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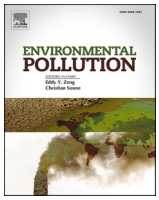

Potential disruption of thyroid hormones by perfluoroalkyl acids in an Arctic seabird during reproduction☆

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ABSTRACT

Arctic marine ecosystems are experiencing rapid change, such as ocean warming and enhanced pollutants. Perfluoroalkyl acids (PFAAs) arriving via long-range transport have been detected in Arctic wildlife, including seabirds which are considered sentinels of marine ecosystem health. There is evidence that PFAA exposure leads to the disruption of thyroid hormones (THs), such as thyroxine (T4) and triiodothyronine (T3), which play important roles in metabolism, incubation, and thermoregulation in seabirds. Here, we investigated relationships between PFAAs and THs [total T4 (TT4), free T4 (FT4), total T3 (TT3) and free T3 (FT3)] in blood plasma collected from 63 thick-billed murres (*Uria lomvia*) at a colony located in northern Hudson Bay (2016–2018). We then tested if PFAAs and TH levels were related to fitness-associated reproductive traits, such as body mass and hatch dates. PFUdA, PFOS, and PFTrDA were the dominant PFAAs in murre blood, accounting for approximately 77% of ∑PFAA. Females had higher PFAAs than males, possibly due to higher trophic feeding. While FT3 increased with PFOS, PFNA, PFDA, PFDoA, PFTeDA, ∑PFCA7, and ∑PFAA in murres, TT3 decreased with PFOS, PFDoA, and PFTeDA in males, but not females, suggesting thyroid disruption. TT3 increased with body mass, whereas several long-chain PFAAs were negatively correlated with body mass. Negative relationships between PFNA, PFDoA, PFTrDA, PFTeDA, and ∑PFAA with hatch dates may be the result of a disruption in incubation behaviour, resulting in earlier hatch dates. Consequently, TT3 concentrations were highest in males and females in 2018, a year in which PFAAs were lowest and hatch dates were delayed relative to 2017. As an Arctic seabird experiencing several indirect effects of climate change, the interaction of PFAAs on thyroid activity may cause additional stress to murres.

1. Introduction

Perfluoroalkyl acids (PFAAs) are terminal and highly stable members of the large class of per-/poly-fluoroalkyl substances (PFAS), which are synthetically produced chemicals used in a variety of commercial and industrial applications. Several long-chain PFAAs such as perfluorinated sulfonic acids (PFSAs) and carboxylic acids (PFCAs) are persistent and biomagnify in food webs, accumulating in top predators at their highest concentrations generally in liver tissue and at substantial levels in the blood (Conder et al., 2008). PFAAs and their precursors have been detected in remote Arctic ecosystems as a result of long-range transport via air and ocean currents (AMAP, 2017; Butt et al., 2010; Letcher et al., 2010; Muir et al., 2019; Wong et al., 2018), with adverse effects reported in Arctic wildlife (Letcher et al., 2010; Pedersen et al., 2016; Routti et al., 2019; Sonne et al., 2021). While restrictions have been placed on the highly bioaccumulative perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and their salts, and have been proposed for long-chain PFCAs and their salts under the Stockholm Convention on Persistent Organic Pollutants (POPs), further assessment on the toxic effects of PFCAs on wildlife is needed for their regulation (UNEP, 2021,

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2019).

Seabirds are long-lived upper trophic predators that are considered sentinels of contaminants in marine ecosystems (Elliott and Elliott, 2013; Furness and Camphuysen, 1997; Furness and Greenwood, 1993). There is evidence that PFAA exposure can lead to the disruption of thyroid hormone homeostasis in seabirds (Ask et al., 2021; Braune et al., 2011; Melnes et al., 2017; Nøst et al., 2012; Sebastiano et al., 2021). PFAAs may disrupt the function of free and total thyroid hormones (THs), thyroxine (T4) and triiodothyronine (T3), which play important roles in avian metabolism and thermogenesis (McNabb, 2007). Additionally, warming temperatures due to climate change are predicted to affect avian thermoregulation and endocrine regulation, especially those processes regulated by thyroid hormones (Ruuskanen et al., 2021).

Thick-billed murres (*Uria lomvia*) are the most abundant seabird in the Canadian Arctic with a circumpolar distribution (Gaston et al., 2012). Murre colonies face several indirect effects of climate change (Gaston et al., 2009, 2005, 2003). In addition, ∑PFCA concentrations have increased in murre eggs and liver in the Canadian Arctic from 1975 to 2011 (Butt et al., 2007; Braune and Letcher, 2013). However, to our knowledge there are no studies on the effects of PFAAs on THs in murres. In Arctic seabirds, T3 is believed to play a critical role in heat production to warm the eggs (Criscuolo et al., 2003), and resting metabolic rates increased with circulating T3 levels in incubating murres (Elliott et al., 2013). Therefore, disruption of TT3 by PFAAs may affect incubation efficiency in murres, ultimately impacting reproductive success.

We measured PFAAs and THs [total T4 (TT4), free T4 (FT4), total T3 (TT3) and free T3 (FT3)] in the blood plasma of breeding murres from 2016 to 2018. First, we examined relationships between PFAAs and THs. Next, we examined relationships between a) THs and b) PFAAs on body condition in murres. Finally, we tested whether a) THs and b) PFAA exposure correlated with reproductive traits. As a top predator, we predicted that long-chain PFCAs would be highest in murres, and THs may decline with long-chain PFCAs as a result of a disruption to thyroid activity. While we predicted TT3 concentrations would be positively associated with fitness, we expected exposure to long-chain PFCAs would have a negative impact on murres.

2. Methods

We collected blood samples (6 mL) for the measurement of thyroid hormones (THs; $n = 139$) and perfluoroalkyl acids (PFAAs; $n = 63$) from breeding thick-billed murres between 3 and 28 July 2016–2018, from the 'West colony' on Coats Island in northern Hudson Bay in Nunavut, Canada (62°56′52.20″N, 82°01′03.70″W). Blood samples were taken from the alar or jugular vein within 3 min post-capture using heparinized syringes with a 25-gauge needle. Samples were centrifuged at 2000 g for 10 min and stored at −80 °C in the field before being shipped to the National Wildlife Research Center (NWRC, Ottawa, Ontario), where they were stored at −80 °C prior to laboratory analysis. Total body mass of murres was measured to the nearest 5 g using a Pesola spring balance. Body mass was used as an index of body condition as it is a better predictor of total body lipids in murres than body mass adjusted for size (Jacobs et al., 2012). There is also no difference in mass between sexes (Gaston and Hipfner, 2020). As our measurements were paired with GPS-deployments used in another study (Esparaza et al., 2022), THs and body mass were measured before and after deployments (mean: 2.42 days) and averaged for analysis. To examine relationships with reproductive success, hatch dates (first date a chick was seen) were recorded for 65 murres and converted to Julian dates.

2.1. Sex identification

Blood samples collected on filter paper were used to determine the genetic sex of the murres (Elliott et al., 2010). Briefly, DNA was extracted from filter paper by excising a 3 mm blood spot and suspending it in 100 μl of 5% Chelex solution (Sigma-Aldrich, St. Louis, MO, USA). Samples were vortexed and incubated in a thermocycler and heated at 95.0 °C for 20 min. Sex was determined by polymerase chain reaction using the P2/P8 primer run with the P2/P8 thermocycler method (Fridolfsson and Ellegren, 2000; Griffiths et al., 1998; National Wildlife Research Centre, 2014; Walsh et al., 1991). In cases where the analysis failed, we determined sex based on a birds' breeding partner.

2.2. Thyroid hormones

At NWRC, plasma was analyzed for free (F) and total (T) triiodothyronine (T3) and thyroxine (T4) in 139 incubating birds ($n = 33$ in 2016, $n = 47$ in 2017, $n = 59$ in 2018; $n = 43$ females, $n = 96$ males). We measured circulating T3 and T4 using commercially available enzyme immunoassay (EIA) kits according to the manufacturer's instructions (Diagnostics Biochem Canada Inc. & Enzo Life Sciences Inc, respectively (Sun et al., 2021)). T3 and T4 concentrations were quantified using standard curves constructed from serial dilutions of the calibration standard. The method detection limits were 0.15 pg/mL (2016: 0.30 pg/mL), 0.08 ng/mL, 0.50 pg/mL and 3.00 ng/mL for FT3, TT3, FT4 and TT4, respectively. Analytical accuracy and precision were assessed using a standard reference material (SRM; human serum-based matrix samples obtained from the Diagnostics Biochem Canada Inc.) and by analyzing duplicate samples. Concentrations are expressed in ng/mL (TT3 and TT4) and pg/mL (FT3 and FT4). Recoveries of standard reference materials ranged from 76.6% to 118% and mean relative percent differences of duplicated samples were 5.1%, 15.9%, 6.8%, 13.8% for TT3, FT3, TT4, and FT4, respectively.

2.3. Perfluoroalkyl acid (PFAA) determinations

PFAAs were measured in 63 incubating birds ($n = 10$ in 2016, $n = 35$ in 2017, $n = 18$ in 2018; $n = 16$ females, $n = 47$ males). PFAA analysis was conducted in the Organic Contaminants Research Lab at NWRC. Extraction methods for blood plasma and instrumental analysis are previously published elsewhere (Chu and Letcher, 2008; Greaves and Letcher, 2013; Sun et al., 2020). Between 0.1 and 0.5 g of plasma samples were weighed into polypropylene tubes and spiked with stable isotope labeled internal standards. The sample was extracted in 3 mL of formic acid acentronitrile/water (0.2%) solution and then vortexed for 1 min. The sample was then centrifuged for 5 min at 5000 rpm. The supernatant was transferred to a tube and the extraction process was repeated. The extract was cleaned-up on methanol and water conditioned SPE WAX cartridges (60 mg × 3 mL; Oasis) and washed with 1 mL of formic acid/water solution (2%; *v:v*) and 2 mL of water. The cartridge was rinsed with 1 mL of methanol, and then PFAAs were eluted with 2 mL of 1% ammonium hydroxide solution. The solvent was then evaporated using nitrogen and reconstituted in methanol, vortexed, and filtered at 6000 rpm for 5 min, before being transferred to injection vials.

A total of 22 PFAAs were quantified using a Xevos TQ-S Ultra performance liquid chromatograph-mass spectrometer system (UPLC-MS/MS; Waters, Mississauga, ON, Canada) by electrospray ionization source operating in multiple reaction monitoring scanning mode. Target compounds included five PFSAs: PFBS, PFHxS, PFEtCHxS, PFOS, and PFDS, and 13 PFCAs: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA, PFHxDA, and PFODA (Table S1). Quality assurance and control procedures included method blanks run every 10 samples, and the analysis of internal standard recovery checks and duplicates, and standard reference checks of a PFAA mixture. PFAAs in the standard reference mixture were within 20% of the expected value, except for PFTrDA, PFTeDA, PFODA, PFHxDA, and PFDS which were over 20% but all below 42%. Mean recoveries for each PFAA ranged from 74 to 107% (Table S1). All samples are reported in ng/g (wet weight).

2.4. Data analysis

For statistical analysis, compounds detected in >70% of individuals were included [PFOS and 7 PFCAs (PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, and PFTeDA)]. Non-detects were replaced with half of the value of the detection limits (see Table S1). In total, 63 birds were analyzed for PFAAs and THs. Inter-annual variation in THs and PFAAs was investigated by comparing the 95% confidence intervals of the means (Ask et al., 2021; Erickson and Rattner, 2020). We were unable to compare inter-annual differences of PFAAs in females due to low sample sizes. Confidence intervals were calculated using the *DescTools* package (Signorelli et al., 2021). Although some differences in PFAAs were detected among years in males, data were pooled across all years to increase our sample size, because the mechanism of action of PFAAs should not vary (Ask et al., 2021).

We investigated the relationships between PFAAs and THs in murres using linear models. As sex-specific effects of PFAAs on THs have been reported in seabirds (Ask et al., 2021; Melnes et al., 2017; Sebastiano et al., 2021), we started with the model TH ~ PFAA × sex to account for sex-specific interactions and proceeded using backward selection. Models with a significant interaction were re-analyzed by each sex separately. An ANOVA was used to assess if removing non-significant terms affected the overall fit of the model, and the most parsimonious model was considered the best. As correlations among PFAAs (Table S2; 0.02 to 0.84) and THs (Table S3; -0.04 to 0.44) were variable, we fitted each PFAAs to each TH and TH ratio (TT3:TT4 and FT3:FT4) separately, to test whether there was an influence of chain length and provide information on the toxic effects of specific PFAAs as required for regulation (UNEP, 2021). We also included ∑PFCA7 and ∑PFAA as predictors. Using the same procedures to account for sex-specific interactions, we then tested relationships between a) THs and b) PFAAs on body mass, and then hatch dates. Linear regressions were assessed for outliers, homogeneity of variance, and normality of residuals using visual plots and tests from the *performance* package (Lüdecke et al., 2021). When appropriate, variables were log-transformed to meet assumptions of normality, outliers, and homogeneity of variances. In cases where transformations did not improve the presence of outliers, we fitted robust linear models using the *MASS* package (Venables and Ripley, 2002). We made all figures using *ggplot2* (Wickham, 2016). All analyses were run using R. 3.6.3 (R Core Team, 2020).

3. Results

3.1. Variation in perfluoroalkyl acids (PFAAs) and thyroid hormones (THs)

Twenty-two PFAAs were analyzed, out of which 8 (PFOS, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA) were detected with sufficient frequency (>70%) in all 63 murres. The dominant isomers in murres were PFUdA, PFOS, and PFTrDA, comprising approximately 77.4% and 77.0% of ∑PFAA in females and males. These three dominant isomers were followed by in decreasing order of concentration, PFDA > PFDoA > PFNA > PFTeDA > PFOA (Table S4).

Male murres in 2018 had lower concentrations of PFOS, PFDoA and ∑PFAA relative to males in 2016 and 2017 (Fig. 1; Table S5). Males in 2018 also had lower concentrations of PFDA than in 2016, and PFTeDA than in 2017. In 2017, females had higher PFTrDA concentrations than males. After pooling all years, females had higher concentrations of PFDoA, PFTrDA, and PFTeDA than males (Table S4). Hormone levels also varied with year (Fig, 1; Table S5, S6). Males and females had

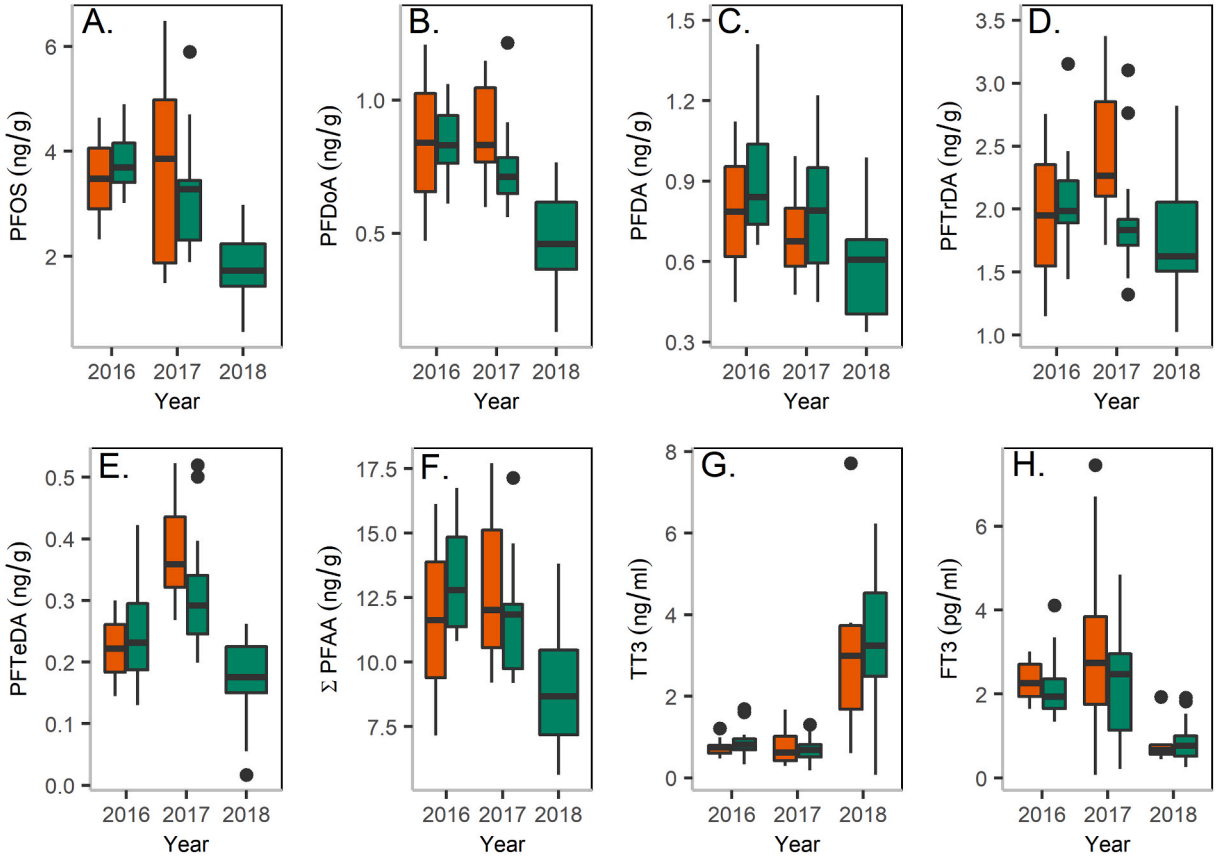

Fig. 1. Boxplots of perfluoroalkyl acid (PFAA) and thyroid hormone (TH) concentrations of male (green; PFAAs $n = 47$; THs $n = 92$) and female (red; PFAAs $n = 16$; THs $n = 42$) thick-billed murres sampled from 2016 to 2018. Only PFAAs and THs that differed among years are shown. No females were sampled for PFAAs in 2018. Black dots represent outliers. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

higher TT3 and lower FT3 concentrations in 2018 relative to 2016 and 2017. There were no differences in THs between sexes (Table S4).

3.2. TH concentrations in relation to PFAAs

Sex-specific interactions were detected with PFOS, PFDoA, and PFTeDA in relation to TT3 (Table S7). TT3 decreased with PFOS, PFDoA, and PFTeDA in males, but not females (Table 1; Fig. 2). While there were no interactions, TT3 decreased with ∑PFAA. For FT3, sex-specific interactions were detected with PFOS and PFNA; FT3 increased with PFOS and PFNA in males, but not females (Fig. 3). While there were no interactions with sex, FT3 increased with PFDA, PFDoA, PFTeDA, ∑PFCA7, and ∑PFAA, whereas TT4 increased with PFOA in murres. There were no relationships between FT4 and PFAA exposure (Table S7).

For TT3:TT4, there were sex-specific interactions with PFOS, as TT3:TT4 decreased with PFOS in males, but not females (Table 1). While there were no interactions with sex, TT3:TT4 decreased with PFOA and PFTeDA, and FT3:FT4 increased with PFOS, PFDA, PFDoA, PFTeDA, ∑PFCA7, and ∑PFAA in murres.

3.3. Body mass in relation to THs and PFAAs

Male murres had the lowest body mass in 2017 (mean: 953.7, 95% CI: 933.7–973.6 g) relative to 2016 (993.7, 976.6–1010.8 g) and 2018 (1007.5, 990.3–1024.7 g). Females had lower body mass in 2017 (942.5, 919.3–965.7 g) relative to 2018 (1010, 972.6–1047.5 g) but not 2016 (985.3, 948.3–1022.4 g). There were no differences in total body mass between sexes.

There were no sex-specific interactions between body mass and THs or PFAAs (Table S7). Total body mass increased with TT3 concentrations, and decreased with FT3 and FT4. Body mass decreased with PFDoA, PFTrDA, PFTeDA, ∑PFCA7, and ∑PFAA (Table 1; Fig. 4).

3.4. Associations between PFAAs and THs with reproductive traits

The median hatch date of murres was significantly later in 2018 (July 29; 95% CI: July 27–29, $n = 26$) than in 2017 (July 18; July 18–20; $n = 36$). Only three hatch dates (median July 19; July 18–21) were available for murres sampled in 2016.

There were sex-specific interactions with TT3 and FT4 in relation to

Table 1
Parameter estimates ($\beta \pm$ standard error) from significant linear regression models explaining the relationships between thyroid hormones (THs; TT3, FT3, TT4, and FT4) and PFAA (perfluoroalkyl acid) exposure (PFOA, PFOS, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA, ∑PFCA7, ∑PFAA) and between THs or PFAAs and fitness-associated traits (body mass, hatch date) of adult thick-billed murres. In cases where sex showed a significant effect on the correlation, results are provided for males (M) and females (F). Robust linear models are indicated with *.

Variable	Predictor	Sex	β	S.E.M	95% CI	Intercept
Log (TT3)	PFOS	M	−0.45	0.09	−0.62,-0.27	1.49 ± 0.3 (0.97,2.00)
		F	0.03	0.08	−0.16,0.21	−0.44 ± 0.3 (-1.18,0.29)
	PFDoA	M	−1.98	0.52	−3.02,-0.93	1.55 ± 0.4 (0.84,2.27)
		F*	0.48	0.81	−1.11,2.08	−0.79 ± 0.8 (-2.26,0.69)
	PFTeDA	M	−4.13	1.00	−6.15,-2.11	1.26 ± 0.3 (0.73,1.80)
		F*	0.04	1.56	−3.00,3.09	−0.35 ± 0.6 (-1.5,0.79)
	∑PFAA	NA	−0.12	0.03	−0.18,-0.05	1.41 ± 0.4 (0.64,2.19)
Log (FT3)	PFOS	M	0.33	0.09	0.15, 0.52	−0.67 ± 0.3 (-1.2,-0.13)
		F	−0.05	0.18	−0.44,0.34	0.96 ± 0.7 (-0.53,2.44)
	PFNA	M	1.13	0.32	0.49,1.77	−0.56 ± 0.2 (-1.05, −0.07)
		F	−0.73	0.86	−2.58,1.13	1.28 ± 0.7 (-0.13,2.69)
	PFDA	NA	1.01	0.47	0.07,1.96	−0.38 ± 0.4 (-1.10,0.34)
	PFDoA	NA	1.81	0.44	0.92,2.69	−0.91 ± 0.3 (-1.57,-0.26)
FT3	PFTeDA	NA	5.83	1.38	3.08,8.59	0.40 ± 0.4 (-0.40,1.19)
Log (FT3)	∑PFCA7	NA	0.14	0.06	0.03, 0.26	−0.81 ± 0.5 (-1.78,0.17)
	∑PFAA	NA	0.11	0.04	0.04,0.19	−0.88 ± 0.4 (-1.75,-0.01)
Log (TT4)	Log (PFOA)	NA	0.58	0.19	0.21,0.96	3.69 ± 0.4 (2.97,4.42)
Log (TT3:TT4)	Log (PFOA)	NA	−0.87	0.27	−1.42,-0.33	−4.18 ± 0.5 (-5.25,-3.11)
	PFOS	M	−0.36	0.13	−0.63,-0.1	−1.31 ± 0.4 (-2.11,-0.51)
		F	0.15	0.12	−0.1, 0.40	−3.68 ± 0.5 (-4.69,-2.67)
	PFTeDA	NA	−3.42	1.11	−5.66,-1.19	−1.56 ± 0.3 (-2.23,-0.89)
Log (FT3:FT4)	PFOS	NA	0.20	0.07	0.05,0.35	−2.41 ± 0.2 (-2.89,-1.94)
	PFDA	NA	0.84	0.42	0.00,1.69	−2.46 ± 0.3 (-3.10,-1.81)
	PFDoA	NA	1.48	0.40	0.67,2.28	−2.88±0.3 (-3.49,-2.28)
	PFTeDA	NA	2.13	0.89	0.36,3.91	−2.41 ± 0.3 (-2.92,-1.90)
	∑PFCA7	NA	0.13	0.05	0.02,0.23	−2.88 ± 0.4 (-3.75,-2.01)
	∑PFAA	NA	0.10	0.03	0.03,0.17	−2.93 ± 0.4 (-3.71,-2.15)
Log (Mass)	Log (TT3)	NA	0.02	0.01	0.01,0.04	6.88 ± 0.0 (6.87,6.89)
Mass	FT3	NA	−0.01	0.00	−0.02,-0.01	6.91 ± 0.0 (6.89,6.93)
	Log (FT4)	NA	−0.03	0.01	−0.06,-0.01	6.96 ± 0.0 (6.90,7.01)
	PFDoA	NA	−108.73	33.53	−175.79,-41.67	1047.63 ± 24.6 (998.48,1096.79)
	PFTrDA	NA	−50.44	13.83	−78.12,-22.77	1071.83 ± 28.4 (1014.98,1128.69)
	PFTeDA	NA	−234.88	63.91	−362.73,-107.04	1035.01 ± 18.7 (997.59,1072.44)
	∑PFCA7	NA	−8.99	4.07	−17.13,-0.86	1045.61 ± 34.3 (976.92,1114.29)
	∑PFAA	NA	−6.02	2.77	−11.57,-0.48	1038.67 ± 31.8 (975.04,1102.29)
Hatch	TT3	M	2.00	0.35	1.30,2.7	200.74 ± 1.1 (198.58,202.91)
		F	0.55	0.50	−0.5,1.61	200.4 ± 1.0 (198.39,202.41)
	FT3	NA	−1.63	0.37	−2.38,-0.89	207.11 ± 0.9 (205.28,208.95)
	FT4	M	−0.76	0.20	−1.17,-0.36	213.34 ± 2.3 (208.77,217.91)
		F	−0.15	0.16	−0.48,0.18	202.75 ± 2.0 (198.54,206.96)
	PFNA	M	−8.54	3.72	−16.42,-0.66	209.56 ± 2.8 (203.68,215.44)
		F	4.11	2.97	−2.5,10.73	197.95 ± 2.3 (192.82,203.07)
	PFDoA	NA	−11.70	3.54	−18.96,-4.45	211 ± 2.7 (205.55,216.45)
	PFTrDA	NA	−3.07	1.48	−6.1,-0.05	209.06 ± 3.2 (202.46,215.67)
	PFTeDA	NA	−25.54	6.32	−38.49,-12.59	210.23 ± 2.0 (206.08,214.38)
	∑PFAA	NA	−0.72	0.31	−1.35,-0.09	210.64 ± 3.5 (203.4,217.88)

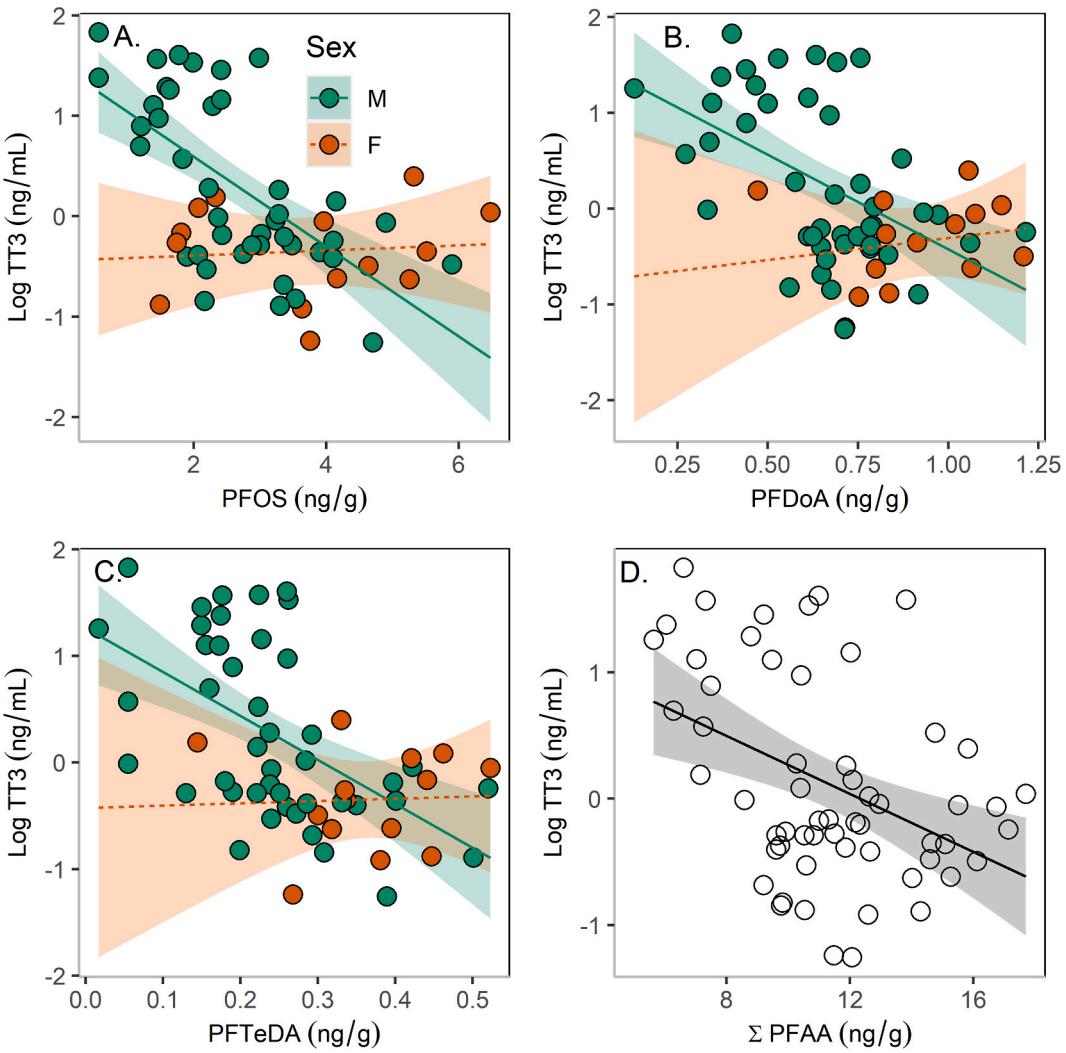

Fig. 2. Linear regressions of the relationships between TT3 and PFAAs in the blood plasma of adult thick-billed murres (n = 58). In cases where sex showed a significant effect on the correlation, results are provided for males (M; n = 44) and females (F; n = 14). The shaded area represents the 95% confidence intervals around the predicted values.

hatch date (Table S7), and hatch date increased with TT3 and decreased with FT4 in males, but not females (Table 1). While there were no interactions, hatch date also decreased with FT3 in murres. For PFAAs, sex-specific interactions were found with PFNA, in which hatch dates decreased with PFNA in males, but not females (Table 1; Fig. 5). While there were no interactions, hatch dates decreased with PFDoA, PFTrDA, PFTeDA, and ∑PFAA concentrations in murres.

4. Discussion

The concentrations of several thyroid hormones (THs) correlated with long-chain perfluoroalkyl acid (PFAA) exposure in adult thick-billed murres. Specifically, TT3 decreased with concentrations of PFOS, PFDoA, and PFTeDA in males, suggesting a possible disruption to thyroid activity and TT3 production. No relationships were found in females, possibly due to low sample size. In contrast, other studies have found positive associations between TT3 and PFAAs in several seabirds (Ask et al., 2021; Braune et al., 2011; Melnes et al., 2017; Sebastiano et al., 2021). While TT3 was positively associated with body mass and hatch date in murres, several long-chain PFAAs were negatively associated with these fitness-associated traits.

4.1. Variation in PFAA concentrations between sexes

From 2016 to 2018, PFUdA, PFOS, and PFTrDA were the dominant PFAAs in the blood plasma of murres. In comparison, from 1975 to 2011, PFOS was the dominant PFAA in murre eggs from the Canadian Arctic, followed by PFUdA and PFTrDA, with the ratio of ∑PFCA:PFOS increasing from 1975 to 2006 (Braune and Letcher, 2013). In contrast to Braune and Letcher (2013), PFUdA was the predominant PFAA in murres from our study, and PFOS concentrations in blood plasma were an order of magnitude lower than in eggs. Decreasing blood-plasma concentrations of PFOS in murres from 2016 to 2018 relative to eggs from 1975 to 2011 may reflect the restriction of PFOS, its salts, and perfluorooctane sulfonyl fluoride in 2009, as listed under Annex B of the Stockholm Convention on POPs (UNEP, 2019). Similarly, PFTrDA and PFUdA were the dominant PFAAs in the blood plasma of black-legged kittiwakes (*Rissa tridactyla*) in Svalbard, Norway (Ask et al., 2021; Costantini et al., 2019; Jouanneau et al., 2021; Tartu et al., 2014) and three gull species in southern France (Sebastiano et al., 2021).

Female murres had higher concentrations of PFDoA, PFTrDA, and PFTeA than males, whereas male kittiwakes had higher concentrations of these PFCAs (Ask et al., 2021; Tartu et al., 2014). We believe differences in long-chain PFCAs between sexes in murres may be the result of differences in foraging ecology. Female murres have higher $\delta^{15}N$ values

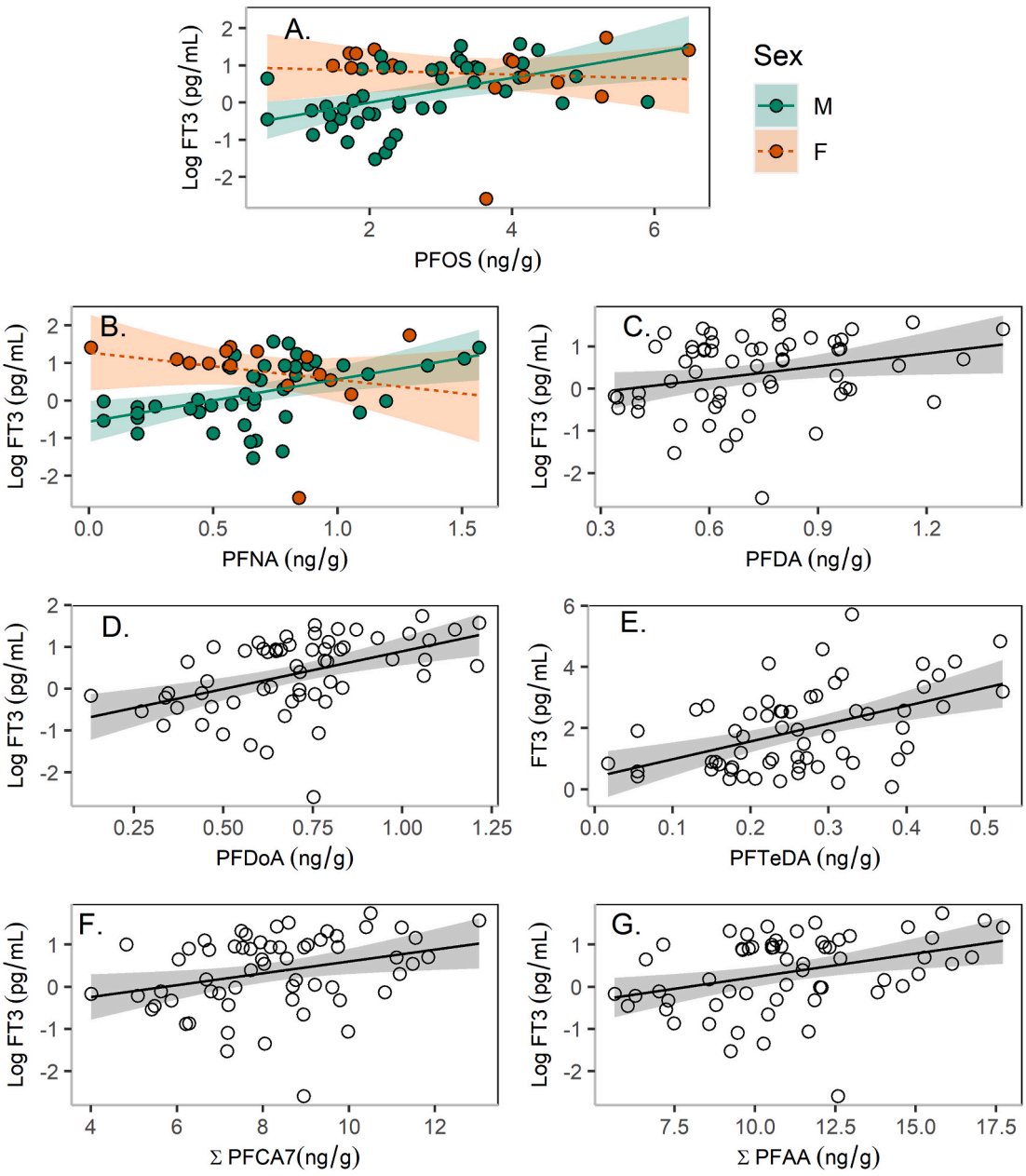

Fig. 3. Linear regressions of the relationships between FT3 and PFAA exposure in the blood plasma of adult thick-billed murres ($n = 60$). In cases where sex showed a significant effect on the correlation, results are provided for males (M; $n = 45$) and females (F; $n = 15$). The shaded area represents the 95% confidence intervals around the predicted values.

than males, indicative of higher trophic feeding (mean females: 15.29‰; males: 14.95‰; Choy et al. in prep).

4.2. Potential disruption of THs by PFAAs

Total T3 was negatively associated with PFOS, PFDoA, and PFTeDA in male murres. In contrast, several seabird studies have found positive associations between PFAAs and TT3 (Ask et al., 2021; Braune et al., 2011; Melnes et al., 2017; Sebastiano et al., 2021), suggested to be the result of a common affinity for protein binding (McNabb, 2007). PFAAs can displace THs from binding proteins (Mortensen et al., 2020; Weiss et al., 2009), such as albumin, which is an important carrier protein for THs (McNabb, 2007) and several PFAAs (Forsthuber et al., 2020; Lau et al., 2007). Total T4 increased with PFOA in murres, a relationship also found in peregrine falcon (*Falco peregrines*) nestlings (Sun et al., 2021). PFOA may alter T4 activity in the thyroid gland, increasing circulating TT4 concentrations (Sun et al., 2021). Decreasing TT3:TT4 ratios with PFOA, PFTeDA, and PFOS suggest disruption of TH homeostasis, as TT3:TT4 ratios are a sensitive bioindicator of contaminant exposure (Peakall, 1992). As T4 is a precursor of T3 through peripheral deiodination (Ishihara et al., 2003; Marsili et al., 2011), the negative association between TT3 and PFAA exposure suggests a disruption to thyroid function (Lau et al., 2007), and possibly reduced conversion of T4 to T3 in murres.

FT3 was positively correlated with PFOS, PFNA, PFDA PFDoA, and PFTeDA in murres. Contrasting relationships between TT3 and FT3 may be the result of these PFAAs disrupting the protein binding of T3, resulting in higher concentrations of circulating FT3. FT3:FT4 ratios also increased with PFOS, PFDA, PFDoA, PFTeDA, ∑PFCA7, and ∑PFAA concentrations. In peregrine nestlings, FT3:FT4 increased with PFOA and PFTeDA, believed to be due to thyroid disruption, and there were no associations between FT4 and PFAAs (Sun et al., 2021).

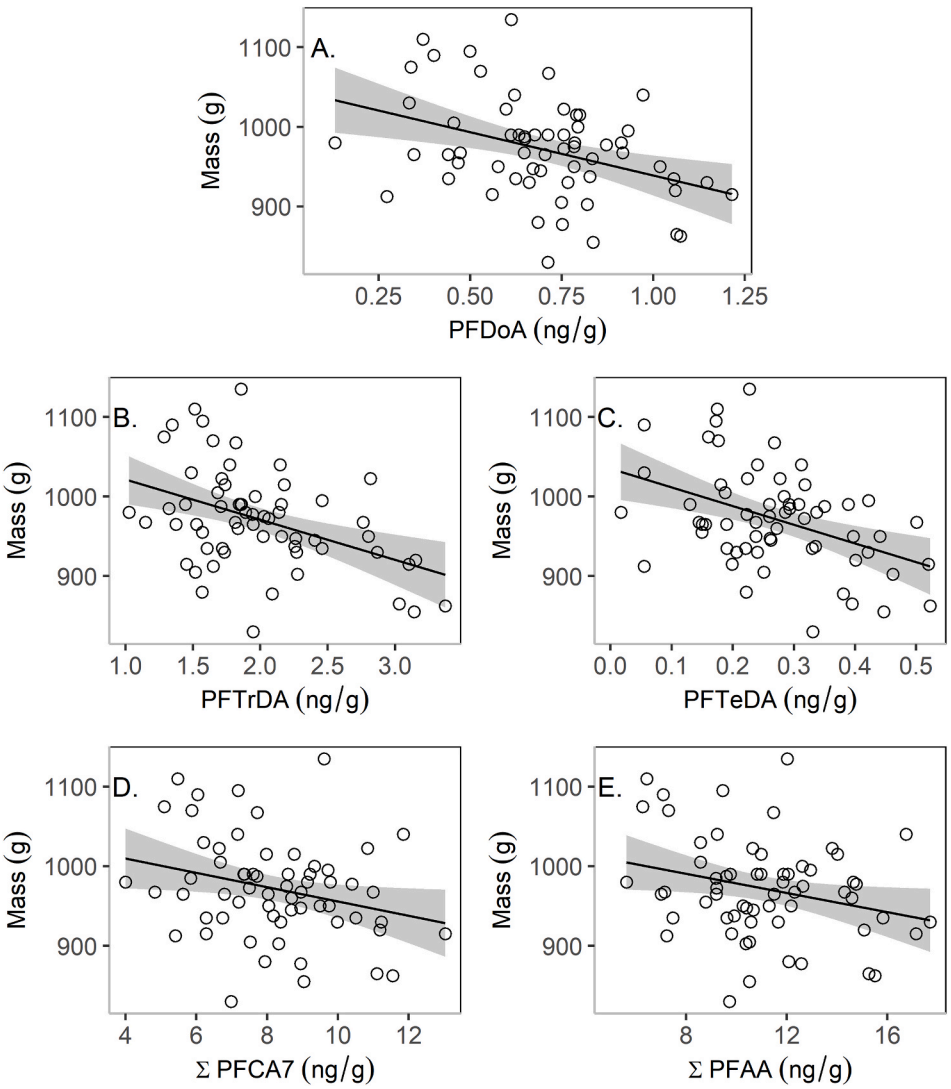

Fig. 4. Linear regressions of the relationships between body mass and PFAA exposure in the blood plasma of thick-billed murres ($n = 62$). The shaded area represents the 95% confidence intervals around the predicted values.

While correlations between THs and PFAAs were found in males, there were no associations found between PFAAs and THs in females, suggesting males may be more susceptible to thyroid disruption. While this may also be due to the low sample size of females, sex differences have been observed in other seabirds (Ask et al., 2021; Melnes et al., 2017), believed to be due to differences in the mechanism of action of THs between sexes. For example, TT3 was positively associated with PFDoA, PFTeDA, and PFTrDA in female kittiwakes (Ask et al., 2021), and with PFOS in glaucous gull (*Larus hyperboreus*) females (Melnes et al., 2017), but not males.

4.3. Associations between THs and PFAAs with body condition

Body mass was positively related to TT3 in murres, but was negatively associated with FT3 and FT4. A positive correlation between TT3 and body condition was found in glaucous gull males, but not females (Melnes et al., 2017), and free-ranging black-legged kittiwakes at the colony but not in the lab (Welcker et al., 2013). These positive correlations were believed to be the result of higher circulating THs in birds in better condition (McNabb, 2000; Melnes et al., 2017).

Body mass decreased with PFDoA, PFTrDA, PFTeDA in murres. We believe that birds feeding at higher trophic levels accumulated higher concentrations of long-chain PFAAs, which had a negative effect on body mass. Previous studies on seabirds have found contrasting relationships between PFAAs and body condition between the sexes, which were attributed to differences in the roles of sex hormones in adipogenesis (Palmer and Clegg, 2013), genetics (Link and Reue, 2017), and dietary differences (Ask et al., 2021). Body condition was positively associated with plasma PFTrDA and PFTeDA in male kittiwakes, attributed to males feeding at higher trophic levels, resulting in higher condition and long-chain PFCAs (Ask et al., 2021). Body condition was negatively associated with PFNA, PFDA, and linear PFOS in great black backed gull (*Larus marinus*) females, and PFDA and PFNA in lesser black backed gull (*Larus fuscus*) males (Sebastiano et al., 2021), but was positively associated with PFNA in male kittiwakes (Tartu et al., 2014). Positive associations between body mass and PFOA, PFHxS, and PFOS in glaucous gulls may be due to higher plasma protein levels as a result of a protein rich diet (Melnes et al., 2017).

4.4. THs and PFAAs in relation to the timing of reproduction

Higher TT3 concentrations were associated with later hatch dates of eggs in males, but not in females possibly due to low sample size. T3 is believed to play a role in incubation energy expenditure, as plasma T3 concentrations increased over the incubation period in female common eiders and were highest at hatching (Criscuolo et al., 2003). TT3

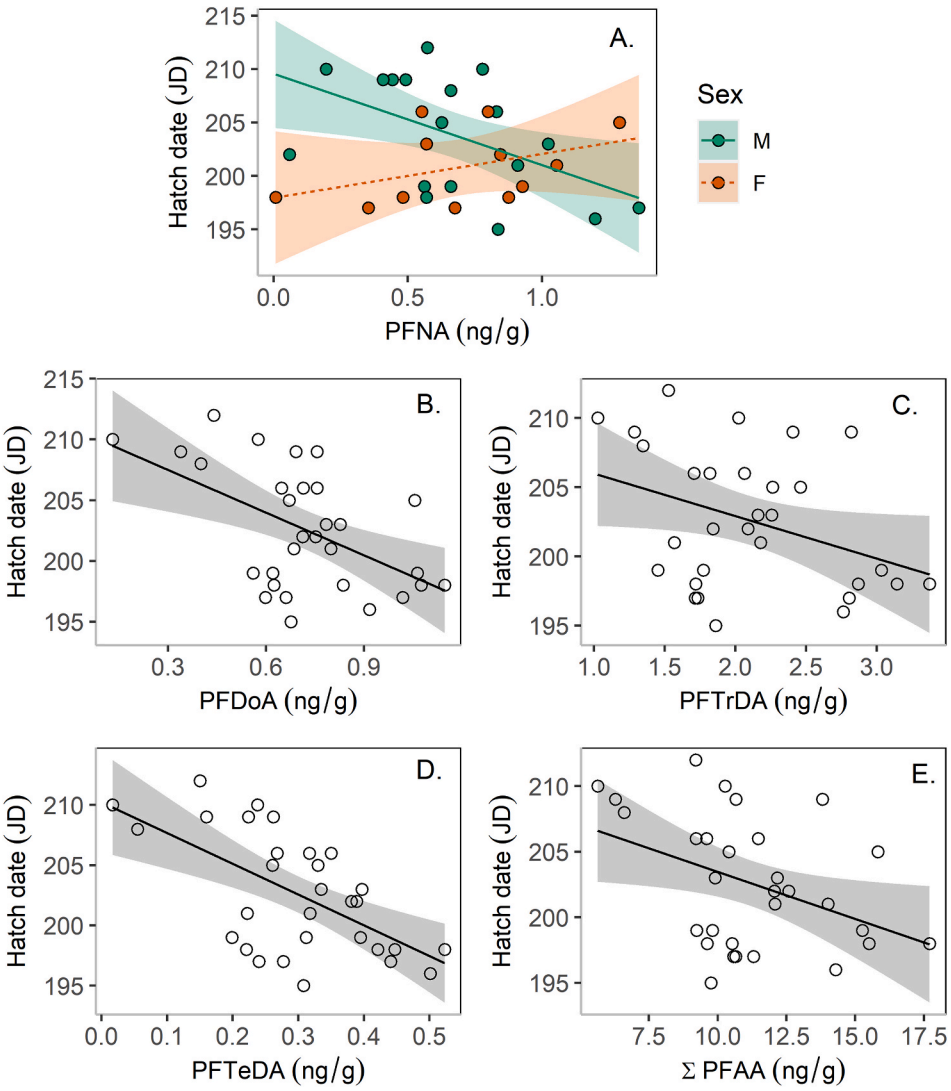

Fig. 5. Linear regressions of the relationships between hatch date and PFAA exposure in the blood plasma of adult thick-billed murres ($n = 30$). In cases where sex showed a significant effect on the correlation, results are provided for males (M; $n = 18$) and females (F; $n = 12$). The shaded area represents the 95% confidence intervals around the predicted values. Hatch dates are listed in Julian dates (JD).

concentrations were higher in murres in 2018 than in 2017, which may be the result of an increased energetic cost of incubation associated with a longer incubation period and later hatch date. The timing of sampling may have also affected TT3 concentrations, as most murres were sampled before the median hatch date in 2018 in comparison to 2017.

In murres, higher concentrations of PFDoA, PFTrDA, and PFTeDA were associated with earlier hatch dates. Reproductive changes in relation to PFAAs have been reported in several seabirds and passerines (Blévin et al., 2020; Custer, 2021; Custer et al., 2014; Tartu et al., 2014). Hatching success of eggs was lower in adult male and female kittiwakes with higher PFDoA concentrations, which may be due to a disruption in incubation behavior and smaller brood patches, resulting in a decline in incubation efficiency (Tartu et al., 2014). A negative relationship between PFTeDA and hatching success was also detected in male kittiwakes (Tartu et al., 2014). As food restrictions decrease T3 (McNabb, 2000), we hypothesize that well-fed birds with higher TT3 concentrations and body masses are able to incubate for longer periods, resulting in later hatch dates. The disruption of TT3 by PFDoA and PFTeDA, and their negative relationships with body mass in adult murres, may have resulted in earlier hatch dates due to a disruption in incubation behaviour.

5. Conclusions

Negative associations between blood plasma PFAAs and TT3 in murres support a potential disruption of thyroid activity, particularly in males. We also provide evidence of contrasting relationships of TT3 and PFAAs in adult murres on individual body condition and hatch dates of their eggs. As correlations do not allow for conclusions on cause-effect relationships, future research should focus on identifying the mechanisms in which PFAAs interact with thyroid activity and fitness in murres. As most toxicity studies have focused on PFOA and PFOS, our study highlights the potential effects of several long-chain PFCAs on Arctic wildlife.

Credit author statement

Emily S. Choy: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing-Reviewing and Editing, Visualization. **Kyle H. Elliott**: Conceptualization, Methodology, Validation, Investigation, Resources, Writing-Reviewing and Editing, Supervision, Project administration, Funding acquisition. **Ilse Esparza**: Writing-Reviewing and Editing. **Allison Patterson**: Investigation, Writing- Reviewing and Editing. **Robert J. Letcher**: Conceptualization, Methodology,

Validation, Resources, Writing-Reviewing and Editing. **Kim J. Fernie**: Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing-Reviewing and Editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2022.119181.

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