



## Mercury exposure in Antarctic seabirds: Assessing the influence of trophic position and migration patterns

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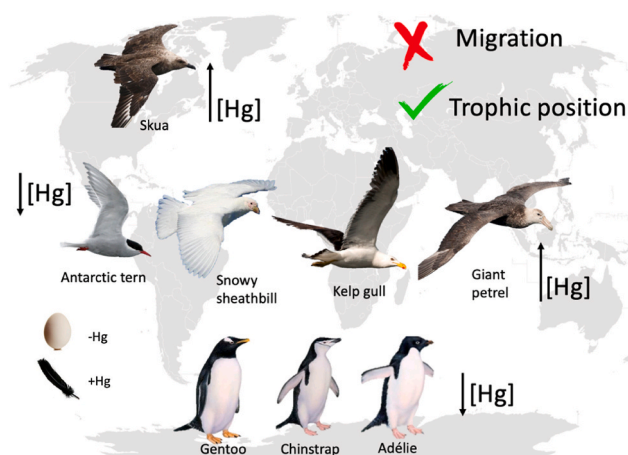
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### HIGHLIGHTS

- Migratory destination does not significantly impact mercury concentrations in Antarctic seabirds.
- Trophic position may play a more significant role in mercury exposure.
- Despite similar diets, species can have different mercury exposure levels due to specific food preferences.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Although naturally present in the environment, mercury (Hg) input is significantly amplified by anthropogenic activities on a global scale, leading to a growing concern about the recent increase in Hg levels observed in Antarctica. This study investigated total mercury (THg) concentrations in feathers and eggs of resident and

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migratory Antarctic seabirds. Stable isotope data ( $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ , and  $\delta^{34}\text{S}$ ) were employed to ascertain the key factors influencing the exposure of these species to Hg. We gathered feathers and eggs from three resident species - Adélie, Gentoo, and Chinstrap penguins, as well as five migratory species - Snowy Sheathbill, Antarctic Tern, Southern Giant Petrel, Kelp Gull, and South Polar Skua. These samples were collected from Admiralty Bay, King George Island, in the Antarctica Peninsula. For all species, THg concentrations were higher in feathers (mean  $\pm$  SD:  $2267 \pm 2480 \text{ ng g}^{-1} \text{ dw}$ ) than in eggs ( $906 \pm 1461 \text{ ng g}^{-1} \text{ dw}$ ). Species occupying higher trophic positions, such as the Southern Giant Petrel ( $5667 \pm 1500 \text{ ng g}^{-1} \text{ dw}$ ) and South Polar Skua ( $4216 \pm 1101 \text{ ng g}^{-1} \text{ dw}$ ), exhibited higher THg levels in their feathers than those at lower positions, like Antarctic Tern ( $1254 \pm 400 \text{ ng g}^{-1} \text{ dw}$ ) and Chinstrap Penguin ( $910 \pm 364 \text{ ng g}^{-1} \text{ dw}$ ). The  $\delta^{15}\text{N}$  values, which serve as a proxy for the trophic position, significantly correlated with THg concentrations. These findings reveal that trophic position influences THg concentrations in Antarctic seabirds. Migration did not appear to significantly affect the exposure of seabirds to THg, contrary to initial expectations. This research highlights the importance of evaluating the impacts of THg contamination on the Antarctic ecosystem by considering a variety of species. This multi-species approach offers critical insights into the factors that may potentially influence the exposure of these species to contaminants.

## 1. Introduction

Mercury (Hg) exists naturally in various forms, including methylmercury (MeHg), inorganic mercury ( $\text{Hg}^{2+}$ ), and gaseous elemental mercury ( $\text{Hg}^0$ ), but its levels can be amplified through both natural and anthropogenic processes (Bargagli, 2008; Lamborg et al., 2014; Polito et al., 2016). Geological events like the weathering of rocks release mercury into soil and water, while volcanic activities emit gaseous forms of mercury, including  $\text{Hg}^0$ , that may undergo atmospheric chemical transformations (Bargagli, 2008; Gaffney et al., 2014; Nriagu and Becker, 2003).  $\text{Hg}^0$  has a residence time of 6–24 months in the atmosphere, facilitating travel over great distances and reaching the Antarctic ecosystem through long-range atmospheric transport (Bargagli, 2008; Bargagli et al., 2007; Lamborg et al., 2014; Zvěřina et al., 2014). Within the Antarctic's aquatic ecosystems, these mercury compounds can undergo further transformation by microbial activity into toxic MeHg, subsequently entering the food web and affecting marine species (Gojkovic et al., 2022; Ruus et al., 2015). Seabirds, such as petrels, and gulls, can act as biovectors due to their tendency to form large colonies and their exposure to more polluted areas (Castro et al., 2022; Cipro et al., 2017). Through their feeding habits and migratory patterns, they may play a critical role in the biotransport process, transporting mercury across different regions and ecosystems (Castro et al., 2022; Cipro et al., 2017; Soares et al., 2023).

In recent years, there has been growing concern over Hg levels in Antarctica, particularly in matrices obtained from seabirds (Carravieri et al., 2017, 2021; Polito et al., 2016; Souza et al., 2020). However, most of the investigations focus on penguins, and studies with migratory seabirds are still scarce (Carravieri et al., 2017, 2021; Polito et al., 2016; Souza et al., 2020). Understanding the exposure of marine birds to mercury is crucial, as this knowledge will assist in evaluating their role as biovectors of pollution in Antarctica. Despite the importance of this issue, it remains insufficiently researched, highlighting the need for additional studies to grasp the full ecological implications of this dynamic.

In marine environments, seabirds are used as sentinels for Hg contamination worldwide, as they are abundant, long-lived, forage in wide areas around the globe, and occupy high trophic positions (Burger and Gochfeld, 2000; Padilha et al., 2018, 2022, 2021; Souza et al., 2020). Penguins and migratory seabirds in Antarctica are exposed to Hg through their diet, primarily composed of krill, fish, and other marine organisms (Polito et al., 2016; Souza et al., 2020). Hg accumulates in organs such as liver and kidneys and is eventually eliminated through various routes, including feathers and egg-laying processes (Bargagli, 2008; Celis et al., 2018).

Feathers and eggs serve as valuable monitoring tools for evaluating avian exposure to contaminants, including Hg, in numerous regions worldwide (Bighetti et al., 2021; Celis et al., 2018; Jerez et al., 2013; Cunha et al., 2015; Padilha et al., 2018, 2021). The incorporation of contaminants in avian feathers and eggs represents exposure across

specific and distinct timeframes (Metcheva et al., 2011). Measuring Hg in feathers is useful as they are the primary elimination route for trace elements, including during their growth phase when contaminants are integrated (Becker et al., 2016; Bighetti et al., 2021; Burger, 1993). The conversion of disulfide bonds in proteins to sulfhydryl groups during feather formation facilitates Hg binding and storage, further highlighting the potential role of feathers in Hg detoxification (Burger, 1993; Metcheva et al., 2011). Conversely, the presence of contaminants in eggs reflects blood levels at the time of oviposition (Burger and Gochfeld, 2003), as the maternal lipids, proteins, and associated contaminants are integrated into the developing eggs during their synthesis (Drouillard and Norstrom, 2001). Furthermore, the high affinity of Hg for proteins causes this element to bind to egg components such as albumin, making this matrix suitable for Hg quantification (Bond and Diamond, 2009).

Mercury is a toxic element that can have adverse effects on the health of humans and wildlife (Bargagli et al., 2007; Bighetti et al., 2021, 2022). This metal can affect all aspects of avian physiology leading to negative impacts on reproduction (e.g., reduces egg production and the number of surviving chicks), behavior (e.g., lethargy, loss of appetite, and motivation to forage) in addition to causing endocrine, immunological, and neurological dysfunction (Brasso and Cristol, 2008; Burger and Gochfeld, 1997; Seewagen, 2009; Whitney and Cristol, 2017). It also makes seabirds more susceptible to diseases and infections (Sebastiano et al., 2022). According to previous literature, high Hg levels ( $1500 \text{ ng g}^{-1}$  in eggs and  $5000\text{--}40000 \text{ ng g}^{-1}$  in feathers) showed a deleterious effect on the breeding success of two skua species (*Stercorarius maccornicki* and *S. lonnbergi*), and wandering albatross (*Diomedea exulans*), ultimately leading to population decline (Burger and Gochfeld, 1997; Goutte et al., 2014a, 2014b).

Despite the well-established role of seabirds as sentinel species for environmental contamination by metals, there is a paucity of information regarding the presence and distribution of Hg in feathers and eggs of Antarctic seabirds (Bargagli et al., 2007; Brasso et al., 2012; Carravieri et al., 2017; Polito et al., 2016; Souza et al., 2020). Furthermore, the limited data on Antarctic bird exposure to Hg and its trophic relationships pose challenges in determining its full extent and the potential impacts on those populations. Ratios of stable isotopes of carbon (C), nitrogen (N), and sulfur (S), serve as valuable tools for identifying foraging habitats, trophic levels, and habitat use of seabirds (Cherel et al., 2014; Connolly et al., 2004; Polito et al., 2016). Carbon ratios (expressed in per mill  $\text{‰} \delta^{13}\text{C}$ ) can be used to differentiate inshore and offshore food items, while nitrogen ratios ( $\delta^{15}\text{N}$ ) indicate trophic positions (Cherel et al., 2014; Dehnhard et al., 2020; Polito et al., 2016). Sulfur ratios ( $\delta^{34}\text{S}$ ) distinguish marine and terrestrial habitats (Connolly et al., 2004). In addition, the utilization of Stable Isotope Bayesian Ellipses (SIBER), in conjunction with Stable Isotope Analysis (SIA), helps to define trophic niches, providing valuable insights in the study of foraging habitats, trophic levels, and habitat use of seabirds (Jackson et al., 2011; Padilha et al., 2021). Despite the considerable potential of SIBER for ecological research, its application to Antarctic bird studies

has been rather limited so far (Padilha et al., 2021). Thus, our study seeks to shed more light on the utility of SIBER in exploring the intricate links between trophic ecology and exposure to pollutants in Antarctic birds. This study aligns with the increasing reliance on Stable Isotope Analysis (SIA) in seabird research, a methodological trend that has significantly propelled our comprehension of the intricate relationship between trophic ecology and exposure to pollutants (Padilha et al., 2021, 2022; Polito et al., 2016).

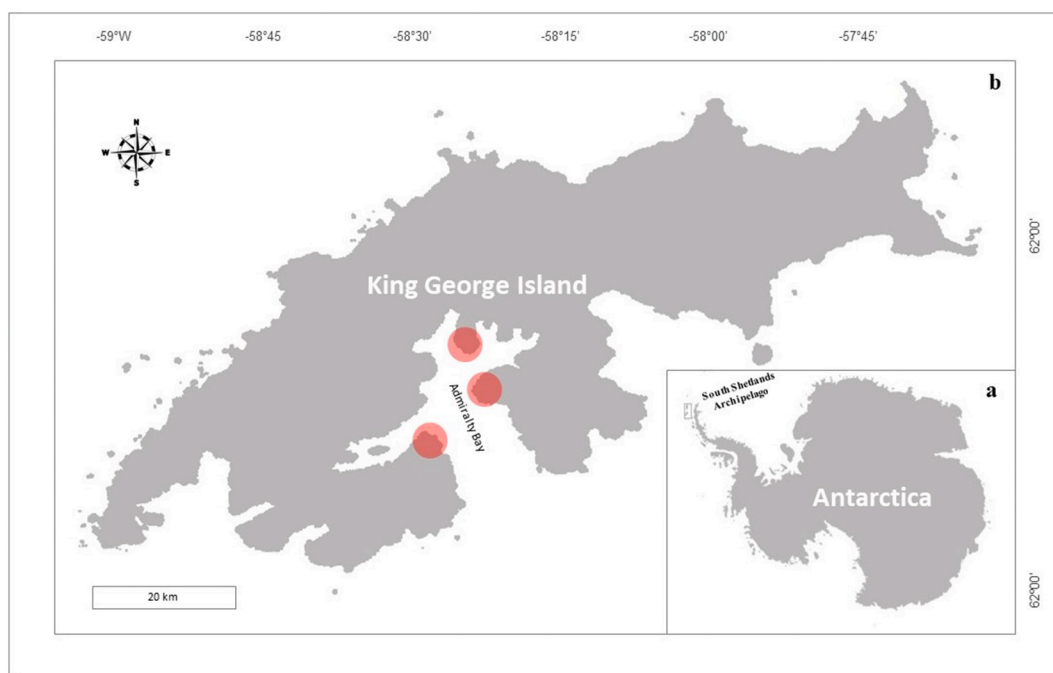
To fill knowledge gaps concerning the influence of trophic interaction and migration on Antarctic birds, our study aims to explore the multifaceted factors affecting the exposure of multiple species of Antarctic seabirds to mercury. While we recognize the intricate dynamics of Hg transport and accumulation, guided by an array of factors such as temperature, chemistry, and oceanographic processes (Bargagli, 2008; Zvěřina et al., 2014), we opt for a more generalized approach to grasp the context within the Antarctic seabirds. Our work draws upon prior research demonstrating that migratory patterns influence Hg exposure, with species migrating to more contaminated areas tending to be more exposed to Hg, a change reflected in their tissues. For example, Fort et al. (2014), observed that little auks wintering in the Northwest Atlantic have twice more Hg concentrations in their feathers than little auks breeding in East Greenland. Additionally, Carravieri et al. (2014a) have documented latitudinal variations in the transfer of Hg to Southern Ocean predators by Wandering Albatrosses, showing that individuals feeding in warmer subtropical waters have higher concentrations of Hg than those feeding in colder subantarctic waters. In our study, specifically, we seek to assess the potential interplay between trophic ecology (characterized by  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ , and  $\delta^{34}\text{S}$  values) and migratory patterns in shaping Hg exposure. To achieve this, we analyzed Hg accumulation patterns in the feathers and eggs of eight Antarctic species. Our hypotheses were as follows: (1) Migratory seabirds, specifically those migrating further north, will exhibit higher Hg concentrations compared to those migrating into the Southern Hemisphere and resident Antarctic birds; and (2) the trophic ecology of Antarctic seabirds, as determined by stable isotope values, will influence their Hg concentrations.

## 2. Materials and methods

### 2.1. Sampling

Eggs of Adélie penguins (*Pygoscelis adeliae*,  $n = 17$ ), Chinstrap penguins (*P. antarcticus*,  $n = 25$ ), Gentoo penguins (*P. papua*,  $n = 13$ ), South polar skua (*Stercorarius maccormicki*,  $n = 20$ ), and Antarctic tern (*Sterna vittata*,  $n = 5$ ), as well as breast feathers of Adélie penguins ( $n = 6$ ), Chinstrap penguin ( $n = 15$ ), Gentoo penguin ( $n = 15$ ), South polar skua ( $n = 17$ ), Snowy sheathbill (*Chionis albus*,  $n = 5$ ), Southern giant petrel (*Macronectes giganteus*,  $n = 17$ ), Kelp gull (*Larus dominicanus*,  $n = 10$ ), and Antarctic tern ( $n = 18$ ), were sampled at Admiralty Bay, King George Island ( $61^{\circ}50'-62^{\circ}15'S$  and  $57^{\circ}30'-59^{\circ}00'W$ ) in the South Shetland Archipelago, Antarctic Peninsula region (Fig. 1), during 2010–2011, 2012–2013 and 2013–2014 austral summers. The feathers from Kelp gulls and Southern giant petrels were collected in their colonies without capturing the birds, following the protocol: 10 to 20 contour feathers (from chest, abdomen, or back) at three distinct points in each colony. Each point collected within the same colony was considered as an independent sample. Skuas were captured using a snare trap, and penguins, snowy sheathbill, and terns were captured during the breeding season with long-handled fish nets. Feathers were packed in individual polyethylene Ziplock bags while non-viable eggs were stored in glass jars and kept frozen until analysis. For the purposes of this study, two groups of birds will be considered: resident species, which consist of the penguins, and migratory species, encompassing all remaining species.

Our study strictly adhered to the established ethical guidelines for research with Antarctic birds. The research was conducted in accordance with Article 3 of Annex II to the Antarctic Treaty Protocol on Environmental Protection and the Antarctic Conservation Measures adopted by the Antarctic Treaty Consultative Meeting. Permits for capture and sample collection were provided to the Penguins and Skua Project (CNPq/MCT: 557049/2009–1) by the Environmental Assessment Group of the Brazilian Antarctic Program (GAAM/PROANTAR) of the Ministry of the Environment (MMA). The species under study are protected by the Antarctic Treaty System, underlining the importance of our non-destructive sampling methods.



**Fig. 1.** Study area: a) Antarctic continent with the South Shetlands Archipelago shown in the rectangle; b) King George Island with sampling locations in Admiralty Bay shown in the circles.

## 2.2. Sample preparation

All feather samples were washed three times with a sequence of Milli-Q ultrapure water (Merck Millipore, USA), 0.01% EDTA (Spectrum, Tedia, USA), and finally Milli-Q ultrapure water (Merck Millipore, USA), for eliminating external contamination, and oven-dried at 50 °C for 24 h before being cut into small pieces using ceramic scissors. Whole eggs were lyophilized and then homogenized.

## 2.3. DMA analysis

Total mercury (THg) concentrations were determined by Atomic Absorption Spectrometry (Direct Mercury Analyzer - DMA 80 Milestone). Approximately 0.02 g of dry powdered feathers and eggs were placed in quartz sample boats for Hg measurements. Blanks were carried through the procedure the same way as the samples, as was the case for the reference materials NIES-1 (human hair; National Institute for Environmental Studies, Japan; n = 4), DORM-2 (fish muscle; National Research Council, Canada; n = 4), and Hg standard (100 ppb). The recovery values of both certified materials and the Hg standard were above 90% (Table S1).

## 2.4. Stable isotopes analysis

The analysis of stable isotopes was conducted with a continuous flow-elemental analysis-isotope ratio mass spectrometry (CF-EA-IRMS) system. This system utilized a Vario MICRO cube C–N–S elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) in conjunction with an IsoPrime100 isotope ratio mass spectrometer (IsoPrime, Cheadle, United Kingdom). Isotopic ratios, expressed conventionally as  $\delta$  concentrations in per mil (‰), were compared against international standards including Vienna Pee Dee Belemnite (carbon), Atmospheric Air (nitrogen), and Vienna Canyon Diablo Troilite (sulfur). Certified reference materials (IAEA-C6, IAEA-N2, and IAEA-S1) from the International Atomic Energy Agency (IAEA, Vienna, Austria) were used as primary analytical standards. Secondary analytical standards were sulfanilic acid. Calibration of isotopic ratios was achieved using primary analytical standards. Measurements of secondary analytical (sulfanilic acid) and lab standards (feathers) were made regularly (every 15 analyses) to ensure consistency. Standard deviations for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values were 0.2‰ and 0.4‰, respectively.

## 2.5. Statistical analyses

Statistical analyses were performed in R Studio (R Core Team, 2022) software. Given the small sample sizes of some species (*Chionis albus*, *Sterna vittata*, and *P. adeliae*) and the non-normal distribution of the data even following log transformation, we employed non-parametric tests. As part of the data preprocessing, outliers were removed to ensure a more robust analysis. A Kruskal-Wallis (KW, Package: ggstatsplot, Patil, 2021) test was used for comparing Hg concentration values among different species. The Dunn test was conducted for verifying pairwise comparisons. Spearman rank correlation tests were used to assess the relationship between Hg concentrations and stable isotope ( $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{34}\text{S}$ ) values. The exploration of ecological niches across various species was conducted using the SIBER (Stable Isotope Bayesian Ellipses in R) method, which incorporated  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  data (Jackson et al., 2011). The ellipse areas were estimated using the SEAc correction, and the SEAb (Standard Ellipse Area Bayesian), a Bayesian-derived estimate of the standard ellipse area, was employed to contrast niche widths among groups, which was predicated on the dimensions of the generated ellipse areas and their predicted posterior distributions. Groups exhibiting similar SEAb values suggest analogous isotopic niche widths, indicating their reliance on a similar assortment of prey species and/or foraging habitats. To achieve this, the SIBER method (Jackson et al., 2011) was executed within the R statistical framework (R Core Team, 2022).

## 3. Results

### 3.1. Mercury in resident and migratory seabirds

THg in feathers and eggs of eight species of nesting seabirds from King George Island are displayed in Table 1 and Fig. 2. THg in feathers (Fig. 2a) ranged as follows (descending order): Southern Giant Petrel > South Polar Skua > Snowy Sheathbill > Kelp Gull > Antarctic Tern > Chinstrap Penguin > Gentoo Penguin > Adélie Penguin. THg in eggs (Fig. 2b) ranged as follows: South Polar Skua > Antarctic Tern > Chinstrap Penguin > Adélie Penguin > Gentoo Penguin.

The Kruskal-Wallis (KW) and pairwise Dunn's Test indicated significant differences in Hg feather concentration among the species (KW = 75.11, Dunn's Test,  $p < 0.001$ ; Fig. 2a). Specifically, the Southern Giant Petrel and South Polar Skua exhibited significantly higher Hg concentrations in their feathers than *Pygoscelis* penguins. Furthermore, the Southern Giant Petrel had significantly higher Hg concentrations than the Antarctic Tern and Kelp Gull.

Concerning egg Hg concentrations, the South Polar Skua displayed a significantly higher THg concentration than the *Pygoscelis* penguins and the Antarctic tern (KW,  $H = 51.47$ , Dunn's Test,  $p < 0.001$ ; Fig. 2b). Notably, the Adélie Penguins showed significantly lower THg values than the Chinstrap Penguins.

When considering only feathers from resident species, Chinstrap Penguins had significantly higher Hg concentrations than both the Gentoo and Adélie Penguins (KW = 15.89, Dunn's Test, Figure S1a). Among the migratory species, the Southern Giant Petrels had significantly higher Hg concentrations than Snowy Sheathbills, Kelp Gulls, and Antarctic Terns (KW = 45.39, Dunn's Test, Figure S1b). Moreover, South Polar Skua exhibited significantly higher Hg concentrations than both Antarctic Tern and Kelp Gull.

### 3.2. Trophic ecology and mercury relationships

Building upon the stable isotope data previously published by Padilha et al. (2022) for the same samples, our analysis revealed significant variations among species in  $\delta^{15}\text{N}$  ( $H = 70.1$ ,  $p < 0.001$ ),  $\delta^{13}\text{C}$  ( $H = 70.6$ ,  $p < 0.001$ ), and  $\delta^{34}\text{S}$  ( $H = 88.8$ ,  $p < 0.001$ ) values. Subsequent post-hoc tests revealed that the *Pygoscelis* species and the Antarctic Tern exhibited significantly lower  $\delta^{15}\text{N}$  values ( $p = 0.009$ ) than the other seabird species.

In terms of  $\delta^{13}\text{C}$  values, South Polar Skuas ( $p < 0.001$ ), Southern Giant Petrels ( $p = 0.02$ ), and Kelp Gulls ( $p = 0.02$ ) had significantly higher values than the rest of the seabird species. With reference to  $\delta^{34}\text{S}$  values, South Polar Skuas ( $p < 0.001$ ) and Chinstrap penguins (0.04) displayed significantly lower values than the Snowy Sheathbills, Southern Giant Petrels, and Antarctic Terns.

A positive and significant correlation was observed between  $\delta^{15}\text{N}$  and THg (Spearman,  $R = 0.44$ ,  $t = 4.41$ ,  $p < 0.001$ , Fig. 3). However, there were no significant correlations between  $\delta^{13}\text{C}$  and THg values (Spearman,  $R = 0.02$ ,  $t = 0.11$ ,  $p = 0.909$ ), or  $\delta^{34}\text{S}$  and THg (Spearman,  $R = 0.08$ ,  $t = 0.47$ ,  $p = 0.643$ ).

The SIBER results (Fig. 4) suggest that the core isotopic niches of *Pygoscelis* penguins were distinctly separated from those of the migratory species, except for the Antarctic Tern. Examining feathers from *Pygoscelis* penguins, a moderate overlap of  $0.38\%^{2}$  (equivalent to 26% of its area) was observed between Chinstrap and Gentoo Penguins. The overlap was considerably lower between the Gentoo and Adélie Penguins ( $0.13\%^{2}$ ), as well as between Chinstrap and Adélie Penguins ( $0.02\%^{2}$ ). The smallest isotopic niche was attributed to the Gentoo Penguin, while the largest was observed in the Antarctic Tern (Figure S2).

**Table 1**

Total mercury concentrations (mean  $\pm$  SD, ng. g<sup>-1</sup>, dry weight) in feathers (F) and eggs (E) of migratory and resident seabirds from King George Island, Antarctic Peninsula, Antarctica. Stable isotope data from Padilha et al. (2022, 2021).

	Species	Matrix	n	Hg	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)	
Migratory	<i>C. albus</i>	F	5	1331 $\pm$ 294	-19.9 $\pm$ 2.26	10.5 $\pm$ 0.67	17.4 $\pm$ 1.36	
		F	17	4216 $\pm$ 1101	-17.6 $\pm$ 0.84	13.2 $\pm$ 1.11	14.6 $\pm$ 1.34	
	<i>L. dominicanus</i>	E	20	1723 $\pm$ 660	-	-	-	
		F	10	1320 $\pm$ 646.2	-18.3 $\pm$ 0.94	11.1 $\pm$ 0.53	16.7 $\pm$ 2.00	
	<i>M. giganteus</i>	F	17	5667 $\pm$ 1500	-21.2 $\pm$ 1.60	12.8 $\pm$ 0.67	17.6 $\pm$ 0.62	
		F	18	1254 $\pm$ 400	-23.8 $\pm$ 2.37	9.28 $\pm$ 1.84	17.4 $\pm$ 0.80	
	<i>S. vittata</i>	E	5	438 $\pm$ 352	-	-	-	
		F	6	285 $\pm$ 147	-24.5 $\pm$ 0.80	9.72 $\pm$ 0.47	14.9 $\pm$ 1.07	
	Resident	<i>P. adeliae</i>	E	17	236 $\pm$ 161	-	-	-
			F	15	910 $\pm$ 364	-25.4 $\pm$ 0.74	9.25 $\pm$ 0.50	14.4 $\pm$ 1.07
<i>P. antarcticus</i>		E	25	263 $\pm$ 241	-	-	-	
		F	15	464 $\pm$ 236	-24.7 $\pm$ 0.69	9.61 $\pm$ 0.46	15.2 $\pm$ 0.97	
<i>P. papua</i>		E	13	218 $\pm$ 318	-	-	-	

## 4. Discussion

### 4.1. Mercury concentrations in Antarctic seabirds: comparing resident and migratory species

Our findings refute our first hypothesis, which posited that (1) migratory seabirds, specifically those migrating further north, will exhibit higher Hg concentrations compared to those migrating into the Southern Hemisphere and resident Antarctic birds. Hg reaches the surface of the ocean mainly by transport and deposition, and the exchange between the atmosphere and ocean leads to its redistribution across the Earth's surface (Lamborg et al., 1999). However, it is known that the Northern Hemisphere (NH) is more contaminated than the Southern Hemisphere (SH) since it concentrates most of the world's population, industries, and, consequently, pollutant emissions (Yan et al., 2011; Sprovieri et al., 2010). For this reason, it was expected that species migrating to the NH would have higher Hg concentrations than species that remain in the SH. However, this does not appear to be the main feature driving Hg concentrations in these species.

Padilha et al. (2023) found that migration appear to influence the concentrations of trace elements such as Li, Be, Ca, As, Mg, Zn, Sn, and Pb in feather and eggs of the same species studied here, as these elements were detected in higher concentrations in birds migrating to the Northern Hemisphere compared to those migrating to the Southern Hemisphere. A similar pattern was observed for organic contaminants such as Perfluoroalkylated compounds using both feather and eggs (Padilha et al., 2022; Roscales et al., 2016). In those previous studies, even though feathers and eggs represent different biological timeframes for the accumulation of these compounds, they exhibit similar patterns of migration-related differences. However, the findings from the present study suggest that Hg concentrations do not seem to be significantly affected by migration. Evidenced by species like the South Polar Skua, which migrate to the Northern Hemisphere, and the Southern Giant Petrel, which during migration remains in the Southern Hemisphere, displaying similar Hg concentrations (Patterson and Hunter, 2000).

Despite these findings, observations of high concentrations of Hg in the South Polar air and in local biota during summer months indicate potential for future concerns (Bargagli, 2008). Associated with the

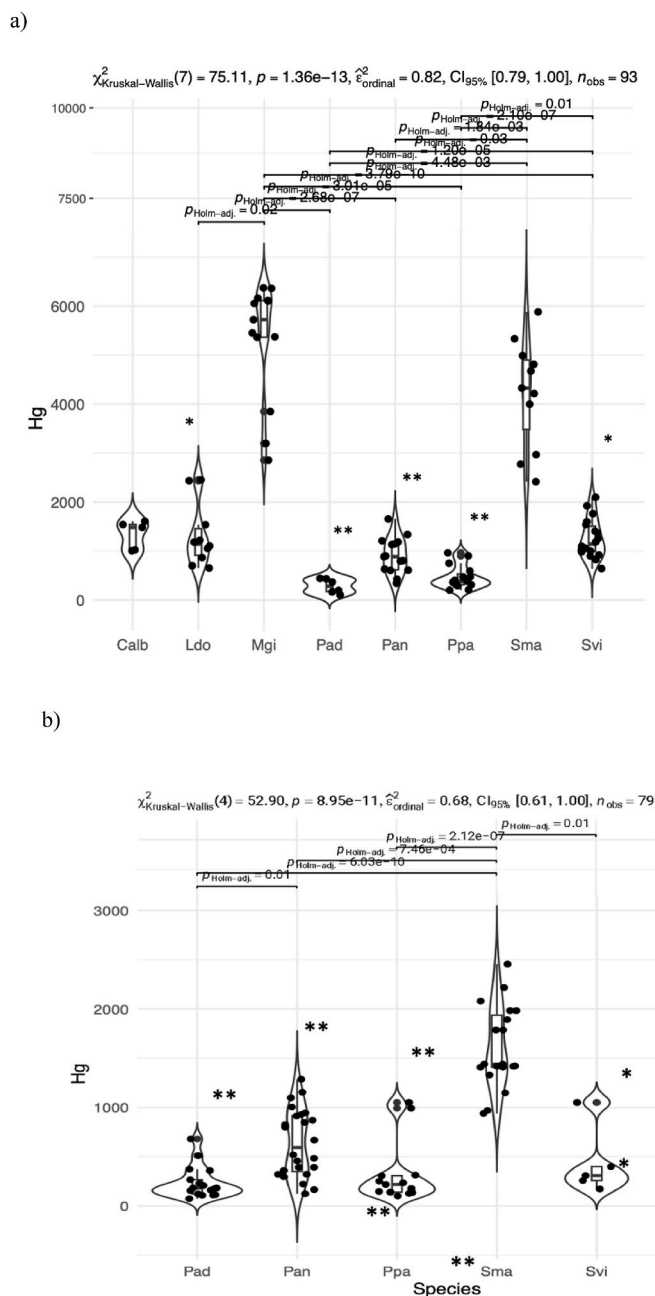
increase in anthropogenic emissions within the Southern Hemisphere (Bargagli, 2008), and potential influences from factors such as temperature, wind patterns, and oceanography, there is an apprehension that Hg released by melting ice in Antarctica may become a substantial Hg source (Li et al., 2020; Matias et al., 2022). This process may be exacerbated with top predators of the Antarctic fauna, which exhibit high Hg bioaccumulation and biomagnification, releasing and remobilizing the accumulated Hg (e.g., through death and decay) back into the local environment (Cipro et al., 2017; Zvěřina et al., 2017). In our study, the Southern Giant Petrel exhibited mean Hg concentrations greater than 5000 ng g<sup>-1</sup>, a level that warrants attention. This finding aligns with previously documented levels that have shown deleterious effects on breeding success in similar species (Burger and Gochfeld, 1997; Goutte et al., 2014a, 2014b). The observed value in the Southern Giant Petrel highlights the importance of continued monitoring and underscores potential ecological concerns.

Complementing our discussion on the first hypothesis - which suggested that migratory Antarctic seabirds would exhibit higher Hg concentrations than resident birds - we found this assumption to be refuted. Migration did not appear to be a decisive factor, given that the penguins and Antarctic Terns exhibited lower concentrations of Hg in both feathers and eggs compared to the other species. Cipro et al. (2017) suggest that the higher levels of THg found in the eggs and feathers of the Skua sp., and the Southern Giant Petrel, as compared to the *Pygoscelis* penguins and the Antarctic Terns, are likely attributed to their higher trophic positions in the food chain.

### 4.2. Comparison with previous studies

Our study results reveal insights into the Hg concentrations in the feathers and eggs of migratory Antarctic seabirds, adding to the limited existing body of research on this subject (refer to Table 2 for a comprehensive comparison) (Bargagli et al., 1998; Cipro et al., 2017; de Moreno et al., 1997; Matias et al., 2022).

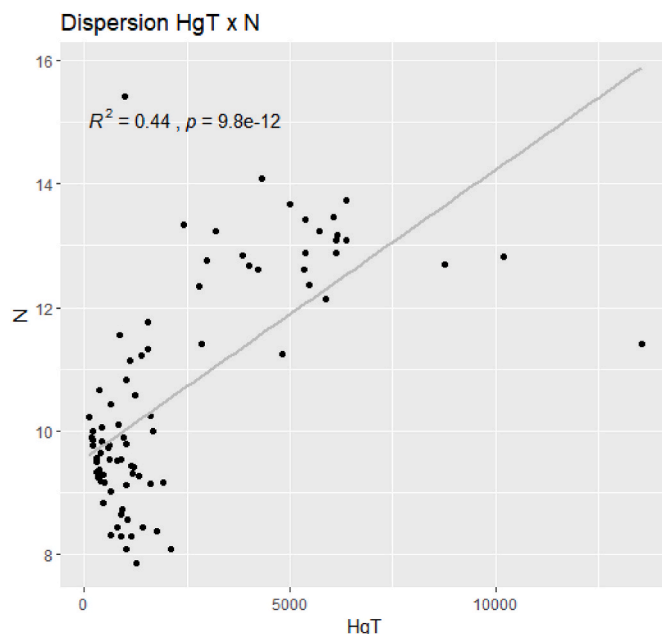
When examining the feathers, our data shows a mean Hg concentration in the South Polar Skua of 4216  $\pm$  1101 (ng.g<sup>-1</sup> dry weight-dw), which is higher than the 2910  $\pm$  1930 (ng.g<sup>-1</sup> dw) observed by Bargagli et al. (1998) in Terra Nova Bay, which emphasizes the need to



**Fig. 2.** Multiple comparison of total mercury feather (a) and eggs (b) concentration values among different species of antarctic seabird (Kruskal-wallis test). *Pygoscelis papua* (Ppa), *P. antarcticus* (Pan), *P. adeliae* (Pad), *Chionis albus* (Calb), *Sterna vittata* (Svi), *Macronectes giganteus* (Mgi), *Stercorarius maccormicki* (Sma), *Larus dominicanus* (Ldo). Each boxplot displays the distribution of mercury levels for a species, with the line in the middle of the boxplot representing the median. Lines at the top of the graph indicate pairwise comparisons between species indicating statistically significant differences after Holm adjustment for multiple testing. \* $p < 0.05$ , \*\* $p < 0.001$ .

continually monitor and reassess Hg levels in this species due to their potential fluctuations over time and across different environments. In the case of the Kelp Gull and the Southern Giant Petrel, our findings display lower mean THg concentrations ( $1320 \pm 646$  and  $5667 \pm 1500$   $ng\ g^{-1}$  dry weight respectively) than [Matias et al. \(2022\)](#) (Table 2), this discrepancy underlines regional variations in mercury exposure and bioaccumulation, presenting a compelling avenue for further research.

Our study represents the first examination of the THg levels in Snowy Sheathbill and Antarctic Tern feathers, providing a valuable baseline for



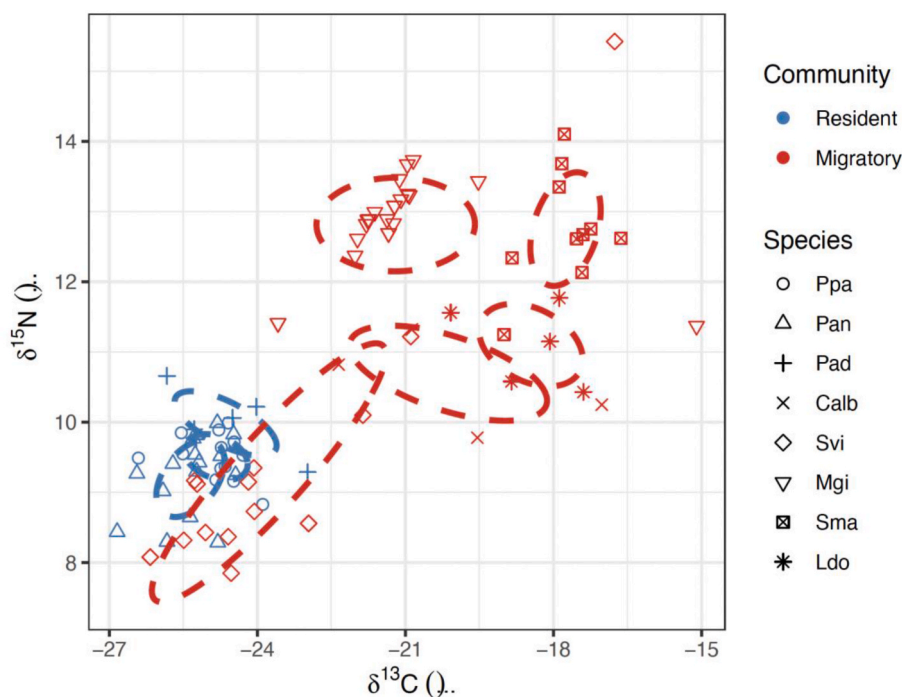
**Fig. 3.** Positive correlation between total mercury (THg  $ng.g^{-1}$  dw) concentrations for all species of Antarctic seabirds (all species together) and  $\delta^{15}N$  values (expressed in per mill, ‰).

future research. These novel findings emphasize the necessity of expanding Hg studies to more species, contributing to a more comprehensive understanding of the environmental impact on Antarctica's unique bird population.

Similarly, the THg concentrations found in the eggs of the species studied here also offer interesting comparisons (Table 2). Our mean Hg concentration in South Polar Skua eggs ( $1723 \pm 660$   $ng\ g^{-1}$ ) aligns closely with previous findings by [Bargagli et al. \(1998\)](#);  $1610 \pm 1220$   $ng\ g^{-1}$ ) and is consistent with the value reported by [Cipro et al. \(2017\)](#) to skuas sp., ( $2520$   $ng\ g^{-1}$ ), reinforcing the consistency of these levels over time (Table 2). When considering the Antarctic Tern eggs, our mean THg concentration ( $438$   $ng\ g^{-1}$  dry weight) falls within the range reported by [de Moreno et al. \(1997\)](#) and [Cipro et al. \(2017\)](#), further confirming our study's alignment with previous research.

As indicated in Table 2, our findings on THg concentrations in *Pygoscelis* penguins' feathers, ranging from 105 to 1654  $ng\ g^{-1}$  dry weight, are well-aligned with prior research. These concentrations are similar to those reported on King George Island, with THg concentrations from 156 to 1648  $ng\ g^{-1}$  ([Souza et al., 2020](#)), and 180–1976  $ng\ g^{-1}$  ([Polito et al., 2016](#)) in *Pygoscelis* penguin's feathers. This consistency trend across studies underscores the stability of THg levels in penguins' feathers, suggesting a common exposure source or comparable bioaccumulation patterns across different penguin populations. The high variability shown in the present and other studies with penguins may be attributed to several factors that were not controlled for in our analysis. Specifically, the penguins in our samples might have varied in age, gender, and even molt stage, all of which are known to influence THg concentrations in feathers ([Carravieri et al., 2014a,b](#); [Souza et al., 2020](#); [Polito et al., 2016](#)). These variations can result in discrepancies across samples, even from the same location or species ([Souza et al., 2020](#); [Polito et al., 2016](#)).

Concerning penguin eggs, we report whole eggs mean concentrations of  $263 \pm 241$ ,  $236 \pm 161$   $ng\ g^{-1}$  and  $218 \pm 318$   $ng\ g^{-1}$  for Chinstrap, Adélie and Gentoo penguins respectively, which are comparable to those found by [Bargagli et al. \(1998\)](#), [Brasso et al. \(2012\)](#), and [Cipro et al. \(2017\)](#). This consistency of THg levels in penguin eggs across different studies further solidifies the notion that mercury exposure and accumulation could be relatively stable in these species.



**Fig. 4.**  $\delta^{13}\text{C}$  vs.  $\delta^{15}\text{N}$  biplot in feathers of adults of Gentoo (Ppa), Chinstrap (Pan) and Adélie (Pad) penguins, representing the corrected Standard Ellipses Areas (SEAc) calculated with the SIBER model in R. *Pygoscelis papua* (Ppa), *P. antarcticus* (Pan), *P. adeliae* (Pad), *Chionis albus* (Calb), *Sterna vittata* (Svi), *Macronectes giganteus* (Mgi), *Stercorarius maccormicki* (Sma), *Larus dominicanus* (Ldo). Blue indicates resident while red indicates migratory species.

**Table 2**

Mean concentrations ( $\pm$  standard deviation) and range (Min-Max) of mercury in feathers and eggs: A comparison with previous studies.

Species	Matrix	Local	THg	Reference
South polar skua	Feather	Terra Nova Bay	2910 $\pm$ 1930	Bargagli et al. (1998)
<i>Pygoscelis</i> penguins	Feather	King George Island	180 to 1976	Polito et al. (2016)
<i>Pygoscelis</i> penguins	Feather	King George Island	156–1648	Souza et al. (2020)
Kelp Gull	Feather	Livingston Island	10800 $\pm$ 6750	Matias et al. (2022)
Southern Giant Petrel	Feather	Livingston Island	12100 $\pm$ 14200	Matias et al. (2022)
Antarctic tern	Egg	Antarctic Peninsula	100	De Moreno et al., 1997
South polar skua (albumen)	Egg	Terra Nova Bay	1610 $\pm$ 1220	Bargagli et al. (1998)
Adélie penguins (albumen)	Egg	Terra Nova Bay	260 $\pm$ 80	Bargagli et al. (1998)
Gentoo penguin (albumen)	Egg	Henry Doorly Zoo in Omaha, NE	162 $\pm$ 29	Brasso et al. (2012)
Adélie penguins	Egg	King George Island	75	Cipro et al., (2017)
Gentoo penguin	Egg	King George Island	133	Cipro et al., (2017)
Chinstrap penguin	Egg	King George Island	599	Cipro et al., (2017)
Skua sp.	Egg	King George Island	2520	Cipro et al., (2017)
Antarctic tern	Egg	King George Island	664	Cipro et al., (2017)

The findings have revealed that while THg levels in both feathers and eggs of various species align with previous studies, they do not appear to be significantly affected by the birds' migratory destinations. Instead, other factors such as trophic position, dietary habits, or regional environmental conditions may play a more significant role. This study also

underscores the need for continuous monitoring of Hg levels in Antarctic seabirds, particularly in light of possible future changes in sea ice coverage and anthropogenic emissions. As our understanding of Hg dynamics in these species deepens, these findings offer valuable insights and directions for future research.

#### 4.3. Stable isotope patterns and their influence on mercury exposure in Antarctic seabirds

The use of stable isotopes of N, C, and S allowed us to infer the trophic level and ecology of the birds, which can affect their Hg exposure (Cipro et al., 2017). The isotope ratio variation among species and locations points to differences in diet and trophic level among the birds (Tables 1 and 2; Padilha et al., 2022). This highlights the importance of considering the diet and trophic level of the birds when assessing their exposure to mercury. Our  $\delta^{15}\text{N}$  values results confirm that the Southern giant petrel and South polar skua are top predators in the Antarctic and sub-Antarctic food webs, relying on a diet of fish, marine mammals, and penguin carcasses (Padilha et al., 2022; Roscales et al., 2016). Studies have shown that the levels of Hg in the feathers of seabirds from Antarctica can be elevated, particularly in species that feed at higher trophic levels (Carravieri et al., 2017; Souza et al., 2020). According to Shirihai (2019), the Antarctic seabirds generally have a diet based on crustaceans such as krill and fish with some species exhibiting scavenging and predatory behaviors. The three *Pygoscelis* species mainly consume krill and some fish, while the South Polar Skua predominantly feeds on fish, particularly *Pleurogramma antarcticum*, along with preying on penguin chicks and eggs. Antarctic Terns feed on small fish and occasionally consume limpets, euphausiids, and crustaceans. Snowy Sheathbills adopt a varied diet, stealing krill and fish from penguins, as well as consuming eggs, small chicks, carrion, algae, invertebrates, and even human refuse. Southern Giant Petrels, functioning as scavengers and predators, often feed on penguin and seal carcasses, while Kelp Gulls scavenge around human settlements, consuming a diverse range of prey including mollusks, fish, birds, and invertebrates. The diversity in dietary habits and trophic levels among these species may contribute to

the observed differences in Hg concentrations.

The comparable Hg and  $\delta^{15}\text{N}$  values in Antarctic Terns and Chinstrap Penguins confirm that, despite the Antarctic Terns' migratory pattern to marine environments in the Southern Hemisphere during winter (i.e., Australia, New Zealand, South Africa, and South America) as noted by Patterson and Hunter (2000), their trophic level is similar to that of penguins, and their dispersal did not result in higher Hg concentrations.

The Stable Isotope Bayesian Ellipses in R (SIBER) results further elucidate the dietary preferences, trophic levels, and consequent mercury exposures of the species under study (Figs. 2 and 4). We discovered that the core isotopic niches of the *Pygoscelis* penguins were distinct from those of the migratory species, excluding the Antarctic Tern. This suggests that the dietary preferences and associated mercury exposures of these penguins differ from those of other species, emphasizing the importance of considering individual species' unique ecology when evaluating mercury exposure.

The overlap in isotopic niches between different *Pygoscelis* penguin species, as highlighted by Padilha et al. (2021), provides further understanding of their shared dietary habits and corresponding mercury exposures. For instance, the moderate overlap between Chinstrap and Gentoo Penguins suggests similar dietary preferences, primarily krill and fish, which is mirrored in their comparable mercury concentrations. However, it is noteworthy that despite these similar diets, Chinstrap Penguins tend to exhibit higher Hg feather levels than both Gentoo and Adélie Penguins. This discrepancy can be attributed to the Chinstrap Penguins' preferential feeding on mesopelagic fish (Polito et al., 2016). These fish have been shown to contain higher concentrations of Hg compared to the epipelagic and benthic fishes that form the staple diet of Gentoo and Adélie Penguins (Polito et al., 2016; Souza et al., 2020). Thus, our findings, in line with previous research, underscore the complexity of the relationship between diet, trophic level, and mercury exposure, where even species with similar dietary habits may exhibit significant differences in mercury exposure due to variations in their specific food preferences.

Our results reinforce the second hypothesis that the trophic ecology of Antarctic seabirds, as determined by stable isotope values, influences their Hg concentrations. The observed positive correlation between  $\delta^{15}\text{N}$  and Hg underscores the significant role dietary factors play in determining Hg exposure. As previously observed by Becker et al. (2016), Antarctic species at higher trophic levels are at greater risk concerning Hg exposure. This study elucidates the complex relationship between stable isotope patterns, dietary habits, and mercury exposure in Antarctic seabirds. Notably, the trophic level and specific dietary preferences of a species may have a greater influence on mercury levels than migratory patterns. For instance, within species occupying the same trophic position, such as *Pygoscelis* penguins, dietary preferences can significantly influence Hg concentrations (Polito et al., 2016). These findings underscore the need for a nuanced understanding of bird ecology when assessing their exposure to mercury. Consideration should extend beyond migratory habits to include trophic ecology, as reflected by stable isotope values. Future research will benefit from further exploration of these intricate relationships, providing a more comprehensive understanding of mercury dynamics in Antarctic bird populations.

#### 4.4. Differences in THg concentrations in eggs and feathers

The findings of the present study confirmed that the feathers of Antarctic seabirds contain higher Hg concentrations than their eggs. This is consistent with previous studies that have found a similar pattern (Burger et al., 2009; Tsipoura et al., 2011). However, this also may be affected on the part of the egg analyzed, since due to the greater affinity of Hg for protein matrices, it could accumulate more in albumin (i.e., richer in protein) than yolk (i.e., richer in lipid) (Bond and Diamond, 2009; Brasso et al., 2012). For example, Bond and Diamond (2009) when investigating Leach's storm petrel from Canada found higher THg

concentrations in feathers ( $4855 \pm 2791 \text{ ng g}^{-1}$ ) compared to whole eggs ( $1170 \pm 297 \text{ ng g}^{-1}$ ). However, although the authors do not discuss the differences between feathers and eggs, albumin concentrations ( $5501 \pm 1535 \text{ ng g}^{-1}$ ) slightly exceeded feather concentrations. Similarly, Brasso et al. (2012) found more THg in albumen (over 90%) than in other components of Gentoo penguin eggs.

Despite these specificities, this study reinforces that feathers serve as a reliable indicator of Hg exposure in birds. Over the years, this matrix has been recommended as an alternative to the use of invasive methods (Burger, 1993; Jaspers et al., 2019; Lodenius and Solonen, 2013; Squadrone et al., 2019), which is particularly needed in the Antarctic environment where the species are under a high degree of protection. This study confirms that using loose feathers collected from reproductive colonies is a dependable method to assess the contamination levels in little-studied species. This approach can help fill the information gap about these species, which are difficult to capture or protect. In this way, in the present study it was possible for the first time to investigate Hg contamination in feather of Snowy sheathbill and Antarctic Tern feathers.

## 5. Conclusion

Contrary to what we hypothesized, (1) the birds that migrate to the NH did not show higher Hg values than those that restrict their migration to the SH, (2) and not all migratory species presented higher levels of mercury than the resident ones, since Antarctic Tern and Chinstrap Penguins have not shown a significant difference in Hg concentration. In addition, (3) not all trophic ecological indicator ( $\delta^{13}\text{C}$ ,  $\delta^{34}\text{S}$ ) had a positive correlation with Hg concentration. It was the case only for  $\delta^{15}\text{N}$ , which is related to trophic position. This study provides new insights into the exposure of Antarctic seabirds to Hg. By using both traditional methods of measuring Hg and stable isotope analysis, we were able to infer the diet and trophic level of the Antarctic seabirds and understand how they relate to Hg exposure. Our findings highlight the need for more research to understand the complex factors affecting Hg accumulation, such as temperature, ocean patterns, wind, and chemistry. While our initial ideas aimed for a general understanding, the complexity of these factors requires a more detailed study. Future research should examine these influences on Hg exposure more closely, helping us better understand how they affect different species. This will guide conservation efforts and improve our understanding of contaminants in the Antarctic environment.

## Credit author statement

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## 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.



## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.chemosphere.2023.139871>.

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