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

343 **5. CONCLUSIONS**

344 Our study demonstrated negative effects of Hg contamination on physiology and breeding
345 parameters in brown skuas on the Antarctic Peninsula. Despite low blood THg
346 concentrations, which are below or similar to those of other Antarctic seabirds, the

347 association between Hg and the physiological and breeding parameters are of concern.
348 The detrimental impact on egg volume highlights the importance of investigating the
349 relationships between blood THg and the hormones that play a role in stress responses
350 and reproductive decisions, as well as, Hg concentration in chicks during development.
351 This is particularly as skuas and other predators in the Antarctic may become more
352 susceptible to pollutants and other environmental stressors, given the evidence for rapid,
353 ongoing climatic changes in the region.

354

355 **CReditT authorship contribution statement**

356 **Andrés E Ibañez:** Conceptualization, Methodology, Investigation, Resources, Formal
357 Analysis, Visualization, Writing – Original Draft. **William F Mills:** Methodology,
358 Investigation, Resources, Writing – Review & Editing. **Paco Bustamante:** Investigation,
359 Writing – Review & Editing. **Lara M Morales:** Investigation, Writing – Review &
360 Editing. **Diego S Torres:** Investigation, Writing – Review & Editing. **Beatriz D´Astek:**
361 Investigation, Resources. **Rocío Mariano-Jelicich:** Investigation, Writing – Review &
362 Editing. **Richard A Phillips:** Conceptualization, Methodology, Investigation, Resources,
363 Supervision, Writing – Review & Editing. **Diego Montalti:** Investigation, Resources,
364 Supervision, Writing – Review & Editing

365

366 **Conflict of interest**

367 The authors declare that they have no known competing financial interests or personal
368 relationships that could have appeared to influence the work reported in this paper.

369

370 **Ethical approval**

371 All applicable international, national, and institutional guidelines for sampling, care and
372 experimental use of animals for the study were followed as established by the Article III,
373 Annex II of the Madrid Protocol, Law 24.216 (Taking, Harmful Intrusion and
374 Introduction of Species) within the framework of the projects evaluated and approved by
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REFERENCES

397 Acero JM, Agraz JL, Aguirre CA (1996) Revisión ambiental de las actividades antárticas
398 en Bahía Esperanza. Instituto Antártico Argentino, pp 1–35.

399

400 Ackerman JT, Eagles-Smith CA, Herzog MP, Hartman CA, Peterson SH, Evers DC,
401 Jackson AK, Elliott JE, Vander Pol SS, Bryan CE (2016) Avian mercury exposure and
402 toxicological risk across western North America: a synthesis. *Sci. Total Environ.* 568:
403 749–769.

404

405 Ackerman JT, Herzog MP, Evers DC, et al. (2020) Synthesis of maternal transfer of
406 mercury in birds: implications for altered toxicity risk. *Environ. Sci. Technol.* 54: 2878–
407 2891.

408

409 Albert C, Renedo M, Bustamante P, Fort J (2019) Using blood and feathers to investigate
410 large-scale Hg contamination in Arctic seabirds: a review. *Environ. Res.* 171: 108588.

411

412 Albert C, Strøm H, Helgason HH, Bråthen VS, Gudmundsson FT, Bustamante P, Fort J
413 (2022) Spatial variations in winter Hg contamination affect egg volume in an Arctic
414 seabird, the great skua (*Stercorarius skua*). *Environ. Pollut.* 314: 120322

415

416 Angelier F, Chastel O (2009) Stress, prolactin and parental investment in birds: a review.
417 *Gen Comp Endocrinol* 163: 142–148.

418

419 Angot H, Dastoor A, De Simone F, Gårdfeldt K, Gencarelli CN, Hedgecock IA, ...
420 Dommergue A (2016) Chemical cycling and deposition of atmospheric mercury in polar
421 regions: review of recent measurements and comparison with models. *Atmosph Chem*
422 *Physics* (16): 10735–10763.

423 Aronson RB, Thatje S, McClintock JB, Hughes KA (2011). Anthropogenic impacts on
424 marine ecosystems in Antarctica. *Ann NY Acad Sci* 1223(1): 82–107.

425 Bargagli R (2008) Environmental contamination in Antarctic ecosystems. *Sci. Total*
426 *Environ.* 400: 212–226.

427

428 Bearhop S, Waldron S, Thompson DR, Furness RW (2000b) Bioamplification of mercury
429 in great skua *Catharacta skua* chicks: the influence of trophic status as determined by
430 stable isotope signatures of blood and feathers. *Mar. Pollut. Bull.* 40: 181–185.

431

432 Bearhop S, Ruxton GD, Furness RW (2000c) Dynamics of mercury in blood and feathers
433 of great skuas. *Environ. Toxicol. Chem.* 19: 1638–1643.

434

435 Bond AL & GJ Robertson (2015) Mercury concentrations in multiple tissues of Arctic
436 Iceland Gulls (*Larus glaucooides*) wintering in Newfoundland. *Arctic Sci.* 1(1): 1-8.

437

438 Borghello P, Torres DS, Montalti D, Ibañez AE (2019) Diet of the Brown Skua
439 (*Stercorarius antarcticus lonnbergi*) at Hope Bay, Antarctic Peninsula: differences
440 between breeders and non-breeders. *Polar Biol.* 42: 385-394.

441

442 Braune BM, Gaston AJ, Hobson KA, Gilchrist HG, Mallory ML (2014) Changes in food
443 web structure alter trends of mercury uptake at two seabird colonies in the Canadian
444 Arctic. *Environ. Sci. Technol.* 48: 13246–13252.

445

446 Bustamante P, Carravieri A, Goutte A, Barbraud C, Delord K, Chastel O, Weimerskirch
447 H, Cherel Y (2016) High feather mercury concentrations in the wandering albatross are
448 related to sex, breeding status and trophic ecology with no demographic consequences.
449 *Environ. Res.* 144: 1–10.

450

451 Bustnes JO, Tveraa T, Varpe Ø, Henden JA, Skaare JU (2007) Reproductive performance
452 and organochlorine pollutants in an Antarctic marine top predator: the south polar skua.
453 *Environ. Int.* 33: 911–918.

454

455 Carneiro APB, Manica A, Trivelpiece WZ, Phillips RA (2015) Flexibility in foraging
456 strategies of Brown skuas in response to local and seasonal dietary constraints. *J.*
457 *Ornithol.* 156:625–633.

458

459 Carneiro APB, Manica A, Clay TA, Silk JRD, King M, Phillips RA (2016) Consistency
460 in migration strategies and habitat preferences of brown skuas over two winters, a decade
461 apart. *Mar. Ecol. Prog. Ser.* 553:267-281.

462

463 Carravieri A, Bustamante P, Tartu S, Meillère A, Labadie P, Budzinski H, Peluhet L,
464 Barbraud C, Weimerskirch H, Chastel O, Cherel Y (2014b) Wandering albatrosses
465 document latitudinal variations in the transfer of persistent organic pollutants and
466 mercury to Southern Ocean predators. *Environ. Sci. Technol.* 48: 14746–14755.

467

468 Carravieri A, Cherel Y, Blévin P, Brault-Favrou M, Chastel O, Bustamante P (2014c)
469 Mercury exposure in a large subantarctic avian community. *Environ. Pollut.* 190: 51–57.

470

471 Carravieri A, Cherel Y, Brault-Favrou M, Churlaud C, Peluhet L, Labadie P, Budzinski
472 H, Chastel O, Bustamante P (2017) From Antarctica to the subtropics: Contrasted

473 geographical concentrations of selenium, mercury, and persistent organic pollutants in
474 skua chicks (*Catharacta spp.*). Environ Pollut 228:464-473.
475

476 Carravieri A, Fort J, Tarroux A, Cherel Y, Love OP, Prieur S, Brault-Favrou M,
477 Bustamante P, Descamps S (2018) Mercury exposure and short-term consequences on
478 physiology and reproduction in Antarctic petrels. Environ. Pollut. 237: 824–831.
479

480 Carravieri A, Warner NA, Herzke D, Brault-Favrou M, Tarroux A, Fort J, Bustamante P,
481 Descamps S (2021) Trophic and fitness correlates of mercury and organochlorine
482 compound residues in egg-laying Antarctic petrels. Environ. Res. 193: 110518.
483

484 Carravieri A, Vincze O, Bustamante P, Ackerman JT, Adams EM, Angelier F, Chastel
485 O, Cherel Y, Gilg O, Golubova E, Kitaysky A, Luff K, Seewagen CL, Strøm H, Will AP,
486 Yannic G, Giraudeau M, Fort J (2022) Quantitative meta-analysis reveals no association
487 between mercury and body condition in birds. Biological Reviews, 97(4): 1253-1271.
488

489 Chételat J, Ackerman JT, Eagles-Smith CA, Hebert CE (2020) Methylmercury exposure
490 in wildlife: a review of the ecological and physiological processes affecting contaminant
491 concentrations and their interpretation. Sci. Tot. Environ. 711: 135117.
492

493 Chitra S, Jayaprakash K (2013) Effect of Mercury on blood components of fresh water
494 edible fish *Labeo rohita*. J. Acad. Indus. Res. 1:12.
495

496 Choi J, Bae S, Lim H, Lim JA, Lee YH, Ha M, Kwon HJ (2017) Mercury Exposure in
497 Association With Decrease of Liver Function in Adults: A Longitudinal Study. J. Prev.
498 Med. Public. Health. 50:377-385.
499

500 Colborn T, Vomsaal FS, Soto AM (1993) Developmental effects of endocrine-disrupting
501 chemicals in wildlife and humans. Environ. Health Perspect. 101: 378–384.
502

503 Core Team R (2015) R: A language and environment for statistical computing. R
504 foundation for statistical computing, Viena, Austria. URL: [http:// www.R- proje ct. org/](http://www.R-project.org/).
505

506 Corsolini S (2009) Industrial contaminants in Antarctic biota. J. Chromatogr. A 1216:
507 598–612.
508

509 Cossa D, Heimbürger LE, Lannuzel D, Rintoul SR, Butler ECV, Bowie AR, Averty B,
510 Watson RJ, Remenyi T (2011) Mercury in the Southern Ocean. Geochem. Cosmochim.
511 Acta 75: 4037–4052.
512

513 Cossa D (2013) Methylmercury manufacture. Nature Geoscience (6): 810–811.
514

515 Coutinho AE, Chapman KE (2011) The anti-inflammatory and immunosuppressive
516 effects of glucocorticoids, recent developments and mechanistic insights. Mol Cell
517 Endocrinol 335(1):2-13.
518

519 de Ferro AM, Mota AM, Canário J (2014) Pathways and speciation of mercury in the
520 environmental compartments of Deception Island, Antarctica. Chemosphere (95): 227–
521 233.
522

523 Descamps S, Tarroux A, Varpe Ø, Yoccoz NG, Tveraa T, Lorentsen S-H (2015)
524 Demographic effects of extreme weather events: snow storms, breeding success, and
525 population growth rate in a long-lived Antarctic seabird. *Ecol. Evol.*5(2): 314–325.
526

527 Denardo DF, Licht P (1993) Effects of corticosterone on social behavior of male lizards.
528 *Horm. Behav.* 27: 184–199.
529

530 Dietz R, et al. (2019) Current state of knowledge on biological effects from contaminants
531 on arctic wildlife and fish. *Sci. Total Environ.* 696: 133792.
532

533 Driscoll CT, Mason RP, Chan HM, Jacob DJ, Pirrone N (2013) Mercury as a global
534 pollutant: sources, pathways, and effects. *Environ. Sci. Technol.* 47: 4967–4983.
535

536 Eagles-Smith CA, Silbergeld EK, Basu N, Bustamante P, Diaz-Barriga F, Hopkins WA,
537 Kidd KA, Nyland JF (2018) Modulators of mercury risk to wildlife and humans in the
538 context of rapid global change. *Ambio* 47: 170–197.
539

540 Eisele K, Lang PA, Kempe DS, Klarl BA, Niemoller O, Wieder T, et al. (2006)
541 Stimulation of erythrocyte phosphatidylserine exposure by mercury ions. *Toxicol. Appl.*
542 *Pharmacol.* 210:116–122.
543

544 Evers DC, Savoy LJ, DeSorbo CR, et al. (2008) Adverse effects from environmental
545 mercury loads on breeding common loons. *Ecotoxicology* 17: 69–81.
546

547 Fallacara DM, Halbrook RS, French JB (2011) Toxic effects of dietary methylmercury
548 on immune system development in nestling American Kestrels (*Falco sparverius*).
549 *Environ. Toxicol. Chem.* 30:1328–1337.
550

551 Finkelstein ME, Grasman KA, Croll DA, Tershy BR, Keitt BS, Jarman WM, Smith DR
552 (2007) Contaminant-associated alteration of immune function in black-footed albatross
553 (*Phoebastria nigripes*), a North Pacific predator. *Environ. Toxicol. Chem.* 26: 1896-1903.
554

555 Fort J, Robertson GJ, Gremillet D, Traisnel G, Bustamante P (2014). Spatial
556 ecotoxicology: migratory arctic seabirds are exposed to mercury contamination while
557 overwintering in the Northwest Atlantic. *Environ. Sci. Technol.* 48: 11560–11567.
558

559 Fridolfsson AK, Ellegren H (1999) A simple and universal method for molecular sexing
560 of non-ratite birds. *J. Avian Biol.* 30: 116–121.
561

562 Gionfriddo CM, Tate MT, Wick RR, Schultz MB, Zemla A, Thelen MP, ... Moreau JW
563 (2016) Microbial mercury methylation in Antarctic sea ice. *Nature Microbiology* (1):
564 16127.
565

566 Goutte A, Barbraud C, Meillère A, Carravieri A, Bustamante P, Labadie P, Budzinski H,
567 Delord K, Cherel Y, Weimeskirch H, Chastel O (2014a) Demographic consequences of
568 heavy metals and persistent organic pollutants in a vulnerable long-lived bird, the
569 wandering albatross. *Proc. Roy. Soc. B* 281: 20133313.
570

571 Goutte A, Bustamante P, Barbraud C, Delord K, Weimerskirch H, Chastel O (2014b)
572 Demographic responses to mercury exposure in two closely related Antarctic top
573 predators. *Ecology* 95: 1075–1086.
574

575 Goutte A, Barbraud C, Herzke D, Bustamante P, Angelier F, Tartu S, Clément- Chastel
576 C, Moe B, Bech C, Gabrielsen GW, Bustnes JO, Chastel O (2015) Survival rate and
577 breeding outputs in a high Arctic seabird exposed to legacy persistent organic pollutants
578 and mercury. *Environ. Pollut.* 200: 1–9.
579

580 Goutte A, Meillère A, Barbraud C, Budzinski H, Labadie P, Peluhet L, Weimerskirch H,
581 Delord K, Chastel O (2018) Demographic, endocrine and behavioral responses to mirex
582 in the south polar skua. *Sci. Total Environ.* 631–632: 317–325.
583

584 Guillette Jr LJ, Cree A, Rooney AA (1995) Biology of stress: interactions with
585 reproduction, immunology and intermediary metabolism. In: Warwirck, C., Frye, F. L.,
586 Murphy, J.B. (Eds.), *Health and Welfare of Captive Reptiles*. Chapman and Hall, London,
587 pp. 32–81.
588

589 Gowda S, Desai PB, Hull VV, Math AA, Vernekar SN, Kulkarni SS (2009) A review on
590 laboratory liver function tests. *Pan. Afr. Med. J.* 3:17.
591

592 Graña Grilli M, Montalti D (2015) Variation in diet composition during the breeding
593 cycle of an Antarctic seabird in relation to its breeding chronology and that of its main
594 food resource. *Polar Biol.* 38:643-649.
595

596 Hamilton BM, Baak JE, Vorkamp K, Hammer S, Granberg M, Herzke D, Provencher JF
597 (2023) Plastics as a carrier of chemical additives to the Arctic: possibilities for strategic
598 monitoring across the circumpolar North. *Arctic Sci* 9: 284–296.
599

600 Ibañez AE, Najle R, Larsen K, Montalti D (2015) Hematology, Biochemistry and Serum
601 Protein Analyses of Antarctic and non-Antarctic Skuas. *Waterbirds* 38(2):153-161.
602

603 Ibañez AE, Graña Grilli M, Figueroa A, Pari M, Montalti D (2018) Declining health status
604 of Brown Skua (*Stercorarius antarcticus lonnbergi*) parents and their offspring during
605 chick development. *Polar Biol.* 41:193–200.
606

607 Ibanez AE, Morales LM, Torres DS, Borghello P, Haidr NS, Montalti D (2020) Plastic
608 ingestion risk is related to the anthropogenic activity and breeding stage in an Antarctic
609 top predator seabird species. *Mar. Pollut. Bull.* 157: 111351.
610

611 Ibañez AE, Mills WF, Bustamante P, McGill RAR, Morales LM, Palacio FX, Torres DS,
612 Haidr NS, Mariano-Jelicich R, Phillips RA, Montalti D (2022a) Variation in blood
613 mercury concentrations in brown skuas (*Stercorarius antarcticus*) is related to trophic
614 ecology but not breeding success or adult body condition. *Mar. Pollut. Bull.* 181: 113919.
615

616 Ibañez AE, Morales LM, Torres DS, Borghello P, Montalti D (2022b) Pellet analysis
617 evidences flexibility in the diet of Brown Skua (*Stercorarius antarcticus*) during breeding
618 at Esperanza/Hope Bay (Antarctic Peninsula). *Polar Biol.* 45: 419–426.
619

620 Keane S, Bernaudat L, Davis KJ, Stylo M, Mutemeri N, Singo P, Twala P, Mutemeri I,
621 Nakafeero A, Etui ID (2023) Mercury and artisanal and small-scale gold mining: review
622 of global use estimates and considerations for promoting mercury-free alternatives.
623 *Ambio* 52: 833–852.
624

625 Kenow KP, Grasman KA, Hines RK, Meyer MW, Gendron-Fitzpatrick A, Spalding MG
626 et al (2007) Effects of methylmercury exposure on the immune function of juvenile
627 common loons (*Gavia immer*). *Environ. Toxicol. Chem.* 26:1460–1469.
628

629 Labocha MK, Hayes JP (2012) Morphometric indices of body condition in birds: a
630 review. *J Ornithol.* 153: 1–22.
631

632 Lemaire J, Bustamante P, Mangione R, Marquis O, Churlaud C, Brault-Favrou M,
633 Parenteau C, Brischoux F (2021) Lead, mercury, and selenium alter physiological
634 functions in wild caimans (*Caiman crocodilus*). *Environ. Pollut.* 286: 117549.
635

636 Lewis CA (2012) Sublethal effects of methyl mercury on the songbird immune response:
637 An experimental study. Master's thesis, The College of William and Mary, Virginia.
638

639 Lewis CA, Cristol DA, Swaddle JP, Varian-Ramos CW, Zwollo P (2013) Decreased
640 immune response in zebra finches exposed to sublethal doses of mercury. *Arch. Environ.*
641 *Contam. Toxicol.* 64:327–336.
642

643 Lim KM, Kim S, Noh JY, Kim K, Jang WH, Bae ON, Chung SM, Chung JH (2010)
644 Low-Level Mercury Can Enhance Procoagulant Activity of Erythrocytes: A New
645 Contributing Factor for Mercury-Related Thrombotic Disease. *Environ. Health. Persp.*
646 118: 7.
647

648 Lim C, Kim N, Lee J, Yoon Y (2022) Potential of Adsorption of Diverse Environmental
649 Contaminants onto Microplastics. *Water* 14: 4086.
650

651 Lumeij JT (1987) The diagnostic value of plasma proteins and non-protein nitrogen
652 substances in birds. *Vet Q* 9:262–268.
653

654 Macirella R, Guardia A, Pellegrino D, Bernabò I, Tronci V, Ebbesson LO, et al. (2016)
655 Effects of two sublethal concentrations of mercury chloride on the morphology and
656 metallothionein activity in the liver of zebrafish (*Danio rerio*). *Int. J. Mol. Sci.* 17(3):361.
657

658 Manceau A, Gaillot A-C, Glatzel P, et al. (2021) *In Vivo* Formation of HgSe
659 Nanoparticles and Hg–Tetraselenolate Complex from Methylmercury in Seabirds:
660 Implications for the Hg–Se Antagonism. *Environ Sci Tech* 55 (3): 1515-1526.
661

662 Malhi H, Guicciardi ME, Gores GJ (2010) Hepatocyte death: a clear and present danger.
663 *Physiol Rev.* 90(3):1165–1194.
664

665 Marí M, Colell A, Morales A, von Montfort C, Garcia-Ruiz C, Fernández-Checa JC
666 (2010) Redox control of liver function in health and disease. *Antioxid. Redox. Signal.*
667 (11):1295–1331.
668

669 Martínez J, Tomás G, Merino S, Arriero E, Moreno J (2003) Detection of serum
670 immunoglobulins in wild birds by direct ELISA: a methodological study to validate the
671 technique in different species using antichickens antibodies. *Funct. Ecol.* 17:700–706.
672

673 Matias RS, Guímaro HR, Bustamante P, Seco J, Chipev N, Fragão J, Tavares S, Ceia FR,
674 Pereira ME, Barbosa A, Xavier JC (2022) Mercury biomagnification in an Antarctic food
675 web of the Antarctic Peninsula. *Environ. Pollut.* 304: 119199.
676

677 McKenzie AC, Silvestro AM, Marti LJ, Emslie SD (2021), Intraspecific Variation in
678 Mercury, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ Among 3 Adélie Penguin (*Pygoscelis adeliae*) Populations in
679 the Northern Antarctic Peninsula Region. *Environ. Toxicol. Chem.* 40: 2791-2801.
680

681 Meyer E, Eagles-Smith CA, Sparling D, Blumenshine S (2014) Mercury exposure
682 associated with altered plasma thyroid hormones in the declining Western Pond Turtle
683 (*Emys marmorata*) from California Mountain Streams. *Environ. Sci. Technol.* 48: 2989–
684 2996.
685

686 Mills WF, Bustamante P, McGill RAR, Anderson ORJ, Bearhop S, Cherel Y, Votier SC,
687 Phillips RA (2020) Mercury exposure in an endangered seabird: long-term changes and
688 relationships with trophic ecology and breeding success. *Proc. R. Soc. B* 287:20202683.
689

690 Mills WF, Ibañez AE, Bustamante P, Carneiro APB, Bearhop S, Cherel Y, Mariano-
691 Jelicich R, McGill RAR, Montalti D, Votier SC and Phillips RA (2022) Spatial and sex
692 differences in mercury contamination of skuas in the Southern Ocean. *Environ. Pollut.*
693 297: 118841.
694

695 Monclús L, Shore RF, Krone O (2020) Lead contamination in raptors in Europe: a
696 systematic review and meta-analysis. *Sci. Total Environ.* 748: 141437.
697
698

699 Phillips RA, Dawson DA, Ross DJ (2002) Mating patterns and reversed size dimorphism
700 in Southern skuas (*Stercorarius lonnbergi*). *Auk* 119: 858–863.
701

702 Phillips RA, Phalan B, Forster IP (2004) Diet and long-term changes in population size
703 and productivity of brown skuas *Catharacta antarctica lonnbergi* at Bird Island, South
704 Georgia. *Polar Biol.* 27: 555–561.
705

706 Pinheiro J, Bates D, DebRoy S, Sarkar D, Eispack S, Heisterkamp, B. Van Willigen
707 (2017) Package “nlme”. *Linear and Nonlinear Mixed Effects Models*.
708

709 Provencher JF, Forbes MR, Hennin HL, Love OP, Braune BM, Mallory ML, Gilchrist
710 HG (2016) Implications of mercury and lead concentrations on breeding physiology and
711 phenology in an Arctic bird. *Environ. Pollut.* 218: 1014e1022.
712

713 Quillfeldt P, Bedolla-Guzmán Y, Libertelli MM, Cherel Y, Massaro M, Bustamante P
714 (2023) Mercury in Ten Storm-Petrel Populations from the Antarctic to the Subtropics.
715 *Arch Environ Cont Toxicol* 85:55–72.
716

717 Reinhardt K, Hahn S, Peter HU, Wemhoff H (2000) A review of the diets of Southern
718 Hemisphere skuas. *Mar. Ornithol.* 28:7–19.

719
720 Renedo M, Amouroux D, Duval B, Carravieri A, Tessier E, Barre J, Beraïl S, Pedrero Z,
721 Cherel Y, Bustamante P (2018a) Seabird tissues as efficient biomonitoring tools for Hg
722 isotopic investigations: Implications of using blood and feathers from chicks and adults.
723 Environ. Sci. Technol. 52: 4227–4234.
724
725 Renedo M, Amouroux D, Pedrero Z, Bustamante P, Cherel Y (2018b) Identification of
726 sources and bioaccumulation pathways of MeHg in subantarctic penguins: a stable
727 isotopic investigation. Sci. Rep. 8(1): 8865.
728
729 Renedo M, Bustamante P, Cherel Y, Pedrero Z, Tessier E, Amouroux D (2020) A
730 ‘seabird-eye’ on mercury stable isotopes and cycling in the Southern Ocean. Sci. Total
731 Environ. 742: 140499.
732
733 Ribarov SR, Benov LC, Benchev IC (1983) On the Mechanism of Mercury-Induced
734 Hemolysis. Gen. Physiol. Biophys.2: 81—84.
735
736 Ritz M, Millar C, Miller G, Phillips R, Ryan P, Sternkopf V, Liebers-Helbig D, Hans-
737 Ulrich P (2008) Phylogeography of the southern skua complex- rapid colonization of the
738 southern hemisphere during a glacial period and reticulate evolution. Mol Phylogenet
739 Evol 49:292–303.
740
741 Robinson SA, Lajeunesse MJ, Forbes MR (2012) Sex differences in mercury
742 contamination of birds: testing multiple hypotheses with meta-analysis. Environ.
743 Sci. Technol. 46: 7094–7101.
744
745 Roos AM, Backlin BMVM, Helander BO, Rigét FF, Eriksson UC (2012) Improved
746 reproductive success in otters (*Lutra lutra*), grey seals (*Halichoerus grypus*) and sea
747 eagles (*Haliaeetus albicilla*) from Sweden in relation to concentrations of organochlorine
748 contaminants. Environ. Pollut. 170: 268–275.
749
750 Tan SW, Meiller JC, Mahaffey KR (2009) The endocrine effects of mercury in humans
751 and wildlife. Crit. Rev. Toxicol. 39: 228–269.
752
753 Tartu S, Goutte A, Bustamante P, Angelier F, Moe B, Clément-Chastel C, Bech C,
754 Gabrielsen GW, Bustnes JO, Chastel O (2013) To breed or not to breed: endocrine
755 response to mercury contamination by an Arctic seabird. Biol. Lett. 9: 20130317.
756
757 Tartu S, Angelier F, Wingfield JC, Bustamante P, Labadie P, Budzinski H, Weimerskirch
758 H, Bustnes JO, Chastel O (2015) Corticosterone, prolactin and egg neglect behavior in
759 relation to mercury and legacy POPs in a long-lived Antarctic bird. Sci. Total Environ.
760 505: 180–188.
761
762 Tartu S, Bustamante P, Angelier F, Lendvai AZ, Moe B, Blévin P, Bech C, Gabrielsen
763 GW, Bustnes JO, Chastel O (2016) Mercury exposure, stress and prolactin secretion in
764 an Arctic seabird: an experimental study. Funct. Ecol. 30: 596–604.
765
766 Tavares S, Xavier JC, Phillips RA, Pereira ME, Pardal MA (2013) Influence of age, sex
767 and breeding status on mercury accumulation patterns in the wandering albatross
768 *Diomedea exulans*. Environ. Pollut. 181: 315–320.

769
770 Ung CY, Lam SH, Hlaing MM, Winata CL, Korzh S, Mathavan S, et al. (2010) Mercury-
771 induced hepatotoxicity in zebrafish: *in vivo* mechanistic insights from transcriptome
772 analysis, phenotype anchoring and targeted gene expression validation. BMC Genomics.
773 11: 212.
774
775 Wadaan MA (2009) Effects of mercury exposure on blood chemistry and liver
776 histopathology of male rats. J Pharmacol. Toxicol. 4(3):126-131.
777
778 Whitney MC, Cristol DA (2017) Impacts of sublethal mercury exposure on birds: a
779 detailed review. Rev. Environ. Contam. Toxicol. 244: 113–163.
780
781 Wingfield JC, Sapolsky RM (2003) Reproduction and Resistance to Stress: When and
782 How. Journal of Neuroendocrinology 15: 711–724.
783
784 Yang G, Zhou Z, Cen Y, Gui X, Zeng Q, Ao Y, Li Q, Wang S, Li J, Zhang A (2015)
785 Death receptor and mitochondria-mediated hepatocyte apoptosis underlies liver
786 dysfunction in rats exposed to organic pollutants from drinking water. Drug. Des.
787 Develop. Ther. 9:4719-33.
788
789 Zolla L, Lupidi G, Bellelli A, Amiconi G (1997) Effect of mercuric ions on human
790 erythrocytes. Relationships between hypotonic swelling and cell aggregation. Biochim.
791 Biophys. Acta. 1328:273–280.
792

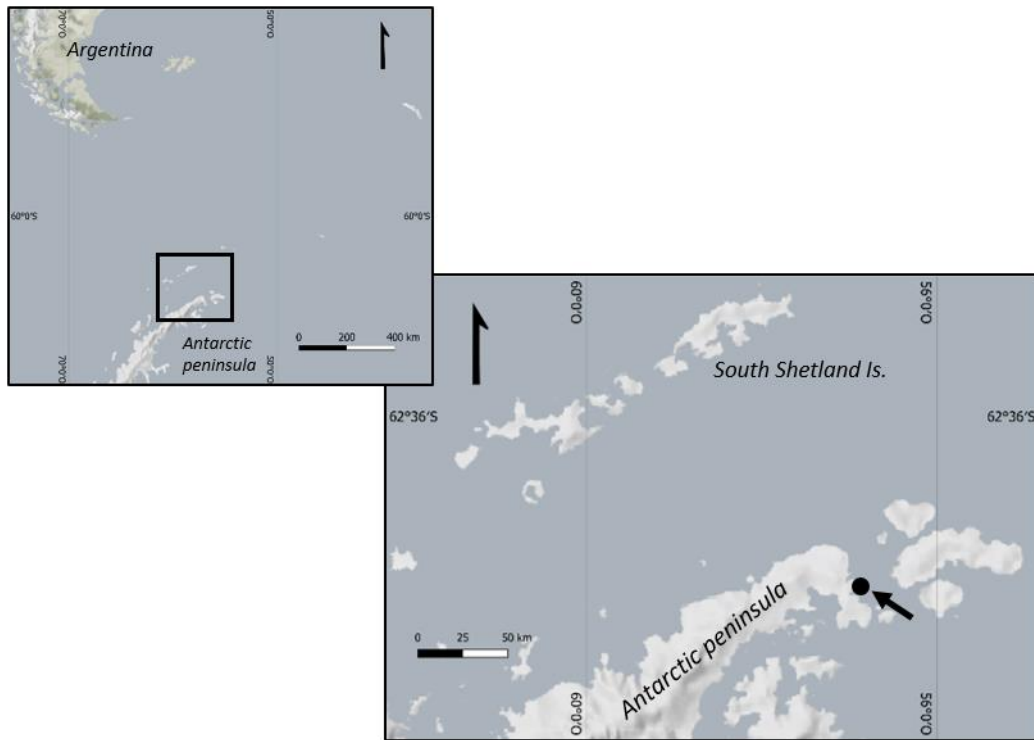


Figure 1. Location of the study site (black circle), Esperanza/Hope Bay, Antarctic Peninsula ($63^{\circ}24' S$, $57^{\circ}01' W$).

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

Year	<i>N</i>	Sex	THg ($\mu\text{g g}^{-1}$ dw)	Range ($\mu\text{g g}^{-1}$ 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 \pm 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 ($p < 0.05$).

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

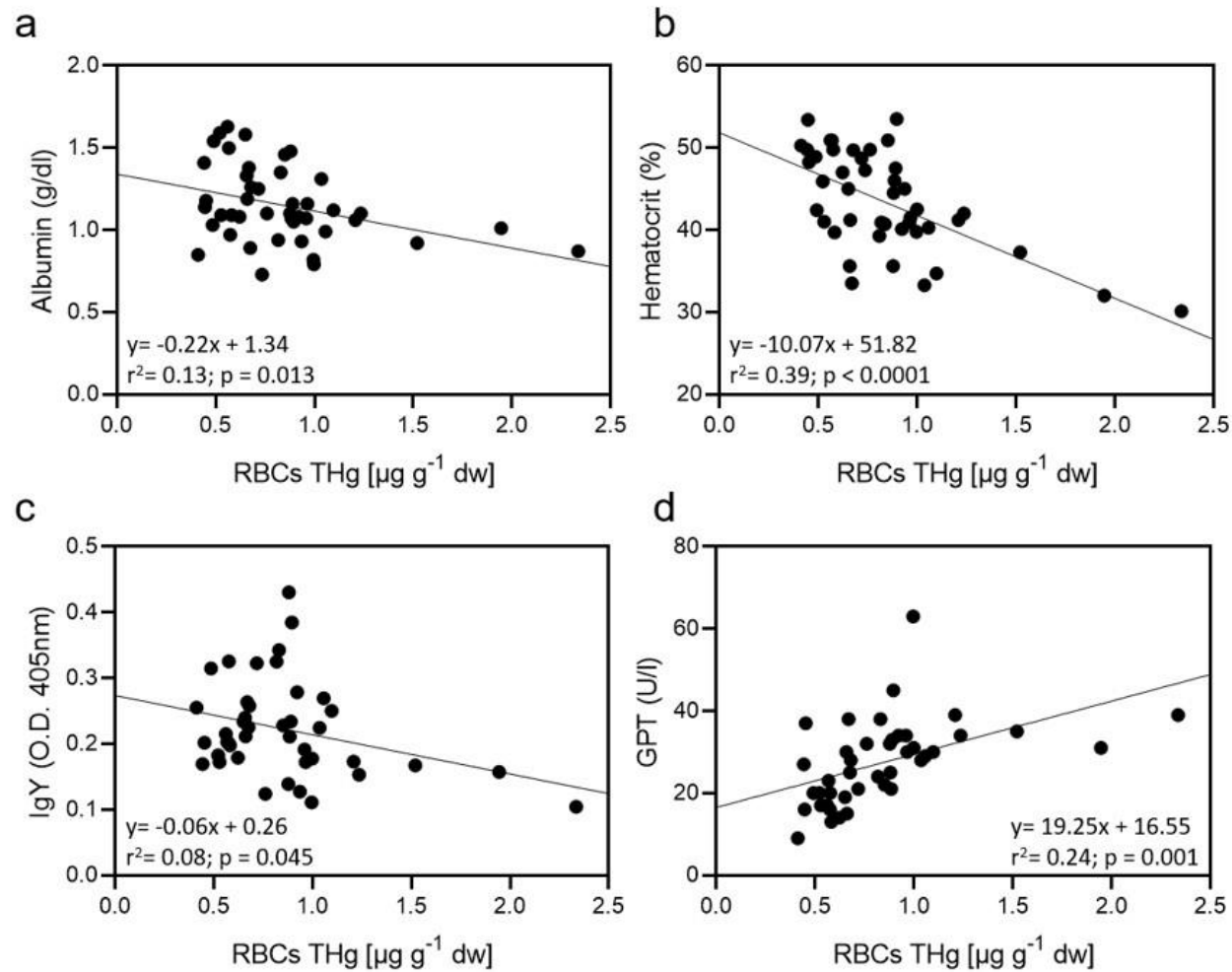


Figure 2. Relationships between red blood cells THg concentrations ($\mu\text{g g}^{-1} \text{ dw}$) and **(a)** albumin (g/dl), **(b)** hematocrit (%), **(c)** IgY (OD 405nm) and **(d)** GPT (U/l) in the blood of adult brown skuas (*Stercorarius antarcticus*) at Esperanza/Hope Bay, Antarctic Peninsula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

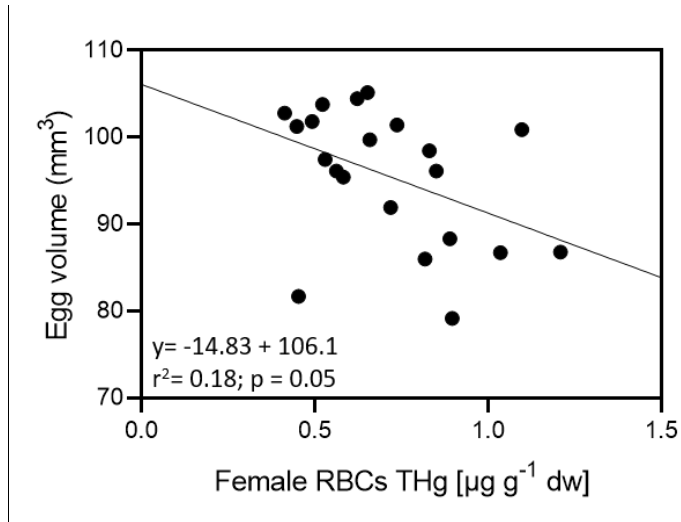


Figure 3. Relationships between red blood cells THg concentrations ($\mu\text{g g}^{-1} \text{ dw}$) and egg volume (mm^3) of female brown skuas (*Stercorarius antarcticus*) at Esperanza/Hope Bay, Antarctic Peninsula ($63^{\circ}24'S$, $57^{\circ}01'W$) in the 2018/19 and 2019/20 breeding seasons.