The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female Black legged kittiwakes

SABRINA TARTU[a,*], FRÉDÉRIC ANGELIER[a], DORTE HERZKE[b], BØRGE MOE[c], CLAUS BECH[d], GEIR W. GABRIELSEN[e], JAN OVE BUSTNES[c], OLIVIER CHASTEL[a]

[a]*Centre d'Etudes Biologiques de Chizé (CEBC), UPR 1934-CNRS, F-79360, France*

[b]*Norwegian Institute for Air Research, FRAM – High North Research Centre on Climate and the Environment, N-9296 Tromsø, Norway*

[c]*Norwegian Institute for Nature Research, FRAM – High North Research Centre on Climate and the Environment, N-9296 Tromsø, Norway*

[d]*Department of Biology, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway*

[e]*Norwegian Polar Research Institute, FRAM Centre High North Research on Climate and the Environment N-9296 Tromsø, Norway*

[*]tartu@cebc.cnrs.fr

Abstract

High levels of environmental pollutants such as persistent organic pollutants (POPs) including PCB and DDT have been found in the Arctic and many of those pollutants may impair reproduction through endocrine disruption. Nevertheless, their effects on stress hormones remain poorly understood, especially in free-ranging birds. Corticosterone, the principal glucocorticoid in birds, can indirectly impair reproduction. The aim of the present study was to examine the relationships between POPs and reproduction through their potential consequences on different reproductive traits (breeding decision, egg-laying date, breeding success) and corticosterone secretion (baseline and stress-induced levels). We addressed those questions in an Arctic population of female black-legged kittiwakes during the pre-breeding stage and measured several legacy POPs (PCBs and pesticides: HCB, p,p'-DDE, CHL) in whole blood. POP levels were not related to breeding decision neither to breeding success, whereas females with high levels of pesticides laid their eggs earlier in the season. We found a negative relationship between POP levels and body condition index in non-breeding females. Black-legged kittiwakes with higher levels of PCB showed stronger adrenocortical response when subjected to a capture-handling stress protocol.We suggest that PCBs may disrupt corticosterone secretion whereas the positive relationship between pesticides and egg-laying date could either originate from a direct effect of pesticides or may be related to other confounding factors such as age or individual's quality. Although no direct negative reproduction output of POPs were found in this study, it is possible that the most contaminated individuals would be more sensitive to environmental stress and would be less able to maintain parental investment than less polluted individuals.

Key words: Persistent organic pollutants, Corticosterone, Stress response, Reproduction, Arctic seabirds

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1. Introduction

Environmental pollutants, such as persistent organic pollutants (POPs: pesticides, PCBs), have received an increasing attention during the last 30 years. The Arctic is considered as a sink for environmental pollution, and for some compounds, levels may exceed that of industrialized cities (Gabrielsen and Henriksen 2001). Because of bioaccumulation into organisms and bio-magnification along the food chain, marine apex predators such as seals, whales and seabirds are particularly vulnerable (Letcher et al. 2010; Vallack et al. 1998). Among free-living vertebrates, highly polluted individuals show decreased breeding capacities, such as abnormal breeding behaviour, reduced fertility or poor breeding success (Bustnes et al. 2003a, 2007; Colborn et al 1993; Gabrielsen 2007; Harrison et al., 1997; Taylor and Harrison 1999; Verreault et al. 2010). Such breeding impairment could originate from the ability of POPs to act as endocrine disruptors and thus, to alter the functioning of major endocrine axes (Ottinger et al. 2013; Tyler et al. 1998). Indeed those substances are able to mimic, antagonize, alter or modify endogenous' hormone functions (e.g. Amaral Mendes 2002). In free-living vertebrates, several studies have found significant relationships between POPs and reproductive hormones such as steroids (Colborn et al 1993; Giesy et al. 2003; Vos et al. 2008) and more recently hypothalamic and pituitary hormones (Verreault et al. 2008). Other hormones, such as those from the hypothalamic-pituitary-adrenal (HPA) axis, and especially glucocorticoids, are however known to affect reproductive behaviors and to mediate major reproductive decisions in vertebrates (reviewed in Wingfield and Sapolsky, 2003). Studies on laboratory mammals have documented a number of effects of chemicals on glucocorticoids (Odermatt and Gumy, 2008) but effects of POPs on stress hormones have been poorly studied in wildlife (Bergman et al. 2012). Hence, the concern for endocrine disruptors should also be directed towards the glucocorticoid system (Dawson 2000; Johansson et al, 1998). Glucocorticoids (cortisol, corticosterone) are released in response to stressful events (food shortage, predation, and pathogens) to adjust life-history strategies in relation to environmental conditions and to individual physiological state (Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003). Indeed, the release of glucocorticoids during stressful events triggers physiological and behavioral adjustments that shift energy investment away from reproduction and redirects it towards survival (Wingfield and Sapolsky, 2003). Stress hormones have therefore a strong connection with fitness traits such as breeding success, individual quality and survival (Angelier et al. 2009, 2010; Bonier et al. 2009; Bókony et al. 2009; Breuner et al. 2008; Goutte et al. 2010b, 2011b; Kitaysky et al. 1999). Importantly, this means that a disruption of glucocorticoid secretion may alter the ability of an individual to adjust breeding decisions (to breed or not, when to breed) to environmental conditions. However, only a few studies have explored the impact of pollutants on both baseline and stress induced glucocorticoid levels, which depict different physiological functions: baseline corticosterone levels (CORT, the major glucocorticoid in birds) mirrors activity, metabolic rate and reflects the ratio between energy available and energy needed (Landys et al. 2006), while stress-induced CORT can be used as an index of the sensitivity to stress of an individual, this value can be modulated in order to maximize either survival, either reproduction (Bókony et al. 2009; Lendvai et al. 2007). Regarding contaminant/HPA axis, the pattern seems clear in fish: i.e. individuals from polluted sites (heavy metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls) are unable to elevate their cortisol levels (reviewed in Hontela 2005). Studies on wild birds remain sparse and the pattern is less clear (reviewed in Verreault et al. 2010), mainly because they are difficult to compare as pollutants have been measured in different tissues (i.e. muscles, liver or feathers). And even when comparing two studies on the effects of POPs on CORT secretion, both measured in blood of Arctic seabirds, no clear pattern appeared. In black-legged kittiwakes Rissa tridactyla (hereafter 'kittiwakes') sampled early in the breeding season (April), increasing blood levels of PCBs were related to an increase of baseline CORT levels, and this relationship did not appear during the incubation period (Nordstad et al. 2012). Whereas in incubating glaucous gulls Larus hyperboreus, increasing blood POP levels (among which PCBs but also several pesticides) resulted in an increase of baseline CORT (Verboven et al. 2010). Moreover, male glaucous gulls subjected to a standardized stress-protocol, had decreased levels of stress-induced CORT with increasing POPs (Verboven et al. 2010). Thus the nature of POPs-CORT relationships could therefore depend on the type of pollutants, gender, types of tissue sampled and the reproductive status of the individuals. Although CORT is considered a key-stone hormone for allocation processes and reproductive effort (Wingfield and Sapolsky, 2003), there is a lack of studies investigating POPs-CORT-fitness in free-living organisms.

The aim of the present study was to consider the relationships between POPs and reproduction through their potential effects on reproductive traits and corticosterone secretion (baseline and stress-induced levels). We addressed those questions on female kittiwakes during the pre-breeding stage and measured POP levels (PCBs and pesticides: HCB, p,p'-DDE, CHL) from whole blood samples. In female kittiwakes CORT predicts breeding decision (Goutte et al. 2010a), egg-laying date (Goutte et al. 2011b) and breeding success (Goutte et al. 2011b). Thus, we investigated if blood POP levels would be related to 1) reproductive traits (the decision to breed or not, egg-laying date and breeding success) and 2) CORT secretion (baseline and stress-induced levels).

2. MATERIALS AND METHODS

2.1. Study area and birds

Our study was conducted in a colony of kittiwakes at Kongsfjorden, Svalbard (78°54'N, 12°13'E), 7 km southeast of Ny-Ålesund, Norway. Kittiwakes are colonial seabirds that breed on cliffs throughout the northern parts of the Pacific and Atlantic, including the Barents Sea region up to the Svalbard

Archipelago (Anker-Nilssen et al., 2000). We studied kittiwakes in one plot of around 117 pairs breeding on cliff ledges at heights of 5-10 m. Female kittiwakes were sampled from 19 May to 7 June 2011, during the pre-laying period (*i.e.* copulations and nest building period), a key period for reproductive decisions during which females kittiwakes appear highly sensitive to stressors (Goutte et al. 2010a, 2011b; Tartu et al. 2013).

2.2. Capture and blood sampling

Forty-seven females were caught on the nests with a noose at the end of a 5 m fishing rod. A first blood sample (*ca.* 0.3 mL) was collected immediately after capture, from the alar vein with a 1 mL heparinised syringe and a 25-gauge needle to assess baseline CORT levels. Bleeding time (i.e. time elapsed from capture to the end of the first blood sample: 2min 55 ± 34 (SD) seconds, on average) did not affect CORT levels (GLM, $F_{1,45} = 1.79$, $p = 0.190$). Kittiwakes were then placed into cloth bags and subsequent blood samples (*ca*. 0.3 mL) were collected from the alar vein at 30 minutes to assess stress-induced CORT levels.

Kittiwakes were individually marked with metal rings and PVC plastic bands engraved with a three-digit code and fixed to the bird's tarsus for identification from a distance. Birds were weighed to the nearest 2 g using a Pesola spring balance, and their skull length (head+bill) was measured to the nearest 0.5 mm with a sliding calliper. For each individual, we calculated an index of body condition by using the residuals from a linear regression of body mass against skull length (GLM, $F_{1,46}=7.05$, $p=0.01$). Kittiwakes were marked with spots of dye on the forehead to distinguish them from their partner during subsequent observation and then released. Using a mirror at the end of an 8 m fishing rod, we checked the whole plot (*ca*. 117 nests) every two days to monitor breeding decision (at least one egg is laid or no egg laid) and egg-laying dates. Then, with same technique, we checked the nest content every 2 or 3 days to monitor the number of chicks that reached at least 12 days of age per active nest (hereafter called ‘breeding success’).

2.3. Molecular sexing and hormone assay

Blood samples were centrifuged, and plasma and red blood cells were separated and stored at -20°C until used respectively in hormone assays or molecular sexing, at the Centre d'Etudes Biologiques de Chizé (CEBC). Molecular sexing was performed as detailed in Weimerskirch et al. (2005). Plasma concentrations of CORT were determined by radioimmunoassay at the CEBC, as described by Lormée et al. (2003). Baseline CORT levels were not related to sampling date (GLM, $F_{1,45} = 0.27$, $p = 0.610$) or time of the day (GLM, $F_{1,45} = 0.33$, $p = 0.571$) and neither were stress-induced levels (sampling date: GLM, $F_{1,45} = 0.55$, $p = 0.460$; time of the day: GLM, $F_{1,45} = 0.72$, $p = 0.399$).The lowest detectable concentration for CORT was 0.53 ng/ml. Only one assay was performed and the intra-assay coefficient of variation was 6.7 % (N = 5 duplicates).

2.4. POPs analyses

POPs were analyzed from 45 whole blood samples at the Norwegian Institute for Air Research (NILU) in Tromsø, for two individuals blood volumes were too low for POP measurements. The following compounds were analysed: the PCBs (CB-28, -52, -99, -101, -105, -118, -128, -138, -153, -180, -183, -187 and -194), and the pesticides (p,p'-DDE, α-, β-, γ-HCH, HCB, oxychlordane, trans-, cis-chlordane, trans-, cis-nonachlor). The compounds chosen for further investigation were the ΣPCBs (CB-99, -105, -118, -128, -138, -153, -180, -183, -187 and -194), and the Σpesticides (p,p'-DDE, HCB, oxychlordane, trans-chlordane, trans-, cis-nonachlor). To a blood sample of 0.5 to 1.5 ml, a 100 µL internal standard solution was added (13C-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 was threefold the signal-to-noise ratio, and for the compounds investigated the limit ranged from 0.4 to 122 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) was run for every 10 samples. The accuracy of the method was within the 70 and 108% range.

2.5. Statistical analyses

All statistical analyses were performed using R 2.8.0 (R Development Core Team 2008). We used generalised linear models (GLM) with a normal, binomial or Poisson error distribution and an identity, logit or log link function, respectively, to test our biological assumptions. To test the relationships between different groups of POPs (pesticides and PCBs), we categorised POP compounds based on chemical structure similarities. We summed their concentrations in four classes as follows: ΣPCBs (n = 10 congeners), ΣCHL (n = 4 compounds), p,p'-DDE and HCH. Because ΣCHL, p,p'-DDE and HCH were all correlated (Pearson correlation coefficients, R=0.70, 0.83 and 0.82) we grouped them into a sum of pesticides (ΣPesticides). To describe the total blood contaminant concentration of individual kittiwakes we used the sum of POPs (ΣPOPs) including ΣPCBs and ΣPesticides as they were also highly correlated (R=0.79). First, we tested the relationships between body condition, organic pollutants (using ΣPOPs, ΣPCBs and ΣPesticides) and breeding decision, egg-laying date and breeding success. We also tested the relationships between organic pollutants and body condition index in non-breeders and breeders separately. Second, we checked for relationships between organic pollutants (using ΣPOPs, ΣPCBs and ΣPesticides), breeding decision and interaction, and baseline and stress-induced CORT. We performed statistical tests by using absolute CORT levels to facilitate the comparison with other published results. Prior to this, we checked for possible statistical effects of bleeding time, sampling date, time elapsed between blood sampling and egg-laying, hour of the day, body condition and breeding decision on baseline and stress-induced CORT levels ($p>0.05$ for all tests). Diagnostic plots were used to assess whether the data sufficiently met the assumptions of the linear model, and dependent continuous variables were log-10 transformed when necessary. Values are mean ± SD.

3. Results

3.1. Relationships between organic pollutants, body condition and reproductive traits

The probability to breed was significantly related to the body condition as non-breeding females had a lower body-condition than breeding females (**Fig.1A; Table 1**). However, the probability of breeding was not related to ΣPOPs, ΣPCBs or ΣPesticides (**Fig.1B; Table 1**). Additionally, in non-breeding females, we found a negative and significant relationship between body condition and ΣPOPs (GLM, $F_{1,17} = 12.90$, $p = 0.002$), the same relationship was found between ΣPCBs (GLM, $F_{1,17} = 12.46$, $p = 0.003$) and ΣPesticides (GLM, $F_{1,17} = 8.85$, $p = 0.008$) on body condition index **(Fig. 2),** while no relationship was observed in breeding females ($p>0.51$ for all tests, **Fig.2**).

In females that bred, egg-laying date was negatively related to ΣPesticides: females that laid early had higher levels of pesticides than females that laid later in June (**Fig.3; Table 1**). This relationship did not appear when considering body condition or ΣPCBs. No relationships were found between body condition, ΣPOPs, ΣPCBs or ΣPesticides and breeding success (**Table 1**).

3.2. Relationships between organic pollutants and CORT secretion

Baseline and stress induced CORT levels (log transformed) were not related to body condition ($p>0.05$ for all tests) or breeding decision (**Table 2**). We did not find any relationship between ΣPOPs, ΣPCBs or ΣPesticides on baseline CORT levels (**Fig.4A**; **Table 2**). However, we found a positive relationship between ΣPCBs and stress-induced CORT levels. The most polluted individuals released more CORT when subjected to a capture-handling stress protocol (**Fig.4B**; **Table 2**), and this relation was not found when using ΣPOPs only or ΣPesticides.

4. Discussion

Although kittiwakes from colonies in Svalbard are exposed to POPs (Nordstad et al. 2012; Savinova et al. 1995), the present study did not reveal any negative impact on the decision to breed or not, and breeding success. Nevertheless, females with the higher levels of pesticides laid eggs earlier. We found a negative relationship between POP levels and body condition index in non-breeding females. We also found that individuals bearing the higher levels of PCB would have a stronger adrenocortical response when subjected to a capture-handling stress protocol, and this did not appear when testing the relationship between ΣPesticides only and CORT secretion.

4.1. Relationships between organic pollutants and reproductive traits

4.1.1. Breeding decision and body-condition index

In kittiwakes, and in many long-lived seabird species, a significant proportion of adult birds will not breed in a given year (Goutte et al. 2010a, 2011c). In a recent study we have shown that moderate levels of mercury could be linked to non-breeding events in kittiwakes from Svalbard (Tartu et al. 2013). In our study, however the decision to breed or not was unrelated to blood POP levels (neither to pesticides nor PCBs). Although Svalbard kittiwakes bear significant amounts of pesticides and PCBs in their blood (Nordstad et al. 2012, this study), their POP levels were about 10–fold lower compared to levels in glaucous gulls, the most polluted arctic seabird species (Borgå et al. 2001; Bustnes et al. 2003b; Gabrielsen et al. 1995). Thus it is possible that POP levels found in pre-laying kittiwakes were not high enough to alter reproduction. Another possibility is that pesticides and PCBs, contrary to mercury, may not interfere with hormonal pathways involved in non-breeding behaviour, such as GnRH (gonadotropin-releasing hormone) and luteinizing hormone (LH, Tartu et al. 2013).

As found in other seabird species females that did not breed had a lower body condition index (Chastel et al. 1995; Goutte et al. 2010a, 2010c). Decreasing condition in non-breeding females was associated with increasing blood POP levels. Organic pollutants are lipophilic; if body fat reserves are low, organic pollutants can be redistributed through the bloodstream and more likely to migrate to sensitive vital organs as brain, kidneys and liver (Fuglei et al. 2007; Henriksen et al. 1996). This pollutant redistribution has been confirmed in several bird species: emaciated individuals had higher levels of POPs in liver, blood and brain than individuals in better body condition (Bogan and Newton, 1977; Bustnes et al. 2010, 2012; Kenntner et al. 2003). In female kittiwakes breeding in northern Norway, average body mass decreases of 20% from the beginning to the end of the breeding period, and this body mass loss was accompanied with a 4-fold increase of PCB levels in the brain (Henriksen et al. 1996). Thus birds in poor body condition are more sensitive to environmental pollution since pollutants are more available in sensitive organs. If vital organs are harmed, detoxification process would be more important, increasing the individual's metabolism. Thus, we can suppose that the most polluted non-breeders may therefore be in lower body condition because they are expending more energy in detoxification than breeders. Our study highlights that in pre-laying female kittiwakes, POPs-condition relationships differ according to breeding decision. However, a question is why such relationship was found in non-breeding birds only although all females despite their breeding decision had, on average, similar blood concentrations of POPs? Maybe non-breeding individuals reached a threshold condition below which POPs/body condition relationships became apparent or non-breeding birds may also be poor quality individuals (Cam et al. 1998), which may be less able to physiologically deal with a given dose of POPs. Also, POPs may act as endocrine disruptors, and according to the physiological state of an individual, hormonal levels vary, especially if individuals chose to breed or not. Because in kittiwakes, endocrine levels differ between breeders and non-breeders (Goutte et al. 2010a; Tartu et al. 2013) this could have an effect on the potential toxicity and threat of endocrine disrupting chemicals. It has been suggested, in studies on rats, that oestrogens have a protective effect on methyl-mercury threat on the brain (Oliveira et al. 2006). Therefore, if some pollutants mimic the effects of sex steroids, and if these latter are endogenously more present in the organism, the pollutants' harm could be less effective. To our knowledge, a protective effect of reproductive hormones on POPs has never been observed. Further studies comparing POP levels in the Hypothalamo-Pituitary-Gonad axis of breeders and non-breeders, which include measurements of sex steroids, would be important to test this supposition.

4.1.2. Egg-laying date and breeding success

In birds, breeding at the right time is one of the most important factors for successful reproduction in a fluctuating environment (e.g. Lack 1968) and in kittiwakes, late breeding is usually associated with low breeding success (Goutte et al. 2011b; Moe et al. 2009). Studies on free-living great black-backed gulls (Larus marinus) and Antarctic skuas (Catharacta maccormicki*)* have reported that the most polluted individuals had a delayed egg-laying date (Bustnes et al. 2007; Helberg et al. 2005). In our study we found the reverse pattern: females with the highest levels of pesticides laid their eggs earlier in the season, as found in some populations of glaucous gulls (Bustnes et al. 2003b) and great black-backed gulls (Bustnes et al. 2008).

This negative relationship between pesticide contamination and egg-laying date may not be causal: e.g females laying early could be of better quality, forage at a higher trophic level and hence be more exposed to pesticide contamination. In the same line of idea, this correlation could be the result of age related processes since in birds old females often lay earlier than young ones (e.g. Goutte et al. 2010c) and those older females may possibly bear higher POP levels. In our study, no birds were of known age, so we were not able to test for a possible influence of age on POP levels. However the few studies that have explored these relationships have failed to find a relationship between age and POPs concentration (Bustnes et al. 2003a; Tartu et al. unpublished data).

In mammals, some studies have described relationships between organochlorine pesticides and preterm birth (Longnecker et al. 2001; Saxena et al. 1981). DDT metabolites are able to impede the binding of some sex steroids (androgen or progesterone) to their receptor through indirect or direct paths, shortening the duration of gestation (Klotz et al. 1997; Lyon and Glenister 1980). Some of the pesticides present in kittiwakes could also mimic or stimulate the secretion of some hormones involved in oviposition. Prostaglandin synthesis, a major hormone involved in oviposition, can be inhibited by p,p'-DDE in ducks (Lundholm, 1997), which is not coherent with our results, but consistent with Bustnes et al. (2007, 2008). However, many pollutants have a non-linear dose-response relationship (Calabrese 2010; Heinz et al. 2012) and several studies have pictured an inverted U-shaped effect (e.g. Love et al. 2003), low dose of pollutant may enhance the synthesis of a hormone while important doses may inhibit it. The amount of DDE measured into the eggs to inhibit prostaglandin in Lundholm (1997) was averagely 3000-fold that of p,p'-DDE levels found in female kittiwakes' blood (this study) and 200-fold that of p,p'-DDE levels found in Svalbard kittiwakes' eggs (Barrett et al. 1996). We may speculate that in kittiwakes low dose of p,p'-DDE may stimulate prostaglandin synthesis, which would in this case initiate an early oviposition. Because p,p'-DDE and prostaglandin are tightly linked (Lundholm 1997) and that the relationship between egg-laying date and p,p'-DDE was statistically the more significant among all other pesticides (i.e. HCB, chlordanes), we suppose there could exist a positive relationship between low levels of p,p'-DDE and prostaglandin secretion. This hypothetically disruption of prostaglandin from pesticides could however be advantageous in Polar Regions. Indeed, in regions where breeding season is short, breeding early is beneficial (Perrins 1970).

Our study did not reveal any negative impact of persistent organic pollutants on breeding success. Average breeding success during our study was quite good, 1.26 ± 0.65 chicks reached more than 12 days old per active nest, against less than 1 chick, 7 years over

11, during the period from 1997 to 2008, excluding 2001 (Moe et al. 2009), suggesting favourable foraging condition at sea. It is therefore possible that birds were able to cope with POPs contamination without visible reproductive penalties.

4.2. Relationships between organic pollutants and CORT secretion

In the present study, stress-induced levels were higher in individuals that had the higher levels of PCBs, and this was not true for pesticides. The adrenal gland is one of the most common target for chemically induced lesions (Rosol et al. 2001). Because of several characteristics as: its large blood supply, its lipophilicity (allowing the accumulation of lipophilic compounds), its high concentration of cytochrome P450 that can also bioactivate toxicants, and its capacity to synthesize all major classes of steroids (Falco et al. 2007; Harvey and Everett 2003; Hinson and Raven, 2006; Rosol et al. 2001). Adrenal cells concentrate a number of toxic agents, as DDT (Lund et al. 1988) and PCB metabolites (Brandt and Bergman, 1987) that may remain inactive caught into the adrenal tissue until a period of particularly high adrenal steroid demand, as the breeding period. During the breeding period, the body mass loss may make available contaminants stored in different organs and body reserves, which at their turn would cause damage. Indeed, reduced food intake enhances biotransformation of halogenated organic contaminants and formation of metabolites which have greater toxicological impacts compared to parent POPs (Routti et al. 2012). In our study, stress-induced CORT levels increased with increasing levels of circulating PCBs. This could reflect a dysfunction coming from the adrenals (e.g. up-regulation of ACTH receptors, pollutants mimicking ACTH etc.) and/or this could mirror a dysfunction coming from the brain (e.g. pituitary loss of negative feedback from CORT on the pituitary). Relationships between PCBs and adrenocortical functions have been experimentally highlighted: PCBs can alter adrenocortical steroidogenesis, down-regulate the number of brain glucocorticoid receptors and some PCB metabolites (i.e. hydroxylor methyl PCBs) can bind competitively to glucocorticoid receptors (Aluru et al. 2004; Johansson et al. 1998; Xu et al. 2006). In addition, it seems that high concentration of PCB126 could sensitize the regulation of ACTH on adrenocortical cells by increasing ACTH receptors levels (Li and Wang, 2005), which could result in an increase of CORT secretion. Thus it is possible that some PCB congeners present in kittiwakes could act similarly by increasing the number of ACTH receptors, thus increasing CORT secretion in the most polluted individuals.

Contrary to our findings on kittiwakes, stress-induced CORT levels in the highly polluted glaucous gull decreased with increasing POP levels (Verboven et al. 2010). As mentioned previously, environmental pollutants can have a non-linear dose-response relationship (Heinz et al. 2012; Love et al. 2003), the lower stress response observed in highly polluted glaucous gulls may also be the consequence of a negative feed-back from higher baseline CORT levels (Verboven et al. 2010) due to an increased allostatic load when resources are allocated to biotransformation, detoxification and excretion of contaminants (Parkinson and Ogilvie 2008). Finally, the lack of relationship between baseline CORT and ΣPCBs in our study compared to that of Nordstad et al. (2012) could be explained by the difference between the two sampling periods: in the study of Nordstad et al. (2012), pre-breeding female kittiwakes were sampled in April, their blood PCB levels were average 14,900 pg/g and baseline CORT levels averaged 7.2 ± 1 ng/ml. In the present study, female kittiwakes were sampled in May-June, their blood PCB levels were on average 23,030 pg/g and baseline CORT levels averaged 6.13 ± 3 ng/ml. Although the compounds entering in the ΣPCBs were not completely identical they were very close (11 PCB congeners in Nordstad et al. (2012), 10 PCB congeners in the present study, among which 8 were similar). Those findings suggest that the relationships between PCBs and CORT secretion may highly depend of the levels of PCBs present in blood, and probably also to the sampling period.

Our study adds new evidence that PCBs are linked to CORT secretion disruption. To elucidate which aspects of the HPA axis are involved in mediating contaminant-related changes in the stress response, experimental ACTH injection would be useful to investigate if contaminant-related enhancement occurs at the level of the adrenal gland, or rather at the level of the pituitary, hypothalamus or perception of the stressor. An enhanced stress response is often the consequence of poor early-life experience across vertebrates as low body mass at birth, food restriction, maternal deprivation (Banerjee et al. 2012; Heath and Dufty, 1998; Kitaysky et al. 2001; Müllner et al. 2004; Phillips and Jones 2006), while in adults it often mirrors poor fitness related traits as poor parental investment (Angelier et al. 2009; Bókony et al. 2009; Goutte et al. 2011a; Lendvai et al. 2007) or an impacted survival (Blas et al. 2007; Goutte et al. 2010b; Romero 2012). Still, as mentioned previously, in our study we failed to relate POP levels to breeding success. Environmental conditions were apparently favourable but in case of poor foraging conditions when CORT secretion is stimulated (Lanctot et al. 2003; Kitaysky et al. 1999), it is likely that the most contaminated individuals would be more sensitive to environmental stress and would be less able to maintain parental investment than less polluted ones.

5. Conclusion

Although in this study we did not find any direct negative reproductive outputs of POPs, no information was available about the chicks or parents' survival. Also, females have been blood sampled during the pre-laying phase, and blood measures performed at the beginning of the breeding season may be less reliable to predict reproductive traits occurring at the end of the season as breeding success (Lanctot et al. 2003). This may be particularly true for POPs as levels vary significantly between the different breeding phases (Nordstad et al. 2012). Moreover a previous study on pre-laying kittiwakes has shown that the decision to defer breeding is highly related to a disruption of GnRH secretion from the hypothalamus by mercury (Tartu et al. 2013). Thus, brought together, all the effects of POPs added to the threat of other environmental pollutants could have important impacts on the long-term, depending on a birds' quality. A point to underline is that our study is correlative; we cannot confirm if there is a causal effect of POPs on reproductive traits or CORT. Feeding different concentrations of POPs to captive individuals (Lundholm 1997; Love et al. 2003) or using silastic tubes filled with PCBs (Van den Steen et al. 2007) are methods that could enlighten on the causal effects of pollutants, yet this would be difficult to perform in free-ranging protected species. Moreover, we only measured legacy POPs, already known to be importantly related to several physiological parameters (Gabrielsen and Henriksen, 2001; Gabrielsen 2007). In the Arctic, several emerging POPs (e.g. Perfluorinated compounds (PFC), brominated flame retardants) show increasing trends (Braune et al. 2007; Braune and Letcher 2013; Butt et al. 2007; de Wit et al. 2006; Verreault et al. 2007). Given this perspective, in top predators those emerging POPs could be in higher levels than legacy POPs (e.g. PFC, Nøst et al. 2012) and their relationships with the parameters we measured in this study may be exacerbated, taking them into account could provide further awareness on the way environmental pollution may affect free-ranging populations.

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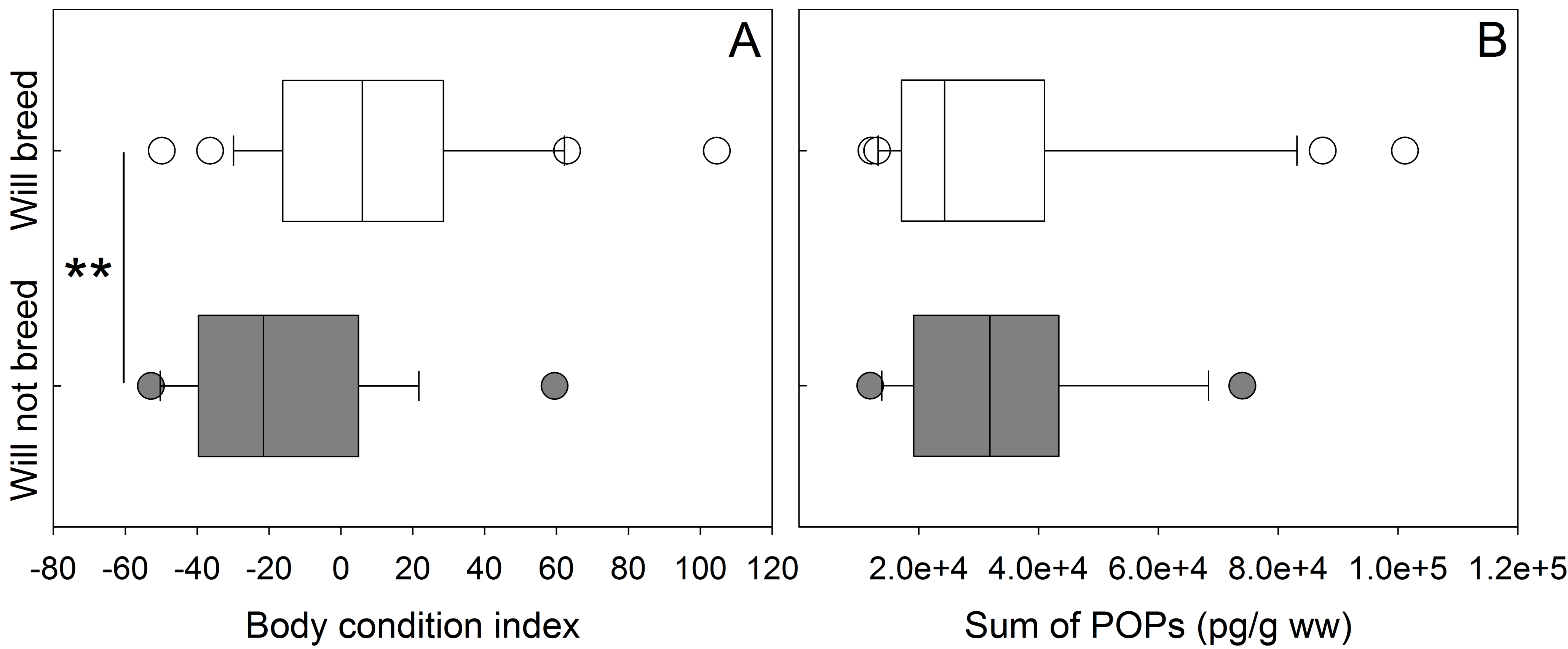
Figures caption:

Figure 1: Body-condition index (A) was higher in breeding (empty boxes) than in non-breeding (filled boxes, **: $p<0.02$) female black-legged kittiwakes, although blood POP levels (pg/g ww, B) did not differ between breeding and non-breeding females.

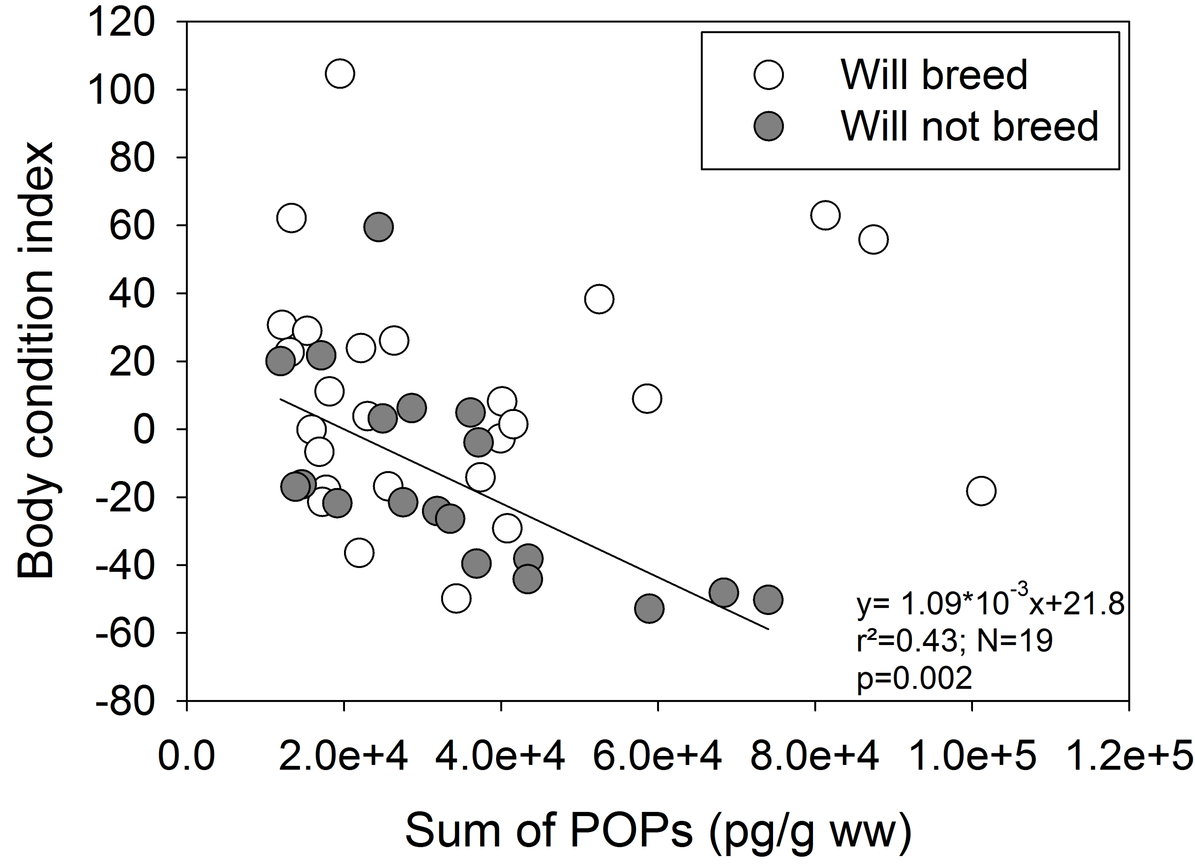
Figure 2: Relationship between blood POPs concentration (ΣPOPs pg/g ww) and body condition index in breeding female black-legged kittiwakes (empty circles) and non-breeding (filled circles). Body condition index decreased with increasing POP levels in non-breeding females (solid line).

Figure 3: Relationship between pesticides concentration in blood (ΣPesticides pg/g ww) and first-egg laying date (days of June) in female black-legged kittiwakes. The females with the higher levels of pesticides (ΣCHL, p,p'-DDE and HCB) laid earlier than those with lower levels.

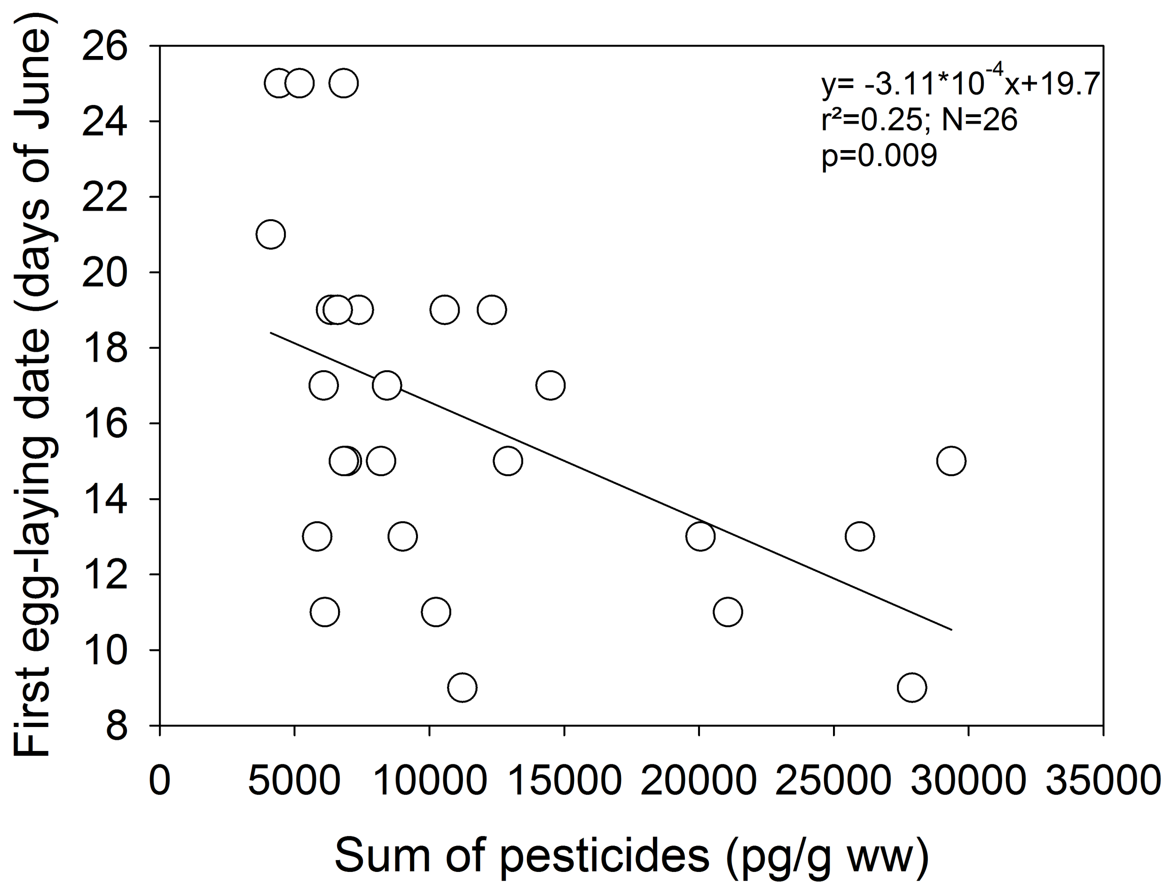
Figure 4: Relationship between PCBs concentration in blood (ΣPCBs pg/g ww) and baseline (A) and stress-induced (B) CORT levels (ng/ml) in female black-legged kittiwakes. Baseline CORT levels were not related to PCB levels whereas stress-induced CORT levels increased with increasing PCB levels. Empty circles refer to breeding females and filled circles to non-breeding females.



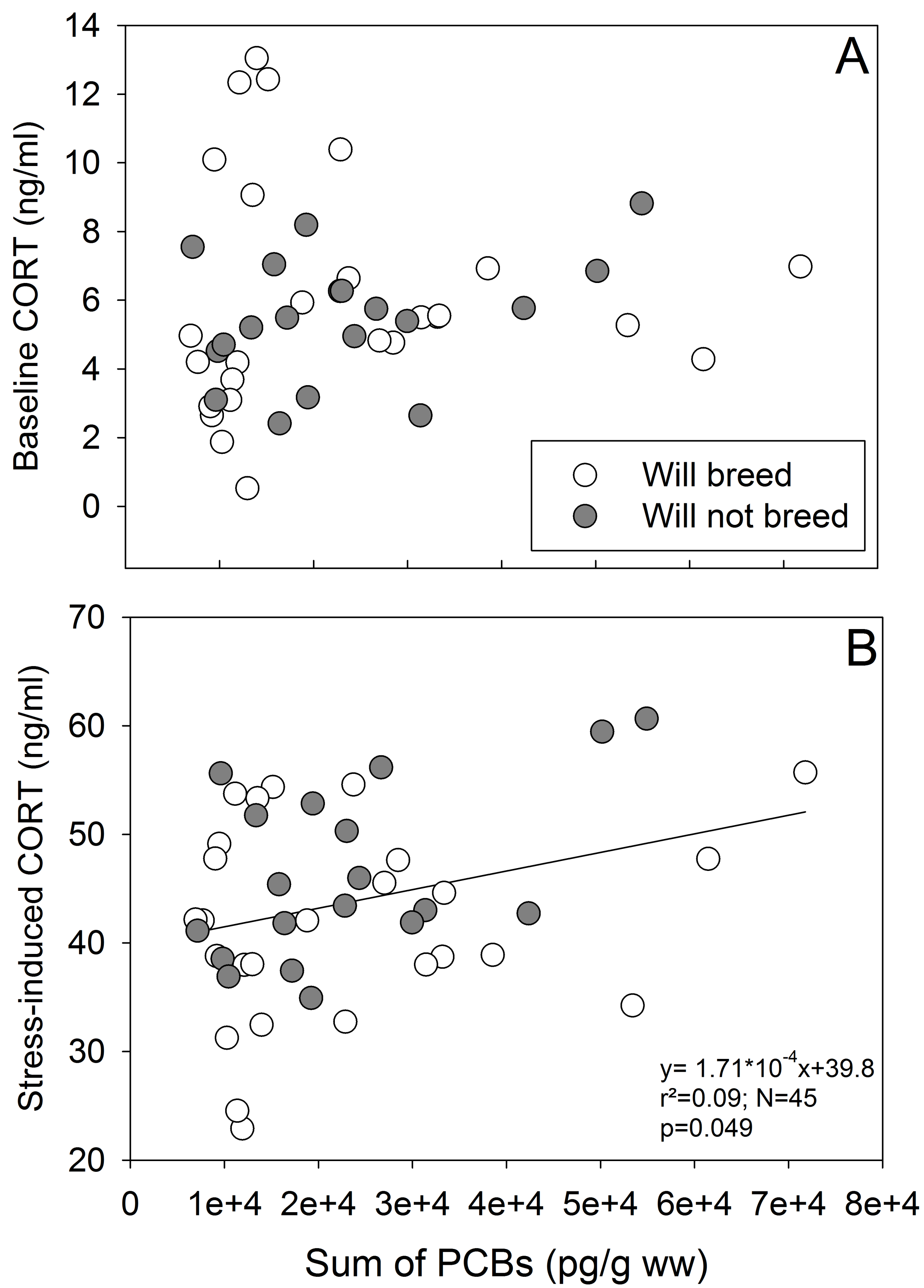
A
B
Will breed
Will not breed
**
-80
-60
-40
-20
0
20
40
60
80
100
120
Body condition index
2.0e+4
4.0e+4
6.0e+4
8.0e+4
1.0e+5
1.2e+5
Sum of POPs (pg/g ww)



Will breed
Will not breed
Body condition index
120
100
80
60
40
20
0
-20
-40
-60
-80
0.0
2.0e+4
4.0e+4
6.0e+4
8.0e+4
1.0e+5
1.2e+5
Sum of POPs (pg/g ww)
y= 1.09*10^-3x+21.8
r²=0.43; N=19
p=0.002



y= -3.11*10^-4x+19.7
r²=0.25; N=26
p=0.009
First egg-laying date (days of June)
26
24
22
20
18
16
14
12
10
8
0
5000
10000
15000
20000
25000
30000
35000
Sum of pesticides (pg/g ww)



A
Baseline CORT (ng/ml)
Will breed
Will not breed
B
Stress-induced CORT (ng/ml)
y= 1.71*10^-4x+39.8
r²=0.09; N=45
p=0.049
Sum of PCBs (pg/g ww)
0
1e+4
2e+4
3e+4
4e+4
5e+4
6e+4
7e+4
8e+4

Table 1: Relationships between body condition index (N=47), organic pollutants (pg/g ww, N=45) as ΣPOPs, ΣPCBs and ΣPesticides and reproductive traits: a) breeding decision (will breed or will not breed), b) egg-laying date (days of June) and c) breeding success in pre-laying female black-legged kittiwakes. Egg-laying dates were available for all breeding females (N=28), while breeding success was not available for two of them. Numbers in bold indicate significant p-values (p<0.05).

	Independant variable	Sum of squares	Df	F or χ^2	p-value
a) Breeding decision	**Body condition**	-	1,45	5.60	**0.018**
	Σ POPs	-	1,43	0.00	0.953
	Σ PCBs	-	1,43	0.01	0.942
	Σ Pesticides	-	1,43	0.14	0.707
b) Egg-laying date (log)	Body condition	0.20	1,26	2.36	0.136
	Σ POPs	0.30	1,24	4.08	0.055
	Σ PCBs	0.21	1,24	2.69	0.114
	Σ Pesticides	0.53	1,24	8.12	**0.009**
c) Breeding success	Body condition	-	1,24	0.19	0.663
	Σ POPs	-	1,22	0.10	0.752
	Σ PCBs	-	1,22	0.07	0.785
	Σ Pesticides	-	1,22	0.16	0.694

F tests were used for models with normal distribution (Egg-laying date)

χ^2 tests were used for models with binomial (Breeding decision) and Poisson (Breeding success) distribution

Table 2: Relationships between organic pollutants (pg/g ww) as ΣPOPs, ΣPCBs and ΣPesticides and a) baseline and b) stress-induced CORT levels (ng/ml) in pre-laying female black-legged kittiwakes. Numbers in bold indicate significant p-values (p<0.05).

Dependant variable	Independant variable	Sum of squares	Df	F	p-value
a) Baseline CORT (log)	Breeding decision	0.00	1,41	0.01	0.931
	Σ POPs	0.63	1,41	1.95	0.170
	Σ POPs × Breeding decision	0.02	1,41	0.07	0.788
	Σ PCBs	0.48	1,41	1.45	0.235
	Σ PCBs × Breeding decision	0.02	1,41	0.05	0.820
	Σ Pesticides	0.95	1,41	3.03	0.089
	Σ Pesticides × Breeding decision	0.03	1,41	0.11	0.741
b) Stress-induced CORT (log)	Breeding decision	0.14	1,42	3.42	0.072
	Σ POPs	0.14	1,42	3.43	0.071
	Σ POPs × Breeding decision	0.03	1,41	0.74	0.395
	Σ PCBs	0.17	1,42	4.11	**0.049**
	Σ PCBs × Breeding decision	0.02	1,41	0.49	0.488
	Σ Pesticides	0.07	1,41	1.60	0.213
	Σ Pesticides × Breeding decision	0.04	1,41	0.97	0.331