Perfluoroalkylated compounds in the eggs and feathers of resident and migratory seabirds from the Antarctic Peninsula

Janeide Padilha a,*, Gabriel O. de Carvalho a, Tim Willems c,e, Gilles Lepoint b, Larissa Cunha a, Adriana R.L. Pessoa a, Marcel Eens d, Els Prinsen e, Erli Costa f, João Paulo Torres a, Paulo Dorneles a, Krishna Das b, Lieven Bervoets c, Thimo Groffen c,d

ARTICLE INFO

Keywords:
- PFSA
- Perfluoroalkylated substances
- PFCAs
- Biomonitoring
- Contamination
- Stable isotopes

ABSTRACT

In this study, we investigated factors that influence the differences in exposure of perfluoroalkyl acids (PFAAs) from eight species of Antarctic seabirds, including Pygoscelis penguins, Stercorarius maccormickii, and Macronectes giganteus. We analyzed the relationship between foraging ecology (based on δ13C, δ15N, and δ34S values) and PFAAs accumulated in eggs and breast feathers. Ten out of 15 targeted PFAAs were detected in eggs compared to eight in feathers. Mean ∑PFAA concentrations in feathers ranged from 0.47 in P. antarcticus to 17.4 ng/g dry weight (dw) in S. maccormickii. In eggs, ∑PFAA concentrations ranged from 3.51 in P. adeliae to 117 ng/g dw in S. maccormickii. The highest concentrations of most PFAAs were found in trans-equatorial migrants such as S. maccormickii, probably due to their high trophic position and higher concentrations of PFAAs in the Northern Hemisphere compared to the Southern Hemisphere. Based on stable isotopes correlations, our results suggest that the trophic position (δ15N) and the foraging area (δ13C and δ34S) influence PFAA concentrations in Antarctic seabirds. Our results point to the possibility that long-distance migratory birds may have as bio-vectors in the transport of pollutants, including PFCAs, in Antarctic environments, although this must be further confirmed in future studies using a mass balanced approach, such as extractable organofluorine (EOF).

1. Introduction

The Antarctic continent is absent of permanent native human residents and industrial activities, but despite this, significant levels of several contaminants have been reported in its ecosystems (Bargagli, 2008; Jerez et al., 2011; Polito et al., 2016). Research stations, tourism, and large-scale krill fishing have intensified the human presence on the continent, generating local anthropogenic impacts in the Antarctic region. However, the main entry way of pollutants into the Antarctic continent, generating local anthropogenic impacts in the Antarctic region. The relative isolation of the polar regions in relation to other ecosystems, combined with shorter food chains, makes them important research sites for studying the environmental behavior of pollutants, including their trophodynamics along the food chain (Gao et al., 2020a).

Among these pollutants, perfluoroalkyl acids (PFAAs) are a widely distributed emerging pollutants, being found in places far from their manufacturing source, such as Antarctica (Gao et al., 2020a; Roscales et al., 2019). Although it is not clear which form of transport is dominant, studies indicate that PFAAs can reach different regions of the globe through the ocean and/or atmospheric currents (Young and Mabury, 2010; Zhao et al., 2012). Among PFAAs, previous studies focused primarily on long-chain perfluoroalkyl carboxylic acids (PFCAs) and...
perfluorooctyl sulfonic acids (PFSAs) (Buck et al., 2011; Groffen et al., 2020). Perfluorooctane sulfonate (PFOS—\(\text{C}_8\text{F}_{17}\text{SO}_2\text{H}\)) and perfluorooctanoic acid (PFOA—\(\text{C}_8\text{F}_{15}\text{COOH}\)) draw special attention for their persistence, potential health effects, and global distribution, and are the most widely detected long-chain PFAAs in the environment, wildlife and humans around the world (Buck et al., 2011; Ericson Jogsten et al., 2012; Groffen et al., 2017). The highest PFOS and PFOA concentrations in humans were reported to be 164 ng/mL from U.S.A and 256 ng/mL from Korea, respectively (Sinclair et al., 2020). So et al. (2006) also measured amounts of PFAS in human breast milk, in which PFOS has been reported at 360 ng/L and PFOA at 210 ng/L. Exposure to PFAAs can cause diseases such as cancer, altered metabolisms, impaired liver function, chronic kidney damage, cardiovascular diseases, probable thyroid, and other hormonal malfunction or imbalances (Poder et al., 2021).

PFOS and PFOA were listed as Persistent Organic Pollutants (POPs) by the Stockholm Convention since 2009 and 2019 respectively, which resulted in regulations that aimed to reduce the levels of these compounds in the environment (Filipovic et al., 2015; Stockholm Convention, 2021). However, since the early 2000s, developing Asian countries have increased their PFAS production and emissions (Xie et al., 2013). Linked to this, the production of other PFAS compounds such as polyfluorinated compounds (e.g., fluorotelomer sulfonate - FTPSA) and short-chain PFASs (e.g., \(\text{C}_4\text{H}_{11}\text{F}_3\text{SO}_3\text{H}\) - perfluorobutane sulfonic acid - PFBS), which are often used as alternatives to PFOS and PFOA, are still increasing, and the impact of these alternative substances on the environment is still not clear (Groffen et al., 2017; Wang et al., 2013).

Seabirds are valuable sentinels of environmental pollution, due to their high trophic position, wide distribution, and longevity. Among the Antarctic birds, penguins constitute the largest avian biomass in the region, presenting populations distributed in Antarctic lands. Therefore they can be useful indicators of local and regional pollution (Espeso et al., 2017; Jerez et al., 2011; Metcheva et al., 2006; Padilha et al., 2021). In addition to resident birds, the migratory ones, such as South Polar Skua (Stercorarius maccormicki) and Antarctic Tern (Sterna vittata), may act as carriers of contaminants to Antarctica, as they migrate during the southern winter and can reach more contaminated regions northwards (Costa et al., 2019).

Eggs provide an useful biomonitoring tool for assessing avian exposure to contaminants, including PFASs, in many regions of the world (Groffen et al., 2017). Regarding feathers, there is still ongoing debate regarding their suitability in PFAAs monitoring, since there is a need for further studies to clarify the correlation of PFAAs with internal organs and the contribution of external contamination in feathers concentrations (Jaspers et al., 2019). Additionally, avian eggs reflect contaminant exposure of maternal tissue, because maternal lipid, protein, and organic contaminants are deposited into eggs during its synthesis (Drouillard and Norstrom, 2001). Feathers are associated with the bloodstream during growth, thus internal contaminants, such as metals and persistent organic pollutants, are incorporated throughout the feather growth period (Costa et al., 2019; Groffen et al., 2020; Jaspers et al., 2006; Lesth et al., 2019). Therefore, feathers constitute a potentially important detoxification pathway for organic and inorganic pollutants.

Little is known about the contamination of seabird species by PFAAs in Antarctica (Larramendi and Soloneski, 2015; Munoz et al., 2017; Roscales et al., 2019). Previous studies on Antarctic environment have shown that the Antarctic Circumpolar Current is responsible for providing a protective barrier against the transport of PFAAs through the water (Bengtson Nash et al., 2010) which may contribute to the low concentrations of many PFASs in Antarctica relative to other continents (Schiavone et al., 2009; Tao et al., 2006). However, the atmospheric transport of PFAAs to the Antarctic environment seems to be relevant, since studies reported air levels of PFASs in Antarctica is similar to northern latitudes (Cai et al., 2012; Dreyer et al., 2009).

The factors that influence the exposure of Antarctic seabirds to PFAAs are not well understood, mainly for migratory species that exhibit marked interspecific differences in their foraging ecology. However, some of the complexities involved in understanding the feeding ecology as well as the migration patterns of seabirds can be clarified using stable isotope analysis (SIA) to infer trophic position (\(\delta^{15}\text{N}\)), foraging areas (\(\delta^{13}\text{C}\)), and origin of food (i.e. benthic vs. pelagic, \(\delta^{27}\text{Si}\)) of these animals (Cherel et al., 2014; Cherel and Hobson, 2007; Herman et al., 2017).

The present study aims to evaluate the species-specific differences in PFAAs accumulation among resident and migratory Antarctic seabirds through feather and eggs analysis of 15 PFAAs (four PFSAs and 11 PFCAs). Feather and eggs were used to observe how the pattern of PFAAs concentrations is distributed across different matrices of Antarctic birds. Simultaneously, stable isotopes were used to test the influence of multiple spatial and ecological factors on these accumulated concentrations, analyzing whether possible interspecific differences are due to habitat contamination, sources of food or differences in trophic positions.

2. Material and methods

2.1. Study area and sampling

All samplings were performed at King George Island (61°50'–62°15'S and 57°30'–59°00'W) in the South Shetland Archipelago, Antarctic Peninsula (Fig. 1, Table 1), during 2010–2011, 2012–2013 austral summers and in the reproductive period of the birds. This study presents two groups of seabirds: resident and migratory. Pygoscelis penguins (Adélie - Pygoscelis adeliae, Chinstrap - P. antarcticus, Gentoo - P. papua) are the resident seabirds in the present study due to their circumpolar distribution (Jerez et al., 2013). As migratory birds in the present study, we have selected species with different migration patterns, since the South Polar Skua (Stercorarius maccormicki) is dispersed by routes through the Atlantic and Pacific oceans, reaching the Northern Hemisphere during the winter (Cruwys, 2008; Kopp et al., 2011). On the other hand, Snowy Sheathbill (Chionis albus), Antarctic Tern (Sterna vittata), Southern Giant Petrel (Macronectes giganteus), and Kelp Gull (Larus dominicanus) disperse in marine environments of the Southern Hemisphere during winter (Patterson and Hunter, 2000; Watson, 1975).

We sampled breast feathers of P. adeliae, P. antarcticus, P. papua, S. maccormicki, S. vittata, and C. albus. The feathers of L. dominicanus and M. giganteus were opportunistically collected from the ground of their colonies following the protocol: 10 to 20 contour feathers (feathers on the chest, abdomen, or back) at three distinct points inside each colony, and each point collected in the same colony was considered as an individual. The Pygoscelis penguins, Sterna vittata, and Chionis albus were captured with long-handled fish nets, and the S. maccormicki were captured using a snare trap. Feathers were packed in individual “zip” type polyethylene bags, and samples were kept at room temperature in dark conditions until the time of analysis. Each captured animal was banded with an aluminum ring, weighed, and measured (wing and tail size, Table S1 of the Supplementary material) with a digital caliper or ruler as described by Sick et al. (1997).

Non-viable eggs of penguins (P. papua, P. adeliae, and P. antarcticus), S. maccormicki, and S. vittata were collected in breeding territories, found abandoned outside the nests. The non-viable eggs were collected and stored in decontaminated jars and kept frozen for later lyophilization.

2.2. Sample preparation

All feather samples were washed three times with a sequence of 1) Milli-Q ultrapure water (Merck Millipore, USA), 2) 0.01% EDTA (Spectrum, Tedia, USA) and 3) Milli-Q ultrapure water (Merck Millipore, USA), for eliminating external contamination, and then the samples were oven-dried at 50 °C for 24 h (Marques et al., 2007). After this procedure the feathers were cut into small pieces using ceramic scissors. For stable isotope measurements, feather samples were additionally...
washed with a chloroform/methanol (2:1, v: v, suprapur Merck, Germany) solution, and dried at 50 °C for 48 h (Padilha et al., 2021). The non-viable eggs were kept frozen until they were lyophilized and stored in PFAAs-free plastic tubes for further analysis. For extraction of PFAAs from the feathers and eggs, protocols described by Groffen et al. (2021) and (Powley et al., 2005) were used respectively. The used abbreviation from the feathers and eggs, protocols described by Groffen et al. (2021) from King George Island in the Antarctic Peninsula during 2010–2013 and 2013–2014 austral summers.

### 2.4. Chemical extraction

For the extraction of PFAAs from the feathers, a protocol described by Groffen et al. (2021) was used with minor modifications as follow. Approximately 100 mg of each sample was weighed and stored in 50 mL polypropylene (PP) tubes. After adding 10 mL of methanol, the samples were vortex-mixed during 1 min and stored for 48 h at room temperature and then centrifuged (4 °C, 10 min, 2400 rpm; 1037 × g, Eppendorf centrifuge 5804 R). The supernatant was transferred into a 15 mL PP tube, spiked with 10 ng of each ISTD and dried completely using a rotational-vacuum-concentrator (Martin Christ, RVC 2–25, Osterode am Harz, Germany). For reconstituting the samples with 2 mL of a 2% ammoniumhydroxide solution in ACN, the samples were vortex-mixed and filtrated through an Ion Chromatography Acrodisc 13 mm Syringe Filter with 0.2 μm Supor (PES) Membrane (VWR International, Leuven, Belgium) into a PP auto-injector vial.

For the extraction of PFAAs from the eggs, a protocol described by Powley et al. (2005) was used with minor modifications as follow. After removal of the shell, the samples were freeze-dried prior to the extractions. Each sample (50 mg) was spiked with 10 ng ISTD, after which 300 μL of Milli-Q and 10 mL of ACN was added. The samples were vortex-mixed, sonicated for 3 min, and filtrated as described above for the feathers. After centrifugation (4 °C, 10 min, 1037 × g, Eppendorf centrifuge 5804 R), the supernatant was reduced until approximately 0.5 mL by using a rotational-vacuum-concentrator (Martin Christ, RVC 2–25, Osterode am Harz, Germany). The concentrated extract and 2 times 250 μL ACN, which was used to rinse the tubes, were added to an Eppendorf tube containing 50 mg graphitized carbon powder (Supelclean ENVI-Carb, Sigma-Aldrich, Belgium) and 50 μL glacial acetic acid. These tubes were vortex-mixed for 1 min and centrifuged (4 °C, 10 min, 10,000 rpm, 9279.4 × g, Eppendorf centrifuge 5415 R). The cleaned-up extracts were dried almost completely and reconstituted in 2 mL of a 2% ammoniumhydroxide solution in ACN and filtrated as described above for the feathers.

### 2.5. UPLC-MS/MS analysis

Ultra-performance liquid chromatography-tandem ES (–) mass spectrometry (UPLC-MS/MS, ACQUITY, TQD, Waters, Milford, MA, USA) was used to analyze the target analytes (i.e., four PFSAs (PFBS, PFHxS, PFOS, and PFDoS) and 11 PFCAs (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, and PseudoD). The individual analytes were separated using an ACQUITY BEH C18 column (2.1 × 50 mm; 1.7 μm, Waters, USA). To retain PFAA contamination from the system, an ACQUITY BEH C18 pre-column (2.1 × 30 mm; 1.7 μm, Waters, USA) was inserted between the solvent mixer and the injector. The mobile phase solvents consisted of 0.1% formic acid in water (A) and 0.1% formic acid in ACN (B). The flow rate was set at 450
μL/min at a partial loop injection of 6 μL. The solvent gradient started at 65% A, decreased to 0% A in 3.4 min and returned to 65% A at 4.7 min. The analytes were identified and quantified using multiple reaction monitoring (MRM) of two diagnostic transitions per analyte. The MRM transitions, cone voltages and collision energy of each target analyte, including the ISTDs, are displayed in Table S3 of the Supplementary material and were validated by Groffen et al. (2019). All results are expressed in dry weight (dw).

2.6. Quality assurance

Calibration curves, with a highly significant linear fit for all target analytes (all p < 0.001; R² > 0.98), have been constructed by (Groffen et al., 2021, 2019). Procedural blanks were added per batch of 20–25 samples as quality control and contained 10 mL of methanol for the feathers and 10 mL of ACN for the eggs. The methanol-blanks contained minor contamination with PFOA (0.05–0.15 ng/g ww), PFDA (<LOQ – 0.28 ng/g ww) and PFUnDA (<LOQ – 0.25 ng/g ww), which were subtracted from concentrations in samples from the same batch. In addition, instrumental blanks (ACN 100%) were analyzed on a regular basis to prevent crossover contamination of the samples. Individual PFAAs were quantified using the most suitable ISTD based on ionization and extraction efficiency (Groffen et al., 2019) and were those closest in terms of functional group and carbon-chain length (Table S3 of the Supplementary material). The individual limits of quantification (LOQs) were determined in matrix based on a signal-to-noise (S/N) ratio of 10 and are displayed in Table S4 of the Supplementary material.

2.7. Stable isotope measurements

Stable isotope measurements were performed via continuous flow - elemental analysis - isotope ratio mass spectrometry (CF-EA-IRMS) using a Vario MICRO cube C–N–S elemental analyzer (Elementar Analysysysteme GmbH, Hanau, Germany) coupled to an IsoPrime100 isotope ratio mass spectrometer (Isoprime, Cheadle, United Kingdom). Isotopic ratios were conventionally expressed as δ concentrations in ‰ (Coplen, 2011) and relative to the international standards: Vienna Pee Dee Belemnite, for carbon; Atmospheric Air, for nitrogen; and Vienna Canyon Diablo Troilite, for sulfur. We used International Atomic Energy Agency (IAEA, Vienna, Austria) certified reference materials IAEA-C6 (δ¹³C values = −10.8 ± 0.5‰; mean ± SD), IAEA-N2, (δ¹⁵N values = 20.3 ± 0.2‰; mean ± SD) and IAEA-S1 (δ¹⁵S values = −0.3% ± 0.01‰; mean ± SD) as primary analytical standards. As secondary analytical standards we used sulfanilic acid (δ¹³C values = −25.9 ± 0.3; δ¹⁵N values = −0.12 ± 0.4; δ³⁴S values = 5.9 ± 0.6; mean ± SD in each case). Isotopic ratios of samples were calibrated using primary analytical standards. Standard deviations on multi-batch replicate measurements of secondary analytical (sulfanilic acid) and lab standards (feathers) analyzed interspersed among samples (one replicate of each standard every 15 analyses) were 0.2‰ for both δ¹³C values and δ¹⁵N values and 0.4% for δ³⁴S values.

2.8. Statistical analysis

The statistical analyses were performed in R (Jackson et al., 2011; “R Core Team (2020). — European Environment Agency,” n. d.) statistical software and STATISTICA software (version 10; StatSoft Inc, USA). For statistical analysis, concentrations < LOQ were replaced to a value equal to f x LOQ where f is the frequency, i.e. the number of samples in which the compound was detected divided by the total number of samples analyzed (Parente et al., 2019). Non-parametric (Spearman rank correlation test-ε, Mann-Whitney, and Kruskal-Wallis) tests were used. A Kruskal-Wallis test was used for comparing PFAA concentration and stable isotope values among different species. The post hoc tests were conducted to test pairwise comparisons. Mann-Whitney U test was used to evaluate the possibility of a significant difference between the concentrations of PFAAs between resident and migratory birds, and to verify possible annual differences. Spearman rank correlation test was used to describe δ¹³N, δ¹⁵C, δ³⁴S values and PFAAs concentration in tissues. We analyzed the relationship between PFAAs concentrations among ten species of resident and migratory seabirds using a principal component analysis (PCA). Before analysis, data was transformed and to deal with values below the limit of quantification (LOQ). When the compounds had almost all the samples below the LOQ they were excluded from the analyses. The PCAs, for both feathers and eggs, included species and habitat as supplementary variables. Feather PCA also included isotopes as supplementary quantitative variables, since the measurements of stable isotopes were only carried out in the feather matrix. The inclusion of isotopes as variables in the PCA was made to observe whether there was also an influence of trophic ecology on the differences in PFAA concentrations between species. Biplots showing δ³⁴S, δ¹³N, and δ¹⁵C values in Antarctic seabirds were made.

3. Results

The dominant compound among PFAAs was PFUnDA in both matrices (Tables 2 and 3; Figs. 2 and 3). More compounds could be detected in eggs than in feathers samples (i.e., ten compounds were detected in eggs compared to eight compounds detected in feathers for a total of 15 compounds measured, Tables 2 and 3; Fig. 2). It was not possible to correlate the egg to the parents in the present study since the non-viable eggs were found abandoned outside the nests. Mean ∑PFAA concentrations in feathers ranged from 0.47 as the lowest concentration to 17.4 ng/g dry weight (dw) as the highest from P. antarcticus to S. maccormicki. In eggs, ∑PFAA concentrations ranged from 3.51 as the lowest concentration to 116.6 ng/g dw as the highest from P. adeliae to S. maccormicki. There was no significant difference between the samples of feathers and eggs collected in different years for each species in the present study (p > 0.05). Additionally, no significant interannual difference was observed for samples of feathers and eggs of S. maccormicki (p > 0.05) and eggs of Pygoscelis penguins (p > 0.05).

3.1. Perfluoroalkyl acids in feathers

The concentrations of PFOA, PFUnDA, PFDoDA, PFTeDA, PFPeA, PFBA, PFHpA, PFNA, PFBS, PFHxS, and PFDS were below the detection limit for all species of seabirds. There was no significant correlation between the morphometric measurements (Table S1 of the Supplementary material) of seabirds and the concentrations of PFAAs (p > 0.05 in all cases).

Comparing migratory with resident seabirds, significantly higher values of PFHxA (U = 352, p < 0.001), PFDA (U = 0, p < 0.001), PFUnDA (U = 1188, p < 0.001), and PFTeDA (U = 2396, p = 0.032) were observed in migratory animals. Profiles based on the relative contribution of the studied compounds to PFAAs were dominated by ΣPFCAs (89–100%), with PFUnDA being the prevalent compound (20–65% of ΣPFAAs) for all species (Fig. 2). PFOS was not detectable in any of the resident species, but it was detectable in one migratory seabird (S. maccormicki). The concentrations of PFBA, PFPeA, PFHpA, PFNA, PFBS, PFHxS, and PFDS were below the detection limit for all species of seabirds. There was no significant correlation between the morphometric measurements (Table S1 of the Supplementary material) of seabirds and the concentrations of PFAAs (p > 0.05 in all cases).
Table 2
Median concentrations and ranges (min-max; ng/g dw) of PFAAs, δ13C, δ15N and δ34S in feathers of resident and migratory seabirds from King George Island, Antarctic Peninsula, Antarctica. The concentrations of PFBA, PFPeA, PFHxS, and PFDS were below the detection limit for all species and therefore omitted from the Table. ∑PFAAs was represented by the average ± standard deviation of the sums of each individual. P-values * < 0.05; ** < 0.01; ***< 0.001.

<table>
<thead>
<tr>
<th>Species</th>
<th>Median concentrations (ng/g dw)</th>
<th>Median ± range (ng/g dw)</th>
<th>Species concentrations (ng/g dw)</th>
<th>Median ± range (ng/g dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stercorarius maccormickii n = 17</td>
<td>&lt;0.97 ± 1.81</td>
<td>1.11-1.74</td>
<td>&lt;0.97 ± 1.81</td>
<td>1.11-1.74</td>
</tr>
<tr>
<td>Larus dominicanus n = 12</td>
<td>&lt;0.87 ± 3.28</td>
<td>0.32-1.69</td>
<td>&lt;0.87 ± 3.28</td>
<td>0.32-1.69</td>
</tr>
<tr>
<td>Macronectes giganteus n = 24</td>
<td>&lt;0.97 ± 0.8</td>
<td>1.11-1.9</td>
<td>&lt;0.97 ± 0.8</td>
<td>1.11-1.9</td>
</tr>
<tr>
<td>Sterna vittata n = 27</td>
<td>&lt;0.97 ± 0.8</td>
<td>1.11-1.9</td>
<td>&lt;0.97 ± 0.8</td>
<td>1.11-1.9</td>
</tr>
</tbody>
</table>

Table 3
Median concentrations and ranges (min-max; ng/g dw) of PFAAs in eggs of resident and migratory seabirds from King George Island, Antarctic Peninsula, Antarctica. PFAA, PFPeA, PFBS, PFHxS, and PFDS concentrations were below the detection limit for all species and therefore omitted from the Table. P-values * < 0.05; ** < 0.01; ***< 0.001.

<table>
<thead>
<tr>
<th>Species</th>
<th>Median concentrations (ng/g dw)</th>
<th>Median ± range (ng/g dw)</th>
<th>Species concentrations (ng/g dw)</th>
<th>Median ± range (ng/g dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pygoscelis adeliae n = 16</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
</tr>
<tr>
<td>P. antarcticus n = 13</td>
<td>&lt;0.97 ± 0.3</td>
<td>0.2-1.19</td>
<td>&lt;0.97 ± 0.3</td>
<td>0.2-1.19</td>
</tr>
<tr>
<td>P. papua n = 15</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.14-1.41</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.14-1.41</td>
</tr>
</tbody>
</table>

Sterna vittata n = 27 | <0.97 ± 0.2 | 0.14-1.41 | <0.97 ± 0.2 | 0.14-1.41 |

<table>
<thead>
<tr>
<th>Species</th>
<th>Median concentrations (ng/g dw)</th>
<th>Median ± range (ng/g dw)</th>
<th>Species concentrations (ng/g dw)</th>
<th>Median ± range (ng/g dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. maccormickii n = 38</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
</tr>
<tr>
<td>S. vitata n = 8</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
</tr>
<tr>
<td>P. adeliae n = 17</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
</tr>
<tr>
<td>P. antarcticus n = 22</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
</tr>
<tr>
<td>P. papua n = 24</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
<td>&lt;0.97 ± 0.2</td>
<td>0.2-1.19</td>
</tr>
</tbody>
</table>
The second principal component (PC2) expressed 25% (Fig. 3A, Table S5) of the variation with the strongest positive contributions from PFHxA (0.68), PFOA (0.62). There is a clear overlap among the PFAAs and stable isotope profiles between migratory and resident seabirds (Fig. 3A).

The pattern of PFAAs contamination in feathers (Table 2) in the present study was as follows: S. maccormicki > C. albus > L. dominicanus > S. vittata > M. giganteus > S. vittata > P. papua > P. adeliae > P. antarcticus.

Comparing the compounds detected in feathers only in migratory seabirds, the concentrations of PFDoDA, PFTrDA, PFTeDA were below the detection limit for PFOA (0.87). There was a clear overlap among the PFAAs and stable isotope profiles between migratory and resident seabirds (Fig. 3A).

The pattern of PFAAs contamination in feathers (Table 2) in the present study was as follows: S. maccormicki > C. albus > L. dominicanus > S. vittata > M. giganteus > S. vittata > P. papua > P. adeliae > P. antarcticus.

Comparing the compounds detected in feathers only in migratory seabirds, the concentrations of PFDoDA, PFTrDA, PFTeDA were below the detection limit for PFOA (0.87). There was a clear overlap among the PFAAs and stable isotope profiles between migratory and resident seabirds (Fig. 3A).

Comparing the compounds detected in feathers only in migratory seabirds, the concentrations of PFDoDA, PFTrDA, PFTeDA were below the detection limit for PFOA (0.87). There was a clear overlap among the PFAAs and stable isotope profiles between migratory and resident seabirds (Fig. 3A).

Comparing the compounds detected in feathers only in migratory seabirds, the concentrations of PFDoDA, PFTrDA, PFTeDA were below the detection limit for PFOA (0.87). There was a clear overlap among the PFAAs and stable isotope profiles between migratory and resident seabirds (Fig. 3A).

The first principal component (PC1) explained 40.6% (Fig. 3B, Table S6) of the total variability in the dataset, with the strongest positive contributions from PFHxA (0.68), PFOA (0.62), and the weakest one from PFHpA (0.18). The second principal component (PC2) expressed 14.5% (Fig. 3B, Table S6), with the strongest positive contributions from PFHxA (0.68), PFOA (0.62). There is a clear overlap between migratory and resident species with a higher tendency of separation than reported in the feathers. However, as in the feathers, the horizontal axis tends to separate Northern Hemisphere migratory birds from Southern Hemisphere birds.

The PFHpA concentrations were above the detection limit only for S. vittata eggs. The PFNA and PFTeDA concentrations were above the detection limit only for S. maccormicki eggs. PFTrDA (H = 56.5, p < 0.001), PFHpA (H = 21.3, p < 0.001), PFOA (H = 39, p < 0.001), PFDA (H = 39, p < 0.001), PFUnDA (H = 25.5, p < 0.001), and PFDoDA (H = 48.6, p < 0.001) concentrations differed among species. The

**Fig. 2.** Sum of compounds and relative contribution of individual PFAAs to ∑PFAAs (ng/g dw) in feathers and eggs of C. albus (Calb), L. dominicanus (Ldo), M. giganteus (Mgi), S. maccormicki (Sma), S. vittata (Svi), P. adeliae (Pad), P. antarcticus (Pan), and P. papua (Ppa) from Antarctic Peninsula.

**Table 2.** Concentrations of individual PFAAs (ng/g dw) in feathers and eggs of C. albus (Calb), L. dominicanus (Ldo), M. giganteus (Mgi), S. maccormicki (Sma), S. vittata (Svi), P. adeliae (Pad), P. antarcticus (Pan), and P. papua (Ppa) from Antarctic Peninsula.

<table>
<thead>
<tr>
<th>Species</th>
<th>Calb</th>
<th>Ldo</th>
<th>Mgi</th>
<th>Sma</th>
<th>Svi</th>
<th>Pad</th>
<th>Pan</th>
<th>Ppa</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFHxA</td>
<td>36.7</td>
<td>8.9</td>
<td>16.1</td>
<td>4.2</td>
<td>5.5</td>
<td>21.3</td>
<td>13.9</td>
<td>5.8</td>
</tr>
<tr>
<td>PFOA</td>
<td>6.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PFDA</td>
<td>3.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PFTrDA</td>
<td>3.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PFTeDA</td>
<td>3.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PFUnDA</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PFOS</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PFHxS</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PFDS</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

The PFHpA concentrations were above the detection limit only for S. vittata eggs. The PFNA and PFTeDA concentrations were above the detection limit only for S. maccormicki eggs. PFTrDA (H = 56.5, p < 0.001), PFHpA (H = 21.3, p < 0.001), PFOA (H = 39, p < 0.001), PFDA (H = 39, p < 0.001), PFUnDA (H = 25.5, p < 0.001), and PFDoDA (H = 48.6, p < 0.001) concentrations differed among species. The
concentrations of PFTrDA were significantly higher in *S. maccormicki* \( (p = 0.04) \) compared to the other analyzed species. The PFOS concentrations were above the detection limit only for *S. maccormicki* and *P. papua*. *S. maccormicki* \( (U = 229, p = 0.004) \) showed significantly higher concentrations of PFOS than *P. papua* eggs.

The concentrations of PFHxA were significantly higher in *P. papua* \( (p = 0.014) \) than in *P. adeliae* \( (p < 0.001) \) and *S. maccormicki* \( (p = 0.001) \). The concentrations of PFOA were significantly lower in *P. antarcticus* \( (p < 0.001) \) than *S. maccormicki* \( (p = 0.002) \) and *P. papua* eggs \( (p < 0.001) \). The PFDA concentrations were significantly higher in *S. maccormicki* \( (p = 0.011) \) than in *P. antarcticus* \( (p = 0.011) \) eggs. The PFUnDA concentrations were significantly higher in *S. maccormicki* \( (p < 0.001) \) than in *P. adeliae* \( (p < 0.001) \) and *P. antarcticus* \( (p < 0.001) \). The concentrations of PFDoDA were significantly higher in *S. maccormicki* \( (p = 0.046) \) and *P. papua* eggs \( (p = 0.012) \) than in the other *Pygoscelis* spp.

3.3. Stable isotope ratios and PFAAs patterns

Spearman rank correlation matrix between PFAAs and stable carbon showed positively significant correlations among \( \delta^{13}C \) values and five compounds (PFDA, PFUnDA, PFTrDA, PFTeDA, and PFOS), and significantly negative between \( \delta^{13}C \) values and PFOA (Fig. 4). Significant positive correlations were found between \( \delta^{15}N \) values and three compounds (PFUnDA, PFTrDA, and PFTeDA), and significantly negative correlations considering \( \delta^{15}N \) values and PFOA (Fig. 4). The \( \delta^{34}S \) values concentrations showed positive and significant correlation with concentrations of PFTrDA, and significantly negative with PFDA (Fig. 4). There is a significantly positive correlation between \( \delta^{13}C \) and \( \delta^{15}N \), but no significant correlation was observed between \( \delta^{15}N \times \delta^{34}S \), and \( \delta^{34}S \times \delta^{13}C \) (Fig. 4).

The values of \( \delta^{15}N \) \( (H = 70.1, p < 0.001) \), \( \delta^{13}C \) \( (H = 70.6, p < 0.001) \), and \( \delta^{34}S \) \( (H = 88.8, p < 0.001) \) differed among species. *Pygoscelis* species \( (p = 0.009) \) and *S. vittata* \( (p = 0.009) \) showed significantly lower \( \delta^{15}N \) Fig. 3. PCA feather (A1 and A2) and egg (B1 and B2) of migratory and resident seabirds from Antarctic Peninsula.

Fig. 4. Spearman rank correlation matrix between PFAAs and C (\( \delta^{13}C \)), N (\( \delta^{15}N \)), and S (\( \delta^{34}S \)) in feathers of seabirds from the Antarctic Peninsula. Significant correlations (rs, \( p < 0.05 \)) are shown in blue (positive) and red (negative). The color intensity is related to the rs value, while non-significant correlations are marked with an X. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
values compared to the other species of seabirds (Table 2, Fig. 5). S. maccormicki (p < 0.001), M. giganteus (p = 0.02), and L. dominicanus (p = 0.02) showed significantly higher δ13C values compared to the other species of seabirds (Table 2, Fig. 5). Regarding δ34S values, S. maccormicki (p < 0.001) and P. antarcticus (0.04) showed significantly lower values compared to C. albus, M. giganteus, and S. vittata (Table 2, Fig. 5).

4. Discussion

Clearly, Antarctic seabirds are exposed to PFAAs concentrations that will rely upon intra-specific and inter-specific driving factors (e.g., tissues, sex, sampling location, biology, ...). Migratory seabirds showed higher concentrations of most PFAAs than resident species. The higher concentrations of PFSAs and PFCAs in migratory seabirds compared to Pygoscelis penguins agree with differences in their trophic positions (δ15N values) and foraging area (δ13C and δ34S values). Trophic ecology (i.e., diet and foraging strategies), metabolic factors (i.e., sex, molting and breeding status), migration patterns (trans-equatorial movements of seabirds), and sources of PFAAs (i.e., point sources and long-range transport) may influence in the interspecific differences found in this study.

4.1. Migration and the PFAAs

Migratory seabirds that migrate to the Northern Hemisphere (S. maccormicki) had higher PFAAs concentrations than seabirds migrating only within the Southern Hemisphere. These data were expected because of the well-known industrialization of the Northern Hemisphere compared to the Southern Hemisphere (Ma et al., 2016; Paul et al., 2009).

The S. maccormicki is a trans-equatorial migrant, disperses widely, reaching the Northern Hemisphere during the southern winter (Cruwys, 2008; Kopp et al., 2011). This top predator is opportunistic, feeding on fish and crustaceans, as well as carrion of seabirds nesting in nearby colonies (Borghello et al., 2019; Cruwys, 2008; Quillfeldt, 2002; Ridoux and Offredo, 1989). Thus, the high trophic position coupled with its migration pattern may explain the high levels of δ13C, δ15N, and PFCAs in S. maccormicki compared to the other Antarctic seabirds in the present study. The literature shows that long-chain PFCAs are mainly noticed in seawater outside of the Antarctic Circumpolar Current (Zhao et al., 2012), being more abundant in seawater from northern compared to southern Atlantic latitudes (González-Gaya et al., 2014; Ma et al., 2016). This may explain the relative high levels of PFTrDA and PFTeDA found in S. maccormicki and low concentrations in penguins. Previous studies about Antarctic seabirds also demonstrate a similar pattern (Roscales et al., 2019; Tao et al., 2006). Tao et al. (2006) detected long-chain PFCAs in liver, serum, and eggs of northern albatrosses, while the PFCAs concentrations were below the limits of quantitation in livers of albatrosses from the Southern Ocean. Roscales et al. (2019) found higher levels of long-chain PFCAs in plasma of seabirds foraging north of Antarctica than in the resident seabirds. Gao et al. (2020b) found lower values of long-chain PFCAs in plasma of seabirds migrating only within the Southern Hemisphere compared to the Northern Hemisphere (Ma et al., 2016; Paul et al., 2009). Tao et al. (2006) observed similar concentrations of PFOS in S. maccormicki eggs (2.5 ng/g ww), which indicate that the concentrations of PFOS have remained constant over the years in the south polar skua. Leat et al. (2013) analyzing S. maccormicki eggs, detected only PFCs with nine or more carbon atoms (PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, and PFTeDA), whereas for PFSAs, the eight-carbon PFOS dominates. Our results showed a similar pattern, since PFDA, PFUnDA, PFDoDA, PFTrDA, and PFTeDA, and PFOS were detected in S. maccormicki eggs. The reported better ability of PFOS, compared to PFCAs, to biomagnificate and bioaccumulate in polar food webs (Kelly et al., 2009; Roscales et al., 2019) could explain the higher values of PFOS in S. maccormicki feathers and eggs in the present study. Thus, migration could result in higher exposure to PFOS, and consequently higher concentrations of PFOS in plasma blood of migratory and resident Antarctic seabirds (Roscales et al., 2019).

Studies performed in birds from Northern Hemisphere have shown a higher exposure to PFSAs compared to the Southern ones. The Great skua (Stercorarius skua) from the North Atlantic showed values of PFOS:

- In feathers: PFDA (22.5 ng/g dw), PFUnDA (17.5 ng/g dw), PFDoDA (15.5 ng/g dw), PFTrDA (13.5 ng/g dw), and PFTeDA (2.5 ng/g dw).
- In eggs: PFDA (15 ng/g dw), PFUnDA (12 ng/g dw), PFDoDA (10 ng/g dw), PFTrDA (8 ng/g dw), and PFTeDA (2 ng/g dw).
- In liver: PFDA (15 ng/g dw), PFUnDA (12 ng/g dw), PFDoDA (10 ng/g dw), PFTrDA (8 ng/g dw), and PFTeDA (2 ng/g dw).

These results are consistent with the hypothesis that long-chain PFCAs are mainly transported from the Northern Hemisphere to the Southern Hemisphere through migratory seabirds.
an order of magnitude greater than (mean range: 0.59 ng/g ww) from the Tibetan Plateau (mean range: 0.59 ng/g ww) from the present study (Leat et al., 2013). The literature has shown PFOS concentrations in plasma of S. skua from Northern Hemisphere (mean 31 ng/g ww) an order of magnitude greater than in brown skuas from the Falklands (Leat et al., 2013; Roscales et al., 2019). Li et al. (2018) observed higher PFAS concentrations in feathers samples of accipiter birds from Tibetan Plateau (mean range: 0.59–6.12 ng/g dw) compared to the present study.

M. giganteus are top predators of the sub-Antarctic and Antarctic food webs, feeding on fish, marine mammals, and penguins' carcasses, as confirmed by our δ15N values results. M. giganteus forages in diverse marine regions during the breeding period, which explains the great variability of δ13C values concentrations (Roscales et al., 2019). Thus, higher concentrations of PFAs in M. giganteus compared to penguins may be explained by their high trophic position and their wide dispersion across southern marine environments. This species can reach the coast of Australia, New Zealand, South Africa and South America, where high levels of PFAs have been reported compared to Antarctica (Gonzalez-Gaya et al., 2014; Patterson and Hunter, 2000).

Despite S. vittata presents δ15N values and diet similar to penguins (Casaux et al., 2008), higher concentrations of PFAs were observed, which may be due to their migratory pattern, since this species migrates to more polluted places such as southern Africa and South America (Cruysw, 2008).

Low PFAs levels were expected in Pygoscelis penguins, owing to their relatively low trophic position, compared to other seabirds in this study, and their resident behavior. In the present study, the concentrations of PFAs in P. antarcticus were lower compared to the other two penguin species. The specialized diet of P. antarcticus, which feeds more on krill, compared to generalist strategy presented by P. papua and the intermediary one presented by P. adeliae (Herman et al., 2017) may explain the significantly lower concentrations of most of PFAs in feather, and δ15N values that P. antarcticus presents compared to other resident and migratory seabirds.

Our results showed the following pattern of PFAs concentrations PFUnDA > PFDA > PFTeDA > PFDoDA for Pygoscelis feather, and PFUnDA > PFOS > PFDoDA > PFHpA for P. papua eggs. This is in line with the findings of (Schiavone et al., 2009) who also observed that among PFCS, PFUnDA was dominant in penguin eggs. On the other hand, previous studies observed short-chain PFHxAs dominated contribution profiles in plasma, guano, and muscle of P. papua (Llorea et al., 2012; Roscales et al., 2019). They suggested that metabolic degradation of long-chain PFCS and direct dietary incorporation might explain this result. This indicates that the PFAs contributions profiles may vary in different tissues of the animal body.

4.2. PFAs and the stable isotopes

In the present study, PFUnDA, PFTeDA, and PFTeDA showed a significant positive correlation with trophic position (δ15N values), indicating a biomagnification potential of these compounds. The literature has shown less or no biomagnification for PFCS compounds (Lescord et al., 2015; Simonnet-Laprade et al., 2019), which contradicts our findings. Unlike (Kelly et al., 2009), who observed a positive and significant correlation between δ13C values and PFOS, we did not observe a correlation between δ13C values and any PFAs (Lopez-Antia et al., 2021), observed, based on δ13C and δ12C values data, that black-backed gulls (Larus fuscus) with a predominantly marine diet have higher exposure to PFOS. Our results also showed that PFOS, PFDA, PFUnDA, PFTrDA and PFTeDA present a correlation with foraging areas (δ12C values). The correlations between PFUnDA and δ13C values in our study suggest an important contribution from coastal or benthic food webs. The latter statement is based on the fact that producers from open marine and pelagic environments typically have higher δ13C values concentrations compared to coastal benthic sediment-associated producers (Connolly et al., 2004).

Hence, our results are in line with the findings of some seabirds (e.g., L. dominicanus, C. albus, and M. giganteus) and logistical limitations for moving to different sampling sites made it impossible to collect egg samples from all studied species. Another limitation is that all resident species were penguins, not allowing to control for phylogenetic characteristics, as there are no migratory penguins in the present study. Therefore, we cannot totally exclude that the reported differences reflect a “penguin effect” rather than a “resident/migrant effect”.

We observed that the migration, especially trans-equatorial migration, has an important role in the exposure of birds to long-chain PFCS, since the lowest concentrations were reported in species residing in Antarctica. This may indicate a certain barrier created by the Antarctic
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.114157.

References


