



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



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HIGHLIGHTS

- We examined relationships between POPs, reproduction and CORT secretion in a seabird.
- POP levels were negatively related to body condition in non-breeding females only.
- Females with high levels of pesticides laid their eggs earlier.
- Females with high levels of PCBs released more CORT when subjected to a stress.
- High POP levels were not associated with poor breeding success.

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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 was 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.

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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.,

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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., 2000) 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 behaviours 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 behavioural 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; Bókonyi et al., 2009; Bonier 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) mirror the activity and metabolic rate and reflect 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, or reproduction (Bókonyi 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). 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 female 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: $2 \text{ min } 55 \pm 34 \text{ (SD) seconds}$, 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 min 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 caliper. 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 $-20 \text{ }^{\circ}\text{C}$ 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

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