Increased adrenal responsiveness and delayed hatching date in relation to polychlorinated biphenyl exposure in Arctic-breeding black-legged kittiwakes (*Rissa tridactyla*)

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Abstract

High levels of environmental contaminants such as polychlorinated biphenyl (PCBs), organochlorine pesticides (OCPs) and mercury (Hg) have been reported in some Arctic top predators such as seabirds. Chronic exposure to these contaminants might alter the response to environmental changes through interference with the regulation of corticosterone (CORT), a glucocorticoid stress hormone released by the hypothalamo-pituitary-adrenal (HPA) axis. Positive and negative relationships between CORT and environmental contaminants have been reported in polar seabirds. However patterns appear inconclusive and it is difficult to attribute these relationships to a dysfunction of the HPA axis or to other confounding effects. In order to explore the relationships between the HPA axis activity and contaminants, we tested whether different aspects of the HPA axis of an Arctic seabird, the black-legged kittiwakes *Rissa tridactyla*, would be related to blood Hg, PCB and OCP concentrations. Male kittiwakes were caught during the incubation period in Svalbard and were subjected to different stress series: 1) a capture-restraint stress protocol, 2) an injection of dexamethasone (DEX) that enabled to test the efficacy of the HPA negative feedback and 3) an injection of adrenocorticotropic hormone (ACTH) that informed on the adrenal responsiveness. The HPA axis activity was unrelated to ΣOCPs and Hg. However, birds with high concentrations of ΣPCBs released more CORT after the ACTH injection. It is suggested that ΣPCBs may increase the number of ACTH-receptors on the adrenals. Also hatching date was delayed in males with higher concentrations of ΣPCBs and ΣOCPs. This study gives new evidence that PCBs and adrenal activity may be related. Thus high PCB burden may make individuals more prone to other stressors such as ongoing climate change.

Key-words: corticosterone; ACTH; dexamethasone; adrenal gland; PCBs; reproduction

1. Introduction

Species breeding in extreme environments, such as Polar Regions, are often subjected to a wide range of stressors (e.g. harsh weather, unpredictable food shortage, presence of predators) and an efficient response to these various stressors is vital to ensure self or brood survival (Wingfield et al. 2011; Wingfield 2013). At the endocrine level, an environmental perturbation will stimulate the hypothalamic–pituitary–adrenal (HPA) axis: specifically corticotropin-releasing hormone (CRH) will be released from the hypothalamus and will then stimulate the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which in turn will activate the synthesis of glucocorticoid hormones (corticosterone in birds, CORT henceforth) from the adrenal cortex (Wingfield 2013). Concurrently, glucocorticoids will provide negative feedback signals for ACTH and CRH release (Wingfield 2013). This hormonal cascade will trigger an array of physiological and behavioral adjustments that shift energy investment away from reproduction and redirect it towards survival (Wingfield and Sapolsky, 2003). In birds, CORT has therefore a strong connection with 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. 2010a, 2011b; Lendvai et al. 2007; Kitaysky et al. 1999; Schultner et al. 2014).

Polar seabirds are top predators which often bear elevated levels of various environmental contaminants (e.g. Gabrielsen 2007; Verreault et al. 2010). Contaminants are well present in aquatic biota (Gabrielsen and Sydnes, 2009) and diet is the principal route of contamination: persistent organic pollutant (POPs) which includes polychlorinated biphenyl (PCBs) and organochlorine pesticides (OCPs) and some heavy metals such as mercury (Hg) can bio-accumulate into individuals and bio-magnify along the food web (e.g. Dietz et al. 2000). Several of these environmental contaminants are endocrine disruptors (Ottinger et al. 2013; Tyler et al. 1998). Indeed those substances are able to mimic, antagonize, alter or

71 modify endogenous hormone functions (e.g. Amaral Mendes 2002). In free-living bird
72 species, several studies have found significant relationships between contaminants and
73 reproductive hormones such as steroids (Colborn et al 1993; Giesy et al. 2003; Vos et al.
74 2008) and more recently with hormones from the HPA axis (Nordstad et al. 2012; Tartu et al.
75 2014, 2015; Verboven et al. 2010). Specifically, in black-legged kittiwakes *Rissa tridactyla*
76 baseline and stress-induced CORT levels were positively associated to ∑PCB concentrations
77 (Nordstad et al. 2012; Tartu et al. 2014; Tartu et al. in press). Also, in the most PCB-exposed
78 Arctic seabird species, the glaucous gull *Larus hyperboreus*, a higher POP burden (including
79 58 PCB congeners, organochlorine pesticides, brominated flame retardants and their
80 metabolically-derived products) was associated with higher baseline CORT levels in both
81 sexes (Verboven et al. 2010). Moreover in incubating snow petrels *Pagodroma nivea*, stress-
82 induced CORT levels increased with increasing ∑POPs (including 7 PCBs congeners and
83 organochlorine pesticides, Tartu et al. 2015). However the mechanisms through which
84 contaminants may influence CORT regulation are poorly known, it is likely that such
85 compounds may disrupt one of the many steps of CORT regulation causing dysfunction of the
86 HPA axis. For instance, energy costs related to detoxification of POPs (Parkinson and
87 Ogilvie, 2008; Preston and Hoffman, 2008) might stimulate CORT secretion. Additionally,
88 the adrenal gland is suspected to be vulnerable to hazardous effects of POPs due to its high
89 lipid content and multiple sites for interference (Hinson and Raven, 2006; Odermatt and
90 Gumy, 2008).

91 When it comes to other environmental contaminants such as Hg, studies of seabirds'
92 eggs from the Canadian Arctic show an increasing trend of Hg levels from 1975 to 2005 in
93 several species (Braune 2007; Mallory and Braune, 2012). Hg is a well-known endocrine
94 disruptor (reviewed in Tan et al. 2009) and some studies on free-ranging birds have reported
95 inconclusive patterns between CORT and Hg concentrations (Franceschini et al. 2009;

96 Herring et al. 2012; Tartu et al. 2015; Wada et al. 2009; Wayland et al. 2002). Specifically, in
97 common eiders *Somateria mollissima borealis* stress-induced CORT levels were not related to
98 liver Hg concentrations (Wayland et al. 2002), in adult tree swallows *Tachycineta bicolor*
99 baseline CORT concentrations were negatively related to blood Hg concentrations
100 (Franceschini et al. 2009) and in chicks sampled in a Hg contaminated area stress-induced
101 CORT levels were lowered compared to less contaminated chicks (Wada et al. 2009). In
102 Forster's tern chicks *Sterna forsteri* fecal CORT metabolites decreased with increasing blood
103 Hg concentrations (Herring et al. 2012) and in snow petrels baseline and stress-induced
104 CORT concentrations were not related to blood Hg concentrations (Tartu et al. 2015).
105 Therefore, it is difficult to draw a general picture of contaminants-CORT relationships.

106 The aim of this study was to investigate the relationships between Hg, PCBs and
107 OCPs and some aspects of the HPA axis of an Arctic seabird, the black-legged kittiwake
108 (hereafter 'kittiwake'). Svalbard kittiwakes bear significant amounts of blood Hg, PCBs and
109 OCPs (Nordstad et al. 2012; Savinova et al. 1995; Tartu et al. 2013, 2014), and three recent
110 studies performed in the same breeding population where the present study was conducted
111 have reported positive relationships between baseline and/or stress-induced CORT
112 concentrations and blood PCB concentrations (Nordstad et al. 2012; Tartu et al. 2014; Tartu et
113 al. in press). However, it is still unknown whether these positive relationships are due to an
114 increased adrenocortical responsiveness, a decreased negative feedback during acute stress, or
115 both.

116 We specifically tested whether different aspects of the HPA axis of incubating male
117 kittiwakes were related to blood Hg, PCBs and OCPs concentrations. Males only were
118 chosen since they are more contaminated than females (Nordstad et al. 2012; Tartu et al.
119 2013), thus hypothetically more susceptible to the hazardous effects of contaminants. To test
120 these relationships, we measured the CORT response to a capture-restraint stress protocol

(Wingfield 1994) and birds were then injected with exogenous dexamethasone (DEX, a potent CORT receptor agonist, Astheimer et al.1994) to test the efficiency of the negative feedback. The purpose of the DEX injection was also to induce inhibitory feedback of the HPA axis, thereby reducing confounding effects of endogenous ACTH release. Whereupon birds were injected with exogenous ACTH, a protocol used in several bird species to test the adrenal responsiveness (Angelier et al. 2009b; Dickens et al. 2009; Rich and Romero 2005; Schmidt et al. 2012). If the exacerbated CORT release is connected to a higher adrenal activity in the most contaminated kittiwakes (Tartu et al. 2014; Tartu et al. in press), we should observe a positive relationship between blood contaminant concentrations and plasma ACTH-induced CORT concentrations. Furthermore, contaminants may represent stressors (Bustnes et al. 2006); we therefore investigated the relationships between contaminant exposure and reproductive outputs such as hatching date and hatching success. If Hg, PCBs or OCPs stimulate CORT secretion, and since reproductive phenology and success are influenced by CORT levels in kittiwakes (Goutte et al. 2011a), we predicted that clutch size, hatching date and hatching success would be negatively associated with blood contaminants.

2. Material and methods

2.1. Ethic statement and study area

The sampling of birds was approved by the Governor of Svalbard and by the Norwegian Animal Research Authority (NARA, permit number 4214). The study was conducted at Kongsfjorden, Svalbard (78°54′N, 12°13′E) from June 19th to July 4th 2012 during the incubating period for kittiwakes.

2.2. CORT stress series: DEX and ACTH injections

Thirty-four male kittiwakes were caught on the nests with a noose at the end of a 5 m fishing rod. A first blood sample (*ca.* 0.2 ml) was collected immediately after capture, from the alar

145 vein with a 1 mL heparinised syringe and a 25-gauge needle to assess baseline CORT
146 concentrations ($CORT_{BL}$ henceforth). Bleeding time (i.e. time elapsed from capture to the end
147 of the first blood sample: 2min 24sec ± 28sec (SD) on average) was not related to $CORT_{BL}$
148 concentrations (GLM, $F_{1,32} = 0.7$, $P = 0.395$). Birds were then placed into a cloth bag and a
149 second blood sample (*ca*. 0.2 ml) was collected from the alar vein 30 minutes later (30min
150 13sec ± 1min 22sec) to assess stress-induced CORT concentrations ($CORT_{30min}$). Immediately
151 following this blood sample, (30min 58sec ± 1min 26sec), 30 birds were injected with DEX
152 then put back into the cloth bag. Fifteen minutes later, we collected a third blood sample
153 ($CORT_{45min}$, at 46min 43sec ± 2min 03sec) and then immediately injected ACTH (47min
154 29sec ± 2min 05sec), after which the birds were placed back into the bag again. Fifteen
155 minutes following the ACTH injection, (at 63min 08sec ± 2min 33sec) we collected a fourth
156 and final blood sample ($CORT_{60min}$). The volume of the final blood sample was 1.5 ml, and
157 this sample was used to measure the concentration of contaminants (see below). All injections
158 were given intramuscularly in the pectoral muscle. Concentrations were adapted to a body
159 mass of 400 g, which was the average body mass of incubating male kittiwakes in 2011. We
160 therefore injected 0.2 ml of DEX (Dexazone 2mg/ml Virbac, France) and 0.1 ml porcine
161 ACTH (Sigma Aldrich, 100IU A6303 dissolved in 0.5 ml Ringer's physiological solution), to
162 obtain doses of 1mg/kg dexamethasone (DEX) and 50 IU/kg ACTH which are considered as
163 sufficient to elicit maximal CORT decrease and increase, respectively in other bird species
164 (Dickens et al. 2009; Rich and Romero 2005; Schmidt et al. 2012).

165 The stress series were shortened compared to those used in laboratory studies: blood samples
166 were collected 15min after each injection versus 45min normally used in song sparrows to
167 elicit maximal CORT responses induced by DEX and ACTH (Schmidt et al. 2012). We
168 shortened the stress series to avoid leaving the nests unattended for a long period (which may
169 increase the risk of the eggs being predated) sampled males were kept for 60min (in total,

170 from baseline sampling to post ACTH injection sample) versus 120min in total in other
171 studies (e.g. Schmidt et al. 2012). Four birds were used as control and injected Ringer's
172 physiological solution to validate the effects of DEX and ACTH injections. We then
173 calculated CORT induced changes following capture/handling protocol, DEX and ACTH
174 injections. Stress-induced CORT: $CORT_{SI}=CORT_{30min}-CORT_{BL}$; $CORT_{DEX}=CORT_{45min}-$
175 $CORT_{30min}$ and $CORT_{ACTH}=CORT_{60min}-CORT_{45min}$. Since absolute CORT concentrations may
176 vary with age as in other seabird species (e.g. Goutte et al. 2010b), we decided to consider
177 relative differences although using absolute or relative CORT values is currently open to
178 debate (Romero 2004).

179 2.3. Clutch size, hatching dates and hatching success

180 Kittiwakes were individually marked with metal rings and PVC plastic bands engraved with a
181 three-letter code and fixed to the bird's tarsus for identification from a distance. Birds were
182 weighed to the nearest 2 g using a Pesola spring balance, and their skull length (head+bill)
183 was measured to the nearest 0.5 mm with a sliding calliper. For each bird we calculated a
184 scaled mass index as a measure of body condition ($M_i\left[\frac{L_0}{L_i}\right]^{bSMA}$, where M_i and L_i are the body
185 mass and the skull length of individual *i* respectively; b_{SMA} is the scaling exponent estimated
186 by the SMA regression of *M* on *L*; L_0 is the average skull length, Peig and Green 2009).
187 Kittiwakes were marked with spots of dye on the forehead to distinguish them from their
188 partner during subsequent observation and then released. Using a mirror at the end of an 8 m
189 fishing rod, we checked the whole plot (*ca*. 117 nests) every two days to monitor the clutch
190 size, the exact hatching date for the first egg (thereafter called "hatching date") and the
191 number of eggs that hatched. The exact hatching date of the first egg laid was obtained for 25
192 individuals (21 birds injected with DEX and ACTH and 4 birds injected with Ringer's
193 physiological solution). For 9 individuals the nest content was not visible for several checks

as the parents would not stand when we approached the mirror. Consequently we were not able to monitor the precise hatching date. Hatching date data could be related to POPs for 19 treated birds (for 2 individuals blood volumes were too small) and to Hg for the 25 birds. We then considered the "hatching success" binomially: 0 = no eggs at all have hatched and 1 = at least one egg has hatched.

2.4. Molecular sexing and hormone assay

For the 34 focal birds, 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 analysis. 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 by radioimmunoassay at UMR 7372 - CNRS-Université de La Rochelle, as previously described (Lormée et al. 2003). The radioimmunoassay used to assay CORT has been validated for kittiwakes (Angelier et al. 2007; Chastel et al. 2005; Goutte et al. 2011a; Nordstad et al. 2012). All samples were run in one assay, to measure intra-assay variation, we included 4 different referents 10 times in the assay and kittiwake plasma samples from previous years. From this, the intra-assay variation was 6.7%. Plasma CORT levels were measured in baseline, stress-, DEX- (or control) and ACTH-induced (or control) CORT samples (**Figure 1**).

2.5. POPs determination in plasma

POPs were analyzed from whole blood of 27 birds injected with DEX and ACTH, for three individuals blood volumes were too small for POP measurements. Analyses were performed at the Norwegian Institute for Air Research (NILU) in Tromsø and the following compounds were search for: the PCBs (CB-28, -52, -99, -101, -105, -118, -138, -153, -180, -183, -187 and

-194), and the organochlorine pesticides (OCPs: *o,p'* DDT, *p,p'* DDT, *p,p'*DDE, *o,p'* DDE, *o,p'* DDD, *p,p'* DDD, α-, β-, γ-HCH, HCB, *trans*-, *cis*-chlordane, oxychlordane, *trans*-, *cis*-nonachlor and mirex). To a blood sample of 0.5 to 1.5 ml, an internal standard solution was added (^{13}C-labelled compounds from Cambridge Isotope Laboratories: Woburn, MA, USA). The sample was extracted twice with 6 ml of n-hexane, after denaturation with ethanol and a saturated solution of ammonium sulphate in water. Matrix removal on florisil columns, separation on an Agilent Technology 7890 GC and detection on an Agilent Technology 5975C MSD were performed as described by Herzke et al. (2009). The limit for detection (LoD) was threefold the signal-to-noise ratio, and for the compounds investigated the limit ranged from 1.1 to 632.7 pg/g wet weights (ww). For validation of the results, blanks (clean and empty glass tubes treated like a sample, 3 in total) were run for every 10 samples, while standard reference material (3 in total, 1589a human serum from NIST) also was run for every 10 samples. The accuracy of the method was between 70 and 108%. For further investigations, concentrations below LoD were assigned LoD value, and only compounds detected in at least 70% of the individuals were included into the statistical analyses (Noël et al. 2009). In incubating male kittiwakes the following PCBs (CB-28, -99, -105, -118, -138, -153, -180, -183 and -187) and OCPs (*p,p'*-DDE, HCB, oxychlordane, *trans*-, *cis*-nonachlor and mirex) were detected in at least 70 % of the individuals (means ± SD and ranges are given in **Table 1**).

2.6. Hg determination in red blood cells

Total Hg was measured for the 34 individuals at LIENSs (La Rochelle), as described by Bustamante et al. (2006) 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

243 hepatopancreas, NRCC; certified value 0.27±0.06 µg/g with recoveries of 98 to 102%). Hg
244 concentrations are expressed in µg/g dry weight (dw).

245 2.7. Statistics

246 All statistical analyses were performed using R 2.13.1 (R Development Core Team 2008). To
247 validate the effects of DEX and ACTH injections on CORT secretion we used generalised
248 linear mixed models (GLMM) with bird identity as a random effect (dependent variable:
249 'CORT'; independent factors 'Time' and 'Treatment'). Then we tested whether CORT would
250 vary following handling stress and injections (controls, DEX, ACTH) and tested whether
251 CORT responses following DEX and ACTH injections were related to body mass and to the
252 hour of the day. We used generalised linear models (GLM) with a normal error distribution
253 and an identity link function to test our biological assumptions. We summed POPs as follows:
254 ΣPCBs (n = 9 congeners) and ΣOCPs (n=6 congeners). First, we tested whether absolute
255 concentration of '$CORT_{BL}$, $CORT_{30min}$, $CORT_{45min}$ and $CORT_{60min}$' and contaminants
256 'ΣPCBs, ΣOCPs and Hg' were related to 'sampling date' and 'scaled mass index'. Second, we
257 tested whether CORT responses to stress series '$CORT_{BL}$, $CORT_{SI}$, $CORT_{DEX}$ and
258 $CORT_{ACTH}$' were related to contaminants 'ΣPCBs, ΣOCPs and Hg' and. Finally, we tested if
259 'clutch size', 'hatching date' and 'hatching success' were related to 'scaled mass index' and
260 contaminants 'ΣPCBs, ΣOCPs and Hg'. Clutch size and hatching success were tested using a
261 GLM with a Poisson/binomial error distribution and a log/logit link function, respectively.
262 Diagnostic plots were used to assess whether the data sufficiently met the assumptions of the
263 model, ΣPCBs and ΣOCPs were log-10 transformed. Values are mean ± SD.

264 **3. Results**

265 3.1. CORT stress series

CORT concentrations were significantly related to the time of blood sampling, the treatment and their interaction (GLMM, time: $F_{3,96}$=340.5, P<0.001; treatment: $F_{1,32}$=6.3, P=0.017; time × treatment: $F_{3,96}$=12.15, P<0.001). Considering the significant relationship between the interaction of time × treatment on CORT: $CORT_{BL}$ and $CORT_{30min}$ were not different between "treated" and "control" birds (GLMM, $CORT_{BL}$: $F_{1,32}$<0.1, P=0.846; $CORT_{30min}$: $F_{1,32}$=0.1, P=0.743) whereas $CORT_{45min}$ and $CORT_{60min}$ were significantly lower in the "treated" birds compared to the "control" birds (GLMM, $CORT_{45min}$: $F_{1,32}$=16.6, P<0.001; $CORT_{60min}$: $F_{1,32}$=15.3, P<0.001). In treated birds, CORT concentrations significantly increased following the capture-restraint protocol (GLMM, $F_{1,29}$=449.3, P<0.001) and CORT concentration significantly decreased over 15 minutes after the DEX injection (GLMM, $F_{1,29}$=160.0, P<0.001). Lastly, ACTH injection did not result in a significant increase of CORT within ~15min of action (GLMM, $F_{1,29}$=1.7, P=0.208). However we observed a large inter-individual variation (**Fig.1A**): for some individuals CORT concentration did not increase or even decreased following ACTH injection, yet in some kittiwakes CORT increased as depicted by positive CORT values on **Figure 2B**. Incubating male kittiwakes were heavier than the average body mass used for DEX and ACTH concentrations (427.2 ± 31g, range: 360-490), however CORT responses were not related to body mass or to its interaction with time (GLMM, body mass: $F_{1,32}$=0.18, P=0.673; time × body mass: $F_{3,96}$=0.76, P=0.519). In control birds CORT significantly increased following the capture-restraint protocol (GLMM, $F_{1,3}$=78.5, P=0.003) then remained steady (GLMM, from $CORT_{30min}$ to $CORT_{45min}$ $F_{1,3}$=2.4, P=0.220, from $CORT_{45min}$ to $CORT_{60min}$, $F_{1,3}$<0.1, P=0.983 **Fig. 1B**). The hour of the day was not related to $CORT_{DEX}$ and $CORT_{ACTH}$ (GLM, $F_{1,28}$=0.4, P=0.526 and $F_{1,28}$<0.1, P=0.986, respectively).

3.2. Contaminants and CORT in relation to sampling date and scaled mass index

290 Absolute concentrations of $CORT_{BL}$, $CORT_{30min}$, $CORT_{45min}$ and $CORT_{60min}$ were not related
291 to sampling date nor to scaled mass index which was used as a measure of body condition
292 (GLM, $F_{1,28}<1.3$, $P>0.269$ for all tests). Hg, ΣPCBs or ΣOCPs were not related to sampling
293 date (GLM, Hg: $F_{1,32}=0.6$, $P=0.428$; ΣPCBs: $F_{1,25}=0.1$, $P=0.745$ and ΣOCPs: GLM, $F_{1,25}=0.2$,
294 $P=0.674$). Hg concentrations were not related to scaled mass index (GLM, $F_{1,32}=0.1$,
295 $P=0.757$), however ΣPCBs and ΣOCPs increased with decreasing scaled mass index (GLM,
296 ΣPCBs: $F_{1,25}=4.3$, $P=0.048$ and ΣOCPs $F_{1,25}=4.9$, $P=0.037$).

297 3.3. Relationships between the HPA activity and contaminants

298 $CORT_{BL}$, $CORT_{SI}$ and $CORT_{DEX}$ (**Fig. 2A)** were not related to contaminants (Hg, ΣPCBs,
299 ΣOCPs, **Table 2**). $CORT_{ACTH}$ was not related to Hg or ΣOCPs (**Table 2**). However
300 $CORT_{ACTH}$ was positively associated to ΣPCBs (**Fig. 2B**, **Table 2**).

301 3.4. Clutch size, hatching dates and hatching success in relation to scaled mass index and
302 contaminants

303 Clutch size (1, 2 or 3 eggs were laid) was not related to scaled mass index (GLM, $\chi^2<0.1$,
304 $P=0.886$) or contaminants (GLM, ΣPCBs: $\chi^2=0.1$, $P=0.721$; ΣOCPs: $\chi^2<0.1$, $P=0.977$ and Hg:
305 $\chi^2=0.4$, $P=0.529$). Hatching date was not related to scaled mass index (GLM, $F_{1,23}=0.2$,
306 $P=0.663$) but was positively related to ΣPCBs (GLM, $F_{1,17}=16.3$, $P<0.001$; **Fig. 3**) and ΣOCPs
307 (GLM, $F_{1,17}=9.2$, $P=0.008$). The relationship between hatching date and Hg was only close to
308 statistical significance (GLM, $F_{1,23}=3.4$, $P=0.076$). With regard to hatching success,
309 individuals with no chicks that hatched tended to have a lower scaled mass index (GLM,
310 $\chi^2=3.7$, $P=0.055$). Hatching success was not related to contaminants (GLM, ΣPCBs: $\chi^2=0.2$,
311 $P=0.635$; ΣOCPs: $\chi^2<0.1$, $P=0.820$ and Hg: $\chi^2<0.1$, $P=0.904$)

312 **4. Discussion**

313 4.1. HPA activity and contaminants

314 The aim of this study was to test whether the positive association between CORT secretion
315 and PCB contamination, which has been repeatedly observed in this Svalbard population of
316 kittiwakes (Nordstad et al. 2012; Tartu et al. 2014; Tartu et al. in press), resulted from an
317 inefficient negative feedback mechanism or from a higher adrenal activity. We also
318 investigated if other contaminants (Hg or OCPs) would be related to some aspect of the HPA
319 axis activity. Contrary to what has been reported in previous studies on kittiwakes from the
320 same breeding colony, we did not find any relationship between contaminants, $CORT_{BL}$ and
321 $CORT_{SI}$ (Nordstad et al. 2012; Tartu et al. 2014; Tartu et al. in press). $CORT_{DEX}$ was not
322 related to contaminants, suggesting that environmental contaminants may not alter the
323 functioning of glucocorticoid-receptors on the hypothalamus or the pituitary. According to
324 our prediction, the adrenal responsiveness ($CORT_{ACTH}$) was positively associated to ΣPCBs in
325 male kittiwakes. Administration of a standardized dose of ACTH is an alternative approach to
326 measure the stress response that is specifically due to variation in the sensitivity of the adrenal
327 cortex to ACTH. Response to exogenous ACTH may also provide a more accurate measure of
328 glucocorticoid production than response to restraint stress (Wada et al. 2007; Schmidt et al.
329 2012). However in some birds ACTH injection was not effective and CORT levels even
330 decreased in birds with lower levels of PCBs. These results are surprising but could be the
331 consequence of a too short time of action of ACTH or maybe the dose of ACTH we used was
332 too low to elicit a maximal CORT release. The time necessary to elicit a maximal CORT
333 release post-ACTH injection in song sparrows was 45min (e.g. Schmidt et al. 2012) and our
334 experimental kittiwakes were only exposed for 15min, also we were not able to previously
335 validate if the ACTH dose we used would elicit a maximal CORT release in kittiwakes.
336 Further studies would be needed to test the dose-response between ACTH injection and
337 CORT secretion in kittiwakes. However, relative CORT differences in response to ACTH

injection ($CORT_{ACTH}$) were significantly related to blood PCB concentrations in incubating male kittiwakes. Consequently, this result suggests that the adrenal activity of male kittiwakes bearing high levels of PCBs is exacerbated. An exacerbated adrenal activity may result from increased number of ACTH-receptors (ACTH-R) on the adrenals. In mammals, ACTH is one of the few polypeptide hormones having a positive trophic effect on its own receptors (Penhoat et al. 1989; Beuschlein et al. 2001). Although there is no evidence for such a relationship in birds, we may assume that a similar effect to what is observed in mammals would occur in birds. Thus, the positive association between adrenal activity and PCB contamination in kittiwakes may be related to an excess of ACTH input to adrenals. In experimental studies, causal effects of POPs on ACTH-R have already been described: PCB126 can increase ACTH-R levels in human adrenocortical cells (Li and Wang, 2005), and a pesticide, the methyl thiophanate, could mimic ACTH and directly activate ACTH-R on the adrenal glands of lizards *Podarcis sicula* (De Falco et al. 2007). The present study and other recent findings on male and female kittiwakes (Nordstad et al. 2012; Tartu et al. 2014; Tartu et al. in press) show that PCBs and not OCPs are associated to CORT secretion in this species and that Hg does not seem related to the adrenocortical response or to the adrenal activity in kittiwakes. Relationships between CORT and PCBs could also be environment-dependent. Indeed, in the here present study $CORT_{BL}$ or $CORT_{SI}$ were not related to PCBs contrary to results found in kittiwakes from the same breeding colony (Nordstad et al. 2012; Tartu et al. 2014; Tartu et al. in press). The nature of the relationship between CORT and PCBs could depend of the level of contamination (Tartu et al. in press). In 2011, where significant relationships were found between CORT (baseline and stress-induced levels) and ΣPCBs, the levels of some CB congeners differed: CB-28 and CB-194 were detected in less and more than 70% of the individuals, respectively. Seven individuals were caught in 2012 (the present study) and in 2011 and when considering indicator PCBs (i.e. ∑CB-28, -52, -101, -118, -138,

363 -153 and -180) which are highly bioaccumulative in a wide range of polar seabird species
364 (Gabrielsen et al. 1995; Savinova et al. 1995), the levels appeared to be significantly lower in
365 2012 than in 2011 (GLMM, $F_{1,6}$=14.21, P=0.009). This result suggests that at lower
366 concentrations PCB may stimulate CORT secretion whereas at higher concentrations the
367 relationship between CORT and PCBs could reach a plateau a thus not be observable. In the
368 present study, ΣPOPs significantly increased with decreasing body-condition as depicted by
369 scaled mass index. Since POPs are stored into fatty tissues it is not surprising that male
370 kittiwakes with poor body reserves show higher concentrations of PCBs and OCPs in their
371 plasma. Such relationships have been observed in several bird species such as kittiwakes
372 (Henriksen et al. 1996; Tartu et al. 2014), glaucous gulls *Larus hyperboreus* (Sagerup et al.
373 2009), common eiders *Somateria mollissima* (Bustnes et al. 2010, 2012), sparrowhawks
374 *Accipiter nisus* (Bogan and Newton, 1977) and white-tailed eagle *Haliaeetus albicilla*
375 (Kenntner et al. 2003). Body-condition in birds predicts a wide range of fitness related traits
376 such as incubation pattern, breeding success or survival (see Labocha and Hayes, 2012 for a
377 review). And because lower body-condition is positively associated to blood POP
378 concentrations, POPs could therefore become more available for more sensitive vital organs
379 as brain, kidneys and liver (Henriksen et al. 1996; Fuglei et al. 2007).

380 4.2. Fitness in relation to contaminants

381 In our study, we observed a positive relationship between ΣPCBs, ΣOCPs and hatching date.
382 The first egg of the most contaminated male kittiwakes hatched later in the season. The same
383 pattern has been found in female south polar skuas *Catharacta maccormicki* (Bustnes et al.
384 2007). A delayed hatching date could be the consequence of an impaired incubation behavior.
385 Previous studies on captive American kestrels *Falco sparverius* and free-ranging glaucous
386 gulls have reported altered incubation behaviors in relation to increasing PCB burden
387 (Bustnes et al. 2001; Fisher et al. 2006; Verboven et al. 2009). Although behavior and egg-

388 laying date were not measured in incubating male kittiwakes, we could assume that high
389 PCBs and OCPs burden may alter incubation behavior leading in a delayed hatching date.
390 Further studies would be needed to support this assumption. Contrary to our prediction, blood
391 contaminants were not negatively associated with hatching success. However, in case of poor
392 foraging conditions when CORT secretion is stimulated (Kitaysky et al., 1999; Goutte et al.
393 2014), it is possible that male kittiwakes bearing high levels of PCBs would be more sensitive
394 to environmental stress and would be less able to properly incubate their eggs than less
395 polluted ones. More generally an exacerbated adrenal responsiveness to stress, as depicted by
396 the CORT response to ACTH, often mirrors poor fitness related traits as poor parental
397 investment (Angelier et al. 2009; Bókony et al. 2009; Goutte et al. 2011b; Lendvai et al.
398 2007) or lower adult survival (Blas et al. 2007; Goutte et al. 2010a; Romero 2012). Thus,
399 although most legacy POPs show decreasing trend in Arctic seabirds (e.g. Helgason 2011),
400 the prevalence of PCBs could make individuals more susceptible to other environmental
401 stressors such as ongoing climate change (Jenssen 2006).

402

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412 **References**

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694 **Figure legends**

695 **Figure 1:** Stress series to characterize the hypothalamo-pituitary-adrenal (HPA) activity in
696 incubating male black-legged kittiwakes. CORT increase from 0 to 30 min indicates the time
697 of exposure to restraint stress. The left panel represents birds that were injected with DEX and
698 ACTH (A) and right panel birds injected with Ringer's physiological solution (B). Each circle
699 denotes an individual and arrows indicate the time of injections.

700 **Figure 2:** $CORT_{DEX}$ and $CORT_{ACTH}$ in relation to ΣPCBs. In incubating male black-legged
701 kittiwakes, $CORT_{DEX}$ (upper panel **A**), was not related to blood ΣPCBs (pg/g ww, log-10
702 transformed). Whereas $CORT_{ACTH}$ (lower panel **B**), was positively associated to ΣPCBs.
703 ACTH injection elicited an increase of CORT in male kittiwakes above dashed line and a
704 decrease of CORT in individuals below. Solid line refers to significant linear regression
705 ($P=0.040$, $R^2=0.16$).

706 **Figure 3:** Hatching date in relation to ΣPCBs. In male black-legged kittiwakes increasing
707 blood ΣPCBs (pg/g ww, log-10 transformed) delayed the hatching of the first egg laid. Solid
708 line refers to significant linear regression ($P<0.001$, $R^2=0.35$).

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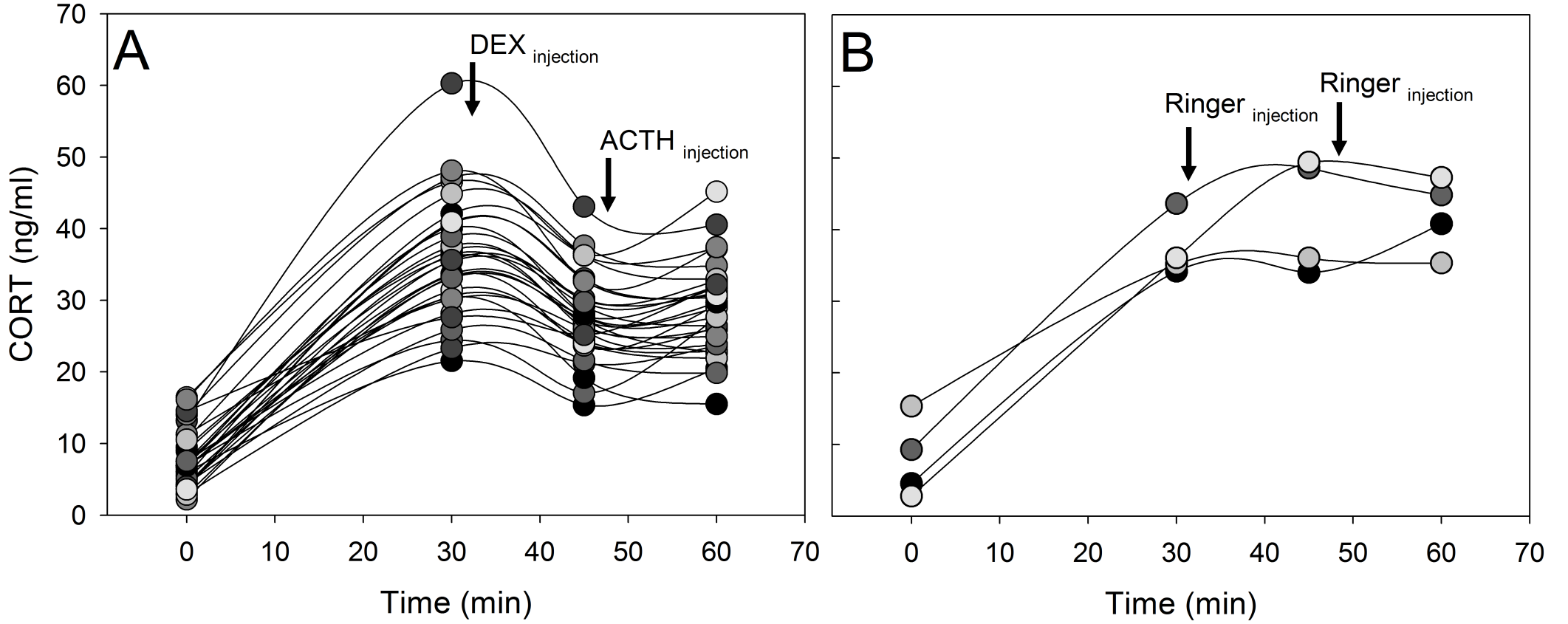
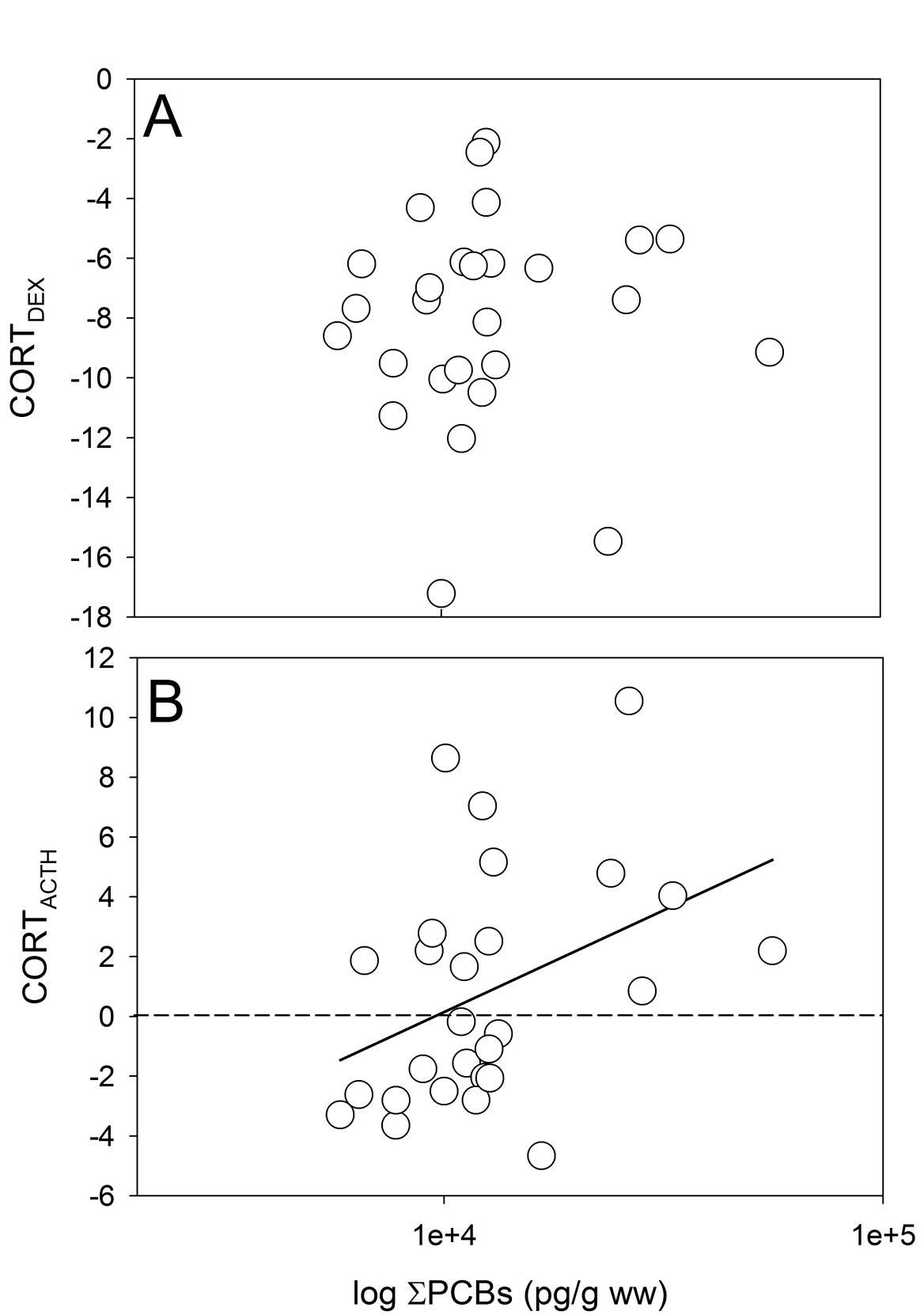

A
DEX injection
ACTH injection
CORT (ng/ml)
Time (min)
B
Ringer injection
Ringer injection
Time (min)


A
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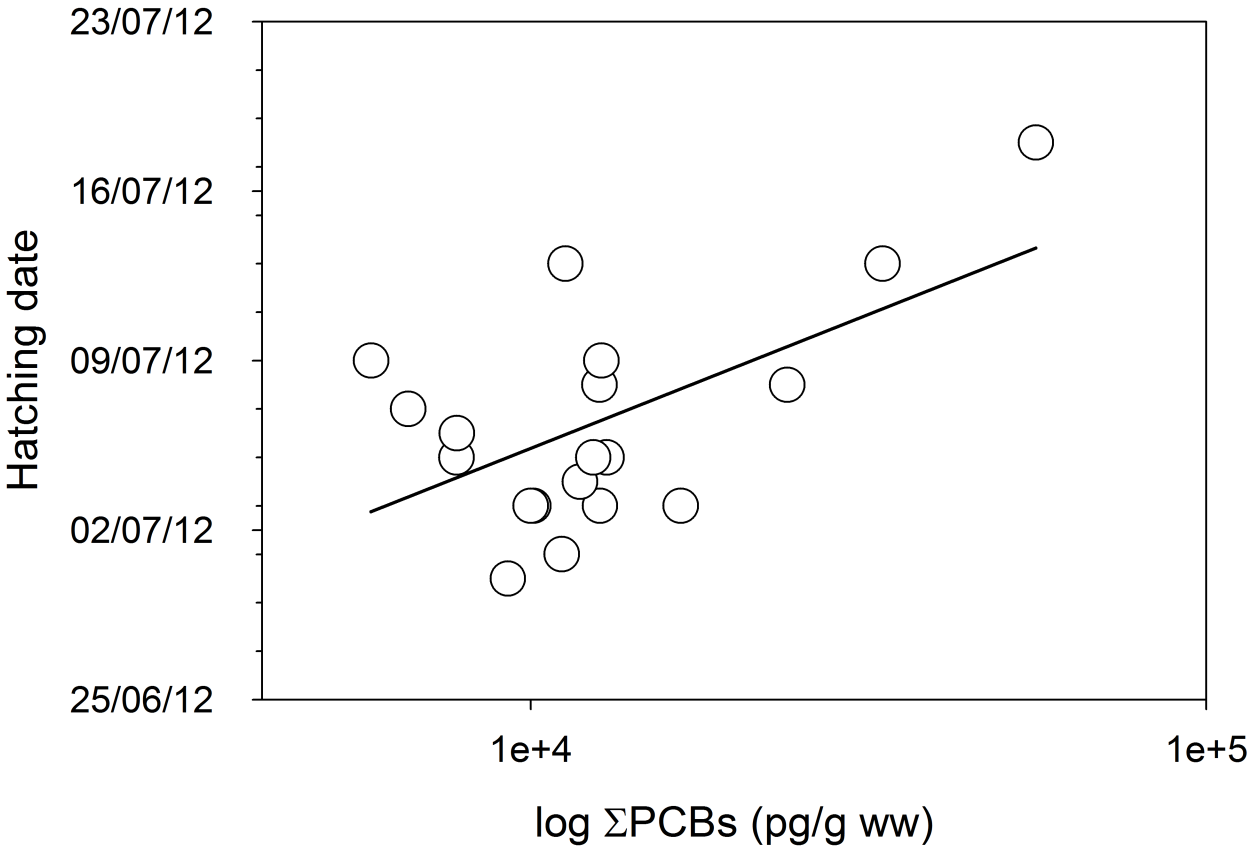
CORT$_{DEX}$
CORT$_{ACTH}$
1e+4
1e+5
log ΣPCBs (pg/g ww)


23/07/12
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Hatching date
1e+4
1e+5
log ΣPCBs (pg/g ww)

Table 1: Concentrations of red blood cells' Hg (µg/g dw) and whole blood persistent organic pollutants (pg/g ww) in incubating male black-legged kittiwakes.

Contaminants	Mean (median)	SD	Minimum	Maximum
Hg	1.80 (1.96)	0.45	0.82	2.96
PCB 28	224 (200)	84	50	478
PCB 99	927 (740)	566	410	2700
PCB 105	323 (267)	222	135	1163
PCB 118	1124 (930)	731	500	3870
PCB 138	3931 (3120)	2865	1550	14750
PCB 153	4968 (3820)	3824	1800	20280
PCB 180	2143 (1550)	1648	730	8490
PCB 183	434 (325)	336	148	1759
PCB 187	756 (591)	519	321	2529
ΣPCBs	14831 (11827)	10694	5801	55951
HCB	2977 (2700)	872	1850	6400
oxy-Chlordane	801.6 (667)	385	359	2120
trans-Nonachlor	38.9 (35)	21	6	98
cis-Nonachlor	35 (32)	16	5	71
Mirex	388 (352)	198	167	959
p,p'-DDE	1265 (1177)	697	306	3506
ΣOCPs	4241 (3700)	1413	2495	9616

Table 2: Parameter estimates for generalized linear models (GLM) assessing the relationships between contaminants (Hg, ΣPCBs and ΣOCPs) and A) [$CORT_{BL}$], B) $CORT_{SI}$, C) $CORT_{DEX}$ and D) $CORT_{ACTH}$ in incubating male black-legged kittiwakes.

Dependent variable	Independent variable	Intercept	Estimate	Df	F	P-value
A) $CORT_{BL}$	Hg	2.07	-0.33	1,28	0.81	0.376
	ΣPCBs	1.73	0.02	1,25	0.01	0.913
	ΣOCPs	6.40	-0.54	1,25	2.19	0.152
B) $CORT_{SI}$	Hg	3.32	-0.03	1,28	0.02	0.882
	ΣPCBs	3.89	-0.06	1,25	0.34	0.562
	ΣOCPs	3.55	0.03	1,25	0.02	0.885
C) $CORT_{DEX}$	Hg	-9.81	3.52	1,28	2.38	0.134
	ΣPCBs	-10.07	0.22	1,25	0.03	0.867
	ΣOCPs	-26.12	2.18	1,25	0.76	0.391
D) $CORT_{ACTH}$	Hg	2.89	-3.55	1,28	1.83	0.187
	ΣPCBs	**-27.10**	**2.96**	**1,25**	**4.68**	**0.040**
	ΣOCPs	-36.12	4.45	1,25	2.64	0.117