Corticosterone, prolactin and egg neglect behaviour in relation to mercury and legacy POPs in a long-lived Antarctic bird

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In preparation for: Science of the total environment

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Tartu, S.; Angelier, F.; Wingfield, J.C.; Bustamante, P.; Labadie, P.; Budzinski, H.; Weimerskirch, H.; Bustnes, Jan Ove; Chastel, O.. Corticosterone, prolactin and egg neglect behavior in relation to mercury and legacy POPs in a long-lived Antarctic bird. Science of the Total Environment 2015 ;Volum 505. s. 180-188 DOI 10.1016/j.scitotenv.2014.10.008

Abstract

Seabirds often have high loads of contaminants. These contaminants have endocrine disrupting properties but their relationships with some endocrine mechanisms are still poorly investigated in free-living organisms. This is the case for the stress response which shifts energy investment away from reproduction and redirects it towards survival. In birds, this stress response is achieved through a release of corticosterone and is also accompanied by a decrease in circulating prolactin, an anterior pituitary hormone widely involved in regulating parental cares. We measured blood concentrations of some legacy persistent organic pollutants (POPs) and mercury (Hg) and examined their relationships with the corticosterone and prolactin responses of known-age (9-46 years old) incubating snow petrels (Pagodroma nivea) to a standardized capture/handling stress protocol. In this Antarctic seabird, we also investigated whether high contaminant burden correlates with a higher occurrence of egg neglect, a frequently observed behaviour in snow petrels. POPs and Hg were unrelated to age. Stress-induced corticosterone concentrations were positively related to POPs in both sexes, and stress-induced prolactin concentrations were negatively related to Hg in males. Egg-neglect behaviour was not related to POPs burden, but males with higher Hg concentrations were more likely to neglect their egg. This suggests that in birds, relationships between age and contaminants are complex and that even low to moderate concentrations of POPs and Hg are significantly related to hormonal secretion. In this Antarctic species, exposure to legacy POPs and Hg could make individuals more susceptible to environmental stressors such as ongoing disturbances in polar regions.

Keywords: Snow petrel, Mercury, Persistent organic pollutants, Age, Reproduction

INTRODUCTION

The parental phase is energy-demanding (Drent and Daan 1980) and individuals adopt different life-history strategies in order to cope with environmental stressors such as food shortage, predation or poor weather. In extreme environments, such as Polar Regions, animals often experience harsh and unpredictable environmental conditions, and as a result long-lived organisms such as seabirds may refrain from breeding or desert their brood (e.g Angelier et al. 2007; Goutte et al. 2011a). At the physiological level, the release of glucocorticoid hormones (cortisol, corticosterone: CORT) during stressful events triggers physiological and behavioral adjustments that shift energy investment away from reproduction and redirects it towards self-preservation and hence survival (Angelier and Wingfield, 2013; Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003). Stress hormones have therefore a strong connection to fitness traits such as breeding success, individual quality and survival (Angelier et al. 2009a, 2010; Bonier et al. 2009; Bókony et al. 2009; Breuner et al. 2008; Goutte et al. 2010, 2011b; Kitaysky et al. 1999). Additionally, the hormone prolactin (PRL) can also mediate this life-history trade-off between reproduction and survival in free-living birds (reviewed in Angelier and Chastel 2009). The release of this anterior pituitary hormone stimulates and facilitates parental behaviour such as egg incubation and brood provisioning (Buntin 1996). In response to acute stress, circulating PRL levels has been shown to decrease in several bird species (Angelier et al. 2013; Chastel et al. 2005), and this could ultimately trigger nest desertion if PRL levels remain low during a prolonged period (Angelier et al. 2007; 2009b; Angelier and Chastel 2009; Heidinger et al. 2010). Importantly, this decrease in PRL levels varies between individuals and life-history stages, suggesting that birds can attenuate their PRL response to acute stress to ensure that reproduction is not inhibited when the fitness value of the current reproductive event is high (the 'brood value hypothesis'; Bókony et al. 2009; Lendvai et al. 2007). Thus, both CORT and PRL are very likely to mediate parental effort and parental

68 investment in birds (Angelier et al. 2007, 2009b, 2013; Chastel et al. 2005; Criscuolo et al.
69 2005; Groscolas et al. 2008; Koch et al. 2004) and any disruption of these major endocrine
70 cascades may alter the ability of an individual to adjust reproductive decisions to
71 environmental conditions (Jenssen 2005; Tartu et al. 2013).

72 In addition to extreme environmental conditions, climate change and anthropogenic
73 disturbances (Clarke and Harris 2003; Moline et al. 2008; Smetacek and Nicol 2005), polar
74 species are subjected to environmental pollution. Indeed, despite their remote location, polar
75 areas are the fall-out region of contaminants which undergo long range transport such as
76 persistent organic pollutants (POPs) and heavy metals (e.g. mercury: Hg). Indeed, because of
77 climate characteristics, contaminants accumulate in the polar environment, where they may be
78 bio-accumulated and for some compounds bio-magnified (Bargagli 2008; Gordeev 2002;
79 Risebrough et al. 1976; Wania and Mackay 1996; Wania 2003). Moreover, long-lived
80 organisms are thought to be highly sensitive to contaminants (Rowe 2008), but there are
81 surprisingly few data on the effect of age on contaminant levels, and it is not clear if seabirds
82 accumulate POPs with increasing age (Bustnes et al., 2003a).

83 Marine apex predators, such as seabirds, are particularly exposed (Gabrielsen 2007; Rowe
84 2008; van den Brink et al. 1997) and several studies have reported breeding impairments in
85 highly polluted seabirds (Bustnes et al. 2001, 2003b, 2007; Tartu et al. 2013; Verboven et al.
86 2009). Such breeding impairments could originate from the ability of contaminants to act as
87 endocrine disruptors and thus, to alter the functioning of endocrine axes (Guillette and
88 Gunderson 2001; Ottinger et al. 2002, 2013; Tan et al. 2009; Tartu et al. 2013, 2014; Tyler et
89 al. 1998). Experimental studies have documented some effects of chemicals on
90 glucocorticoids (Love et al. 2003; Odermatt and Gumy, 2008), but the effects of
91 contaminants on stress hormones in free-living organisms such as seabirds have rarely been
92 studied (Bergman et al. 2013; Nordstad et al. 2012; Tartu et al. 2014; Verboven et al. 2010). It

93 is therefore difficult to draw a general pattern of the relationships between contaminants and
94 stress hormones.

95 Regarding the effects of contaminants on PRL, the knowledge is even poorer and only one
96 study (Verreault et al. 2008) has addressed relationships between PRL secretion and POPs. In
97 glaucous gulls (Larus hyperboreus) baseline PRL levels and the rate of decrease in PRL levels
98 tended to vary negatively with organohalogen contaminants in males only (Verreault et al.
99 2008). Furthermore, numerous compounds are potential environmental contaminants (e.g.
100 heavy metals, POPs), which may have different effects on hormones of the hypothalamo-
101 pituitary-adrenal (HPA) axis, such as CORT but also PRL secretion. There is thus a need to
102 determine whether different environmental contaminants can disrupt these hormones in free-
103 living organisms. The aim of this study was to investigate the potential roles of environmental
104 contaminants such as Hg and some legacy POPs (i.e. polychlorinated biphenyls: PCBs;
105 organochlorine pesticides: OCPs; and polybrominated diphenyl ethers: PBDEs) on two major
106 endocrine mechanisms: stress hormones from the HPA axis: CORT, and a key pituitary
107 'parental hormone': PRL. The snow petrel (Pagodroma nivea) a contaminated Antarctic
108 seabird (Xie et al. 2008; Corsolini et al. 2011; Goutte et al. 2013, Tartu et al. unpublished
109 data) provides an ideal species to address these questions. In this long-lived species (until ~50
110 years old, Chastel et al. 1993), CORT and PRL responses to acute stress are modulated in
111 relation to parental investment and incubation commitment (Angelier et al. 2007; Goutte et al.
112 2011c). For example, low stress-induced PRL levels are associated with a high probability of
113 egg neglect, a frequently observed behaviour in snow petrels (Angelier et al. 2007). Further,
114 thanks to an exceptional long-term banding survey (1964-present; Barbraud and
115 Weimerskirch 2001; Chastel, et al. 1993), many snow petrels are of known age, making it
116 possible to address the effect of age on contaminant burden.

In that context, we investigated if POPs and/or Hg concentrations were related to 1) age, 2) CORT and/or PRL secretion and 3) parenting through egg-neglect behaviour. We predicted that POPs and/or Hg: 1) would increase with increasing age as a result of bio-accumulation; 2) would increase CORT and decrease PRL secretion; 3) would be higher in individuals that neglected their egg.

MATERIALS AND METHODS

Ethics statement

Animals were cared for in accordance with the guidelines of the ethics committee of the Institut Polaire Français Paul Emile Victor (IPEV) that specifically approved this study (Program no. 109, H. Weimerskirch).

Study site, blood sampling and body-condition

Snow petrels are Antarctic seabirds with a delayed sexual maturity (~10 years of age), a low fecundity (one egg per clutch and a maximum of one clutch per year) and a long lifespan (~50 years old) (Chastel et al. 1993). Adult males and females were handled during the 2010 late incubation period (8-21 January). A total of 49 birds (27 males and 22 females) were caught in 49 different nests and age was known for 47 of them (9-46 years old). Birds were captured by hand and were then bled according to the standardized capture/restraint stress protocol described by Wingfield (1994). Immediately after capture (i.e. within 3 min), an initial blood sample (300 µl) was collected from the alar vein with a 1-mL heparinized syringe and a 25-gauge needle. These initial blood samples were considered to reflect baseline levels of CORT and PRL (Chastel et al. 2005; Romero and Reed 2005; thereafter called 'baseline' sample). After collection of the initial blood samples birds were placed into cloth bags, and a subsequent sample (300 µl) was collected 30 min after capture (thereafter called 'stress-induced' sample). During handling of the adult birds, their eggs were covered with cotton and

kept warm. After these blood samples, each bird was put back in its nest. Snow petrels are tame and usually resume parental duties as soon as returned to their nest (e.g. Angelier et al. 2007). After this acute stress protocol, petrels were left undisturbed at their nest for 20 min and were then captured again and blood sampled within 3 min of recapture (thereafter called 'post-stress' sample) to monitor how quickly hormone levels may return to baseline after a stressor. This blood sample was taken before CORT and PRL concentrations returned to normal, allowing us to effectively monitor the stress recovery. All birds were weighed to the nearest 2 g using a spring balance and their skull length (head + bill) was measured to the nearest 0.5 mm. Body condition index (thereafter 'body condition') was calculated as the residuals between body mass and skull length (regression: $F_{1,47} = 20.28$, $p < 0.001$, $R^2=0.35$). After capture and blood sample, each nest was monitored twice a day until the manipulated petrel was relieved by its mate. We were therefore able to know whether a bird neglected its egg during the incubation bout following capture/restraint stress protocol (thereafter called 'egg neglect behaviour'). Leaving eggs unattended temporarily is common in Procellariiform birds (Boersma and Wheelwright 1979; Chaurand and Weimerskirch 1994). Distant foraging and unpredictable weather increase the probability to delay an individual's returning to relieve its incubating partner (Boersma and Wheelwright, 1979). Eggs left unattended for a long period are less likely to hatch successfully (Boersma and Wheelwright, 1979, Angelier et al. 2007). In snow petrels were egg-neglect is often observed (Angelier et al; 2007), both parents incubate the single egg four bouts lasting ca. 4 to 8 days while the partner is feeding at sea (Ryan and Atkins 1989). In two sampled birds, the egg was predated during the incubation bout following the capture/restraint stress protocol. Egg-neglect data were available for 47 birds.

Molecular sexing and hormone assay

Blood samples were centrifuged, and plasma was decanted and stored at −20°C until assayed. After centrifugation, red cells were kept frozen for molecular sexing as well as for Hg determination. The sex was determined by polymerase chain reaction amplification of part of two highly conserved genes (CHD) present on the sex chromosomes at UMR 7372 - CNRS-Université de La Rochelle, as detailed in Weimerskirch et al. (2005). Plasma concentrations of CORT were determined first by radioimmunoassay at UMR 7372 - CNRS-Université de La Rochelle, as previously described (Lormée et al. 2003). Plasma concentrations of PRL were determined with the remaining plasma by a heterologous radioimmunoassay at UMR 7372 - CNRS-Université de La Rochelle, as detailed in Cherel et al. (1994). The PRL assay has previously been validated in snow petrels (Angelier et al. 2007). All samples were run in one assay for both hormones. To measure intra-assay variation, we included 4 different referents 10 times in the CORT and PRL assays. From this, the intra-assay variation was 6.7% for total CORT and 7.8% for PRL. CORT and PRL concentrations were measured in baseline, stress-induced and post-stress samples.

Organic pollutants determination in plasma

POPs were measured in plasma samples collected from 15 females and 21 males only, since in 13 birds the remaining plasma volumes were too low. The targeted compounds included 7 indicator PCBs (CB-28, -52, -101, -118, -138, -153 and -180), 11 OCPs (HCB, Gamma HCH, Heptachlore, cis-chlordane, trans-nonachlor, 2,4' DDE, 4,4’ DDE, 4,4' DDD, 2,4' DDT, 4,4' DDT and mirex) and two PBDE (BDE-47 and BDE-99). Certified solutions containing all analytes in isooctane at 2 $ng \bullet \mu L^{-1}$ each were obtained from LGC Standards (Molsheim, France). To a plasma sample of 100 µL, internal standards (1 ng each) were added gravimetrically: CB-30, -103, -155 and -198 were used to quantify PCBs, p,p’-DDT-d8 was used to quantify OCPs and F-BDE-47 was used to quantify BDE-47 and BDE-99; standards were provided by either Dr Ehrenstorfer GmbH, Cambridge Isotope Laboratory (via Cluzeau

Info Labo, Sainte-Foy-La-Grande, France) or Chiron (via BCP Instruments, Irigny, France). POPs were extracted with 1 mL of pentane:dichloromethane (90:10; v/v) ; after centrifugation (2000 rpm, 2min at 4°C), the organic layer was collected and the operation was repeated. Both extracts were combined and purified on an acid silica gel column (40% H_2SO_4). After extract loading, analytes were eluted with 3 x 5 mL of pentane/dichloromethane (90/10; v/v). Extracts were then concentrated using a RapidVap vacuum evaporation system from Labconco (Kansas City, MO, USA) to a volume of 1 mL and further concentrated under a gentle stream of nitrogen (40°C) after addition of 100 µL of isooctane as solvent keeper. Octachloronaphtalene (1 ng) was finally added to determine the recovery rate for each internal standard, for each sample (68-108%). Final extracts were analysed by gas chromatography coupled with electron capture detection (GC-ECD) as described elsewhere (Tapie et al. 2011).

Quality control consisted in the analysis of standard solutions (NIST SRM 2261 and SRM 2262) and of procedural blanks (clean and empty glass tubes treated like a sample, one blank for 8 samples). Recoveries for standard solutions ranged from 89 to 104 % with standard deviations lower than 13 % (n=4). Chicken plasma samples (Sigma-Aldrich, S^t Quentin Fallavier, France) spiked with all analytes (3 $ng \bullet g^{-1}$ each) were analysed; the recovery rates were in the range 77-103 % with coefficients of variation lower than 17 % (n=5), except for CB-52 (22%) and mirex (29%). POP concentrations were blank corrected and the detection limit (LoD) was set at two times the mean blank value; for analytes that were not detected in blanks, LoD was determined as the concentration with a signal to noise ratio of 3 in spiked chicken plasma samples. Overall, LoDs ranged from 0.03 to 0.34 $ng \bullet g^{-1}$ wet weight (ww). Additionally, plasma total lipids were measured on an aliquot of 10 µL by the *sulfo-phospho-vanillin* (*SPV*) method for colorimetric determination (Frings et al. 1972).

Hg determination in blood cells

214 Total Hg was measured as described in details in Bustamante et al. (2006). Briefly, from
215 freeze-dried and powdered red blood cells (hereafter called 'blood') in an Advanced Hg
216 Analyzer spectrophotometer (Altec AMA 254). At least two aliquots ranging from 5 to 10 mg
217 were analyzed for each individual and quality assessment was measured by repeated analyses
218 of certified reference material TORT-2 (lobster hepatopancreas, NRCC; certified value
219 0.27±0.06 $\mu g \bullet g^{-1}$; with recoveries of 98 to 102%) and blanks, empty sample container, run
220 every 20 samples. Hg concentrations are expressed in $\mu g \bullet g^{-1}$ dry weight (dw).

221 **Statistical analyses**

222 All analyses were performed using R 2.13.1 (http://r-project.org/). We first tested inter-
223 correlations between the different families of POPs detected by using linear models (LM).
224 Second, we used generalized linear model (GLM) with normal errors and an identity link
225 function to test whether ΣPOPs or Hg were influenced by sex, body-condition and age
226 (dependent variable: ΣPOPs and Hg, independent factor and variables: sex, body-condition
227 and age). Third, we tested whether CORT and PRL kinetics differed between male and
228 females by using repeated measures GLM with the time of sampling (baseline, stress-induced
229 and post-stress levels) as the repeated measures; (dependent variable: CORT and PRL
230 concentrations, independent factors: sex, time and their interaction). Fourth, we tested
231 whether CORT concentration (baseline, stress-induced and post-stress) was related to ΣPOPs
232 and Hg (dependent variable: CORT, independent factor and variables: ΣPOPs, sex, age, Hg,
233 body-condition and their interaction with sex). For PRL we analysed males and females
234 separately (dependent variable: PRL, independent variables: ΣPOPs, age, Hg, body-condition)
235 because in incubating snow petrels, females bear higher PRL concentrations than males
236 (Angelier et al. 2007). Finally, we tested if the probability of neglecting the egg was related to
237 ΣPOPs and Hg in males and females separately (dependent variable: egg neglect; independent
238 variables: ΣPOPs, age, Hg, body-condition). To test the relationships between contaminants

and egg-neglect behaviour (yes or no) we used GLM with binomial error and logit link. Dependent continuous variables were previously tested for normality with a Shapiro–Wilk test and were log-transformed when necessary. Selected models were then checked for assumptions, that is, constancy of variance and residual normality. We performed all our model selection starting from the most general model that included all the variables/factors of interest and their interactions and we removed step by step the non-significant interactions, variables or factors. For POPs statistical analyses, concentrations below LoD were assigned LoD value, and only compounds detected in at least 70% of the individuals were included into the sum of POPs (Noël et al. 2009).

RESULTS

Contaminants: concentrations and compounds

Out of the 20 POP targeted compounds, 15 could be detected but the concentrations of four OCPs (cis-chlordane, trans-nonachlor, heptachlor and 2,4'-DDE) and one PBDE (BDE-99) were systematically below LoD. The most abundant compounds were the PCBs, with the PCBs 101 and 118 reaching the highest concentrations, closely followed by CB-138 and CB-153 (**Table 1**). Of the OCPs, HCB had the highest concentrations followed by 4,4'-DDE (**Table 1**). Only four PCBs (-CB101, -118, -138 and -153) and four OCPs (HCB, gamma HCH, 4,4' DDE and 2,4' DDT) were detected in at least 70% of the individuals and were thus included into the analyses. ΣPCBs and ΣOCPs were positively correlated (PCBs vs. OCPs: LM, $F_{1,34}$= 18.1, $p<0.001$, $R^2 = 0.35$). Thus the global pollutant burden was described as a sum of POPs (hereafter 'ΣPOPs'). In incubating snow petrels, blood Hg averaged 1.91 ± 0.75 $\mu g \bullet g^{-1}$dw, specifically in males 1.94 ± 0.77 $\mu g \bullet g^{-1}$dw (range: 0.89 - 4.01) and in females 1.87 ± 0.73 $\mu g \bullet g^{-1}$dw (range: 0.74 - 3.70).

Relationship between contaminants, sex, body-condition and age

During the incubation period, ΣPOPs was not statistically different between male and female snow petrels (GLM, $F_{1,34}$= 3.21, p = 0.082). Hg concentrations were not related to sex neither (GLM, $F_{1,47}$= 0.12, p = 0.734). ΣPOPs and Hg concentration, respectively, were unrelated to the body-condition index (ΣPOPs: $F_{1,34}$= 1.05, p = 0.313, interaction with sex: $F_{1,33}$= 2.18, p = 0.149; Hg: $F_{1,46}$= 3.30, p = 0.076, interaction with sex: $F_{1,45}$= 0.14, p = 0.714). ΣPOPs were not related to age ($F_{1,34}$= 0.88, p = 0.355, interaction with sex: $F_{1,33}$= 0.61, p = 0.441), and neither were Hg concentrations ($F_{1,45}$= 2.05, p = 0.159 interaction with sex: $F_{1,44}$= 0.16, p = 0.693). Finally, ΣPOPs and Hg were not related ($F_{1,34}$= 2.09, p = 0.157).

CORT and PRL kinetics: response and recovery to acute stress protocol

CORT concentrations significantly increased over 30 min from 4.7 ± 3.4 to 37.8 ± 8.6 ng•ml^{-1} and then declined 20 min post-stress to 23.6 ± 12.6 ng•ml^{-1} (GLMM, time as factor, $F_{2,94}$= 269.98, p <0.001), without sex difference (sex: $F_{1,47}$ = 0.28, p = 0.600; time × sex interaction: $F_{2,94}$= 0.08, p = 0.926). PRL concentrations significantly decreased over time (GLMM, time as factor, $F_{2,94}$ = 144.26, p <0.001) and females had higher PRL concentrations than males (sex: $F_{1,47}$ = 152.72, p <0.001; time × sex interaction: $F_{2,94}$= 3.79, p = 0.026). In females, PRL decreased from 239.5 ± 36.3 to 169.4 ± 32.1 ng•ml^{-1} after 30 min and until 165.4 ± 30.8 ng•ml^{-1} 20 min post-stress. In males PRL concentrations decreased from 139.5 ± 30.7 to 90.9 ± 24.7 over 30 min and they reached 83.4 ± 24.0 ng•ml^{-1} 20 min post-stress.

Relationships between contaminants and CORT concentrations

CORT absolute concentrations (baseline, stress-induced and post-stress) were not related to sex, neither to age, Hg, body-condition and their interaction with sex (**Table 2**). ΣPOPs were not related to baseline CORT (**Fig. 1.A, Table 2**), but increasing concentration of ΣPOPs was positively related to increasing concentration of stress-induced and post-stress CORT (**Fig. 1.B-C, Table 2**).

Relationships between contaminants, PRL concentrations and egg-neglect

In females we did not find any relationship between PRL (baseline, stress-induced and post-stress) and ΣPOPs, age, Hg, or body-condition ($p>0.08$ for all tests). In males baseline and post-stress PRL concentrations were not related to ΣPOPs, age, Hg, or body-condition ($p>0.07$ for all tests), but increasing blood Hg concentration was related to decreasing stress-induced PRL concentration: i.e. after 30 min restraint the most contaminated males were less likely to maintain high concentrations of PRL (GLM, $F_{1,25}=5.6$, $p=0.0263$; **Fig. 2).** Eleven females and eight males were observed neglecting their egg, out of 21 and 26, respectively. Blood Hg concentration was higher in males that were more likely to neglect their egg (GLM, $\chi^2=$, $p = 0.019$, **Fig. 3**) a relationship not found in females (GLM, $\chi^2=0.1$, $p = 0.796$, **Fig. 3**). Finally, egg neglect behaviour was not related to ΣPOPs, age or body-condition in any sex ($p>0.4$ for all tests).

DISCUSSION

The present study is the first to report plasma POP concentrations in the long-lived, Antarctic snow petrel. Firstly, there were no relationships between plasma ΣPOPs or blood Hg and age, suggesting that long-lived seabird are able to eliminate much of their contaminant burden. Secondly, ΣPOPs and Hg seem related to different hormonal pathways involved in reproductive decisions; ΣPOPs may disrupt the HPA axis whereas Hg was related to PRL secretion in males and consequently to egg-neglect behaviour.

Contaminants and age

Although snow petrels are very long-lived and thus exposed to contaminants over many years, no evidence was found that contamination was age-related, neither for ΣPOPs nor Hg. POPs and Hg measured in blood (plasma and red blood cells, respectively) can be correlated to levels found in storage organs and also adipose tissues, in birds but also chelonians and

311 humans (Henriksen et al. 1998; Henny et al. 2002; ; Pauwels et al. 2000; Wayland et al. 2001;
312 Keller et al. 2004; van de Merwe et al. 2010; Szumiło et al. 2013; Fromant et al. unpublished
313 data). Thus, blood contaminant concentration may be a good proxy of contaminant burden in
314 other organs.

315 The relationship between Hg and age in seabirds is often contradictory, for example liver Hg
316 was found to decrease, increase or be unrelated to age (Furness and Hutton, 1979; Hutton
317 1981; Thompson et al. 1991). For blood, the relationship between Hg contamination and age
318 is also not clear: no relationship was found between age and Hg contamination (Gonzáles-
319 Solís et al. 2002; Tavares et al. 2013) but in pre-breeding snow petrels and incubating cape
320 petrels (Daption capense), a negative relationship was found between blood Hg and age
321 (Tartu et al., unpublished data). This relationship was, however more likely the result of an
322 age-related change in feeding ecology. With regard to POPs, it seems that in seabirds,
323 concentrations in different tissues and blood increase until a steady-state is reached, often
324 before the age of breeding (Donaldson et al. 1997; Newton et al. 1981; van den Brink et al.
325 1998), and for breeding birds, most studies have not observed any age-related POP
326 accumulation (Bustnes et al. 2003; Newton et al. 1981). In this study, all snow petrels were
327 breeders, and presumably they had already reached their steady-state levels.

328 Besides, seabirds can biotransform PCBs and eliminate POPs through their preen gland
329 (Borgå et al. 2005; Henriksen et al. 1996; Solheim 2010), in the same line Hg can be excreted
330 through feather growth (Bearhop et al. 2000). These mechanisms could partially explain the
331 lack of association between blood contaminants and increasing age. Also we have to remain
332 cautious as we hypothesized that in snow petrels, as in other bird species, blood contaminants
333 would represent levels in internal tissues (Henny et al. 2002; Szumiło et al. 2013; Wayland et
334 al. 2001; Henriksen et al., 1998; Fromant et al. unpublished data). However, we have no
335 evidence for this relationship in snow petrels. Additionally, following food intake or lipid

mobilization, contaminant levels in blood may fluctuate more than those in adipose tissues or liver and this could mask a hypothetical contaminant/age relationship.

POPs: Concentrations and relationship with the CORT stress response

Very few studies have examined blood concentrations of POPs in adult Antarctic seabirds, and among the few studies, comparisons are made difficult due to different analytical methods. Indeed, POPs are often described as a "sum of compounds" and the compounds taken into the sum vary among studies. However, some OCPs are often reported individually since their detrimental effects have been well identified. This is the case of the HCB a relatively volatile compound (Calamari et al. 1991) principally used in fungicide formulations (Barber et al. 2005). Higher concentrations of this compound are commonly found in species restricted to the Antarctic region than those in temperate regions (e.g. van den Brink, 1997). HCB was the OCP with the highest concentrations in incubating snow petrels, but much lower than the concentrations found in other high-Antarctic species such as south polar skuas (Catharacta maccormicki) from Svarthamaren (71° 53' S, 05° 10' E) in Dronning Maud Land (Antarctica): i.e. HCB concentrations were 10-fold lower, and mirex concentrations 100-fold lower (Bustnes et al. 2006, 2007). These results could be the consequence of a different trophic level, toxicokinetic factors (e.g. metabolism, clearance rate), compound-specific physicochemical properties (K_{ow}, half-life) or depend on the bio-availability of contaminants in the breeding area (Walker et al. 2012).

The Arctic is more contaminated by POPs than Antarctica (Bustnes et al. 2006; Choi et al. 2008), HCB concentrations in snow petrels were slightly lower than those measured in plasma of incubating black-legged kittiwakes (Rissa tridactyla) from Svalbard: 1.85 ± 1.41 $ng \bullet g^{-1}$ ww versus 2.5 ± 0.44 $ng \bullet g^{-1}$ ww respectively (Tartu et al. unpublished data). In comparison, incubating glaucous gulls (Larus hyperboreus) had HCB concentrations in plasma much

higher than snow petrels (Verreault et al. 2005): on average ~400 $ng \bullet g^{-1}$ lipid weight whereas in snow petrels concentrations given in lipid weight average ~200 $ng \bullet g^{-1}$. In both black-legged kittiwakes and glaucous gulls there is evidence of CORT disruption by POPs (Nordstad et al. 2012; Verboven et al. 2010). Indeed, in both species increasing POP concentrations were related to higher baseline CORT concentrations and for male glaucous gulls, higher POP concentrations were related to decreasing stress-induced CORT concentrations. In the present study, increasing ΣPOPs were not related to baseline CORT, but to stress-induced and post-stress CORT concentrations. Hence, the most polluted birds released more CORT when subjected to a handling stress protocol, and those concentrations remained high 20 minutes post-stress. These results are in accordance with the recent finding that POPs, and especially PCBs are associated with a higher adrenocortical response to an acute stress in pre-laying female black-legged kittiwakes (Tartu et al. 2014). However, although post-stress CORT concentrations were admittedly higher in the most contaminated snow petrels they did not decrease more slowly than in less polluted birds, indicating that negative feedback from CORT on the hypothalamus and the pituitary was functional.

One possible explanation for the over-release of CORT could be related to an increase of the number of adreno-corticotrophic-hormone (ACTH) receptors (ACTH-R) on the adrenals. ACTH is one of the few polypeptide hormones having a positive trophic effect on its own receptors (Beuschlein et al. 2001; Penhoat et al. 1989). Thus, an increase of ACTH-R in the most POP contaminated snow petrels may be the consequence of an excess of ACTH input to adrenals. This suggests that ΣPOPs may alter the functioning of the pituitary by stimulating ACTH release and/or that ΣPOPs may mimic ACTH and bind to ACTH-R, which in that case would mobilize more ACTH-R from the adrenals of the most contaminated individuals. However this study is correlational, we cannot confirm without experimental support that the observed relationship is not the consequence of other intrinsic or extrinsic factors. Yet, an

385 exacerbated secretion of CORT in response to a stressful event often mirrors poor fitness
386 related traits as lowered parental investment (Angelier et al. 2009a; Bókony et al. 2009;
387 Goutte et al. 2011b; Lendvai et al. 2007) or an impacted survival (Blas et al. 2007; Goutte et
388 al. 2010; Romero 2012). Nevertheless we did not find any relationship between POPs and
389 parenting in terms of PRL concentration contrary to the study of Verreault et al. (2008) or
390 egg-neglect behaviour.

391 **Hg: concentrations and relationships with stress-induced PRL and egg neglecting**

392 Hg concentrations in incubating snow-petrels were within the range of those measured in the
393 blood of south polar skuas breeding in Adélie land (2.15 ± 0.17 $\mu g \bullet g^{-1}$ dw, Goutte et al.
394 2014). In comparison with an Arctic breeding seabird, we also found comparable
395 concentrations in incubating black-legged kittiwakes, (average 1.6 ± 0.5 $\mu g \bullet g^{-1}$ dw, Tartu et
396 al. unpublished data). Contrary to other studies on free-ranging birds (Franceschini et al.
397 2009; Herring et al. 2012; Wada et al. 2009), we did not find any relationship between Hg and
398 CORT secretion. Hg is well-known for its negative effects on breeding (reviewed in Tan et al.
399 2009). However, to the best of our knowledge, no studies have described relationships
400 between Hg and PRL in free-living organisms. In humans, urinary Hg concentration was
401 negatively correlated to plasma PRL (De Burbure and Bernard 2006; Lucchini et al. 2002,
402 2003). In the present study, we found a similar relationship in incubating male snow petrels:
403 increasing Hg concentrations were related to decreasing stress-induced PRL concentrations.

404 PRL is an anterior pituitary hormone, and a previous study on polar seabirds has described
405 relationships between Hg and another anterior pituitary hormone: luteinizing hormone (LH,
406 Tartu et al. 2013, Tartu et al. unpublished data). Hg seemed to disrupt LH secretion via a lack
407 of Gonadotropin-Releasing-Hormone (GnRH) input from the hypothalamus (Tartu et al.
408 2013). GnRH release is controlled by an area of the hypothalamus called zona incerta (Ben-

409 Jonathan and Hnasko, 2001). Interestingly, this area also participates in the secretion of
410 dopamine, a neuro-transmitter which is the principal antagonist of PRL (reviewed in Ben-
411 Jonathan and Hnasko, 2001). Moreover, it has been well established that organic and
412 inorganic Hg can stimulate the spontaneous release of dopamine in laboratory rodents (Faro et
413 al. 1997, 2000, 2007; Minnema et al. 1989) but also in wild larvae of a fish (Fundulus
414 heteroclitus, Zhou et al. 1999) and in wild American minks Mustela vison, where Hg induced
415 a decrease of dopaminergic receptors and ligand affinity interpreted as an adaptive mechanism
416 to prevent the hyper-stimulation of the dopaminergic system (Basu et al. 2005). Additionally,
417 when subjected to a stress, dopamine concentrations in blood increase (e.g. Finlay and
418 Zigmond, 1997). Stress-induced dopamine synthesis in male snow petrels may thus be
419 enhanced by Hg contamination, and result in a decrease of stress-induced PRL concentrations
420 but not baseline or post-stress PRL concentrations. The fact that the most polluted birds
421 quickly decrease their PRL concentrations when exposed to stress may highly affect their
422 parental investment: they would be more likely to neglect their egg than less polluted birds.
423 This goes together with the fact that in males, where PRL concentrations were lower than in
424 females, the most polluted individuals were more likely to neglect their egg. In females, PRL
425 concentrations and egg-neglect behaviour were not related to Hg, maybe their PRL
426 concentrations remained sufficiently high to prevent egg-neglect, a behaviour associated with
427 poor hatching success and chick mortality (Boersma and Wheelwright 1979; Angelier et al.
428 2007).

429 **CONCLUSION**

430 In conclusion, there were no relationships between age and POPs or Hg, which is in line with
431 most other studies. However we report significant relationships between contaminants and
432 hormones involved in reproductive decisions. Over time, the action of POPs and Hg may
433 jeopardize the maintenance of long-lived species populations. Indeed in long-lived species,

that are expected to maximize their own survival rather than that of their brood, an exacerbated stress response as a consequence of POPs contamination and a decrease of PRL for the most Hg polluted males, are additional threats that may encourage individuals to refrain from breeding or desert their brood. To confirm the reported relationships, this study would greatly benefit from further experimental support.

Role of the funding source

This project was funded by Agence Nationale de la Recherche (ANR PolarTop, O. Chastel), the French Polar Institute (IPEV, Program 109, H. Weimerskirch) and the 7th research program of the European Community FP7/2007–2013 (Marie-Curie Fellowship, no. 237034, F. Angelier). The funders played no part in the design, analysis, or reporting of the study.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

The authors thank M. Kriloff, S. Lucchini, F. Amélineau, E. Cottin, and A.M. Thierry for their help in the field, C. Trouvé, C. Parenteau, S. Ruault, C. Churlaud, A. Carravieri, L. Peluhet and P. van Delft for their assistance in hormone, Hg and POPs assays and three anonymous referees for their very useful comments on a previous version of the manuscript.

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824 **Figure legends:**

825 **Figure 1:** Relationships between plasma ΣPOP concentrations ($ng \bullet g^{-1}$ wet weight) and
826 plasma CORT concentrations ($ng \bullet ml^{-1}$) in incubating snow-petrels (A) baseline, (B) stress-
827 induced and (C) post-stress. White circles denote females and black triangles denote males.
828 The solid line refers to statistically significant linear regression.

829 **Figure 2:** Relationship between total blood Hg ($\mu g \bullet g^{-1}$ dry weight) and stress-induced plasma
830 PRL concentrations ($ng \bullet ml^{-1}$) in incubating snow-petrels. White circles denote females and
831 black triangles denote males. The solid line refers to statistically significant linear regression
832 for males.

833 **Figure 3:** Relationship between blood Hg ($\mu g \bullet g^{-1}$ dry weight) and egg-neglect behaviour in
834 incubating snow-petrels. The empty boxes denote females and the filled boxes denote males
835 (*: $p<0.02$).

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837

838 **Table 1:** Concentrations of plasma persistent organic pollutants and blood Hg (μg•g^{-1}) of
839 incubating female and male snow petrels. For POPs, concentrations are given in wet-weight
840 (ng•g^{-1} ww) in the first rows and lipid-weight (ng•g^{-1} lw) in the second rows.

	Females			Males		
	Mean	Standard deviation	Range (min-max)	Mean	Standard deviation	Range (min-max)
Lipids (%)	0.68	0.1	(0.48 - 0.88)	0.7	0.1	(0.50 - 0.94)
CB-50/28	0.4	0.6	(< 0.10 - 2.1)	1.2	2.5	(< 0.10 - 10.9)
	65.2	92.1	(< 14.4 - 322.7)	152.2	286.7	(< 14.4 - 1160.8)
CB-52	3.8	9.5	(< 0.04 - 36.6)	10.0	16.3	(< 0.04 - 63.1)
	617.1	1494.2	(< 5.2 - 5719.4)	1300.2	1953.0	(< 5.2 - 6709.3)
CB-101	6.9	14.4	(< 0.02 - 57.4)	24.0	40.5	(< 0.02 - 167.9)
	1082.1	2251.5	(< 3.1 - 8976.5)	3151.6	5019	(< 3.1 - 20730.2)
CB-118	6.6	19.0	(< 0.04 - 73.9)	22.7	38.4	(< 0.04 - 150.6)
	1045	2960.4	(< 5.4 - 11543.5)	2963.4	4802.2	(< 5.4 - 18587.5)
CB-138	2.8	6.8	(< 0.02 - 26.5)	12.1	17.5	(< 0.02 - 52.4)
	441.5	1067	(< 2.5 - 4136.9)	1601.4	2261.5	(< 2.5 - 6469.2)
CB-153	2.6	6.3	(< 0.04 - 24.6)	9.8	13.9	(< 0.04 - 44.5)
	414.9	988.0	(< 6.0 - 3845.6)	1297.4	1780.2	(< 6.0 - 5488.8)
CB-180	0.2	0.6	(< 0.02 - 2.2)	1.1	1.6	(< 0.02 - 5.4)
	37	88.4	(< 3.2 - 338.8)	145.8	204.3	(< 3.2 - 666.0)
ΣPCBs*	12.6	28.0	(1.6 - 110.7)	47.0	72.0	(1.8 - 270.2)
	1975.5	4379.2	(211.4 - 17297.8)	5394.6	8427	(222.2 - 33354.2)
HCB	1.3	1.0	(< 0.01 - 3.2)	2.2	1.6	(< 0.01 - 4.8)
	192.6	153.2	(< 1.2 - 395.0)	296.6	223.9	(< 1.2 - 779.1)
gamma HCH	0.1	0.1	(< 0.02 - 0.3)	0.2	0.1	(< 0.02 - 0.5)
	16.9	19.8	(< 2.1 - 47.8)	21.7	16.8	(< 2.1 - 56.5)
4,4'-DDE	0.6	1.0	(< 0.01 - 4.0)	0.4	0.4	(< 0.01 - 1.2)
	92.7	168	(< 1.7 - 669.3)	53.9	51.9	(< 1.7 - 192.3)
4,4'-DDD	0.1	0.1	(< 0.05 - 0.4)	0.1	0.2	(< 0.05 - 0.9)
	8.9	17.5	(< 7.3 - 65.7)	18.2	27.9	(< 7.3 - 114.7)
2,4'-DDT	0.3	0.7	(< 0.03 - 2.8)	1.1	1.9	(< 0.03 - 6.6)
	47.4	114.9	(< 4.6 - 433.5)	155.9	266.8	(< 4.6 - 1018.7)
4,4'-DDT	0.3	0.6	(< 0.02 - 2.2)	1.3	2.5	(< 0.02 - 9.0)
	40.2	92.0	(< 2.7 - 337.2)	176.3	350.8	(< 2.7 - 1388.9)
Mirex	0.2	0.3	(< 0.02 - 1.2)	0.6	1.0	(< 0.02 - 3.4)
	26.8	49.3	(< 2.6 - 190.4)	78.1	124.2	(< 2.6 - 420.5)
ΣOCPs**	2.3	1.8	(0.3 - 6.2)	3.9	2.6	(0.3 - 10.4)
	349.5	286.7	(33.1 - 1027.7)	531.9	375.3	(43.6 - 1593.1)
BDE-47	0.1	0.2	(< 0.03 - 0.7)	0.2	0.3	(< 0.03 - 0.9)
	10.2	29.8	(< 4.3 - 115.4)	27.4	35.5	(< 4.3 - 95.7)
ΣPOPs***	14.9	29.0	(2.3 - 116.1)	50.8	73.5	(3.9 - 275.3)
	2325.1	4533.2	(244.5 - 18134.5)	5926.5	8645.2	(456.1 - 33985.0)
Hg	1.8	0.7	(0.7 - 3.7)	1.9	0.8	(0.9 - 4.0)

*ΣPCBs = CB101+CB138+CB153+CB180; **ΣOCPs = HCB+ɣ-HCH+4,4'-DDE+2,4'-DDT; ***ΣPOPs = ΣPCBs + ΣOCPs

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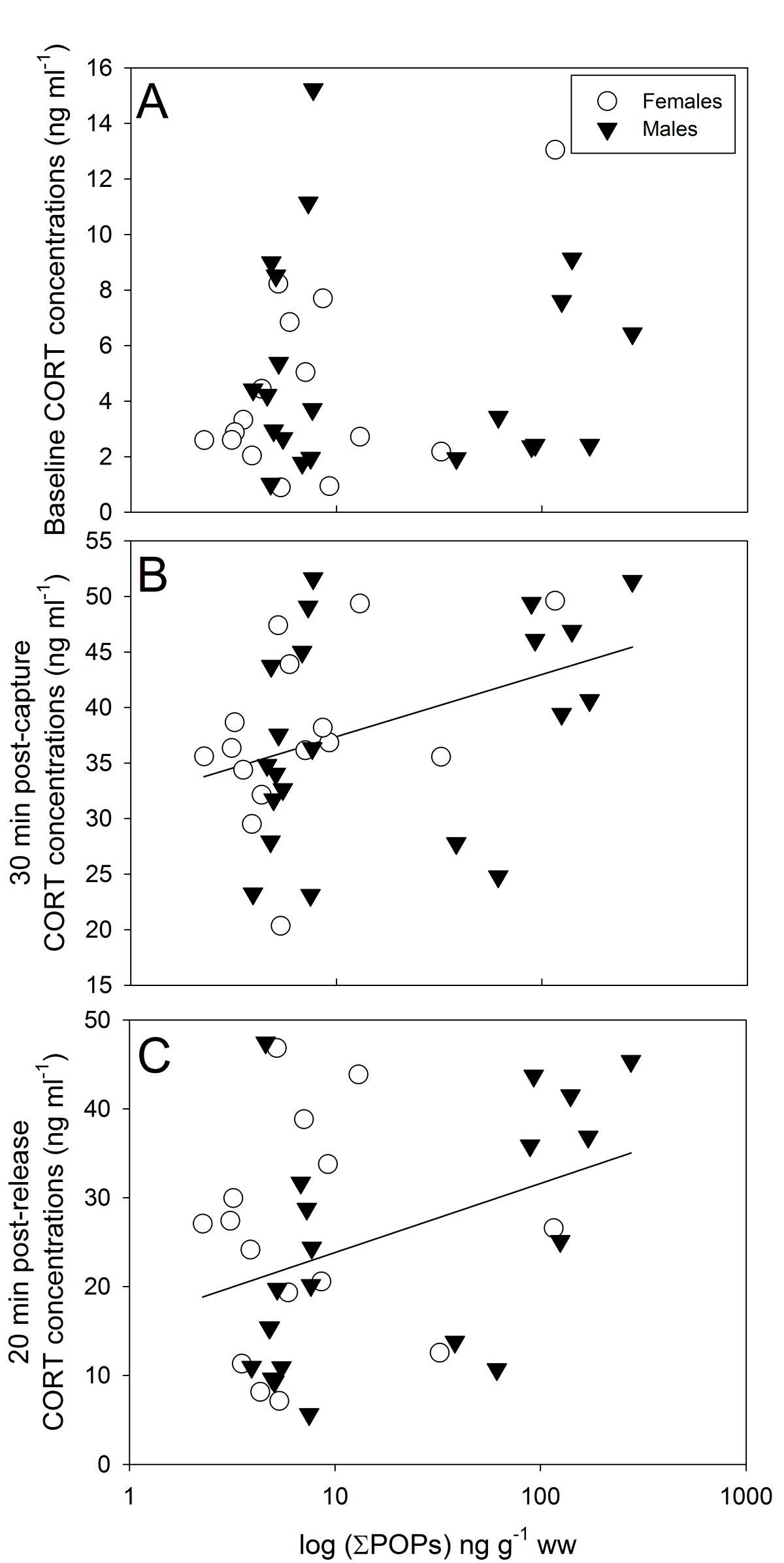
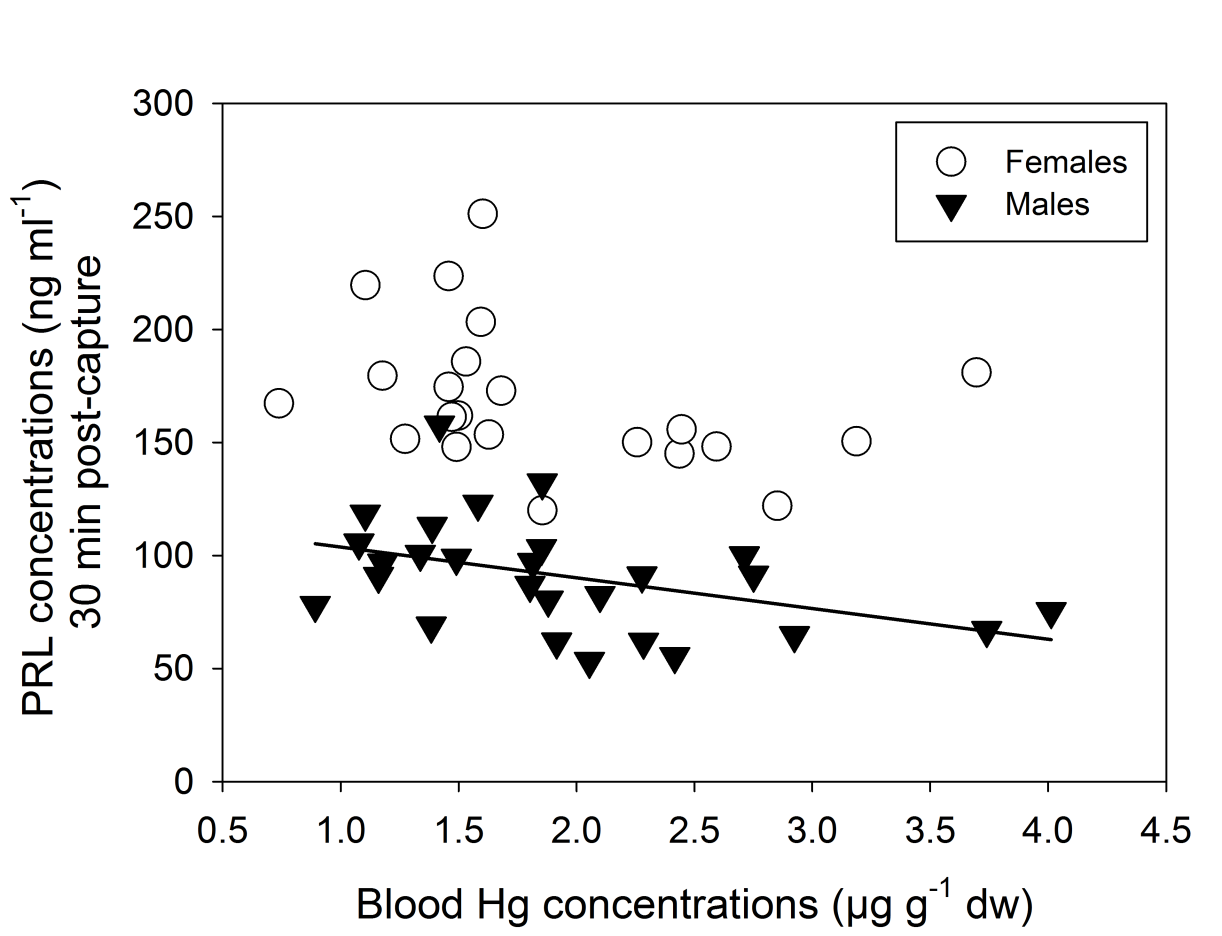
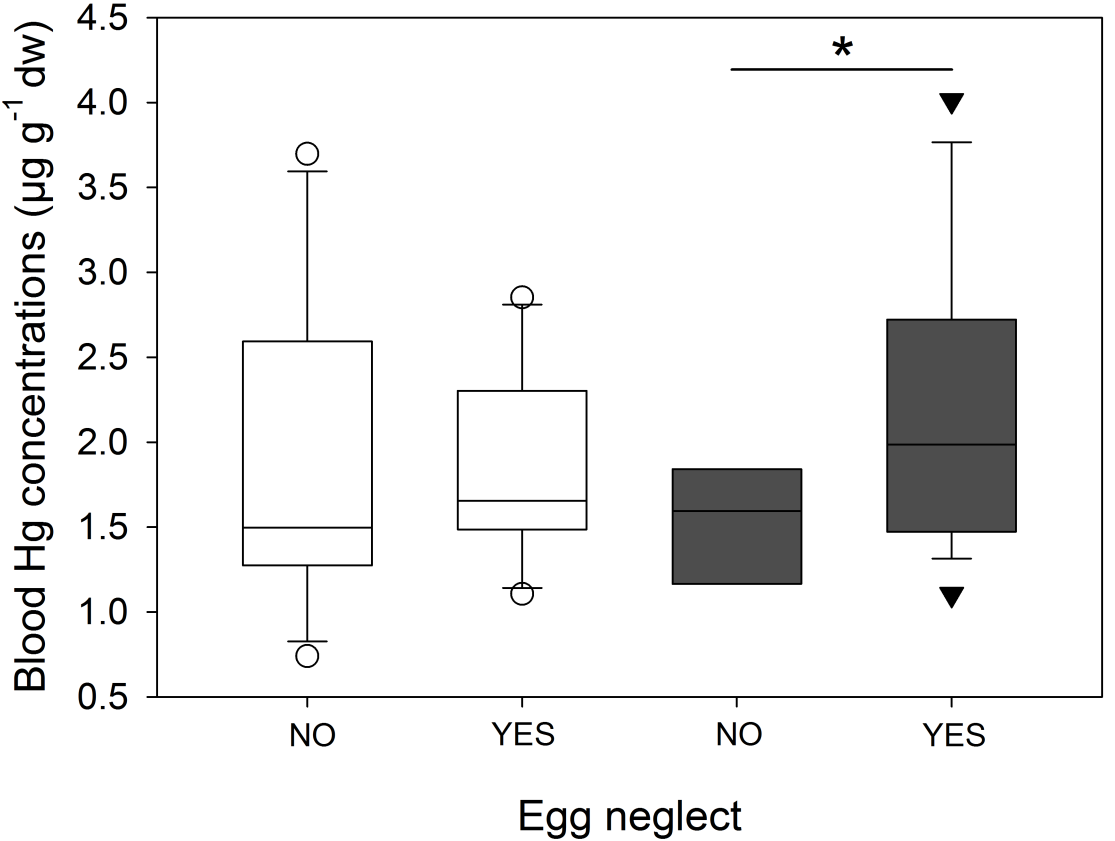
Table 2: Relationships between contaminants (ΣPOPs and Hg), sex, age, body-condition and interaction with sex as a function of CORT concentrations ($ng \bullet ml^{-1}$) in incubating snow petrels: a) baseline, b) stress-induced and c) post-stress. N is the number of birds of each variable. Degrees of freedom vary between measures of CORT because model selection was performed by starting from the most general model that included all the variables/factors of interest and their interactions and we removed step by step the non-significant interactions, variables or factors.

Dependent variables	N	Independent variables	*Df*	F	*p-value*
a) Baseline CORT	36	log (ΣPOPs)	1,27	0.14	0.715
	49	Sex	1,47	1.2	0.279
	47	Age	1,44	0.2	0.66
	49	Hg	1,42	0.09	0.767
	48	Body condition	1,39	1.73	0.195
	36	log (ΣPOPs) × Sex	1,26	0.46	0.505
	47	Age × Sex	1,43	3.49	0.068
	49	Hg × Sex	1,41	3.29	0.077
	49	Body condition × Sex	1,38	0.05	0.819
b) Stress-induced CORT	36	**log (ΣPOPs)**	**1,34**	**6.1**	**0.019**
	49	Sex	1,33	0.48	0.495
	47	Age	1,30	0.02	0.9
	49	Hg	1,28	0.01	0.915
	48	Body condition	1,31	2.82	0.103
	36	log (ΣPOPs) × Sex	1,26	0	0.989
	47	Age × Sex	1,29	0.64	0.429
	49	Hg × Sex	1,27	0.21	0.653
	49	Body condition × Sex	1,32	3.97	0.055
d) Post-stress CORT	36	**log (ΣPOPs)**	**1,34**	**5.17**	**0.029**
	49	Sex	1,33	1.2	0.282
	47	Age	1,30	0.33	0.57
	49	Hg	1,27	0.03	0.863
	48	Body condition	1,31	1.37	0.25
	36	log (ΣPOPs) × Sex	1,32	1.51	0.228
	47	Age × Sex	1,29	1.34	0.257
	49	Hg × Sex	1,26	0	0.955
	49	Body condition × Sex	1,28	0.18	0.676


A
Females
Males
Baseline CORT concentrations (ng ml-1)
16
14
12
10
8
6
4
2
0
B
30 min post-capture
CORT concentrations (ng ml-1)
55
50
45
40
35
30
25
20
15
C
20 min post-release
CORT concentrations (ng ml-1)
50
40
30
20
10
0
1
10
100
1000
log (ΣPOPs) ng g-1 ww


300
250
200
150
100
50
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
PRL concentrations (ng ml-1)
30 min post-capture
Blood Hg concentrations (µg g-1 dw)
Females
Males


Blood Hg concentrations (µg g-1 dw)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
*
NO
YES
NO
YES
Egg neglect