Science of the Total Environment 505 (2015) 180–188

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

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HIGHLIGHTS

- We measured legacy POPs and Hg in the blood of adult snow petrels from Antarctica.
- POPs or Hg concentrations were not related to age.
- Stress-induced CORT concentrations were positively related to POP concentrations.
- Stress-induced PRL concentrations were negatively related to Hg concentrations.
- Hg concentrations were higher in males that neglected their egg.

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 1 October 2014
Accepted 2 October 2014
Available online 14 October 2014

Editor: F. Riget

Keywords:
Snow petrel
Mercury
Persistent organic pollutants
Age
Reproduction

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 behavior 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 behavior 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.

1. 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

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http://dx.doi.org/10.1016/j.scitotenv.2014.10.008
0048-9697/

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

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

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

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

2. Materials and methods

2.1. 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).

2.2. 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 behavior'). 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 Watkins, 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.

2.3. 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.

2.4. 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 $2\ \text{ng}\cdot\mu\text{l}^{-1}$ 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, 2 min 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 × 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 analyzed 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, St. Quentin Fallavier, France) spiked with all analytes ($3\ \text{ng}\cdot\text{g}^{-1}$ each) were analyzed; 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 $0.34\ \text{ng}\cdot\text{g}^{-1}$ 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).

2.5. Hg determination in blood cells

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

2.6. Statistical analyses

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

3. Results

3.1. 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. PCBs and OCPs 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 ‘ POPs’). In incubating snow petrels, blood Hg averaged 1.91 ± 0.75 μg·g^{-1} dw, specifically in males 1.94 ± 0.77 μg·g^{-1} dw (range: 0.89–4.01) and in females 1.87 ± 0.73 μg·g^{-1} dw (range: 0.74–3.70).

3.2. Relationship between contaminants, sex, body-condition and age

During the incubation period, POPs 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, $F_{1,47} = 0.12$, $p = 0.734$). POPs and Hg concentration, respectively, were unrelated to the body-condition index (POPs: $F_{1,34} = 1.05$, $p = 0.313$, interaction with sex: $F_{1,33} = 2.18$, $p = 0.149$; Hg: $F_{1,46} = 3.30$, $p = 0.076$, interaction with sex: $F_{1,45} = 0.14$, $p = 0.714$). POPs 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, POPs and Hg were not related ($F_{1,34} = 2.09$, $p = 0.157$).

3.3. 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, $F_{2,94}$ =

Table 1
Concentrations of plasma persistent organic pollutants and blood Hg (μg·g^{-1}) of incubating female and male snow petrels. For POPs, concentrations are given in wet-weight (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	(<3.1–20,730.2)
CB-118	6.6	19.0	(<0.04–73.9)	22.7	38.4	(<0.04–150.6)
	1045	2960.4	(<5.4–11,543.5)	2963.4	4802.2	(<5.4–18,587.5)
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)
PCBs*	12.6	28.0	(1.6–110.7)	47.0	72.0	(1.8–270.2)
	1975.5	4379.2	(211.4–17,297.8)	5394.6	8427	(222.2–33,354.2)
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)
OCPs**	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)
POPs***	14.9	29.0	(2.3–116.1)	50.8	73.5	(3.9–275.3)
	2325.1	4533.2	(244.5–18,134.5)	5926.5	8645.2	(456.1–33,985.0)
Hg	1.8	0.7	(0.7–3.7)	1.9	0.8	(0.9–4.0)

* PCBs = CB101 + CB138 + CB153 + CB180.
** OCPs = HCB + -HCH + 4,4′-DDE + 2,4′-DDT.
*** POPs = PCBs + OCPs.

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.

3.4. 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). POPs were not related to baseline CORT (Fig. 1A, Table 2), but increasing concentration of POPs was positively related to increasing concentration of stress-induced and post-stress CORT (Fig. 1B–C, Table 2).

3.5. 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 POPs, age, Hg, or body-condition ($p > 0.08$ for all tests). In males baseline and post-stress PRL concentrations were not related to POPs, 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, $\chi^2 = 5.4$, $p = 0.019$, Fig. 3) a

Table 2
Relationships between contaminants (POPs and Hg), sex, age, body-condition and interaction with sex as a function of CORT concentrations (ng·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 (POPs)	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 (POPs) × 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 (POPs)**	**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 (POPs) × 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 (POPs)**	**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 (POPs) × 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

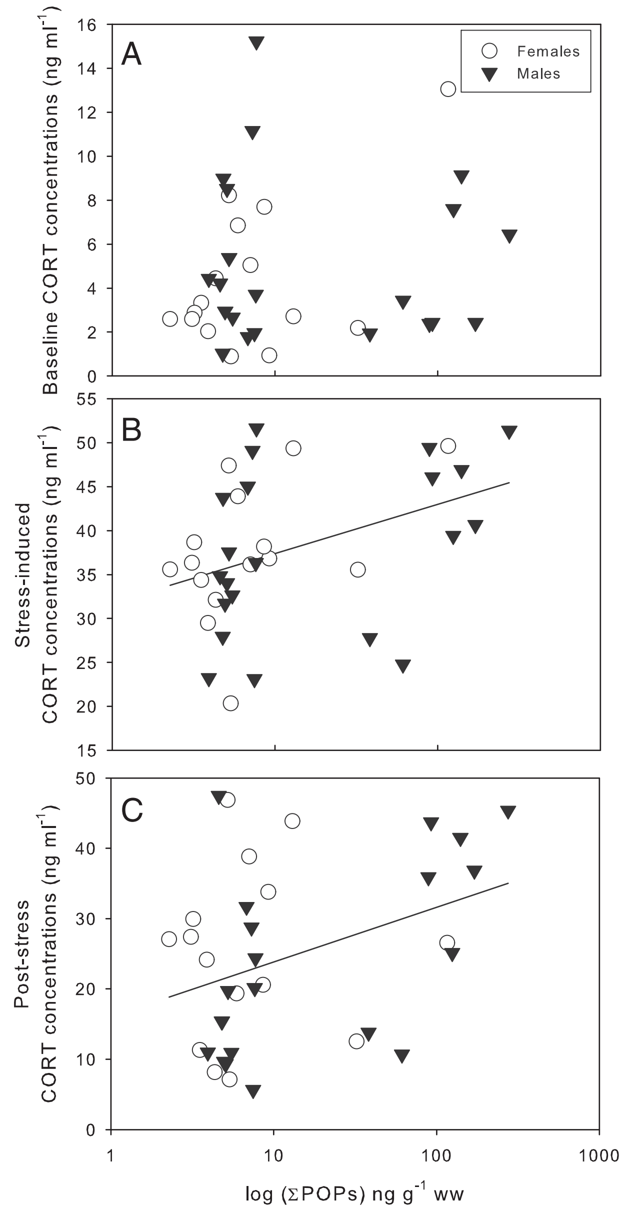

Fig. 1. Relationships between plasma POP concentrations (ng·g^{-1} wet weight) and plasma CORT concentrations (ng·ml^{-1}) in incubating snow-petrels (A) baseline, (B) stress-induced and (C) post-stress. White circles denote females and black triangles denote males. The solid line refers to statistically significant linear regression.

relationship not found in females (GLM, $\chi^2 = 0.1$, $p = 0.796$, Fig. 3). Finally, egg neglect behavior was not related to POPs, age or body-condition in any sex ($p > 0.4$ for all tests).

4. 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 POPs or blood Hg and age, suggesting that long-lived seabird are able to eliminate much of their contaminant burden. Secondly, POPs and Hg seem related to different hormonal pathways involved in reproductive decisions; POPs may disrupt the HPA axis whereas Hg was related to PRL secretion in males and consequently to egg-neglect behavior.

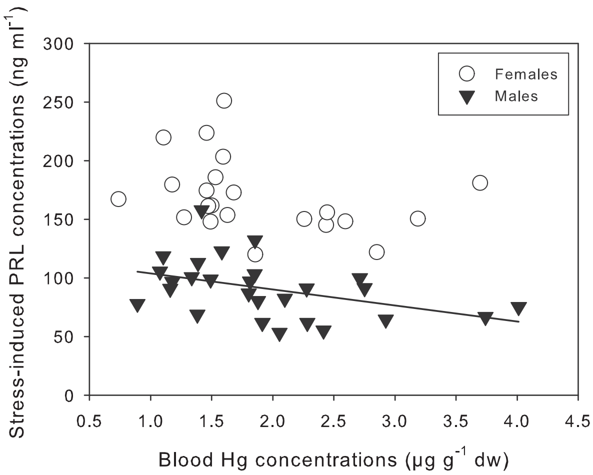

Fig. 2. Relationship between total blood Hg (μg·g^{-1} dry weight) and stress-induced plasma PRL concentrations (ng·ml^{-1}) in incubating snow-petrels. White circles denote females and black triangles denote males. The solid line refers to statistically significant linear regression for males.

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

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

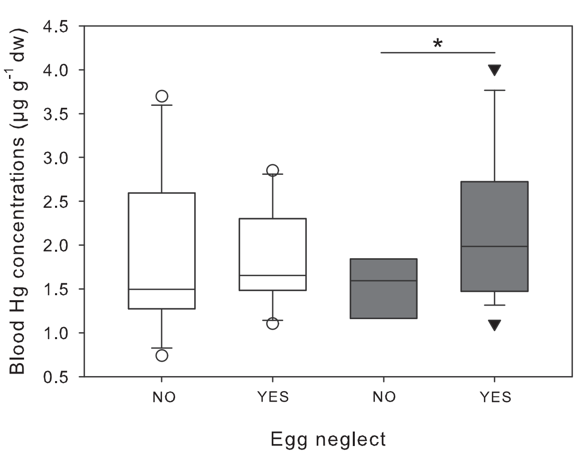

Fig. 3. Relationship between blood Hg (μg·g^{-1} dry weight) and egg-neglect behavior in incubating snow-petrels. The empty boxes denote females and the filled boxes denote males (*: $p < 0.02$).

Besides, seabirds can biotransform PCBs and eliminate POPs through their preen gland (Borgå et al., 2005; Henriksen et al., 1996; Solheim, 2010), in the same line Hg can be excreted through feather growth (Bearhop et al., 2000). These mechanisms could partially explain the lack of association between blood contaminants and increasing age. Also we have to remain cautious as we hypothesized that in snow petrels, as in other bird species, blood contaminants would represent levels in internal tissues (Henny et al., 2002; Szumiło et al., 2013; Wayland et al., 2001; Henriksen et al., 1998). However, we have no 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.

4.2. 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·g^{-1} ww versus 2.5 ± 0.44 ng·g^{-1} ww respectively. 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·g^{-1} lipid weight whereas in snow petrels concentrations given in lipid weight average ~200 ng·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 POPs 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 min 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 POPs may alter the functioning of the pituitary by stimulating ACTH release and/or that POPs 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 exacerbated secretion of CORT in response to a stressful event often mirrors poor fitness related traits as lowered parental investment (Angelier et al., 2009a; Bókony et al., 2009; Goutte et al., 2011b; Lendvai et al., 2007) or an impacted survival (Blas et al., 2007; Goutte et al., 2010; Romero, 2012). Nevertheless we did not find any relationship between POPs and parenting in terms of PRL concentration contrary to the study of Verreault et al. (2008) or egg-neglect behavior.

4.3. Hg: concentrations and relationships with stress-induced PRL and egg neglecting

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

PRL is an anterior pituitary hormone, and a previous study on polar seabirds has described relationships between Hg and another anterior pituitary hormone: luteinizing hormone (LH, Tartu et al., 2013). Hg seemed to disrupt LH secretion via a lack of Gonadotropin-Releasing-Hormone (GnRH) input from the hypothalamus (Tartu et al., 2013). GnRH release is controlled by an area of the hypothalamus called *zona incerta* (Ben-Jonathan and Hnasko, 2001). Interestingly, this area also participates in the secretion of dopamine, a neuro-transmitter which is the principal antagonist of PRL (reviewed in Ben-Jonathan and Hnasko, 2001). Moreover, it has been well established that organic and inorganic Hg can stimulate the spontaneous release of dopamine in laboratory rodents (Faro et al., 1997, 2000, 2007; Minnema et al., 1989) but also in wild larvae of a fish (*Fundulus heteroclitus*, Zhou et al., 1999) and in wild American minks *Mustela vison*, where Hg induced a decrease of dopaminergic receptors and ligand affinity interpreted as an adaptive mechanism to prevent the hyper-stimulation of the dopaminergic system (Basu et al., 2005). Additionally, when subjected to a stress, dopamine concentrations in blood increase (e.g. Finlay and Zigmond, 1997). Stress-induced dopamine synthesis in male snow petrels may thus be enhanced by Hg contamination, and result in a decrease of stress-induced PRL concentrations but not baseline or post-stress PRL concentrations. The fact that the most polluted birds quickly decrease their PRL concentrations when exposed to stress may highly affect their parental investment: they would be more likely to neglect their egg than less polluted birds. This goes together with the fact that in males, where PRL concentrations were lower than in females, the most polluted individuals were more likely to neglect their egg. In females, PRL concentrations and egg-neglect behavior were not related to Hg, maybe their PRL concentrations remained sufficiently high to prevent egg-neglect, a behavior associated with poor hatching success and chick mortality (Boersma and Wheelwright, 1979; Angelier et al., 2007).

5. Conclusion

In conclusion, there were no relationships between age and POPs or Hg, which is in line with most other studies. However we report significant relationships between contaminants and hormones involved in reproductive decisions. Over time, the action of POPs and Hg may 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.

Acknowledgments

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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