Environmental Science & Technology

Article

pubs.acs.org/est

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

Sabrina Tartu,*[,†] Geir W. Gabrielsen,[‡] Pierre Blévin,[†] Hugh Ellis,[§] Jan Ove Bustnes,[∥] Dorte Herzke,[⊥] and Olivier Chastel[†]

[†]Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372-ULR CNRS, Villiers-en-Bois F-79360, France

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

[§]Department of Biology, University of San Diego, San Diego, California 92110, United States

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

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

Supporting Information

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),[1−4] 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,[5] but ecotoxicological data on these compounds are lacking.[6] 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[7] 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.[8,9] Nowadays, human exposure to PFASs measured in the blood reaches the highest values observed by an exogenous chemical.[9] The occurrence of PFASs have been described in polar region such as the Arctic[10] and concentrations of some PFASs tend to increase over time in several Arctic mammal and seabird species.[11−13] In Arctic regions long-chained perfluoroalkyl carboxylic acids (PFCAs) are prevalent[11] and acute toxicity of PFCAs increases with chain length.[9] 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

Received: July 8, 2014
Revised: October 1, 2014
Accepted: October 22, 2014
Published: October 22, 2014

Table 1. PFASs Mean Concentrations ± Standard Deviation (pg/mL ww), LOD and LOQ in the plasma of female and male chick-rearing adult kittiwakes

				females					males				
	n° C	LOD	LOQ	*N* > LOD	mean		SD	range	*N* > LOD	mean		SD	range
Perfluoroalkyl Sulfonates (PFSA)													
PFBS	4	18.5	55.5	0	nd				0				
PFHxS	6	10.7	32.0	5				[<10.7; 216]	4				[<10.7; 130]
PFOSlin	8	704	2111	10	9299	±	2611	[6804; 13581]	10	10 233	±	2685	[7002; 15183]
Perfluoroalkyl carboxylates (PFCA)													
PFBA	4	918	2754	0	nd				0				
PFPA	5	36.2	109	0	nd				0				
PFHxA	6	6.2	18	0	nd				0				
PFHpA	7	91.2	274	0	nd				0				
PFOA	8	26.5	80	2				[<26.5; 122]	2				[<26.5; 167]
PFNA	9	40.9	123	10	967	±	704	[805; 3047]	10	1241	±	547	[787; 2593]
PFDcA	10	61.9	186	10	1705	±	464	[1301; 2764]	10	2162	±	528	[1233; 3123]
PFUnA	11	83.0	249	10	10 449	±	2636	[7712; 16618]	10	11 413	±	2808	[7853; 17546]
PFDoA	12	109	327	10	2188	±	709	[1472; 4014]	10	2658	±	662	[1893; 3815]
PFTrA	13	360	1079	10	12 960	±	7330	[4495; 29735]	10	18 156	±	4022	[11217; 23055]
PFTeA	14	235	706	10	1167	±	840	[289; 3258]	9	1798	±	532	[<235; 2712]
∑7PFASs					47 947	±	11 213	[29172; 66048]		41 339	±	11 967	[24336; 71204]

energy investment away from reproduction and redirects it toward self-preservation and hence survival.[17,18] CORT is very likely to mediate parental effort and parental investment in birds[19,20] and any disruption of this hormone may alter the ability of an individual to adjust reproductive decisions to environmental conditions.[21,22] 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.[23−25] Arctic seabirds are top predators particularly at risk given the biomagnification properties of some PFASs along the trophic web.[26] The aims of this paper are to (1) describe the concentrations of plasma PFSAs (perfluoroalkyl 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.[16]

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 calliper. For each bird we calculated its scaled mass index[27] 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).[28] Plasma concentrations of CORT were determined by radioimmunoassay[29] 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), perfluorohepta-

noate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDcA), perfluoroundecanoate (PFUnA), perfluorododecanoate (PFDoA), perfluorotridecanoate (PFTrA), and perfluorotetradecanoate (PFTeA). The method was described in detail along with instrumental settings in previous studies.[30,31] In short, a sample (0.5 mL) spiked with internal standards was extracted in acetonitrile (1 mL) by repeated sonication and vortexing. The supernatant was cleaned up using ENVI-Carb graphitized carbon absorbent and glacial acetic acid. Extracts were analyzed by UPLC/MS/MS. Recovery of the internal standards ranged between 45% and 120%. The deviation of the target concentrations in the SRMs (NIST Human serum 1958) were within the laboratory's accepted deviation from target concentrations (<40%) (n = 4). All blanks contained concentrations below the instrument detection limits (three times chromatographic noise). For each compound, limit of detection (LOD) and limit of quantification (LOQ) are given in Table 1.

Statistical analyses. For statistics, only samples with concentrations over the analyte-specific LODs and detected in more than 70% of the samples were included. All statistical analyses were performed using R 2.13.1 and generalized linear models (GLM) with a normal/binomial error distribution and an identity/logit link function were used to test our biological assumptions. First, we tested the effects of "sex" on "body-condition", "CORT" and "PFASs". Second we checked for relationships between "PFASs" and "CORT". Third, we tested if PFAS were related to body-condition in males and females separately. Finally, we tested whether "CORT" and "PFASs" affected "hatching success" and 'breeding success'. Since only one bird had a null hatching success and thus a null breeding success it was removed from statistical analyses, we thus performed analyses with a binomial response, hatching success (1 or 2 eggs have hatched) and breeding success (1 or 2 chicks have reached 12 days of age) . Model selection was performed by a step-down approach starting from the global model including all the independent variables, these latter were log-10 transformed when necessary and statistical significance was fixed to $\alpha < 0.05$. In all models we tested the effect of each compound separately.

RESULTS AND DISCUSSION

Compounds and levels of PFASs. Fourteen PFASs were analyzed of which six (PFOSlin, PFNA, PFDcA, PFUnA, PFDoA, PFTrA) were detected in the 20 captured kittiwakes and PFTeA was detected in 19 kittiwakes. PFHxS and PFOA were detected in 9 and 4 birds, respectively (Table 1). They were thus excluded from statistical analyses. The dominating compound was PFTrA closely followed by PFUnA and PFOSlin then by decreasing order PFDoA > PFDcA > PFTeA > PFNA (Table 1). Most studies on PFASs have concentrated on PFOS and PFOA, as they are often the most present compounds in vertebrates.[32] As a consequence there is limited information available on the toxicological effects and risk of PFCAs with longer chains than PFOA.[32,33] Contrary to kittiwake chicks, where PFOSlin was the dominant compound,[16] in adult chick-rearing kittiwakes, odd numbered longer chained PFCAs (C11 and C13) were the dominant fluorinated compounds. This difference in PFASs profile could originate from PFOS regulations taken in 2009 by the Stockholm Convention on POPs, indeed the study on chicks[16] occurred 6 years before the present one. Another explanation could be a diet difference between chicks and adults.

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 PFCAs with longer chains (i.e., PFDoA, PFTrA, 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

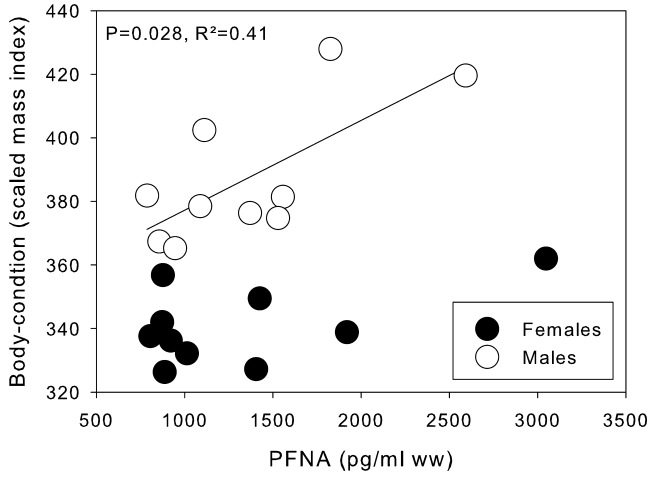

Figure 1. In male black-legged kittiwakes (open circles), body-condition (scaled mass index) was positively related to plasma PFNA concentrations. A relationship not found in females (closed circles). The solid line refers to a statistically significant linear regression.

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 PFASs 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 PFTrA and PFTeA (Figure 2, Table 2). No relationships were found with the other PFASs, and no

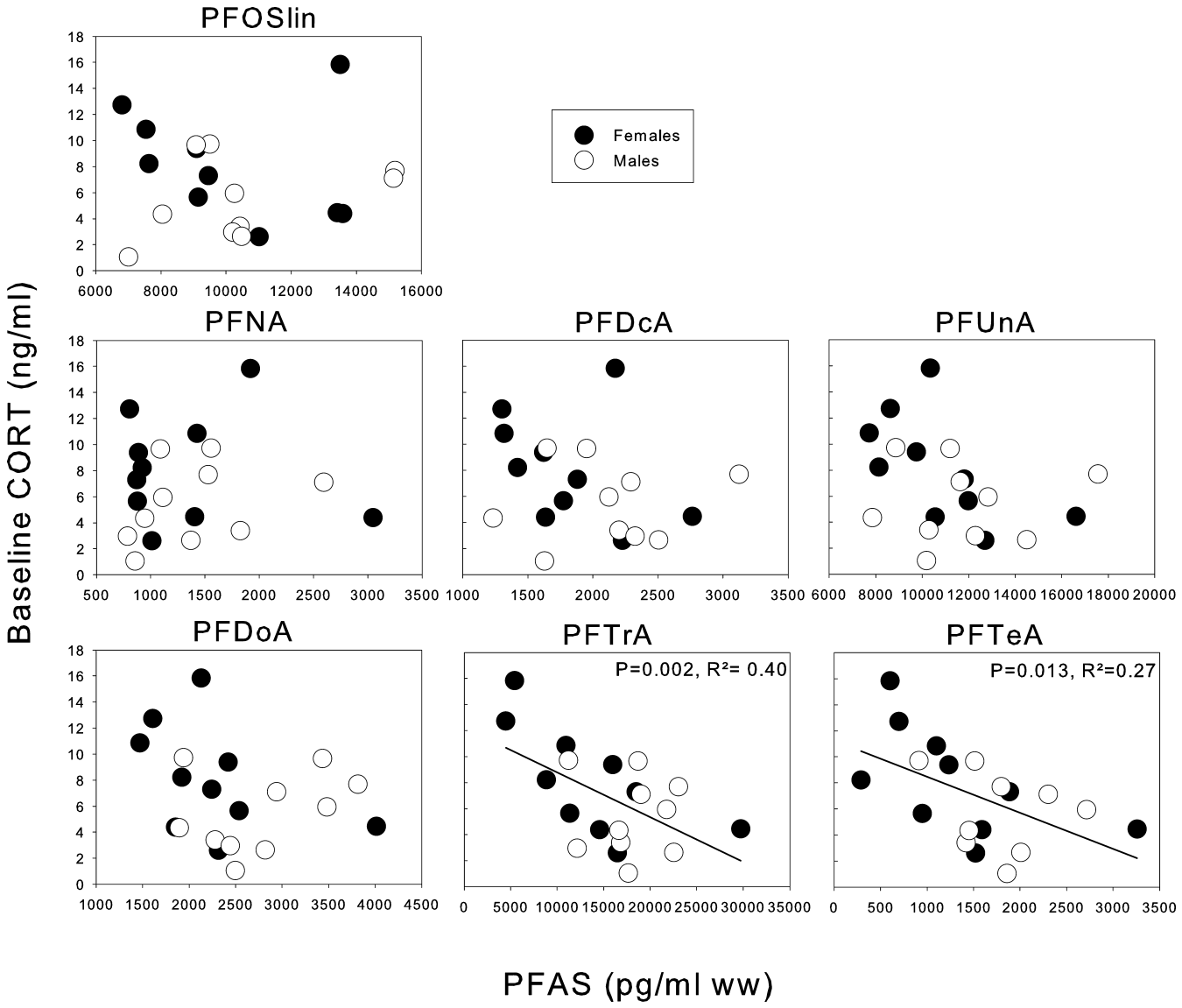

Figure 2. Baseline CORT concentrations in relation to seven PFASs. Baseline CORT decreased with increasing plasma PFTrA and PFTeA concentrations, in male (open circles) and female (closed circles) chick-rearing black-legged kittiwakes. The solid line refers to a statistically significant linear regression.

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. 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.[8] Because of their high affinity for proteins, it has been suggested that PFASs could easily bind to transport proteins and cause hormone displacement.[8] 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

Table 2. Modelling the Relationship Between PFASs and (A) Baseline and (B) Stress-Induced CORT Concentrations in Chick-Rearing Black-Legged Kittiwakes

dependent variable	independent variable	SS	Df	F	Pr(>F)
(A) baseline CORT	linear PFOS	0.6	1.18	0.0	0.838
	PFNA	2.9	1.18	0.2	0.668
	PFDcA	18.6	1.18	1.3	0.266
	PFUnA	36.3	1.18	2.8	0.114
	PFDoA	23.8	1.18	1.7	0.207
	PFTrA	117.2	1.18	13.5	0.002
	PFTeA	80.1	1.17	7.7	0.013
(B) stress-induced CORT	linear PFOS	0.5	1.16	0.0	0.905
	PFNA	2.5	1.16	0.1	0.795
	PFDcA	0.5	1.16	0.0	0.909
	PFUnA	0.6	1.16	0.0	0.899
	PFDoA	1.9	1.16	0.1	0.819
	PFTrA	1.0	1.16	0.0	0.868
	PFTeA	46.1	1.15	1.3	0.267

(PFTrA and PFTeA) are still of concern. These results should be interpreted cautiously and would greatly benefit from experimental support. It would thus be interesting to measure free CORT and GR in relation to PFTrA and PFTeA. Another interesting point is that the observed pattern in the present study is the opposite of what has been found in previous studies with regard to legacy POPs and CORT in Arctic seabird species.[44−46] In these latter studies, baseline[44,45] or stress-induced[46] CORT increased with increasing legacy POPs. The lower concentrations of CORT in relation to PFTrA and PFTeA, could have interfered with the adaptive weight loss observed in seabirds and consequently with chick feeding.[47,48] However, in the present study PFTrA and PFTeA were not related to body-condition or reproductive success.

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 (χ^2 < 1.11, p > 0.29). Hatching success was significantly lower in birds with higher concentrations of PFDoA (GLM, PFDoA: χ^2 = 4.2, p = 0.040; sex: χ^2 = 0.4, p = 0.528 PFDoA × sex: GLM, χ^2 = 0.1, p = 0.72; Figure 3, SI) and was significantly related to the interaction between PFTeA and sex (PFTeA × sex: GLM, χ^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, χ^2 = 3.4, p = 0.064) and no relationship was found in females (GLM, χ^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 ≥150 ng/g.[24,25] In lesser black-backed gulls *Larus fuscus* no relationships were found between PFASs and life-history traits.[23] 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 eggs[39] 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

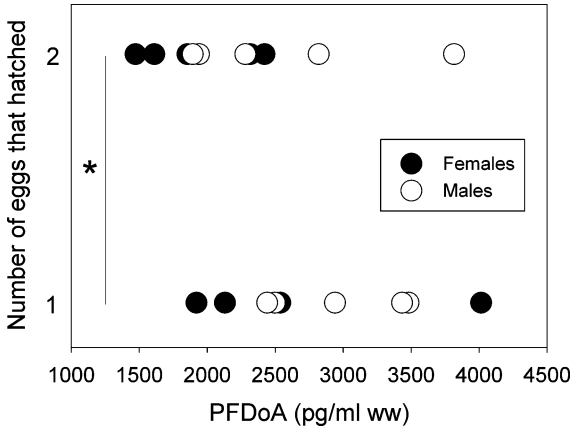

Figure 3. Hatching success (1 or 2 eggs have hatched) was lower in black-legged kittiwakes with high concentrations of PFDoA (*: p = 0.040) open circles denote males and closed circles denote females.

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.[33] Additionally, PFCAs show dramatic increasing trends in Arctic seabird eggs,[13,57] 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/.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: tartu.sabrina@gmail.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This project was supported by Institut Polaire Français (IPEV project 330 to O. Chastel), Agence National de la Recherche (ANR project PolarTop to O. Chastel), INSU- EC2CO 2014 (to O. Chastel) and COPOL (GW Gabrielsen & JO Bustnes). This study was approved by the French and Norwegian Ethic committees and by the Governor of Svalbard. The authors thank C. Parenteau, C. Trouvé, S. Dano, for their excellent technical assistance in hormones assays and molecular sexing, Linda Hanssen and the staff of the NILU laboratory in Tromsø for their assistance during the chemical analyses and P. Duncan for his help with a previous version of the paper.

■ REFERENCES

(1) Colborn, T.; vom Saal, F. S.; Soto, A. M. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ. Health Perspect* **1993**, *101*, 378−384.

(2) Guillette, L. J.; Crain, D. A.; Rooney, A. A.; Pickford, D. B. Organization versus activation: The role of endocrine-disrupting contaminants (EDCs) during embryonic development in wildlife. *Environ. Health Perspect* **1995**, *103*, 157−164.

(3) Jimènez, B. Environmental effects of endocrine disruptors and current methodologies for assessing wildlife health effects. *TrAC, Trends Anal. Chem.* **1997**, *16*, 596−606.

(4) Tyler, C. R.; Jobling, S.; Sumpter, J. P. Endocrine disruption in wildlife: A critical review of the evidence. *Crit. Rev. Toxicol.* **1998**, *28*, 319−361.

(5) la Farré, M.; Pérez, S.; Kantiani, L.; Barceló, D. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. *TrAC, Trends Anal. Chem.* **2008**, *27*, 991−1007.

(6) Petrovic, M.; Eljarrat, E.; Alda, M. J. L. de; Barceló, D. Endocrine disrupting compounds and other emerging contaminants in the environment: A survey on new monitoring strategies and occurrence data. *Anal Bioanal Chem.* **2004**, *378*, 549−562.

(7) Muir, D. C. G.; de Wit, C. A. Trends of legacy and new persistent organic pollutants in the circumpolar arctic: Overview, conclusions, and recommendations. *Sci. Tot. Environ.* **2010**, *408*, 3044−3051.

(8) Jones, P. D.; Hu, W.; De Coen, W.; Newsted, J. L.; Giesy, J. P. Binding of perfluorinated fatty acids to serum proteins. *Environ. Toxicol. Chem.* **2003**, *22*, 2639−2649.

(9) Jensen, A. A.; Leffers, H. Emerging endocrine disrupters: perfluoroalkylated substances. *Int. J. Androl.* **2008**, *31*, 161−169.

(10) Haukås, M.; Berger, U.; Hop, H.; Gulliksen, B.; Gabrielsen, G. W. Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. *Environ. Pollut.* **2007**, *148*, 360−371.

(11) Butt, C. M.; Mabury, S. A.; Muir; Braune, B. M. Prevalence of long-chained perfluorinated carboxylates in seabirds from the Canadian Arctic between 1975 and 2004. *Environ. Sci. Technol.* **2007**, *41*, 3521−3528.

(12) Butt, C. M.; Berger, U.; Bossi, R.; Tomy, G. T. Levels and trends of poly- and perfluorinated compounds in the arctic environment. *Sci. Tot. Environ.* **2010**, *408*, 2936−2965.

(13) Helgason, L. B., Sagerup, K., Gabrielsen, G. W. Temporal trends and contaminant profiles of persistent organic pollutants (POPs) in seabird eggs from Northern Norway and Svalbard. In *Global Contamination Trends of Persistent Organic Chemicals*; Loganathan, B. G., Lam, P. K. S., Eds.; CRC Press, 2011.

(14) Liu, C.; Du, Y.; Zhou, B. Evaluation of estrogenic activities and mechanism of action of perfluorinated chemicals determined by vitellogenin induction in primary cultured tilapia hepatocytes. *Aquat. Toxicol.* **2007**, *85*, 267−277.

(15) Benninghoff, A. D.; Bisson, W. H.; Koch, D. C.; Ehresman, D. J.; Kolluri, S. K.; Williams, D. E. Estrogen-like activity of perfluoroalkyl acids in vivo and interaction with human and rainbow trout estrogen receptors in vitro. *Toxicol. Sci.* **2011**, *120*, 42−58.

(16) Nøst, T. H.; Helgason, L. B.; Harju, M.; Heimstad, E. S.; Gabrielsen, G. W.; Jenssen, B. M. Halogenated organic contaminants and their correlations with circulating thyroid hormones in developing Arctic seabirds. *Sci. Tot. Environ.* **2012**, *414*, 248−256.

(17) Wingfield, J. C.; Sapolsky, R. M. Reproduction and resistance to stress: When and how. *J. Neuroendocrinol.* **2003**, *15*, 711−724.

(18) Ricklefs, R. E.; Wikelski, M. The physiology/life-history nexus. *Trends Ecol. Evol.* **2002**, *17*, 462−468.

(19) Lendvai, Á. Z.; Giraudeau, M.; Chastel, O. Reproduction and modulation of the stress response: An experimental test in the house sparrow. *Proc. R. Soc. B* **2007**, *274*, 391−397.

(20) Angelier, F.; Clément-Chastel, C.; Welcker, J.; Gabrielsen, G. W.; Chastel, O. How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. *Funct. Ecol.* **2009**, *23*, 784−793.

(21) Jenssen, B. M. Endocrine-disrupting chemicals and climate change: A worst-case combination for Arctic marine mammals and seabirds? *Environ. Health Perspect.* **2005**, *114*, 76−80.

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

(23) Bustnes, J. O.; Erikstad, K. E.; Lorentsen, S.-H.; Herzke, D. Perfluorinated and chlorinated pollutants as predictors of demographic parameters in an endangered seabird. *Environ. Pollut.* **2008**, *156*, 417−424.
(24) Custer, C. M.; Custer, T. W.; Schoenfuss, H. L.; Poganski, B. H.; Solem, L. Exposure and effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA. *Reprod. Toxicol.* **2012**, *33*, 556−562.
(25) Custer, C. M.; Custer, T. W.; Dummer, P. M.; Etterson, M. A.; Thogmartin, W. E.; Wu, Q.; Kannan, K.; Trowbridge, A.; McKann, P. C. Exposure and effects of perfluoroalkyl substances in tree swallows nesting in Minnesota and Wisconsin, USA. *Arch. Environ. Contam. Toxicol.* **2014**, *66*, 120−138.
(26) Kelly, B. C.; Ikonomou, M. G.; Blair, J. D.; Surridge, B.; Hoover, D.; Grace, R.; Gobas, F. A. P. C. Perfluoroalkyl contaminants in an Arctic marine food web: Trophic magnification and wildlife exposure. *Environ. Sci. Technol.* **2009**, *43*, 4037−4043.
(27) Peig, J.; Green, A. J. New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. *Oikos* **2009**, *118*, 1883−1891.
(28) Weimerskirch, H.; Lallemand, J.; Martin, J. Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. *J. Anim. Ecol.* **2005**, *74*, 285−291.
(29) Lormée, H.; Jouventin, P.; Trouve, C.; Chastel, O. Sex-specific patterns in baseline corticosterone and body condition changes in breeding Red-footed Boobies *Sula sula*. *Ibis* **2003**, *145*, 212−219.
(30) Powley, C. R.; George, S. W.; Ryan, T. W.; Buck, R. C. Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrixes. *Anal. Chem.* **2005**, *77*, 6353−6358.
(31) Rylander, C.; Phi, D. T.; Odland, J. Ø.; Sandanger, T. M. Perfluorinated compounds in delivering women from south central Vietnam. *J. Environ. Monit.* **2009**, *11*, 2002−2008.
(32) Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicol. Sci.* **2007**, *99*, 366−394.
(33) Martin, J. W.; Smithwick, M. M.; Braune, B. M.; Hoekstra, P. F.; Muir, D. C. G.; Mabury, S. A. Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. *Environ. Sci. Technol.* **2004**, *38*, 373−380.
(34) Vanden Heuvel, J. P. V.; Thompson, J. T.; Frame, S. R.; Gillies, P. J. Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: A comparison of human, mouse, and rat peroxisome proliferator-activated receptor-α, -β, and -γ, liver X receptor-β, and retinoid X receptor-α. *Toxicol. Sci.* **2006**, *92*, 476−489.
(35) Wolf, C. J.; Takacs, M. L.; Schmid, J. E.; Lau, C.; Abbott, B. D. Activation of mouse and human peroxisome proliferator−activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. *Toxicol. Sci.* **2008**, *106*, 162−171.
(36) Nelson, J. W.; Hatch, E. E.; Webster, T. F. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. *Environ. Health Perspect.* **2009**, *118*, 197−202.
(37) Lin, C.-Y.; Wen, L.-L.; Lin, L.-Y.; Wen, T.-W.; Lien, G.-W.; Chen, C.-Y.; Hsu, S. H.; Chien, K.-L.; Sung, F.-C.; Chen, P.-C.; et al. Associations between levels of serum perfluorinated chemicals and adiponectin in a young hypertension cohort in Taiwan. *Environ. Sci. Technol.* **2011**, *45*, 10691−10698.
(38) Halldorsson, T. I.; Rytter, D.; Haug, L. S.; Bech, B. H.; Danielsen, I.; Becher, G.; Henriksen, T. B.; Olsen, S. F. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: A prospective cohort study. *Environ. Health Perspect* **2012**, *120*, 668−673.
(39) Gebbink, W. A.; Letcher, R. J. Comparative tissue and body compartment accumulation and maternal transfer to eggs of perfluoroalkyl sulfonates and carboxylates in Great Lakes herring gulls. *Environ. Pollut.* **2012**, *162*, 40−47.
(40) Fang, X.; Zhang, L.; Feng, Y.; Zhao, Y.; Dai, J. Immunotoxic effects of perfluorononanoic acid on BALB/c mice. *Toxicol. Sci.* **2008**, *105*, 312−321.
(41) Breuner, C. W. Stress and reproduction in birds. *Horm. Reprod. Vertebr.* **2011**, *4*, 129−151.
(42) Breuner, C. W.; Orchinik, M. Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J. Endocrinol* **2002**, *175*, 99−112.
(43) Malisch, J. L.; Breuner, C. W. Steroid-binding proteins and free steroids in birds. *Mol. Cell. Endocrinol.* **2010**, *316*, 42−52.
(44) Verboven, N.; Verreault, J.; Letcher, R. J.; Gabrielsen, G. W.; Evans, N. P. Adrenocortical function of Arctic-breeding glaucous gulls in relation to persistent organic pollutants. *Gen. Comp. Endrocrinol.* **2010**, *166*, 25−32.
(45) Nordstad, T.; Moe, B.; Bustnes, J. O.; Bech, C.; Chastel, O.; Goutte, A.; Sagerup, K.; Trouvé, C.; Herzke, D.; Gabrielsen, G. W. Relationships between POPs and baseline corticosterone levels in black-legged kittiwakes (*Rissa tridactyla*) across their breeding cycle. *Environ. Pollut.* **2012**, *164*, 219−226.
(46) Tartu, S.; Angelier, F.; Herzke, D.; Moe, B.; Bech, C.; Gabrielsen, G. W.; Bustnes, J. O.; Chastel, O. The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. *Sci. Tot. Environ.* **2014**, *476−477*, 553−560.
(47) Moe, B.; Langseth, I.; Fyhn, M.; Gabrielsen, G. W.; Bech, C. Changes in body condition in breeding kittiwakes *Rissa tridactyla*. *J. Avian Biol.* **2002**, *33*, 225−234.
(48) Angelier, F.; Clément-Chastel, C.; Gabrielsen, G. W.; Chastel, O. Corticosterone and time−activity budget: An experiment with black-legged kittiwakes. *Horm. Behav.* **2007**, *52*, 482−491.
(49) Kato, H.; Fujii, S.; Takahashi, M.; Matsumoto, M.; Hirata-Koizumi, M.; Ono, A.; Hirose, A. Repeated dose and reproductive/developmental toxicity of perfluorododecanoic acid in rats. *Environ. Toxicol.* **2014**, DOI: 10.1002/tox.21996.
(50) Fisher, S. A.; Bortolotti, G. R.; Fernie, K. J.; Bird, D. M.; Smits, J. E. Behavioral variation and its consequences during incubation for American kestrels exposed to polychlorinated biphenyls. *Ecotoxicol. Environ. Saf.* **2006**, *63*, 226−235.
(51) Bustnes, J. O.; Bakken, V.; Erikstad, K. E.; Mehlum, F.; Skaare, J. U. Patterns of incubation and nest-site attentiveness in relation to organochlorine (PCB) contamination in glaucous gulls. *J. Appl. Ecol.* **2001**, *38*, 791−801.
(52) Verboven, N.; Verreault, J.; Letcher, R. J.; Gabrielsen, G. W.; Evans, N. P. Nest temperature and parental behaviour of Arctic-breeding glaucous gulls exposed to persistent organic pollutants. *Anim. Behav.* **2009**, *77*, 411−418.
(53) Bailey, R. E. *Incubation Patch of Passerine Birds*. Doctoral dissertation, University of California, Berkeley, 1951.
(54) Shi, Z.; Zhang, H.; Liu, Y.; Xu, M.; Dai, J. Alterations in gene expression and testosterone synthesis in the testes of male rats exposed to perfluorododecanoic acid. *Toxicol. Sci.* **2007**, *98*, 206−215.
(55) Shi, Z.; Ding, L.; Zhang, H.; Feng, Y.; Xu, M.; Dai, J. Chronic exposure to perfluorododecanoic acid disrupts testicular steroidogenesis and the expression of related genes in male rats. *Toxicol. Lett.* **2009**, *188*, 192−200.
(56) Shi, Z.; Zhang, H.; Ding, L.; Feng, Y.; Xu, M.; Dai, J. The effect of perfluorododecanonic acid on endocrine status, sex hormones and expression of steroidogenic genes in pubertal female rats. *Reprod. Toxicol.* **2009**, *27*, 352−359.
(57) Braune, B. M.; Letcher, R. J. Perfluorinated sulfonate and carboxylate compounds in eggs of seabirds breeding in the Canadian Arctic: Temporal trends (1975−2011) and interspecies comparison. *Environ. Sci. Technol.* **2013**, *47*, 616−624.