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Short Communication

Per- and polyfluoroalkylated substances (PFAS) in the feathers and excreta of Gentoo penguins (*Pygoscelis papua*) from the Antarctic Peninsula

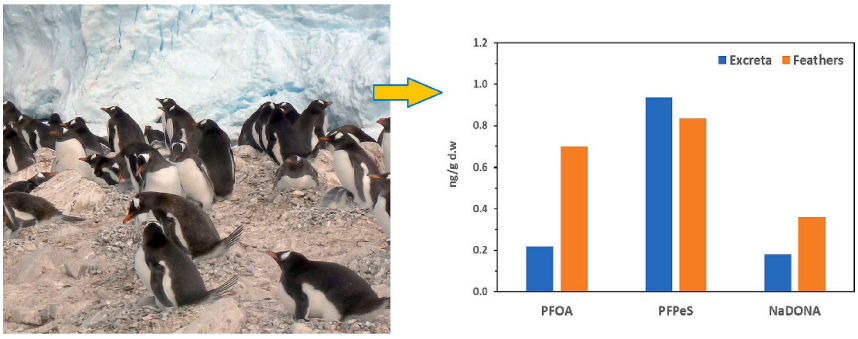
José E. Celis [a], Winfred Espejo [b,*], Thimo Groffen [c], Lieven Bervoets [c], Janeide Padilha [d], Flávia V. Mello [e], Marco Sandoval [b], Gustavo Chiang [f,g]

[a] *Department of Animal Science, Facultad de Ciencias Veterinarias, Universidad de Concepción, Av. Vicente Méndez 595, Chillán, Chile*
[b] *Department of Soils & Natural Resources, Facultad de Agronomía, Universidad de Concepción, Av. Vicente Méndez 595, Chillán, Chile*
[c] *ECOSPHERE, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020, Antwerp, Belgium*
[d] *CBMA–Centre for Molecular and Environmental Biology/ ARNET-Aquatic Research Network, Department of Biology, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal*
[e] *IPMA, Instituto Português do Mar e da Atmosfera, Divisão de Aquacultura e Valorização, I.P, Lisboa, Portugal*
[f] *Centro de Investigación para la Sustentabilidad (CIS-UNAB) & Department of Ecology and Biodiversity, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago 8370251, Chile*
[g] *Centro de Resiliencia, Adaptación y Mitigación (CReAM), Universidad Mayor, Av. Alemania 281, Temuco, Chile*

HIGHLIGHTS

- There is bioaccumulation of PFAS compounds in penguin feathers
- NaDONA is found here for the first time in Antarctica
- PFAS levels are lower than those in aquatic birds from the Northern Hemisphere
- Penguins excrete PFAS through feathers and guano

GRAPHICAL ABSTRACT



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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) exhibit widespread global distribution, extending to remote regions including Antarctica. Despite potential adverse effects on seabirds, PFAS exposure among Antarctic penguins remains poorly studied. We investigated the occurrence of 29 PFAS compounds in feathers and excreta of Gentoo penguins (*Pygoscelis papua*) from Fildes Bay, Antarctica. Sample collection was conducted during the austral summer (February 2015) and analyzed by Ultra-performance liquid chromatography-tandem ES (−) mass spectrometry. The results showed that adults tend to accumulate more PFOA, PFPeS and NaDONA than chicks, with PFOA emerging as the predominant compound in feathers. The compounds PFHxA, PFDoDA, PFBS, PFOS, 4:2 FTS, 6:2 FTS, and PFEESA were only detected in penguin excreta, indicating that they are not absorbed into the organism. The detection of PFAS in penguin feathers and excreta not only indicates local contamination but also reaffirms the far-reaching impact of anthropogenic pollutants. This study presents the first documented

* Corresponding author.
E-mail address: winfredespejo@udec.cl (W. Espejo).

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occurrence of NaDONA in Antarctica, despite its status as a regulatory-compliant alternative to legacy PFAS compounds—a finding that needs deeper attention. The data can serve as a base for further research to understand the full extent of PFAS contamination and its implications for Antarctic wildlife and ecosystems.

1. Introduction

Anthropogenic activities and economic pressures are increasingly threatening the world's coastlines, posing potential risks to both people and ecosystems (Gross, 2014; Liu et al., 2017). Considering the resistance to degradation, wide distribution, and bioaccumulation potential of most anthropogenic chemicals, their possible effects constitute an issue of concern worldwide (Noyes et al., 2009; Fang et al., 2014; Yao et al., 2020). Among these contaminants, *per-* and polyfluoroalkyl substances (PFAS) represent a particularly problematic class of ubiquitous organic pollutants, which are extensively utilized in industrial and consumer applications, including textiles, leather goods, paper products, metal plating and etching, floor polishes, insulators, Teflon pans, among others (ITRC, 2020). For over 40 years, industries and consumers have widely used these compounds due to their chemical stability and lipid-hydrophobic properties (Ericson et al., 2012). PFAS are now considered priority pollutants, due to their chemical persistence, and their pervasive presence in water, food products, animals, and even humans (Van den Bergh et al., 2020). Animal exposure to these toxicants has been linked to numerous adverse health problems, including cancer, metabolic disorders, liver dysfunction, chronic kidney damage, cardiovascular conditions, and endocrine disruption affecting thyroid and other hormonal systems (Podder et al., 2021). These effects highlight the risks PFAS pose to both environmental and human health (Shin et al., 2011; Sznajder-Katarzyńska et al., 2019).

The environmental impact of PFAS is due to their exceptional mobility and dispersal capabilities, thus they can be detected in remote locations, including polar regions, far from their sources (Gao et al., 2020; Padilha et al., 2024). Research has demonstrated that PFAS can be dispersed via oceanic and atmospheric currents worldwide (Young and Mabury, 2010; Llorca et al., 2012; Zhao et al., 2012; Rigét et al., 2013). Previous investigations have primarily focused on long-chain perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) (Buck et al., 2011; Groffen et al., 2020). Particular attention has been directed toward perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), owing to their exceptional persistence, stability, potential health risks, and ubiquitous global presence (ATSDR, 2018). These two compounds were declared as persistent organic pollutants by the Stockholm Convention in 2009 and 2019, respectively, and remain among the most frequently detected long-chain PFAS in environmental samples, wildlife, and human populations worldwide (Buck et al., 2011; Groffen et al., 2017; Padilha et al., 2024).

Current knowledge regarding PFAS contamination in Antarctic seabird species remains limited (Larramendy and Soloneski, 2015; Munoz et al., 2017; Roscales et al., 2019; Padilha et al., 2022, 2024). Penguins, particularly Antarctic species, represent the region's largest avian biomass and occupy high trophic positions with considerable longevity, making them ideal bioindicators of local and regional pollution (Espejo et al., 2017). Non-invasive sampling methods utilizing feathers and excreta have proven effective for monitoring bioaccumulation of both trace elements and toxic compounds (Rudolph et al., 2016; Chiang et al., 2021; Celis et al., 2022, 2024) as well as PFAS (Padilha et al., 2022; Wells et al., 2024). The keratin-rich composition of feathers facilitates PFAS accumulation due to these compounds' protein affinity (Padilha et al., 2022). Elevated chemical concentrations in penguin excreta suggest an active detoxification mechanism, though this may also indicate limited intestinal absorption (Espejo et al., 2017). Studies have shown that Gentoo penguins exhibit specific bioaccumulation responses to certain pollutants, correlating with their higher trophic position (Rudolph et al., 2016). The potential application of penguin guano and feathers for continuous PFAS monitoring could serve both chemical exposure assessment and species conservation objectives (Kuo et al., 2022).

Given the significant avian biomass represented by Gentoo penguins in Antarctica, understanding the fate and potential effects of these pollutants is crucial. To date, no studies have examined age-related PFAS accumulation in feathers of this species or compared PFAS levels between feathers and excreta. Therefore, this study aims to: i) determine PFAS presence in feathers across different age groups (chicks and adults) of Gentoo penguins, ii) assess PFAS levels in adult Gentoo penguin excreta, and iii) compare PFAS accumulation patterns between feathers and excreta in adult Gentoo penguins to evaluate whether these compounds are predominantly absorbed or excreted.

2. Methods

Gentoo penguin colonies from Fildes Bay of King George Island (Fig. 1) were sampled by February of 2015, during the seasonal summer in Southern Hemisphere. Feathers were obtained from adult and chick birds, while excreta samples from adult birds. We sampled 450 and 300 feathers from different adults and chicks, respectively. For feathers, 40 individuals were captured using a long-handled net during moulting and before foraging. The penguins were contained between two trained people wearing gloves and eye protection. Once the bird was captured a black blanket or hood was placed over its head, with the beak and nostrils exposed so that the birds could breathe easily (Dr. Daniel González, personal communication). For excreta, 16 samples were collected using disposable plastic spatulas directly from nesting sites.

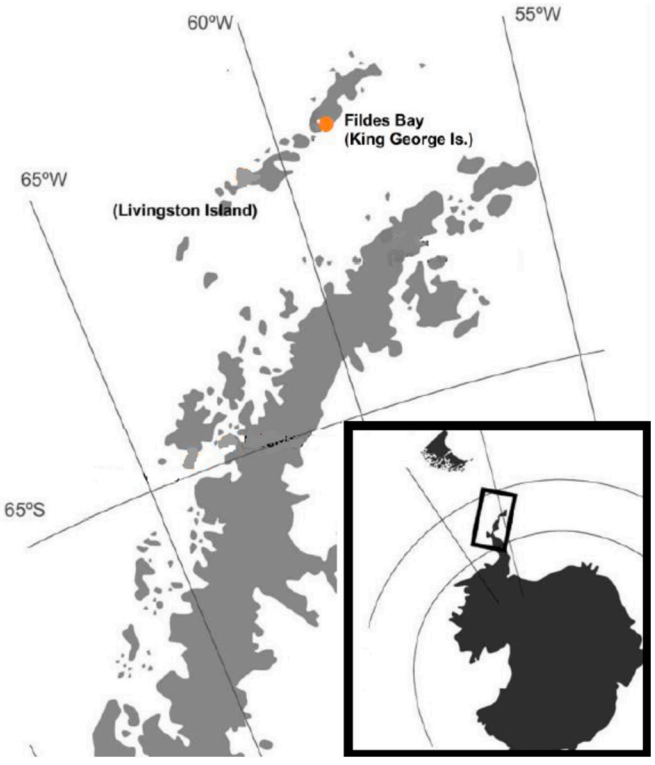


Fig. 1. Map highlighting in red circle the sampling location, Fildes Bay (62°15′S, 58°51′W), King George Island (South Shetland Islands, Antarctic Peninsula area).

Excrement samples were collected from the ground at different points within the colony, taking special care not to repeat each point collected again. All samples were collected using disposable plastic gloves, kept in sealed plastic bags and stored at −4 °C for transport until their analysis in the laboratory.

In the laboratory, samples were washed with deionised water, acetone, 2 % nitric acid and again deionised water to remove external contamination (Burger, 1993). Then, all samples were freeze-dried until dry masses were constant. Following, feathers were cut into smaller pieces (ceramic scissors) and along with excreta were homogenized into a fine powder using a ceramic mortar. The protocol described by Groffen et al. (2021) was used to extract PFAS from feathers. About 200 mg of dried feathers were weighed and stored in 50 mL polypropylene (PP) tubes. After adding 10 mL of methanol (MeOH) and 10 ng of mass-labeled internal standards (MPFAC-MXA, Wellington Laboratories, Guelph, Canada), the samples were vortex-mixed for at least 1 min and left in the dark at room temperature for 24 h. After centrifugation (4 °C, 1037 xg, 10 min, Eppendorf centrifuge 5804R), the supernatant was transferred into a 15 mL PP tube and dried completely using a rotational vacuum centrifuge (Martin Christ, RVC 2–25, Osterode am Harz, Germany). Hereafter, the samples were reconstituted with 0.2 mL of a 2 % ammonium hydroxide solution (diluted in acetonitrile (ACN)), vortex-mixed for at least 1 min, and filtered through an Ion Chromatography Acrodisc 13 mm Syringe filter with 0.2 μm Supor polyethersulfone (PES) membrane (VWR International, Leuven, Belgium) into PP auto-injector vials. Excreta samples (± 200 mg of dried material) were extracted following a protocol described by Powley et al. (2005) with minor modifications. The samples were spiked with 10 ng of the aforementioned internal standard mixture and 10 mL of ACN, after which they were vortex-mixed, sonicated for 3 × 10 min (with vortex-mixing in between) and stored on a shaking plate overnight (135 rpm, room temperature). After centrifugation (4 °C, 1037 xg, 10 min, Eppendorf centrifuge 5804R), the supernatant was transferred into a 15 mL PP tube and dried to approximately 0.5 mL, which was then transferred into PP Eppendorf tubes that contained 0.1 mL of graphitized activated carbon powder (Supelclean ENVI-Carb, Sigma-Aldrich, Belgium) and 50 μL of glacial acetic acid. The 15 mL PP tubes were rinsed twice with 250 μL of ACN which was also transferred into the Eppendorf tubes. Hereafter, samples were vortex-mixed for at least 1 min and centrifuged (4 °C, 9279.4 xg, 10 min, Eppendorf centrifuge 5415R). The supernatants were dried almost completely and reconstituted and filtered as described above for the feathers.

At the ECOSPHERE laboratory of the University of Antwerp (Belgium), ultra-performance liquid chromatography-tandem ES (−) mass spectrometry (UPLC-MS/MS, ACQUITY, TQD, Waters, Milford, MA, USA) was used to analyze 29 targeted PFAS (Table 1S, Supplementary material). The individual analytes were separated using an ACQUITY BEH C18 column (2.1 × 50 mm; 1.7 μm, Waters, USA) and an ACQUITY BEH C18 pre-column (2.1 × 30 mm; 1.7 μm, Waters, USA) was inserted between the solvent mixer and injector to retain any PFAS contamination from the system. The mobile phase solvents consisted of 0.1 % formic acid in water and 0.1 % formic acid in ACN. The flow rate was set at 450 μL/min at a partial loop injection of 6 μL. The solvent gradient started at 65 % of 0.1 % formic acid in water, decreased to 0 % of this solvent in 3.4 min, and returned to 65 % 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 2S (Supplementary material) and validated by Groffen et al. (2021). Procedural blanks (10 mL of MeOH for feathers ($N = 3$) and 10 mL of ACN for excreta (N = 3)) were included to test for any contamination that may have occurred during the extraction and analysis. No contamination (i.e., concentrations of all PFAS were < LOQ) was detected in these blanks. As instrumental blanks, ACN was regularly injected to prevent cross-over contamination between injections. The limits of quantification (LOQ) of each analyte were determined in the matrix as the concentration corresponding to a signal-to-noise ratio of 10 (Table 1S, Supplementary material). Calibration curves were prepared by adding a constant amount of the ISTD to varying concentrations of an unlabeled PFAS mixture. A linear regression function with highly significant linear fit (all $R^2 > 0.98$; all $p < 0.001$) described the relationship between the ratio of concentrations of unlabeled and labeled PFAS and the ratio of the peak areas of the unlabeled and labeled PFAS.

Nonparametric statistical methodologies were employed because the data did not meet the assumptions of normality and homoscedasticity even after applying a log transformation. The differences between the levels of the chemical elements were achieved by Kruskal-Wallis analysis. Post hoc tests were conducted with the critical differences of mean rank. A significance level of $p < 0.05$ was used to determine statistical significance. Recoveries of the internal standards varied between 83 % and 97 % in the feather samples, and between 86 % and 93 % in the excreta, depending on the targeted analyte. The data was analyzed using the SPSS 27.0 software.

Table 1
Mean ± standard error, ranges (min-max), and median values of PFAS in feathers of Gentoo penguins from Fildes Bay (King George Island, Antarctic Peninsula). Values expressed in ng/g dry weight. The n-value represents the analytical quantification (3 readings) on the basis of 5 and 3 pooled samples collected per adults and chicks, respectively.

Compounds	Age of Gentoo penguins	
	Adults ($n = 15$)	Chicks ($n = 9$)
PFOA	0.70 ± 0.28	0.31 ± 0.01
	(0.27–1.49)	(0.29–0.32)
	0.51	0.31
PFPeS	0.84 ± 0.01	<LOQ
	(0.81–0.86)	-
	0.84	-
NaDONA	0.36 ± 0.11	0.16 ± 0.02
	(0.19–0.81)	(0.14–0.19)
	0.26	0.16

LOQ = limit of quantification. The contents of PFBA, PFPeA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS, PFDS, 4:2 FTS, 6:2 FTS, 8:2 FTS, HFPO-DA, 9Cl-PF3ONS, 11Cl-PF3OUdS, PFEESA, FBSA, PF4OPeA, PF5OHxA and 3,6-OPFHpA were below LOQ for all adults and chicks.

Table 2
Concentrations of PFAS in excrements of adult Gentoo penguins ($n = 16$) from Fildes Bay (King George Island, Antarctic Peninsula).

Compounds detected (ng/g dry weight)	Mean	Std. error	Min	Max	Median
PFHxA	1.55	0.26	0.77	2.33	1.56
PFOA	0.22	–	–	–	0.22
PFDoDA	0.94	0.08	0.80	1.07	0.94
PFBS	7.74	0.46	6.09	8.56	8.15
PFPeS	0.94	0.23	0.37	1.55	0.98
PFOS	0.72	0.20	0.24	1.65	0.66
4:2 FTS	2.91	0.82	1.17	4.87	2.80
6:2 FTS	2.52	0.86	1.08	4.94	1.55
NaDONA	0.18	0.01	0.15	0.20	0.18
PFEESA	0.87	0.02	0.84	0.91	0.87

The contents of PFBA, PFPeA, PFHpA, PFNA, PFDA, PFUnDA, PFTrDA, PFTeDA, PFHxS, PFHpS, PFDS, 8:2 FTS, HFPO-DA, 9Cl-PF3ONS, 11Cl-PF3OUdS, FBSA, PF4OPeA, PF5OHxA and 3,6-OPFHpA were below LOQ (limit of quantification).

3. Results and discussion

Out of the 29 congeners measured in feathers of Gentoo penguins, only PFOA, PFPeS, and NaDONA were above LOQ (Table 1). PFPeS was exclusively found in adult specimens. The highest levels corresponded to PFOA (1.49 ng/g) in the feathers of adult Gentoo penguins. A significant

accumulation of NaDONA and PFOA in feathers of adult penguins was found as compared to chick penguins ($p < 0.05$; Fig. 2). Analysis of 29 PFAS congeners in Gentoo penguin feathers revealed quantifiable levels of only PFOA, PFPeS, and NaDONA (Table 1). PFPeS was exclusively detected in adult specimens. Adult penguins exhibited the highest PFOA concentrations (1.49 ng/g) and demonstrated significantly higher accumulation of both NaDONA and PFOA compared to chicks ($p < 0.05$; Fig. 2). This finding in adults is consistent with previous observations in long-lived seabirds, where adults typically show higher feather contaminant burden than chicks (Bond and Diamond, 2009; Squadrone et al., 2016). Multiple factors may influence PFAS deposition, including feather physiology, dietary seasonal variations, external contamination, metabolic rate, and exposure duration (Behrooz et al., 2009; Dauwe et al., 2005; Eulaers et al., 2011). While the mechanisms of organic pollutant binding to feathers remain incompletely understood, evidence suggests certain contaminants integrate permanently into the feather structure (Pacyna-Kuchta, 2023).

It is not easy to establish a comparison with previous studies because there are few studies on the same matrices. In feathers, the PFOA values in adult Gentoo penguins (Table 1) are lower than those found in cape petrel (*Daption capense*) and Gentoo penguin feathers during 2010–2015 from the Antarctic Peninsula (1–8 ng/g dw, Gao et al., 2020; Padilha et al., 2022). Compared with other species of aquatic birds from the Northern Hemisphere, the concentrations of PFASs in the Gentoo penguin feathers found in the present study are much lower than those in different waterbird feathers (3–58 ng/g dw, Sun et al., 2019; Monclus et al., 2022; Huang et al., 2022; Mo et al., 2024). These findings are consistent with a study conducted by Padilha et al. (2022), which highlighted that resident seabird, such as *Pygoscelis* penguins, have lower levels of PFAS than Antarctic migratory birds. This is attributed to their lower trophic position compared to other seabirds in the study and their resident behavior. Penguins' localized foraging habits and relatively low trophic levels contribute to their reduced exposure to PFAAs.

In Table 2 and Fig. 1S (Supplementary material) are indicated the concentrations of PFHxA, PFOA, PFDoDA, PFBS, PFPeS, PFOS, 4:2 FTS, 6:2 FTS, NaDONA and PFEESA found in the excreta of adult Gentoo penguins. The higher levels found in excreta corresponded to PFBS, 4:2 FTS and 6:2 FTS. The compounds only detected in the excrement of Gentoo penguins (PFHxA, PFDoDA, PFBS, PFOS, 4:2 FTS, 6:2 FTS, and PFEESA) may imply that these birds do not absorb them from diet and therefore do not retain them. This indicates the ubiquity of these compounds in penguin diet, an issue that requires more understanding. The highest concentration corresponded to PFBS with 8.56 ng/g (dw), whereas the lowest was for NaDONA (0.15 ng/g dw). A study performed in 2010 in excreta of adult Gentoo penguins from different locations of the Antarctic Peninsula (Llorca et al., 2012) presented higher concentrations of PFOS (150–550 ng/g), PFHxA (25–240 ng/g), PFBS (10–50 ng/g), PFOA (5–10 ng/g) and PFDoDA (1–2 ng/g) than the levels of these compounds found herein. The lower compound levels found in our study could be explained as Gentoo penguins present great variation in diet between years and among breeding locations (Garcia borboroGlu and Boersma, 2013), a topic that needs to be further investigated.

The presence of PFAS in penguin excreta and feathers indicates that these compounds are incorporated into the bird organism through diet, which is highly influenced by the increasing anthropogenic activities in the Antarctic Peninsula area, as has been previously reported for chemicals like POPs and metals in this area (Hale et al., 2008; Rudolph et al., 2016; Espejo et al., 2017; Celis et al., 2023). The concentrations of PFOA and NaDONA were significantly higher in penguin feathers compared to their excreta (Fig. 3). In contrast, PFPeS concentrations were similar in both sample types, with no statistically significant differences observed. These findings suggest that PFOA and NaDONA are being absorbed by the penguin organism, with feathers serving as the primary elimination of these compounds. For PFPeS, both feathers and excreta appear to be effective detoxification routes. Future studies should assess the concentration of PFOA and NaDONA in other organs, such as blood, liver, and kidneys, to determine the extent of bioaccumulation of these toxins in this protected species.

Due to their environmental persistence, bioaccumulation, and potential biological toxicity, PFOS, PFOA, and PFHxS are classified as critical control pollutants (Mo et al., 2024). The novel detection of NaDONA in Antarctica is particularly noteworthy. Along with GenX (HFPO-DA), and PFNA, NaDONA belongs to the new-generation short-chain PFAS which are used as alternatives to replace PFOS and PFOA from industrial processes because they are highly toxic to fauna (Khan et al., 2023; Vendl et al., 2024). Its presence in remote regions such as Antarctica reveals that NaDONA has the potential for long-range transport, posing potential environmental concerns that may require a strategy for its regulation in the near future. Although there are not many PFAS congeners detected in the present work, this situation might change if there is an increasing level of contamination by anthropogenic activities in the Southern Hemisphere, which could be worsened by a future global climate crisis (Deng et al., 2022). Increasing temperatures

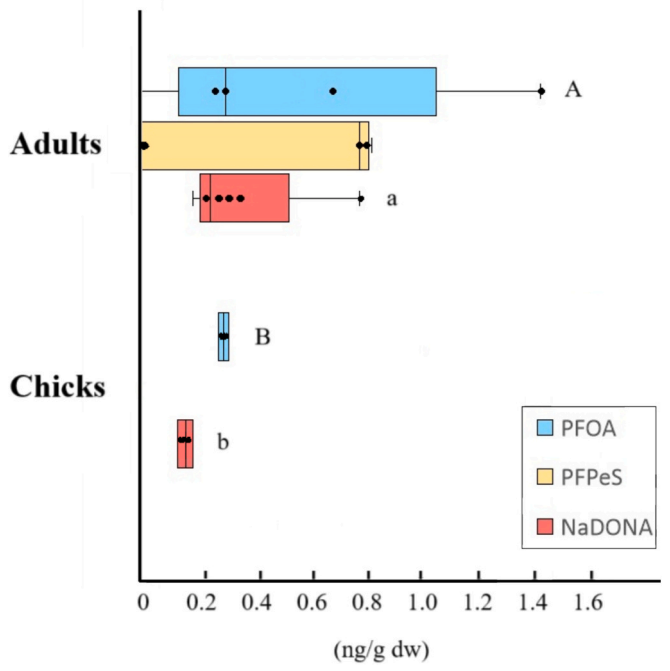


Fig. 2. Accumulation of PFAS in feathers of adult and chick Gentoo penguins from Fildes Bay, King George Island. Different letters indicate significant differences between age groups for each compound ($p < 0.05$): PFOA (AB) and NaDONA concentrations (ab). PFPeS was not detect in chicks.

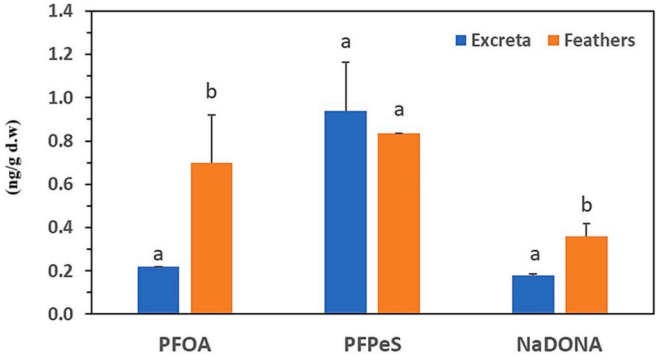


Fig. 3. Comparison between the mean levels of PFAS detected in feathers and excrements of adult Gentoo penguins from Fildes Bay, King George Island. Different letters indicate significant differences between the matrices for each compound ($p < 0.05$).

can enhance the mobility and spread of contaminants, potentially leading to higher bioavailability and uptake in local wildlife (Kemp et al., 2022). Moreover, climate crisis may result in changes to the Antarctic ecosystem, including alterations in food web dynamics and prey availability, which could further influence contaminant exposure and bioaccumulation patterns in penguins and other seabirds (Caputi et al., 2020). Investigating the sources and pathways of PFAS into the Antarctic environment, assessing the long-term effects on wildlife health, and exploring mitigation strategies to reduce contamination are crucial steps. Additionally, understanding how climate change may exacerbate these issues is essential for developing comprehensive conservation strategies to protect vulnerable species and ecosystems in the Antarctic. Climate change can trigger changes in the ocean with an increase of chemicals in sea biota, thus impacting on food sources (Wang et al., 2023). This is an issue that needs deeper attention.

4. Conclusions

The present study provides valuable insights into PFAS bioaccumulation in Gentoo penguins from the Antarctic Peninsula, including the novel detection of NaDONA, which requires further studies to confirm this finding. An age-dependent bioaccumulation of PFAS compounds in the feathers was observed, as higher PFAS-levels were found in adults as compared to chicks. While adult penguins exhibited higher PFAS accumulation than chicks, with PFOA emerging as the dominant compound in feathers, PFBS, 4:2 FTS, and 6:2 FTS predominated in excreta. This pattern differs from typical avian PFAS profiles, where PFOS, PFUnDA, and PFTrDA usually dominate in eggs (Schiavone et al., 2009; Khan et al., 2023). The higher levels of PFOA, PFPeS and NaDONA in feathers suggest that these compounds are primarily absorbed and then deposited into the feathers, with guano serving as a secondary route of elimination of these chemicals.

These findings serve as an early warning indicator, emphasizing the need for vigilant monitoring of novel chemical entities in Antarctica. Future research priorities should include investigating contaminant sources, pathways, and biological effects to develop effective conservation strategies. As human-wildlife interactions intensify, ecotoxicological studies become increasingly crucial for understanding anthropogenic impacts on vulnerable Antarctic species and their ecosystems.

CRediT authorship contribution statement

José E. Celis: Writing – original draft, Resources, Project administration, Investigation. **Winfred Espejo:** Writing – original draft, Resources, Investigation, Conceptualization. **Thimo Groffen:** Writing – review & editing, Methodology, Formal analysis. **Lieven Bervoets:** Writing – review & editing, Resources. **Janeide Padilha:** Writing – review & editing. **Flávia V. Mello:** Writing – review & editing. **Marco Sandoval:** Writing – review & editing. **Gustavo Chiang:** Writing – review & editing, Resources, Investigation.

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.2024.178333.

Data availability

Data will be available upon request.

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