Endocrine and Fitness Correlates of Long-Chain Perfluorinated Carboxylates Exposure in Arctic Breeding Black-Legged Kittiwakes

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ABSTRACT: Increasing levels of poly- and perfluorinated alkyl substances (PFASs) have recently been described in Arctic biota. These emerging substances are of concern given their resistance to degradation and metabolization. Some studies have reported endocrine disrupting effects for some PFASs. However, there is a gap of knowledge on the potential relationships between PFASs and hormones mediating the life-history trade-off between reproduction and survival, such as glucocorticoids. The aims of this study were to (1) describe the concentrations of plasma perfluoroalkyl sulfonates and perfluoroalkyl carboxylates in Svalbard black-legged kittiwakes (Rissa tridactyla) in relation to gender and body-condition, (2) explore the relationships between PFASs and corticosterone (the major glucocorticoid in birds), and (3) assess the consequences of PFAS exposure for reproductive success. Perfluorononanoate was positively related to body-condition in male kittiwakes; perfluorotridecanoate and perfluorotetradecanoate to decreased baseline corticosterone in both sexes; and perfluorododecanoate was related to lower hatching success. These results underline the importance of considering each compound separately when investigating the hazardous effects of PFASs on wildlife.

INTRODUCTION

While most attention was directed toward the endocrine disrupting effects of legacy persistent organic pollutants (POPs),1,2 the so-called emerging POPs came into focus in the late 90s. Many emerging POPs are not regulated and comprise a wide array of everyday life products,3 but ecotoxicological data on these compounds are lacking.4 Among them, poly- and perfluorinated alkyl substances (PFASs) are used as surface-active agents in a multitude of manufactured and consumer products (e.g., fire-fighting foam and impregnation agent for carpets, papers, and textiles). PFASs are particularly alarming, because of their special properties: they are thermally and chemically stable, have no route of degradation and cannot be metabolized by vertebrates under normal environmental conditions which makes them extremely persistent in the environment. Moreover, PFASs consist of perfluorinated carbon chains that are hydrophobic and lipophobic, so they can accumulate in the blood, liver and kidney.5,6 Nowadays, human exposure to PFASs measured in the blood reaches the highest values observed by an exogenous chemical.7 The occurrence of PFASs have been described in polar region such as the Arctic8 and concentrations of some PFASs tend to increase over time in several Arctic mammal and seabird species.9–11 In Arctic regions long-chained perfluoroalkyl carboxylic acids (PFCAs) are prevalent11 and acute toxicity of PFCAs increases with chain length.7 With regard to the potential endocrine disrupting properties of PFASs, laboratory studies have shown that some PFAS possess estrogenic, androgenic and thyroid-like activity.5,9,14,15 However, to date very few studies have investigated the relationships between hormones and PFAS in free living species.16 Furthermore, the possible influence of PFAS on some major endocrine axes has only been investigated in a few studies. This is especially the case for the hypothalamo-pituitary-adrenal (HPA) axis. The HPA axis plays an important role in mediating the life-history trade-off between reproduction and survival across the release of stress hormones such as glucocorticoids.17 However, little is known about the disruption of PFASs on glucocorticoids. The release of glucocorticoid hormones (cortisol, corticosterone: CORT) during stressful events triggers physiological and behavioral adjustments that shift...
Twenty birds (10 males and 10 females) were caught on 20 July 12th to July 26th 2012 during the chick-rearing period. We investigated these relationships in chick-rearing adult Rissa tridactyla (black-legged kittiwake) species in relation to gender and body-condition, (2) explore the ability of an individual to adjust reproductive decisions to environmental conditions. Because of increasing prevalence of PFASs in the environment, especially of the most toxic long-chained PFCAs in the Arctic, and because of the pivotal role of the HPA axis, PFASs should therefore become the focus of research toward self-preservation and hence survival. CORT is very energy investment away from reproduction and redirects it toward self-preservation and hence survival. CORT is very likely to mediate parental effort and parental investment in birds, and any disruption of this hormone may alter the ability of an individual to adjust reproductive decisions to environmental conditions. Because of increasing prevalence of PFASs in the environment, especially of the most toxic long-chained PFCAs in the Arctic, and because of the pivotal role of the HPA axis, PFASs should therefore become the focus of interest as emerging endocrine disruptors for wildlife. There is also very limited information on the potential negative impact of PFASs on fitness (e.g., reproductive success, survival) of free-living organisms.

Arctic seabirds are top predators particularly at risk given the biomagnification properties of some PFASs along the trophic web. The aims of this paper are to (1) describe the concentrations of plasma PFASs (perfluoralkyl sulfonic acids) and PFCAs in an Arctic seabird species in relation to gender and body-condition, (2) explore their relationships with the HPA axis, and especially with plasma CORT concentrations, (3) assess the consequences of PFAS exposure on fitness traits like reproductive success. To do so, we investigated these relationships in chick-rearing adult black-legged kittiwakes (Rissa tridactyla) which in Svalbard are known to be exposed to PFASs.

### EXPERIMENTAL SECTION

#### Study Area and Sampling Collection.

The sampling of birds was approved by the Governor of Svalbard, and national guidelines for ethical treatment of experimental animals were followed (NARA, FOTS id 4214, RIS 2961). The study was conducted at Kongsfjorden, Svalbard (78°54’N, 12°13’E) from July 12th to July 26th 2012 during the chick-rearing period. Twenty birds (10 males and 10 females), were caught on 20 different 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 concentrations. Bleeding time (i.e., time elapsed from capture to the end of the first blood sample: 2 min 27s ± 31s (SD), on average) did not affect CORT concentrations (GLM, $F_{1,18} = 0.39, p = 0.538$). Eighteen kittiwakes (10 males and 8 females) were then placed into a cloth bag and a second blood sample (ca. 2.5 mL) was collected from the alar vein at 30 min from capture (30 min 13 s ± 1 min 02 s) to assess stress-induced CORT and PFAS concentrations. 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 bird we calculated its scaled mass index as a measure of body-condition. Kittiwakes were marked with spots of dye on the forehead to distinguish them from their partner during subsequent observation and then released. Prior to the beginning of the sampling period, using a mirror at the end of an 8 m fishing rod, we checked the whole plot (ca. 117 nests) every 2 days to monitor the clutch size, the number of chicks that hatched (thereafter ‘hatching success’) and those that reached at least 12 days of age per active nest (thereafter called “breeding success”). All birds studied and sampled had a clutch of two eggs.

#### Molecular Sexing and Hormone Assay.

Blood samples were centrifuged and stored at −20 °C until used respectively in hormone assays or molecular sexing, at the Centre d’Etudes Biologiques de Chizé (CEBC). The sex was determined by polymerase chain reaction (PCR) amplification of part of two highly conserved genes (CHD) present on the sex chromosomes at the Centre d’Etudes Biologiques de Chizé (CEBC). Plasma concentrations of CORT were determined by radioimmunoassay at the CEBC. The lowest detectable concentration for CORT was 1.05 ng/mL. Only one assay was performed and the intra-assay coefficient of variation was 6.7% ($N = 5$ duplicates).

#### Chemical Analyses.

Analyses for perfluorinated compounds in plasma samples were performed at the Norwegian Institute of Air Research (NILU, Tromsø, Norway). We searched for 14 PFASs: perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), linear perfluorooctanesulfonate (PFOSlin), perfluorobutanoate (PFBA), perfluoropentanoate (PFPA), perfluorohexanoate (PFHxA), perfluorohexafluoro-...
Hormones and PFASs in Relation to Sex and Body-Condition. CORT concentrations (baseline and stress-induced) and PFASs were not related to sex (GLM, $F < 2.7$, $p > 0.11$, SI) however for the PFCA with longer chains (i.e., PFDoA, PFTeA, and PFTeA), plasma concentrations tended to be higher in males than in females ($F > 3.88$, $p < 0.064$, SI). Body-condition was higher in males than in females (GLM, $F_{1,18} = 38.7$, $p < 0.001$). CORT concentrations were not related to body-condition in males or in females ($F < 3.01$, $p > 0.133$). PFNA was positively related to body-condition in males only (GLM, $F_{1,8} = 7.19$, $p = 0.028$; Figure 1) and no relationships were found between the other PFASs and body-condition in males or females ($F < 2.43$, $p > 0.158$, SI). In the results presented here, male kittiwakes with higher concentrations of PFNA were in better body-condition. Body-condition as measured by scaled mass index is a reliable predictor of body fat and proteins$^{27}$ and PFASs have high affinity for proteins.$^8$ The positive relationship observed between PFNA and body-condition in male kittiwakes could be related to the structural resemblance of PFASs to fatty acids and their capability to bind to nuclear receptors which play a key role in lipid metabolism and adipogenesis.$^{34,35}$ Activation is greater as carbon backbone length increases, and carboxylates (PFOA and PFNA) have higher activation properties.$^35$ In humans, PFNA can be associated with increased cholesterol and adiponectin concentrations$^{36,37}$ and PFOA levels correlate with body weight but also insulin and leptin concentrations.$^{38}$ All these hormones are strongly associated with obesity and food intake; it is possible that similar mechanisms to those reported in humans could occur in birds. However, we should be cautious in interpreting this result, as we have no evidence that PFNA disrupts lipid metabolism in birds, and the reason why no relationship was found in females remains unexplained. It may result from the ability of females to transfer elevated amounts of PFAs into their eggs$^{13,39}$ however in the present study PFNA concentrations were not lower in females.

Relationships between PFASs and Hormones. In adult chick-rearing kittiwakes baseline CORT concentrations were negatively related to PFTeA and PFTeA (Figure 2, Table 2). No relationships were found with the other PFASs, and no
relationship was found between PFASs and stress-induced CORT concentrations (Table 2, SI). This negative relationship between PFTrA, PFTeA, and CORT could be the result of several mechanisms: a negative feedback due to other hormones, hormone displacement due to high protein affinity, or a disruption of the HPA axis that has resulted in a lower ability to secrete proper baseline CORT. Some experimental studies have reported effects of PFCAs on CORT: for example in PFNA treated mice, the group receiving the higher dose had increased concentrations of both ACTH and cortisol. 40 However, in kittiwakes PFNA concentrations were not related to CORT. Some experimental studies have reported effects of PFCAs on CORT: for example in PFNA treated mice, the group receiving the higher dose had increased concentrations of both ACTH and cortisol. 40 However, in kittiwakes PFNA concentrations were not related to CORT. One possible interpretation to explain these relationships between CORT, PFTrA, and PFTeA comes from the ability of PFASs to displace hormones by binding to proteins. 41 Because of their high affinity for proteins, it has been suggested that PFASs could easily bind to transport proteins and cause hormone displacement. 42 CORT is protein dependent to ensure its specific role by binding to CORT-binding-globulin (CBG). 41 This binding of CORT to CBG may serve as a tissue buffer against potentially deleterious effects of elevated circulating CORT. 41,42 Measuring the quantity of CBG and the number of glucocorticoid receptors (GR) could inform on the effective activity of CORT in kittiwakes contaminated by PFASs. Indeed, the observed decrease of baseline CORT concentrations with increasing PFTrA and PFTeA in kittiwakes could be a response to an increase of GR and/or a decrease of CBG. Indeed, the action of CBG is supposed to make CORT inactive, 43 so if most of the CBG are bounded with PFTrA and PFTeA, more CORT will remain free and active. Very high levels of active CORT could have an important impact on health, thus a decrease in the production of CORT from the adrenals may be an adaptation to keep free CORT concentration within the normal physiological ranges. It has been suggested 8 that “given the current environmental concentrations of PFOS, it was unlikely that PFOS would cause displacement of hormones from serum proteins in wildlife”; indeed though no relationships between PFOS and CORT were found in the present study, the relationships with longer chain PFCAs

![Figure 2. Baseline CORT concentrations in relation to seven PFASs. Baseline CORT decreased with increasing plasma PFTrA and PFTeA concentrations, in male (open circles) and female (closed circles) chick-rearing black-legged kittiwakes. The solid line refers to a statistically significant linear regression.](image-url)
Induced CORT increased with increasing legacy POPs. The signifi-
0.528 PFDoA was observed in seabirds and consequently with chick feeding.47,48 PFTeA, could have interfered with the adaptive weight loss of black-legged kittiwakes with high concentrations of PFDoA (Figure 3).

Traits. Relationships between PFASs and reproductive traits. Hatching success and breeding success were not related to baseline or stress-induced CORT concentrations, or with the interactions with sex ($\chi^2 < 1.11, p > 0.29$). Hatching success was significantly lower in birds with higher concentrations of PFDoA (GLM, PFDoA: $\chi^2 = 4.2, p = 0.040$; sex: $\chi^2 = 0.4, p = 0.528$ PFDoA x sex: GLM, $\chi^2 = 0.1, p = 0.72$; Figure 3, SI) and was significantly related to the interaction between PFTeA and sex (PFTeA x sex: GLM, $\chi^2 = 4.0, p = 0.045$, SI). However, when analyzing males and females separately, the negative relationship between hatching success and PFTeA was only close to statistical significance in males (GLM, $\chi^2 = 3.4, p = 0.064$) and no relationship was found in females (GLM, $\chi^2 = 0.6, p = 0.455$). All the other compounds were unrelated to hatching success ($p > 0.35$ for all tests, SI). Breeding success was not related to PFASs ($p > 0.07$ for all tests, SI). In this study, male and female kittiwakes bearing the higher concentrations of PFDoA were more likely to hatch one egg instead of two in a two eggs clutch. In mammals, some effects of PFDoA on reproduction and development have been observed. For example, in male rats fed PFDoA lower spermatid and spermatozoa counts were observed in reproductive organs and in female rats PFDoA administration resulted in death or in the delivery of dead pups.49 Relationships between PFASs and fitness have rarely been investigated for wildlife. In tree swallows Tachycineta bicolor, a similar apparent reproductive impairment was observed as in the present study, although the associated PFASs differed: PFOS concentration measured directly in eggs was negatively associated with hatching success, with PFOS concentration $\geq 150$ ng/g.44 In lesser black-backed gulls Larus fuscus no relationships were found between PFASs and life-history traits.33 However, in lesser black-backed gulls, whole blood concentrations for long chain PFCAs were slightly lower than in kittiwakes’ plasma (1.4 ng/g versus 2.5 ng/g, respectively, for PFDoA). The relationships between PFDoA and hatching success could either be the result of nonviable embryos or less efficient incubation behavior. In oviparous vertebrates, females transfer a large amount of PFASs to their eggs50 which may result in nonviable embryos for the most contaminated females. However, in the present study high PFDoA concentrations measured in male kittiwakes were also related to lower hatching success. Consequently the lower hatching success observed in birds with the higher concentrations of PFDoA is more likely to result from disrupted incubating behavior. Regarding legacy POPs, an experimental study conducted on American kestrels Falco sparverius, reported that PCB administration resulted in altered incubation behaviors:50 the incubation bouts realized by female and male American kestrels were not synchronized and the eggs were left unattended for longer periods.50 In free-ranging glaucous gulls Larus hyperboreus, the proportion of time absent from the nest site when not incubating and the number of absences were related to blood PCB,51 and the most contaminated glaucous gulls were less able to maintain an optimal nest temperature.52 However, we have no evidence for such effects of PFDoA. Another possible explanation that could relate PFDoA to hatching success would be a disruption of the hormonal control of brood patches. Brood patches are highly vascularized featherless patches placed on the ventral surface of both male and female birds when biparental care is provided.53 These patches enable the egg to be kept at an optimal temperature: if incubation patches are too small, one egg at least would probably be less exposed to parental heat. The feather loss and vascularization of those patches are under hormonal control, particularly through a synergetic association of prolactin and estrogen.53 In rats, PFDoA administration reduces serum estradiol concentration in males and the expression of estrogen receptors in the ovaries of females.54–56 If PFDoA reduces estradiol expression in kittiwakes, this could lead to reduced brood patches, less efficient incubation and nonviable embryos. Again, further studies are needed to test

![Figure 3](image-url)
these hypotheses, such as measurement of estrogen and monitoring incubation behavior in relation to PFDoA. Another, important point is that we did not measure PFASs in the partners of our focal birds. Because kittiwakes provide biparental care to the brood, measuring PFASs in the partner would provide important data to explain the observed interaction between PFDoA and hatching success.

This study underlines the importance of considering each PFAS separately and their relationships with sex. To the best of our knowledge this is the first study which shows relationships between long chain PFCAs (PFNA, PFDoA, PFTra, and PFTeA), body-condition, baseline CORT concentrations and hatching success in a free-ranging seabird. Most toxicity studies of PFASs have concentrated on PFOS and PFOA, hence limited information is available on the toxicological effects and risk of other PFASs. Additionally, PFCAs show dramatic increasing trends in Arctic seabird eggs, given their hazardous effects on hormones and fitness related traits, more studies are needed. The small amount of data available makes interpreting the statistical results difficult; additionally this study is correlational; it is thus difficult to draw conclusions on the causality of these relationships, some of them could be the result of unmeasured chemicals or parameters that could confound these associations.

ASSOCIATED CONTENT

■ Supporting Information

Figures depicting the nonsignificant relationships between PFASs, body-condition and hatching success are given in Supporting Information. Additionally, we also included tables with statistics concerning the relationships between (1) sex, PFASs, CORT and body-condition, (2) PFASs and body-condition, (3) PFASs and reproductive traits. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(22) Tartu, S.; Gouette, A.; Bustamante, P.; Angelier, F.; Moe, B.; Clément-Chastel, C.; Bech, C.; Gabrielsen, G. W.; Busnæs, J. O.; Chastel, O. To breed or not to breed: Endocrine response to mercury contamination by an Arctic seabird. Biol. Lett. 2013, 9, 20130317.

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