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Per- and poly-fluoroalkyl substances (PFAS) do not accumulate with age or affect population survival in ruddy turnstone (*Arenaria interpres*)

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HIGHLIGHTS

- PFAS pollution, survival and juvenile recruitment studied in wild ruddy turnstones
- Concentrations of PFAS in blood of turnstones are very low
- Repeatedly sampled individual wild birds showed no evidence of PFAS bioaccumulation
- No trend of survival or juvenile recruitment in turnstone population over 15 years

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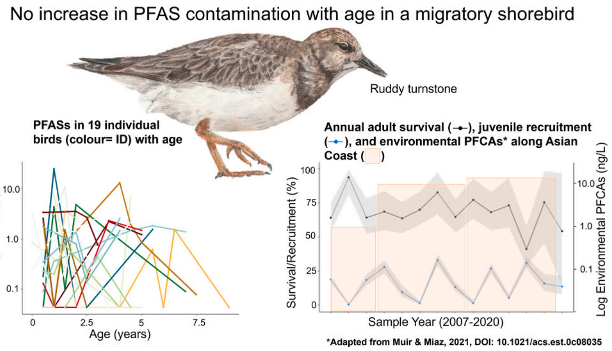
GRAPHICAL ABSTRACT



ABSTRACT

Per- and poly-fluoroalkyl substances (PFAS) may threaten wildlife due to their high environmental persistence, toxicity potential and potential to bioaccumulate. Bioaccumulation may be particularly profound in long-lived animals inhabiting higher trophic niches. To date, there is a paucity of data on PFAS bioaccumulation potential in individual wild birds over their lifetime. In this study, we analysed within-individual PFAS contamination in a declining long-distance migratory shorebird, the ruddy turnstone (*Arenaria interpres*), and the variation in PFAS contamination with age by repeatedly sampling 19 individuals throughout their lives between 2007 and 2022. We found blood-sampled turnstones on their non-breeding grounds in King Island, Tasmania, exhibited no variation of PFAS contamination with age, with low overall circulating PFAS concentrations (<0.015–25 ng/g, median: 0.78 ng/g). Moreover, irrespective of the increased PFAS usage along the East Asian Australasian Flyway over the past two decades, ruddy turnstone survival remained consistent throughout the 15-year sampling period, with no temporal trend in percentage of juveniles in the population. From a conservation perspective, low concentrations of PFAS found in this study are good news as they suggest PFAS alone do not seem to threaten

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turnstone survival. However, the unknown effects of exposure to mixtures of pollutants may yet threaten turnstones.

1. Introduction

Per- and poly-fluoroalkyl substances (PFAS) are a group of pollutants known to be stable, persistent and mobile in the environment (Wang et al., 2017). Consisting of a chain of carbon-fluorine bonds with a functional group at the end, PFAS molecules have both lipo- and hydrophobic properties at opposing ends of the molecules (Buck et al., 2011), and are additionally proteinophilic (Kelly et al., 2009). The properties of PFAS make them very versatile, used in textiles, cosmetics, pesticides and fire-retardant foam. Recent estimates suggest over 14,000 PFASs are on the market globally (US Environmental Protection Agency, 2022). Yet, such profligate application is also the cause of these compounds' ubiquity in the global environment (Ahrens and Bundschuh, 2014; Cousins et al., 2022; Evich et al., 2022; Lee et al., 2020; Muir et al., 2019; Szabo et al., 2022; Wild et al., 2022; Yao et al., 2017). PFAS with carbon chains over 6 atoms long have been shown to be highly persistent in the environment (De Silva et al., 2021). Even with adequate regulation of PFAS, there is the tendency for PFAS precursor compounds to also be released into the environment where they can be bio-transformed into PFAS, resulting in environmental contamination higher than inputs would suggest (Wang et al., 2017). As a result, PFAS have also been found in humans as well as many species of wildlife (Chen et al., 2021; De Silva et al., 2021; Giesy and Kannan, 2001; Muir et al., 2019; Sunderland et al., 2019).

Continued exposure to PFAS can cause their accumulation in the tissues of animals, particularly in high-protein tissues, including blood (Forsthuber et al., 2020). The major aim of this study was to investigate accumulation of PFASs within individuals. Relatively little is yet known about the bioaccumulation dynamics and health impacts in birds, especially at a within-individual level. High PFAS concentrations can cause acute toxicity, but sublethal concentrations can also result in impacts on immune function (Castaño-Ortiz et al., 2019), endocrine systems and reproduction (Custer et al., 2014; Dietz et al., 2019) at tissue concentrations as low as 50 ng/g (Dennis et al., 2021). Studies have found associations between some PFAS and decreased embryonic heart rate and increased liver mass in chickens (1.08–2.17 mg/g wet weight in liver, Briels et al., 2018), decreased hatching success in northern bobwhite (*Colinus virginianus*) (89.2–613 ng/g wet weight in liver, Dennis et al., 2021) and wild tree swallows (*Tachycineta bicolor*) (150–200 ng/g wet weight in eggs, Custer et al., 2014) though no other effects have been observed in swallows due to PFAS exposure (Custer et al., 2019). High concentrations of PFAS (median liver concentration 294 ng/g wet weight, max 11,300 ng/g) have been observed in raptors which feed at high trophic levels, thereby indicating biomagnification (Barghi et al., 2018), the potential for which is high when birds are repeatedly exposed to the pollutants (Tarazona et al., 2015).

Migratory shorebirds (Order Charadriiformes, particularly families Scolopacidae and Charadriidae) represent a promising taxon in which to study bioaccumulation dynamics in birds (Ma et al., 2022) for four reasons. First, shorebirds are positioned at relatively high trophic levels (Bocher et al., 2014) so are likely to experience biomagnification when exposed to PFAS (Hong et al., 2014). Second, shorebirds are often benthic foragers, inhabiting coastal environments where they target prey species living in recently deposited sediments, habitats which often accrue PFAS at concentrations higher than observed in the surrounding water (Stark, 1998). Third, shorebirds tend to be highly site faithful (Christie et al., 2009) and any exposure to pollutants is likely to occur year upon year. Finally, shorebirds are relatively long-lived. Many individuals reach ages in excess of 10+ years (Conklin et al., 2017), showing high survival rates (Méndez et al., 2018), and thus have considerable potential of bioaccumulation of pollutants over their lifetime.

Arctic breeding shorebirds undertake some of the most long-distance migrations on the planet, with some undertaking transhemispheric flights in excess of 10,000 km via the east coast of Asia (e.g. Battley et al., 2012; Minton et al., 2013). Migrating shorebirds deposit large body stores at stopover sites along their flyway to fuel their migrations, sometimes doubling in mass (Lindström et al., 2011). Shorebirds of the East Asian-Australasian Flyway (EAAF) frequent coastal habitats that are among the most heavily impacted by human activity in the world (Halpern et al., 2008). Human activity impacts include agricultural and industrial practices known to emit PFASs into the environment (De Silva et al., 2021). Indeed, PFAS pollution in China, especially in the Yellow Sea stopover sites, has increased over the last 15 years, resulting in the world's highest observed PFAS concentrations in coastal waters (Muir and Miaz, 2021). Accordingly, shorebirds in the Yellow Sea have been found to have high concentrations of PFAS (Sun et al., 2023). Migratory flight can increase the heat stress of birds due to exertion from their intense workload. Such exertion may subsequently exacerbate the effects of any pollution shorebirds experience, as many pollutants have been shown to have higher toxicity in animals experiencing thermal stress (Gordon et al., 2014). Pollution exposure with concomitant fitness consequences in these shorebirds would add to already identified threats including tidal flat conversion at stopover sites (Murray et al., 2018) and climate change effects on their breeding grounds (van Gils et al., 2016), making shorebirds among the most threatened avian taxa in the world. Yet, very few studies have thus far examined PFAS in shorebirds (Ross et al., 2023, 2024; Sun et al., 2023).

In this study, we examined the within-individual dynamics of PFAS pollution in known-age, wild ruddy turnstones (*Arenaria interpres*) throughout their lives, repeatedly sampled at their Australian non-breeding sites on King Island. Ruddy turnstones are a medium-sized shorebird with an Arctic, circumpolar breeding distribution (Conklin et al., 2014). Migrating turnstones in the EAAF regularly use the Yellow Sea as one of several stopover sites on their migrations (Zhao, 2016). Only adult birds visit the Yellow Sea; ruddy turnstones exhibit delayed maturity where upon arrival, young birds spend approximately one and a half years on the non-breeding grounds before embarking on their first northward migration as adults (Rogers, 2006). Like many other shorebirds, ruddy turnstones have experienced a steep population decline with a reduction of up to 50 % of their EAAF population in the quarter century prior to 2011 (Garnett et al., 2011) at an estimated decline of up to 3 % per year (Clemens et al., 2016). Ruddy turnstones forage on benthic prey such as amphipods and gastropods (Whitfield, 1990), and are highly site faithful to their non-breeding habitats (Christie et al., 2009; Hoye et al., 2021), allowing repeated captures of the same individual turnstones. Using samples from repeatedly captured wild turnstones, we examined within- and among-individual variations in red blood cell concentrations of 15 different PFAS over time, to gain insight into any temporal dynamics of PFAS pollution. We conducted a 15-year mark-recapture survival analysis on this population of turnstones and measured annual juvenile percentages, to gauge whether there are impacts on mortality and fecundity in the population, reflective of a putative increased exposure to PFAS over the same period (Baluyot et al., 2021; Muir and Miaz, 2021). Our study thus provides data on lifetime PFAS exposure in known-age, repeatedly sampled turnstones, and investigates any potential effects on the overall survival of the population. Our insights are of importance to evaluate the role of current PFAS pollution as a potential threat to ruddy turnstones and other shorebirds of the EAAF.

2. Materials and methods

2.1. Sample collection

Between 2007 and 2022, ruddy turnstones were captured by cannon-net along the west coast of King Island (Fig. 1). King Island is situated in the Bass Strait approximately halfway between Victoria and Tasmania. Captures generally occurred twice yearly, in March/April each year before the birds departed north, and November/December once the birds arrived back in Australia. When captured, birds were fitted with an Australian Bird and Bat Banding Scheme band, then aged and (where possible) sexed based on plumage characteristics outlined by Higgins and Davies (1996). Juvenile birds are characteristically duller than adults and have a scaled pattern to their contour feathers. Juveniles also have a uniform moult and wear of feathers when compared to the routine primary moult of adults. In breeding plumage, adult birds have much brighter colours with rufous patterning, and males in particular show a stark black and white pattern on their heads. Where birds were first captured as juveniles, their exact age is known. When a bird was captured in spring upon arrival in Australia, age was recorded as a whole number (e.g. 2 years old). After spending the non-breeding season in Australia, the ages of birds captured in autumn were recorded as a fraction (e.g. 2.5 years old). Between 2010 and 2022, blood samples were taken from all birds captured, under Deakin University Animal Ethics Permits A113–2010, B37–2013 and B43–2016, as well as Tasmania Animal Ethics 20/2013/14, 1/2017–18 and 5/2019–20. Blood is often an ideal matrix in sampling birds as it is non-lethal, allows birds to be resampled over time, and can be used in studies for a wide range of factors, from pollution to health biomarkers (Pacyna-Kuchta, 2023). When bled, approximately 200 μL of whole blood was taken from the brachial vein of each individual bird using Microvette® 200 Serum CAT capillary tubes (Sarstedt, Nümbrecht, Germany). Blood samples were left to clot while refrigerated, and then centrifuged to separate red blood cells (RBC) from serum, resulting in approximately 100 μL of RBC. The separated blood components were then stored at −20 °C until analysis. In this study, we used a total of 122 RBC samples for PFAS analyses (serum was unavailable as these components had already been used for other studies). Samples were selected based on whether birds were sampled at least three times or only once over the study period. Our selection criteria resulted in two pools of samples thereby allowing us to compare birds that are known to have survived between sampling events, with those with no other records. 19 individual birds of known age were repeatedly caught and sampled at least three times yielding 82 samples (i.e., on average 4.3 samples per individual). An additional 40 samples were included from birds that were sampled only once. Of the 19 repeatedly sampled birds, 11 were female (10 first sampled as juveniles), and 8 were males (5 first sampled as juveniles). Of the 40 birds sampled only once, 18 were juveniles, 22 were adults. However, we had

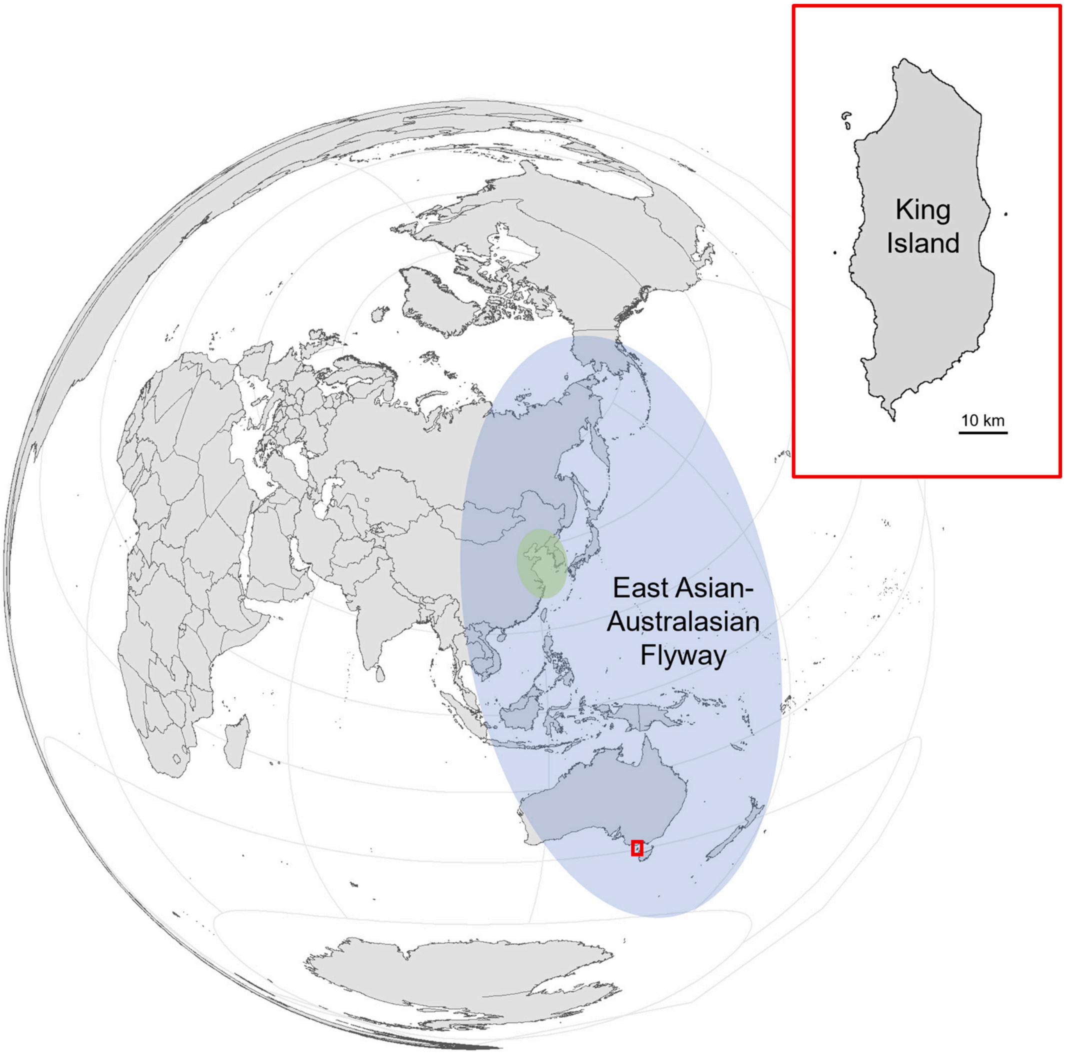

Fig. 1. Map of the East Asian-Australasian Flyway (blue oval). Red rectangle and inset is King Island, the west coast of which was the sample area for this study. Highlighted in green within the flyway is the Yellow Sea, a key stopover region for shorebirds and a putatively polluted region.

sex data for only 14 of these birds, constituting 10 females and 4 males. Seasons of samples are shown in Table S1.

2.2. Pollutant analysis

PFAS analyses on avian RBC samples were conducted at the Norwegian University of Science and Technology (NTNU) targeting 12 different PFAS compounds. Blood is generally an indicator of recent exposure to pollution, as opposed to long-term storage of pollutants as seen in adipose or liver tissues (Burger and Gochfeld, 2004). However, pollutant concentrations in blood and body tissues have been shown to reach an equilibrium, so that increases of pollution in body tissues can also be reflected by increases in blood pollution concentrations (van de Merwe et al., 2010). Some variation in concentrations of pollution within individuals was expected from factors beyond our control, such as how recently the bird ate/drank.

Target PFAS included two categories: carboxylates (hereafter referred to as ΣPFCAs, consisting of PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA; full names and details of chemicals provided in Tables 1 and S2) and sulfonates (hereafter referred to as ΣPFSAs, consisting of PFBS, PFHxS, PFOS, see Tables 1 and S2). We also calculated total PFAS contamination for each bird (hereafter referred to as ΣPFAS, consisting of all the aforementioned compounds). Extraction of these RBC samples were conducted using similar methods to Trimmel et al. (2021) and Sait et al. (2023), using hybrid-SPE. Sample masses ranged between 0.009 g and 0.097 g, at an average of 0.033 g. Briefly, samples were weighed in 1.5 mL PP tubes and 10 ng of the 13C-isotope labelled PFOS and PFOA IS-mixture was added. Then the samples were extracted by 0.3 mL methanol containing 1 % ammonium formate in an ultrasonicator for 30 min. After centrifugation, the supernatant was purified by Hybrid SPE cartridge (1 cc/30 mg) which was precleaned by 1 mL methanol also containing 1 % ammonium formate. Extracts were then transferred to amber vials with insert for UPLC-MS/MS analysis.

To quantify the concentrations of the samples, calibration curves with the range of 0.01–50 ng/mL were made for all 12 compounds, and the correlation coefficients for all compounds exceeded 0.99. The extraction method efficiency was evaluated by spiking known concentrations of the compounds pre- and post-extraction, with recoveries (Trimmel et al., 2021) ranging between 75 % and 120 % (Honda et al., 2018). Method limits of quantification (mLOQ) were based on the lowest concentration detected in the linear range of the calibration curve and the average sample weight and method limit of detection (mLOD) is mLOQ/0.33 (Table S3). In addition, solvent blanks consisting of pure methanol injected during sample detection were utilized during the detection to check the carryover of the instrument and no PFAS were found in solvent blanks. Procedure blanks monitored potential contamination during sample extraction. Both solvent and procedure blanks served as negative controls and only PFOA, PFNA, and PFDoA were found in one of the four procedure blanks with the concentrations of 0.30, 0.55 and 0.56 ng/g, respectively.

2.3. Data analysis

All statistical analyses were conducted using R Version 3.6.3, in RStudio Version 1.2.5033. Initially, non-detections of each compound (below method level of detection, mLOD, see Table S3 for compound-specific values) were stored as missing data. All compounds were then summed into their respective categories (ΣPFCAs, ΣPFSAs, as well as ΣPFAS). Once these had been summed, when no compound was detected (for both individual compounds and groups), the missing data was populated with p*mLOD, with p being the proportion of the compound data found above mLOD (Briels et al., 2019). Detection frequencies for each compound (shown as percentages), in each group of birds (individuals sampled repeatedly, or once) were calculated by dividing the number of detections by the total number of samples in the group, and multiplying by 100. The mLOD for grouped compounds was set as the lowest value of all compounds in the group. All PFAS concentrations were log-transformed to remove skewness and increase homogeneity in variances. An additional binary variable was created, where non-detections were marked as 0, and detections were marked as 1.

First, we analysed the samples from individually re-sampled individual turnstones. Binomial generalized linear mixed-effects models from R package lme4 (Bates et al., 2015) were run on the binary detected/non-detected data for each compound/group of compounds, with multiple testing accounted for by a Bonferroni correction. The binomial generalized linear mixed-effects models modelled pollutant detection status against the age of the birds, as well as their sex given females' additional potential ability to excrete pollutants via eggs (Newsted et al., 2005). Individual bird, denoted by a unique band number, was included as a random effect. For all compounds/groups of compounds with a detection frequency >30 %, we then ran linear mixed-effects models of the pollution concentrations, using the same structure as the earlier binomial models. We calculated confidence intervals for the estimate of each variable's effect to determine statistical significance.

Finally, we determined whether repeatedly sampled individuals (and

Table 1

Concentrations of PFAS in ruddy turnstone red blood cells ($n = 82$ samples from 19 individual birds; for resampled birds. $N = 40$ samples from 40 individual birds, for those sampled only once), sampled between 2007 and 2022. For estimated relative serum concentrations, see Table S4.

			Repeatedly Sampled Individuals (3+ times)				Singularly Sampled Individuals (Once only)			
Compound Name	Group	Code	Detection frequency	Median	Min	Max	Detection frequency	Median	Min	Max
Perfluorooctanosulfonic acid	Sulfonate	PFOS	47.5	<LOD	<LOD	4.4	47.5	<LOD	<LOD	0.94
Perfluorobutanesulfonic acid	Sulfonate	PFBS	9.8	<LOD	<LOD	0.64	7.5	<LOD	<LOD	1.73
Perfluorohexane sulfonic acid	Sulfonate	PFHxS	1.2	<LOD	<LOD	0.74	0.0	<LOD	<LOD	<LOD
Perfluoropentanoic acid	Carboxylate	PFPA	2.4	<LOD	<LOD	8.98	0.0	<LOD	<LOD	<LOD
Perfluorohexanoic acid	Carboxylate	PFHxA	17.1	<LOD	<LOD	1.8	5.0	<LOD	<LOD	9.37
Perfluoroheptanoic acid	Carboxylate	PFHpA	1.2	<LOD	<LOD	5.2	0.0	<LOD	<LOD	<LOD
Perfluorooctanoic acid	Carboxylate	PFOA	20.7	<LOD	<LOD	7.78	12.5	<LOD	<LOD	8.47
Perfluorononanoic acid	Carboxylate	PFNA	14.6	<LOD	<LOD	17.1	2.5	<LOD	<LOD	2.57
Perfluorodecanoic acid	Carboxylate	PFDA	12.2	<LOD	<LOD	1.68	17.5	<LOD	<LOD	1.40
Perfluoroundecanoic acid	Carboxylate	PFUnA	6.1	<LOD	<LOD	0.76	0.0	<LOD	<LOD	<LOD
Perfluorododecanoic acid	Carboxylate	PFDoA	19.5	<LOD	<LOD	0.74	10.0	<LOD	<LOD	0.87
Perfluorotridecanoic acid	Carboxylate	PFTrA	19.5	<LOD	<LOD	2.58	12.5	<LOD	<LOD	3.66
Total sulfonates		ΣPFSAs	51.2	0.07	<LOD	4.4	52.5	0.04	<LOD	1.73
Total carboxylates		ΣPFCAs	60.9	0.23	<LOD	24.9	47.5	<LOD	<LOD	9.48
Total ALL PFAS		ΣPFAS	71.9	0.78	<LOD	25.2	70.0	0.45	<LOD	9.61

The above table includes the abbreviations and indicates the detection frequency of each compound (% of samples in which the compound was detected) and summed compound classes, with their median, minimum and maximum concentrations, in ng/g. Where a concentration is recorded as '<LOD', it was below the method level of detection.

therefore potentially long-surviving birds) had disproportionately low concentrations of pollutants when compared to birds only caught and sampled once (thus potentially being shorter-lived). To do so, we log-transformed our grouped PFAS concentration data for both resampled individuals and singularly sampled individuals to approximate normally distributed data. We then took the average concentration of each PFAS group in each resampled individual and compared these concentrations with those seen in birds sampled once, using a Welch's *t*-test, to determine if there were any significant difference in PFAS concentrations between groups.

2.4. Population survival analyses and breeding success

Banding data from King Island were gathered from 2007 to 2022, during which time 3964 adult and 815 juvenile ruddy turnstones were captured. The data were used to estimate annually varying adult survival rates and average juvenile survival rates over the study period. Additionally, annual juvenile percentages were used as an indicator of breeding success. For estimating survival rates we employed a Cormack-Jolly-Seber (CJS) model following Kéry and Schaub (2011) using WinBUGS14 and R-package R2WinBUGS (Sturtz et al., 2005). To justify differential survival between juveniles and adults, we considered that adults and juveniles exhibit different migratory behaviours. After their first arrival to King Island as juveniles, young birds do not migrate north again until their second year of life. Aiming at building a realistic but also parsimonious and therewith statistically powerful survival model, we considered that a) data from first-year juveniles is limited compared to that of adults, and b) environmental conditions on King Island have remained relatively stable over the past decades, thereby warranting the assumption of a single, constant juvenile annual survival prior to their first northward migration across our study period. In the face of major environmental changes along the remainder of the flyway over the same period, we assumed a variable among-year annual survival for adults. In our CJS model, recapture rate was assumed to be identical for both age classes but variable among years, to accommodate different catching efforts across years. Six Markov chain Monte Carlo (MCMC) simulations of 50,000 iterations each were run, discarding the first 25,000 and using a thinning factor of 30 to result in a saved 5000 iterations. The saved iterations were used to describe the posterior distribution of both adult and juvenile survival and annual recapture rates, and present a 95 % Bayesian credible interval for each. $\widehat{R}$ was calculated for each model parameter, and used to assess model convergence thereby ensuring the reliability and accuracy of survival and recapture rate estimates. In ideal circumstances, $\widehat{R}$ should approach 1. All our model parameters had $\widehat{R}$ values between 1.000 and 1.006. To evaluate the presence of any significant temporal trend in either of adult survival and juvenile catch percentages, we conducted a linear model of each percentage (adult survival or juvenile catch percentage) against year. In all analyses and data presentations “year” denotes the calendar year in which our annual non-breeding research season started (e.g., our last catching season of 2021–2022 is denoted by 2021, when it started). Survival was calculated from year to year+1. Thus, our last research season allowed to calculate survival for 2020, as the last transition between years in our CJS model was inestimable due to the time-dependence in both survival and probability of detection in our model.

3. Results

All but one concentration of ΣPFAS (and therefore the nested groups of ΣPFCAs and ΣPFSAs, as well as the most common PFSA, PFOS) in our sampled ruddy turnstones were below 15 ng/g (median ΣPFAS concentration 0.78 ng/g) with the maximum concentration recorded at 25.2 ng/g in a male (Fig. 2). Frequency of detection for grouped sulfonates and carboxylates were around 50 % for both individuals sampled once (47.5–52.5 %) and individuals sampled repeatedly (51.2–60.9 %), but median concentrations for individual compounds were consistently less than levels of detection (Table 1). Calculated relative serum concentrations are presented in Table S4, based on the approximately 3:1 relationship between serum and RBC concentrations reported by Ross et al. (2024).

Our binomial generalized linear mixed-effects models with Bonferroni-corrected *p*-values, testing detects/non-detections yielded no significant effect of either sex or age in our sampled turnstones (Table 2). Due to low detection frequencies, we ran linear mixed effects models on only ΣPFAS, ΣPFCAs, and PFOS. ΣPFSAs were overwhelmingly driven by PFOS detection, so we did not run analyses on this group, opting

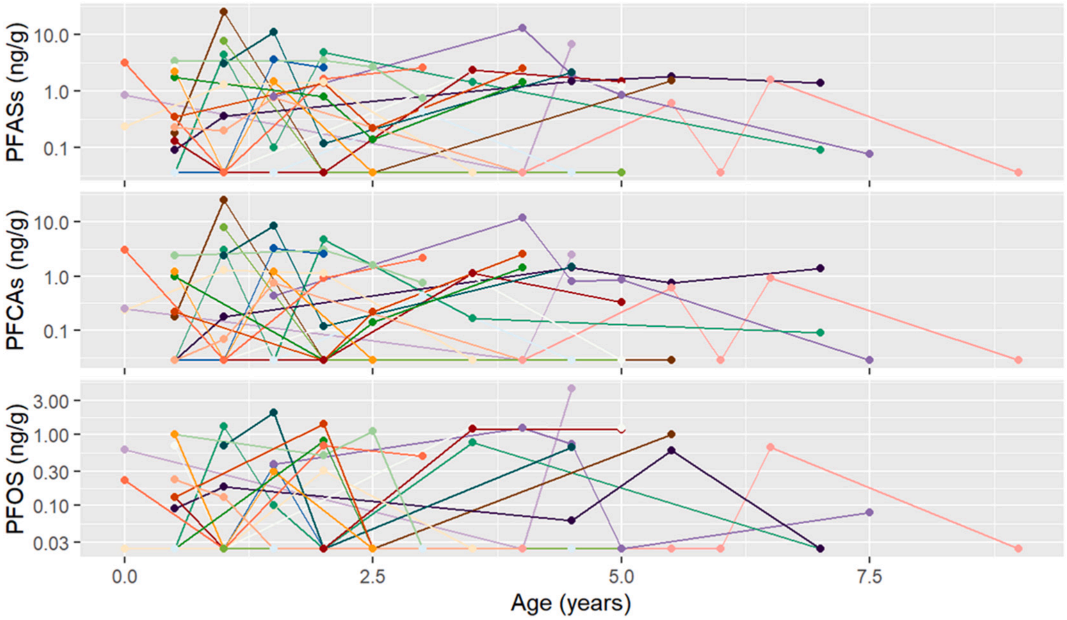

Fig. 2. Repeated samples of individual ruddy turnstones (*Arenaria interpres*) showing red blood cell pollutant concentration for each grouping of PFAS as a function of the individuals' age (82 samples from 19 individuals). Each colour/line represents one individual bird. Y-axis values are shown on a log-10 scale, and the minimal value on the y-axis represents the level of detection. There are no trends in PFAS concentrations within individuals as they age, for any of ΣPFAS, ΣPFCAs nor PFOS.

Table 2

Summaries of binomial generalized linear mixed-effects model results for detect/non-detect values of PFAS in repeatedly sampled individual ruddy turnstones, where detection of compounds was marked as a 1, and non-detections as a 0. Groupings are as follows: ΣPFAS includes all compounds, ΣPFSAs are PFOS, PFBS and PFHxS, and ΣPFCAs are PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, and PFTrA. Values in boldface are statistically significant (below $\alpha = 0.05$), and adjusted p-values were calculated using a Bonferroni correction.

Dependent Variable	Explanatory Variable	Estimate	Standard Error	p-value	Adjusted p-value
PFOS	Intercept	−0.167	0.422	0.693	1.000
	Sex(male)	0.696	0.507	0.170	1.000
	Age	−0.098	0.119	0.413	1.000
PFBS	**Intercept**	**−3.769**	**1.087**	**0.001**	**0.008**
	Sex(male)	2.270	1.105	0.040	0.599
	Age	0.004	0.177	0.984	1.000
PFHxS	Intercept	−11.78	73.546	0.873	1.000
	Sex(male)	−5934	10,890,000	1.000	1.000
	Age	−9.927	88.01	0.910	1.000
PFPA	Intercept	−32.58	2,058,000	1.000	1.000
	Sex(male)	30.07	2,058,000	1.000	1.000
	Age	−0.135	0.359	0.706	1.000
PFHxA	Intercept	−1.459	0.512	0.004	0.066
	Sex(male)	−0.569	0.625	0.363	1.000
	Age	0.044	0.152	0.770	1.000
PFHpA	Intercept	−29.14	1264	0.982	1.000
	Sex(male)	26.61	1264	0.983	1.000
	Age	−0.497	0.758	0.512	1.000
PFOA	Intercept	−0.793	0.485	0.102	1.000
	Sex(male)	0.226	0.564	0.689	1.000
	Age	−0.280	0.167	0.094	1.000
PFNA	**Intercept**	**−1.949**	**0.562**	**0.001**	**0.008**
	Sex(male)	−0.339	0.657	0.606	1.000
	Age	0.121	0.154	0.435	1.000
PFDA	Intercept	−1.745	0.601	0.004	0.055
	Sex(male)	0.276	0.688	0.688	1.000
	Age	−0.148	0.188	0.432	1.000
PFUnA	Intercept	−3.881	1.440	0.007	0.105
	Sex(male)	−0.572	1.214	0.638	1.000
	Age	0.288	0.246	0.242	1.000
PFDoA	**Intercept**	**−2.023**	**0.680**	**0.003**	**0.044**
	Sex(male)	0.434	0.651	0.506	1.000
	Age	0.104	0.153	0.497	1.000
PFTrA	Intercept	−1.462	0.585	0.012	0.186
	Sex(male)	0.308	0.697	0.658	1.000
	Age	−0.089	0.166	0.592	1.000
ΣPFSAs	Intercept	0.081	0.398	0.838	1.000
	Sex(male)	0.857	0.473	0.070	1.000
	Age	−0.162	0.117	0.169	1.000
ΣPFCAs	Intercept	0.569	0.405	0.160	1.000
	Sex(male)	0.006	0.465	0.991	1.000
	Age	−0.047	0.115	0.682	1.000
ΣPFAS	Intercept	1.021	0.486	0.036	0.536
	Sex(male)	0.223	0.574	0.697	1.000
	Age	−0.048	0.132	0.713	1.000

instead just for PFOS. No significant effect was found for sex in any of ΣPFAS, ΣPFCAs or PFOS (Table 3). We also found no significant effect of age on any grouped PFAS concentrations, nor PFOS (Fig. 2). Furthermore, our comparisons between resampled birds and birds only sampled once found no significant differences between these groups (see Table S5, Fig. S1). Collectively, we found no evidence of PFAS contamination increasing with age in any of our repeatedly sampled 19 individual birds.

Furthermore, our survival analyses found that adult turnstone survival fluctuated annually. Apparent survival probabilities ranged from

Table 3

Summaries for linear mixed effect models for total per/poly-fluoroalkyl substances (ΣPFAS), total carboxylates (ΣPFCAs), and PFOS (the most prevalent sulfonate of all 12 individual PFAS measured in our samples).

Dependent variable	Explanatory variables	Estimate	SE	t value	95 % CI
ΣPFAS (log-transformed)	Intercept	−0.367	0.165	−2.225	(−0.690, −0.046)
	Sex (male)	0.135	0.190	0.707	(−0.236, 0.505)
	Age	−0.021	0.047	−0.446	(−0.113, 0.071)
ΣPFCAs (log-transformed)	Intercept	−0.555	0.172	−3.231	(−0.889, −0.221)
	Sex (male)	0.083	0.198	0.418	(−0.302, 0.468)
	Age	−0.032	0.049	−0.652	(−0.128, 0.064)
PFOS (log-transformed)	Intercept	−1.076	0.145	−7.434	(−1.354, −0.798)
	Sex (male)	0.278	0.170	1.638	(−0.049, 0.606)
	Age	−0.013	0.041	−0.314	(−0.093, 0.065)

The models included sex, and age as explanatory variables and individual bird as a random effect. Pollutant concentrations were log-transformed prior to analysis to ensure homogeneity of variances.

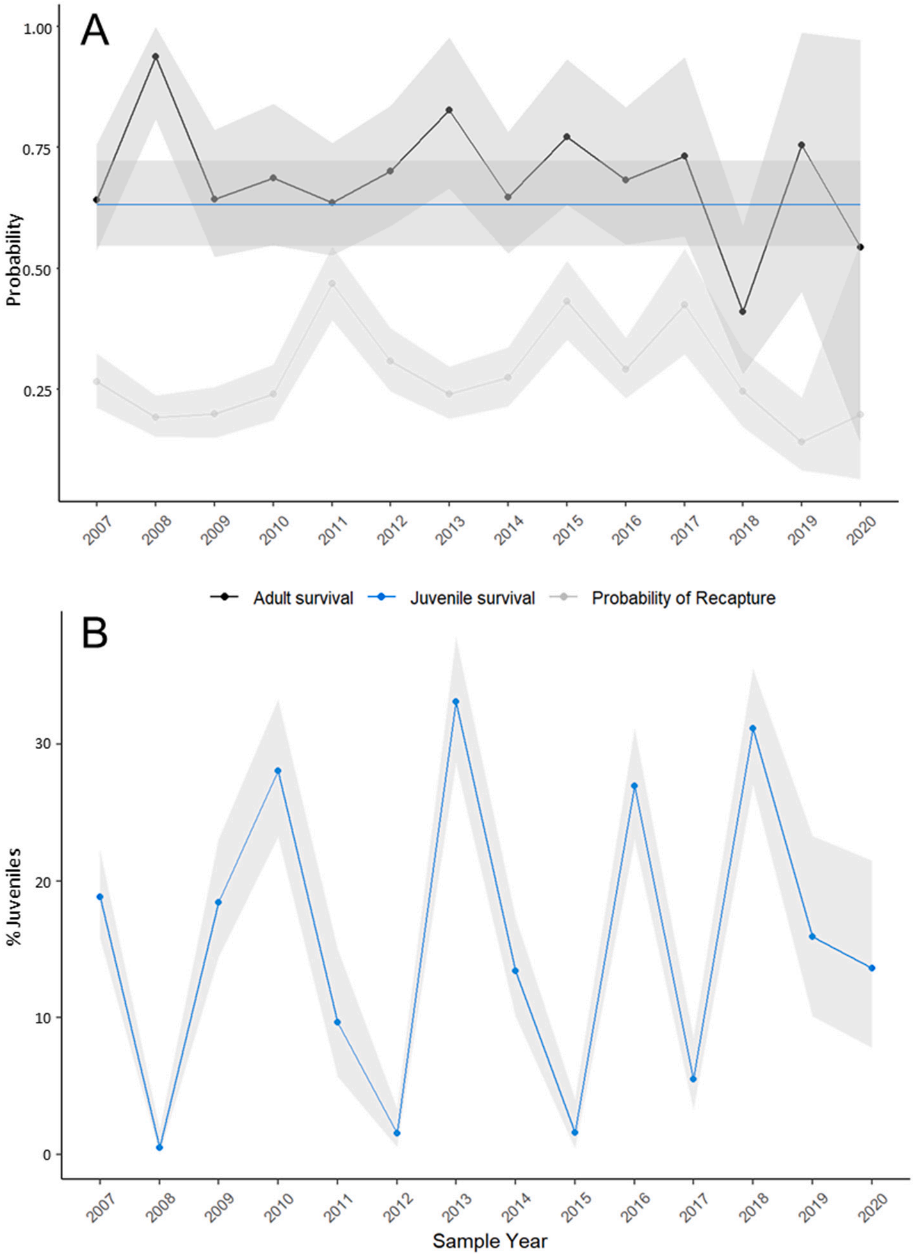

Fig. 3. **A)** Annual adult survival (black dots and line), juvenile survival (blue line; assumed constant over time) as well as recapture rate (grey dots and line) of ruddy turnstones on King Island from 2007 to 2020 including their 95 % Bayesian credible interval indicated by grey shaded areas. **B)** Annual juvenile percentages in catches with binomial 95 % Bayesian credible interval indicated by grey shaded area. While considerable annual variation existed in both adult survival and juvenile percentage, no temporal trends were apparent in either.

0.441 to 0.937 (average 0.697) in the 15 years sampled, and recapture probability ranged from 0.141 to 0.468 (average 0.280). Average juvenile survival probability was 0.631 (95 % CI 0.547–0.722). The annual fluctuations in adult survival across our study period from 2007 to 2022 did not show any significant temporal trend ($p = 0.215$ when testing for linear trend) (Fig. 3a). Juvenile percentage varied greatly from year to year, and also showed no significant linear trend over time ($p = 0.726$, Fig. 3b).

4. Discussion

Collectively, our results found no evidence to suggest that bioaccumulation of PFAS is occurring in ruddy turnstones. Concentrations were very low overall, with the majority of pollutants exhibiting a detection frequency of lower than 50 %. Low levels of pollutants are consistent with our lack of evidence for long-term declines in fecundity or survival in migratory turnstones, despite the inclusion of the highly PFAS-polluted Yellow Sea in their migration routes. Combined, our results provide no evidence to suggest PFAS currently pose a threat to the population survival of ruddy turnstone in Australia.

4.1. *PFAS pollution is low in ruddy turnstones*

Compared to known metrics such as the established ‘predicted no-effects concentration’ (PNEC) values for PFOS put forth by Newsted et al. (2005) and ‘lowest observable adverse effects levels’ (LOAELs) by Dennis et al. (2021), our observed PFAS concentrations in ruddy turnstones are very low. Newsted et al. (2005)’s PNECs for physiological effects in birds for PFOS in blood serum were 2400 ng/g for both males and females pre-egg laying. For females after egg-laying the study used

150 ng/g. Newsted et al. (2005)'s serum concentrations equate to 800 ng/g and 50 ng/g respectively in red blood cells, based on the above relationships presented by Ross et al. (2024) or 600 ng/g and 38 ng/g in red blood cells based on the 4:1 ratio presented by Gebbink and Letcher (2012). Conversely, Dennis et al. (2021) suggested LOAELs could be as low as 50 ng/g in liver tissues of young birds. While unable to directly compare due to differences in sample matrix (blood vs liver), it is important to consider as liver tissues tend to have higher concentrations than blood (Gebbink and Letcher, 2012). Nevertheless, all of our observed concentrations were also below this threshold, yet the presence of any PFAS in our study attests to the environmental ubiquity of these compounds even in remote Australian locations (Szabo et al., 2022).

Compared to red-necked stint (*Calidris ruficollis*) and curlew sandpiper (*C. ferruginea*) studied in the vicinity of Melbourne (Ross et al., 2023, median RBC concentrations 85 ng/g wet weight, max 836 ng/g), turnstones appear to be substantially less polluted, which is likely due to their different foraging ecology. While still benthic foragers, turnstones have short bills and do not probe deeply in sediment; they take their prey from the surface of the substrate and often prefer rocky coasts and foraging among kelp. Red-necked stint and curlew sandpiper often favour more heavily sedimented environments and forage on buried benthos (Conklin et al., 2014) thereby potentially exposing the two species to higher levels of deposited pollutants (Stark, 1998). Turnstone foraging behaviour, coupled with migratory behaviours that render the species less reliant on the Yellow Sea than curlew sandpiper (and thus may ostensibly limit their exposure to pollutants) (Minton et al., 2011), may explain why we did not see similar pollution patterns to those observed in resident plover species in China (Sun et al., 2023). Furthermore, as mentioned earlier, the low concentrations may also be related to analysis in RBC, which carry lower concentrations of PFAS than do serum or plasma (Gebbink and Letcher, 2012). Yet, our median concentration of all summed PFAS was 0.78 ng/g. Therefore, even at the 4:1 plasma:RBC ratio presented by Gebbink and Letcher (2012), the concentrations in the turnstones from the current study would still be very low regardless of our choice of matrix.

4.2. No evidence of PFAS variation with age in ruddy turnstones

Our finding that turnstones exhibit no variation in PFAS concentration with age is interesting, as PFAS have previously been discovered to accumulate and biomagnify in birds and mammals (Houde et al., 2011), including humans (Sunderland et al., 2019). Previous studies of within-individual PFAS pollution have been limited to captive species (e.g. domesticated birds, see Tarazona et al., 2015), whereas we have studied wild, free-living birds. Our study's unique ability to examine PFAS concentrations over time at a within-individual level in wild birds provides us with exceptional high-resolution data on the temporal pollution dynamics in this long-distance migrant. Despite the profligate application of PFAS along the flyway and consequent growth in PFAS pollution particularly in waterways around China and notably the Yellow Sea (Baluyot et al., 2021; Muir and Miaz, 2021), our PFAS concentrations in turnstones do not reflect this. We propose five possible reasons to explain our observed lack of increasing PFAS concentrations with age in ruddy turnstones.

First, the lack of effect of age on any group of PFAS may be a product of our sample size of 19 birds. While very little literature exists on age dynamics of PFAS concentrations in shorebirds, a previous study by Ross et al. (2023) found higher PFCAs concentrations in adult birds than juveniles, from a sample size of 77 juveniles and 71 adults. However, Ross et al. (2023) did not find significant effects of age on any other grouped PFAS, suggesting that there is the possibility that our results are representative of the patterns seen in the wider population.

Second, the birds in this study were caught on their non-breeding grounds in Australia and not on their Asian stopover sites, so the concentrations in blood may reflect the concentrations at their putatively cleaner non-breeding sites rather than sites along the flyway. Nevertheless, the low PFAS concentrations match with the results of our survival analysis that did not indicate any declining recruitment rate and survival rate in adult turnstones in our study period despite our study period coinciding with the period of increases in PFAS use. Therefore, sampling at the southern terminus of the migration may not be the only reason we observed such low concentrations.

Third, concentrations of PFAS to which birds are exposed may be so low that concentrations do not appear above the mLOD in RBC, which have a lower affinity for PFAS than do other components of blood (Gebbink and Letcher, 2012). In this case, the lack of observable bioaccumulation may be partly an artifact of the high numbers of non-detections we have seen in our samples. Furthermore, blood pollutant concentrations may be easily affected by recent ingestion of food items and hydration status of the individual birds (Beuchat and Chong, 1998), their body condition (Hughes et al., 2019) or mere variation of exposure over time, which could explain some of the within-individual variation we observed. However, the birds were only caught when actively foraging during daytime and processed rapidly (within 2 h of capture). Thus, any variation due to hydration status would be minimal, and concentrations are likely to be consistently low.

Fourth, it is possible that selective mortality of individuals with high loads of contaminants is occurring, thus only minimally polluted individuals survive repeated migration. Variation in pollution between individuals may be stochastic or could stem from other factors such as variations in migration route. Turnstones undertake highly variable migratory routes with most individuals following a coastal route hugging the east Asian coast while some use a more easterly route involving long legs across the Pacific Ocean (Minton et al., 2011), thereby skipping the more polluted areas. The number and location of stopover sites along these routes also vary across individuals. However, neither our survival analyses nor our observed PFAS concentrations suggest such selective mortality because of pollution is occurring. Such a result is further supported by our comparisons between repeatedly sampled individuals and singularly sampled individuals. Indeed, these comparisons showed that singularly sampled individuals, which might have lived shorter than repeatedly sampled individuals, had no significant difference in pollutant burden than did the repeatedly sampled individual turnstones. Given the overall increase in PFAS pollution over the past 15 years, particularly around the Yellow Sea and east coast of Asia (Muir and Miaz, 2021), we would have expected to see an increase in pollution concentrations in turnstones over time, and a decline in population survival. Neither of these patterns apply. While showing large year-to-year variation in annual adult survival (ostensibly due to fluctuating migratory conditions or conditions on the breeding grounds), our survival analyses show no definitive survival trend over the 15 years covered by this study, thereby rendering pollution mediated selective mortality unlikely.

A fifth and final explanation is that birds possess excretory pathways by which they can reduce their internal pollutant concentrations, and thus the concentrations we see are effectively the steady-state concentrations of PFAS in ruddy turnstone blood. Birds can, for instance, deposit pollutants in their feathers when these are being grown (Groffen et al., 2021). Feathers are shed regularly and mostly annually throughout a bird's life, thereby providing an avenue for the bird to lower its pollutant levels. Shorebirds in particular moult primarily on their non-breeding grounds (Battley, 2006). As a result, both adult and juvenile birds have been shown to have much faster excretion of PFOS than do mammals, and thus recover faster (Newsted et al., 2005). Female birds can also sequester pollutants in their protein-rich eggs due to the proteinophilic properties of PFAS (Newsted et al., 2005), and such deposition could potentially reduce hatching success (Custer et al., 2014). Yet, we did not see any differences in PFAS contamination between sexes, and based on our analysis of juvenile recruitment (Fig. 3b), and other data on turnstone juvenile percentages (Minton et al., 2020), fecundity appears annually fluctuating but stable. Instead, environmental conditions on the breeding grounds may affect annual variations in fecundity and survival. One such example of fluctuating conditions may be lemming cycles that may occur on a 2–3 year periodicity in parts of the Arctic. When lemming populations increase, this allows for high breeding success in shorebirds as predators such as arctic fox (*Vulpes lagopus*) primarily target lemmings. Poorer success can be attributed to the decrease in lemming populations leading predators to prey-switch to shorebirds instead (Aharon-Rotman et al., 2015; Ehrich et al., 2020; Summers et al., 1998).

Observed low concentrations of pollutants may also be attributable to the possibility of other excretion pathways, e.g. preen oil (Kocagöz et al., 2014) or unknown biotransformation pathways, but these remain speculative at present. Nevertheless, the established means by which birds can eliminate pollutants from their body may be substantial enough to obscure any potential bioaccumulation. Hence, the lack of obvious bioaccumulation patterns in our study may occur through a combination of low PFAS retention in RBC associated with variable concentrations in blood, generally low environmental exposure to PFAS pollution, paired with excretion pathways in birds that are efficient enough to prevent such low exposure from accumulating in the birds over time.

4.3. Future work

While the relatively low concentrations of PFAS in RBC of ruddy turnstones as observed here are evidently good news, it is important to continue to monitor PFAS concentrations in shorebirds. If anything, it is expected that these compounds will continue to increase in the global environment and ultimately these increasing concentrations may potentially further hasten their population decline. Moreover, despite the low concentrations found here, there is evidence that mixes of PFAS have additive or synergistic detrimental effects on wildlife (Flynn et al., 2019; Ojo et al., 2021). Any such ‘cocktail’ effect warrants concern given the already precarious status of ruddy turnstones and other shorebirds (Clemens et al., 2016). Documented negative effects on endocrinology and reproduction even at low concentrations in other species as documented by Blévin et al. (2017) and Tartu et al. (2014) suggest that even our observed low levels of PFAS may yet pose a threat to turnstones. Furthermore, PFAS tend to accumulate in protein-rich tissues (Forsthuber et al., 2020), which may pose a particular risk to long-distance migrants including many shorebirds. While adipose tissue remains the primary fuel source for migratory flight, long-distance migrants also catabolise other lean, protein-rich tissues during migration (Klaassen et al., 2000) which could result in a sudden, disproportionately large dose of now-mobilised pollutants. Such an increase in pollution may have an additional synergistic effect in combination with the high physiological stress of migration, analogous to increased effects of pollutants in heat stressed animals (Gordon et al., 2014), posing a greater health threat than the PFAS concentrations would normally entail.

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CRediT authorship contribution statement

Tobias A. Ross: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Junjie Zhang:** Writing – review & editing, Methodology, Investigation. **Tonje G. Skaalvik:** Methodology, Investigation. **Robyn Atkinson:** Project administration, Investigation. **Roz Jessop:** Project administration, Investigation. **Alexandros G. Asimakopoulos:** Writing – review & editing, Methodology, Investigation. **Veerle L.B. Jaspers:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Marcel Klaassen:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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 to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176790.

Data availability

Data will be made available on request.

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