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Factors that influence trace element levels in blood and feathers of Pygoscelis penguins from South Shetland Islands, Antarctica

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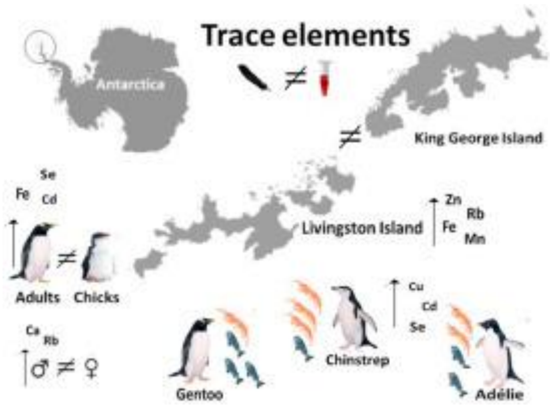
Abstract :

Contaminant levels are lower in Antarctica than elsewhere in the world because of its low anthropogenic activities. However, the northern region of the Antarctic Peninsula, is close to South America and experiences the greatest anthropogenic pressure in Antarctica. Here, we investigated, in two Antarctic Peninsula islands, intra and interspecific factors that influence the concentrations of 17 trace elements (TEs) in blood and feathers of three penguin species breeding sympatrically in relation to their trophic ecology assessed via a stable isotopic approach (C, N and S). Geographical location, foraging zone (delta C-13 and delta S-34) and diet influences the interspecific difference, and sex and maturity stage diet influence the intraspecific difference of Pygoscelis penguins. Penguins from Livingston showed higher values (mean, ng. g(-1), dry weight - dw) of Zn (103), Mn (0.3), and Fe (95) than those from King George Island (Zn: 80, Mn: 1.9, and Fe: 11). Gender-related differences were observed, as males showed significantly higher values (mean, ng. g(-1), dw) of Rb (3.4) and delta N-15 in blood of gentoo, and Ca (1344) in Adelie feathers. Chicks of gentoo and Adelie presented higher Zn, Mg, Ca, and Sr and lower C-13 values in blood than adults. The highest concentrations (mean, ng. g(-1), dw) of Cd (0.2) and Cu (26), and the lowest delta N-15 values were found in chinstrap. Geographical, intraspecific (i.e., ontogenetic and gender-related) and interspecific differences in feeding seemed to have influenced TE and stable isotope values in these animals. The TE bioaccumulation by penguins may have also been influenced by natural enrichment in environmental levels of these elements, which seems to be the case for Fe, Zn, and Mn. However, the high level of some of the TEs (Mn, Cd, and Cr) may reflect the increase of local and global human activities.

Graphical abstract



Highlights

► Trace elements (TEs) levels are influenced by sex and maturity stage of penguins. ► Foraging zone and geographical location mainly explain TE levels in *Pygoscelis* spp. ► Trophic position ($\delta^{15}N$) poorly explain TE levels in *Pygoscelis* penguins.

Keywords : Marine pollution, Heavy metal, Antarctic seabird, Stable isotopes

1. Introduction

The contamination of Antarctic environments largely reflects the use of chemicals in the southern half of the planet, a hemisphere with comparatively little land mass and smaller human population (Nash, 2011). This, combined with the shorter food chains of the Southern Ocean and the absence of subsisting human populations on the Antarctic continent, results in lower theoretical chemical risk for Antarctic biota (Abrams, 1985; Metcheva et al., 2010).

Despite the low environmental concentrations of pollutants in Antarctica these have been increasing over time, at global level, due to chemical pollution and to the global transport of persistent, bioaccumulative and toxic substances (PBTs) in the atmosphere and through oceanic circulation (Das et al., 2017; Jerez et al., 2011). In addition, at regional level, impacts due to the increase of research facilities and tourist activities, that occur mainly in the summer, has been detected in the region over the years (Bargagli, 2008; Jerez et al., 2011; Tin et al., 2009). From 1989-1990 (3,146 tourists) to 2018-2019 (55,489 tourists) there was a considerable increase in tourism in Antarctica ("Data & Statistics," 2021). This escalation in human presence over the years increases environmental concentrations of pollutants as trace elements (TEs), which are contaminants of concern due to their toxicity and bioaccumulative nature (Nordberg et al., 2014). In addition to tourism, few studies have investigated the contribution of the scientific stations operations and logistics for the accumulation of TEs (Hong et al., 2002; Kakareka et al., 2020; Tin et al., 2009). Naval operations (ballast water, fuel combustion), land-based activities (transport, maintenance of the research station), and the inefficient sewage management practices at several scientific stations contribute to local pollution of PBTs, that has the capacity to damage the local fauna and flora (Dobaradaran et al., 2018; Tin et al., 2009). Previous studies observed higher content of TEs in snow near human impacted areas when compared with the ice sheet (Kakareka et al., 2020) and higher TEs levels in feather samples of penguins from places with more anthropogenic influence (Jerez et al., 2011).

In addition to anthropogenic influence, the literature has shown a natural enrichment of TEs in Antarctic food webs through local volcanism, algal bloom, and upward flux of TE-rich waters. Antarctica is surrounded by the Antarctic Circumpolar Current, and the overturning circulation in the Southern Ocean replace superficial waters with deep waters from the surrounding oceans (Atlantic, Indian and Pacific), which can carry TEs with them (Bargagli et al., 1996; Bengtson Nash et al., 2010; Deheyn et al., 2005; Jiankan et al., 1999). However, the main source of pollutants for the Antarctic environment is global, not local (Bargagli, 2008).

Our study area, the northern region of the Antarctic Peninsula, is close to South America and experiences the greatest anthropogenic pressure in Antarctica. It is therefore vulnerable to the increase in contaminant concentrations (Espejo et al., 2017; Tin et al., 2009). TEs levels in sediments from South Shetland Islands and the northern zone of the Antarctic Peninsula has increased, and this seems to result from a growth in local and global anthropogenic activities (Celis et al., 2012, 2015; Espejo et al., 2017). King George Island, located in Antarctic Peninsula, presents a great concentration of anthropogenic activities, where most Antarctic scientific stations in the region are located, being one of the favorite destinations for tourists as well (Jerez et al., 2011; Tin et al., 2009).

Penguins are valuable sentinels of environmental pollution, due to their abundance and longevity, which is approximately 20 years (Bargagli, 2008; Burger and Gochfeld, 2000; Herman et al., 2017; Jerez et al., 2011; Metcheva et al., 2010). Antarctic penguins provide an important contribution to total avian biomass in the Southern Ocean (Bargagli, 2008; Metcheva et al., 2006). *Pygoscelis* penguins have a circumpolar distribution, and as a result, their tissues are matrices of choice for contaminant biomonitoring in Antarctica (Jerez et al., 2013, 2011; Metcheva et al., 2006). Generally, chinstrap (*Pygoscelis antarcticus*) and Adélie (*Pygoscelis adeliae*) penguins forage primarily on Antarctic krill further offshore in pelagic areas (Trivelpiece et al., 1987). Gentoo penguins (*Pygoscelis papua*) forage on a mix of krill and fish, in deeper benthic habitats (Herman et al., 2017; Miller et al., 2010), but the literature reported geographical variations in the diet among *P. papua* due to dissimilarities in prey availability at different breeding locations (Deheyn et al., 2005). Differences in foraging habitat use, prey preferences, larger-scale migration, and dispersal strategies can expose seabirds breeding in the same location to different TEs concentrations (Carravieri et al., 2014; Polito et al., 2015).

Little is known about polar seabirds contamination by TEs, which is highly variable among taxa; thus, there is a need for further studies to better understand the accumulation patterns of TEs in seabirds' bodies (Espejo et al., 2017; Jerez et al., 2013a; Metcheva et al., 2010). Previous studies have basically focused on TEs in feathers of adults and on interspecific differences between *Pygoscelis* penguins (Jerez et al., 2011; Metcheva et al., 2006). Although diet represents the main source of TEs for consumers, factors other than trophic position, ontogenetic and sex-related differences, foraging habitat, or movements have been suggested to drive accumulation patterns in wildlife and still poorly understood (Colominas-Ciuró et al., 2018; Herman et al., 2017). Therefore, a better understanding of the presence of TEs in polar seabirds can help in an assessment of the sources and fate of these pollutants in remote regions and shed new light on the global transport and distribution of TEs.

To fill this gap, we measured the concentrations of 17 TEs and the stable isotopes compositions (C, N, S) in blood and feather of *Pygoscelis* penguins (*P. adeliae*, *P. antarcticus, P. papua*) breeding in sympatry in the South Shetland Islands to investigate individual and populational differences in trace element concentrations, and to assess how their trophic ecology can influence their exposure to TEs. In addition, we explored small scale geographical differences between King George and Livingston Islands, in order to better understand how natural sources and/or anthropogenic pressures can influence TEs values.

2. Material and methods

2.1 Sampling

Feather and blood sampling were performed at King George (61° 50' S - 57°30' W) and Livingston (62° 39' S - 60° 35' W) Islands in the South Shetland Archipelago, Antarctic Peninsula region, during the 2012-2013 and 2013-2014 austral summers (Figure 1). Adult and juvenile penguins were captured during the breeding season with long-handled fish nets. Each captured animal was banded with an aluminum ring, weighed, and measured (beak size, wing, tail) with digital caliper or ruler and freed after measurements and sampling. Breast feather samples of all species were cut close to their base with stainless steel scissors. Blood samples (1 mL) were taken from each individual using disposable syringe and needle, stored into identified Eppendorfs, and kept frozen at -80 °C until being freeze-dried prior to TEs measurements. The number of samples per location, species, gender, state of maturity, and tissue are presented in table 1.

2.2 Sample preparation

Breast 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) again, for eliminating external contamination, and oven-dried at 50 °C for 24 h (Marques et al., 2007) before being grounded into a fine powder using stainless steel scissors. For trace element measurements, aliquots of approximately 0.1 g of dry powdered feathers and freeze-dried blood samples were subjected to acid digestion in the microwave, in Teflon vessels, with the addition of 5 mL of nitric acid (HNO_3, 65% suprapur Merck, Germany), 2 mL of hydrogen peroxide (H_2O_2, 30 % suprapur Merck, Germany) and 1 mL of Milli-Q ultrapure water (Merck Millipore, USA). For stable isotopes 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.

2.3 ICP-MS analysis

Lithium (Li), Be, Mg, Ca, Cr, Fe, Mn, Ni, Cu, Zn, Se, Rb, Sr, Cd, Sn, Ba and Pb concentrations were determined by inductively coupled plasma - mass spectrometry (ICP-MS), using a Perkin Elmer Elan 9000 spectrometer following the methodology described in Lehnert et al. (2016). Blanks were carried through the procedure in the same way as the samples, as it was the case for the reference materials NIES-1 (human hair) and SERONORM L-3 (whole blood). Reference material results were in good agreement (recovery between 90 and 110%) with the values certified by the National Institute for Environmental Studies (NIES). The detection limits of the method, in $\mu g.\ g^{-1}$, were: 0.046 for Li; 0.049 for Be; 0.38 for Mg; 1.247 for Ca; 0.007 for Cr; 0.917 for Fe; 0.01 for Mn; 0.27 for Ni; 1.063 for Cu; 0.12 for Zn; 0.034 for Se; 0.005 for Rb; 0.005 for Sr; 0.006 for Cd; 0.047 for Sn; 0.004 for Ba; and 1.329 for Pb.

Stable isotope measurements

Stable isotopes 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 Analysensysteme GmBH, Hanau, Germany) coupled to an IsoPrime100 isotope ratio mass spectrometer (Isoprime, Cheadle, United Kingdom). Isotopic ratios were conventionally expressed as δ values 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 ($\delta^{13}C$ = -10.8 ± 0.5 ‰; mean ± SD), IAEA-N2, ($\delta^{15}N$ = 20.3 ± 0.2 ‰; mean ± SD) and IAEA-S1 ($\delta^{34}S$ = -0.3 ‰; mean) as primary analytical standards, and sulfanilic acid ($\delta^{13}C$= -25.9 ± 0.3; $\delta^{15}N$ = -0.12 ± 0.4; $\delta^{34}S$= 5.9 ± 0.6; mean ± SD in each case) as secondary analytical standards. 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 (blood and feathers) analyzed interspersed among samples (one replicate of each standard every 15 analyses) were 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ and 0.4‰ for $\delta^{34}S$.

2.4 Molecular determination of sex

Molecular analyzes of sex were performed at the Laboratory of Marine Genetics, at the Department of Genetics, at the State University of Rio de Janeiro (UERJ) using the molecular technique of the CHD gene developed by Griffiths et al. (1998). Not all adult samples could be determined by gender, so those that could are listed in table 2 and 3.

2.5 Statistical analysis

For statistics, non-parametric (Mann-Whitney U test, Spearman correlation test-r and Kruskal-Wallis) tests were used. We analyzed the relationship between trace element concentrations and stable isotopes among three species of penguins using a principal component analysis (PCA). Linear regression analyses were used to assess the relationship between TEs concentrations and stable isotopes ($\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$) values. Statistical analyses were performed in R (R Core Team, 2019) statistical software and Statistica 7.

Individuals were grouped by species (*P. adeliae*, *P. antarcticus*, *P. papua*), life stage (adult and chick), location (King George and Livingston islands) and sample type (blood and feathers). Ecological niches across different species were explored using the SIBER (Stable Isotope Bayesian Ellipses in R) method (Jackson et al., 2011). The ellipse areas were estimated using the SEA_C correction, as well as the Bayesian modelling (SEA_b, 106 iterations) for intergroup pairwise comparisons (Jackson et al., 2011). The SEA_b (Bayesian estimate of the standard ellipse area) can be used to compare niche widths between groups, based on the size of simulated ellipse areas and their estimated posterior distributions. Groups with similar SEA_b have similar isotopic niche width, i.e., rely on a similar diversity of prey items and/or feeding habitats. For this purpose, the SIBER 2.1.4 method (Jackson et al., 2011) was run in R ("R Core Team (2020). — European Environment Agency," 2020) statistical environment.

3. Results

Essential (Mg, Ca, Fe, Mn, Cu, Zn, Se) and nonessential (Li, Be, Cr, Rb, Sr, Cd, Sn, Ba and Pb) trace element concentrations in blood and feathers of gentoo (*P. papua*), chinstrap (*P. antarcticus*) and Adélie (*P. adeliae*) penguins from King George and Livingston islands are given in Table 2.

No significant correlation was found between blood and feather TE concentrations except for a negative correlation for Rb ($r^2 = 0.65$; $p = 0.003$) in *P. antarcticus*, as well as for a positive correlation for Cu in *P. papua* ($r^2 = 0.49$; $p = 0.039$).

The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values for blood and feathers of *P. papua*, *P. adeliae* and *P. antarcticus* from King George and Livingston islands are shown on Table 3. There was no significant correlation between feather and blood isotope values in any of the three penguin species.

3.1 *Geographical differences*

Significant differences in trace element concentrations in blood and feathers between sampling locations were observed only for *P. papua* adults (Figure S1 of the Supplementary Material). Blood samples from King George Island presented lower Rb concentrations ($p < 0.01$) than those from Livingston. Feather samples from Livingston Island presented higher Zn, Mn, Fe and Rb concentrations ($p < 0.01$) than those from King George Island.

3.2 *Intraspecific differences*

Gender-related differences: Feather and blood concentrations of several TEs were significantly different between males and females ($p > 0.001$), and these sex-related differences were found in distinct patterns for the three studied species (Figure S2 of the Supplementary Material). In *P. papua*, significantly higher blood Rb concentrations were found in males than in females, while in *P. antarcticus*, blood Ca and Zn concentrations were significantly higher in females than in males. In *P. adeliae*, significantly higher Ca values in feathers were found in males than in females. In *P. antarcticus*, Sr values in feathers were significantly higher in females than in males.

Regarding sexual differences in stable isotope ratios, males of *P. papua* showed significantly higher $\delta^{15}N$ values than females for both, blood ($U_{-2.47} = 33$; $p = 0.013$) values. On the other hand, females presented higher $\delta^{13}C$ ($U_{-2.32} = 22$; $p = 0.020$) values in feathers than males.

Ontogenetic differences: Significant differences in blood concentrations of several TEs were observed between adults and chicks, and such dissimilarities were verified for *P. papua* and *P. adeliae* (Figure 2). It was not possible to perform this comparison for *P. antarcticus*, as only adults of this species were sampled. Concerning *P. papua*, chicks showed significantly higher Zn, Mg, Ca, and Sr concentrations than adults, while concentrations of Fe, Se and Cd were significantly higher in adults than in chicks. A similar pattern was observed for *P. adeliae* (Figure 2), *i.e.*, chicks showed significantly higher Zn, Mg, Ca, and Sr concentrations than adults. Still concerning *P. adeliae*, blood Fe and Se concentrations were significantly higher in adults than in chicks. Using weight for investigating the possible occurrence of TE bioaccumulation, significant negative correlations were observed between the weight (g) and two elements, Se (R = -

0.65, $p < 0.001$) and Cu (R = -0.32, $p = 0.008$). In addition, a significant positive correlation was found between the weight (g) and Ca levels (R = 0.30, $p = 0.01$).

For stable isotope, chicks of *P. adeliae* and *P. papua* presented ^{13}C-depleted blood values in comparison to adults ($H_{75.11} = 43.94$; $p < 0.005$ and $H_{80.62} = 43.94$; $p < 0.001$, respectively).

3.3 *Interspecific differences*

Regarding blood concentrations (Fig. 3A), principal component 1 (PC1, 31.6%) had negative loadings of Mg (−0.45), Zn (−0.44), Ca (−0.45) and had positive loadings of Fe (0.30), with the weakest contribution from Cd (0.06). PC1 tended to separate chicks from adults (Fig. 3A). PC2 explained 16.2% of the overall variation, with the strongest positive contributions from Mn (0.43) and Rb (0.43) and the weakest one from Ca (0.05). Regarding feather values (Fig. 3B), principal component 1 (PC1, 29%) had positive contributions from Ca (0.41), Sr (0.38) and Mg (0.34) and the weakest one from Cs (-0.03). PC2 explained 16.4% of the overall variation, with the strongest positive contributions from Rb (0.39) and Zn (0.38) and the weakest one from Mg (−0.43). Nevertheless, there was a clear overlap among the multivariate TE profiles in adults of the three studied species.

The highest blood Li concentrations (H = 20.81, $p < 0.001$) were found in *P. adeliae.* The highest blood values of Mn (H = 8.74, $p = 0.03$), Se (H = 82.46, $p < 0.001$), and Cd (H = 37.16, $p < 0.001$) were found in *P. antarcticus*. The highest feather concentrations of Cu (H = 31.12, $p < 0.001$), Zn (H = 13.31, p < 0.05) and Rb (H = 14.45, $p < 0.05$) were found in *P. antarcticus*; as well as the highest Se concentrations were found in *P. adeliae* (H = 37.52, $p < 0.001$). In addition, Se (H = 41.55, $p < 0.001$)

concentrations were significantly higher in feather of *P. antarcticus* than in *P. papua*, as it was the case for Sr as well (H = 7.28, $p < 0.05$).

3.5 *Stable isotope ratios and trace element patterns*

Regarding feather samples from King George, *P. antarcticus* showed significantly lower values of both $\delta^{15}N$ and $\delta^{13}C$ than *P. adeliae* ($U_{2.50} = 81$, $p = 0.012$ for $\delta^{15}N$; $H_{39.76} = 13.55$, $p = 0.019$ for $\delta^{13}C$) and *P. papua* ($U_{-2.26} = 178$, $p = 0.024$ for $\delta^{15}N$; $H_{44.13} = 13.55$; $p = 0.007$ for $\delta^{13}C$). Regarding $\delta^{34}S$, *P. papua* from King George Island showed significantly higher values than *P. antarcticus* ($H_{28.96} = 13.86$; $p = 0.043$) and *P. papua* ($H_{14.83} = 13.86$; $p = 0.007$) from Livingston Island.

Correlation analyses between stable isotope ratios and TEs concentrations in blood and feathers of *P. papua*, *P. adeliae* and *P. antarcticus* are presented on Figure 4. Significant negative correlations were found between $\delta^{15}N$ and four elements (Cr, Zn, Cd, and Rb) for blood, as well as $\delta^{15}N$ and six elements (Mg, Ca, Cr, Sr, Cd, and Fe) for feather. Positive correlations were found in feathers between $\delta^{15}N$ and five elements (Se, Mg, Ca, Se and Sr). Significant negative correlations were found between $\delta^{13}C$ and six metals (Mg, Ca, Cr, Zn, Cu and Sr) for blood, as well as between $\delta^{13}C$ and three elements (Zn, Cd and Se) for feathers. Significant positive correlations were found between $\delta^{13}C$ and five elements (Fe, Mn, Se, Cd and Cs) in blood samples. Significant negative correlations were found between $\delta^{34}S$ and six elements (Cr, Fe, Mn, Zn, Rb and Ba) for feathers.

3.6 Stable isotope ellipses

SIBER results (Figure 5) suggest that the core isotopic niches of the chicks of *P. papua* and *P. adeliae* were markedly separated from the groups of adults. Regarding feathers from adults, the overlap between the *P. adeliae* and *P. papua* from King George Island was 0.84‰^{2} (i.e., 53% of its area). The overlap between *P. papua* in feathers of adults from King George and Livingston islands was considerable for carbon and nitrogen (0.86‰^{2}, i.e., 64% of its cumulative area). Concerning blood, a moderate overlap was observed in *P. antarcticus* from King George and Livingston islands (0.35‰^{2}, i.e., 22% of its area), and a weak overlap was found in *P. papua* from King George and Livingston islands (0.05‰^{2}, i.e., 7% of its area).

Areas of the standard ellipses associated with each penguin group varied in a narrow range for feathers and a moderate one for blood, with SEAc values ranging from 1.09‰^{2} to 1.33‰^{2} for feathers and from 0.28‰^{2} to 2.21‰^{2} for blood (Figure 6). *P. papua* and *P. adeliae* from King George Island showed smaller isotopic niches than *P. antarcticus* for blood (99.8% and 97.4% of model solutions, respectively) and *P. adeliae* chicks showed the largest isotopic niche (>99% of model solutions).

The three penguin species had similar isotopic niche sizes in King George Island for feathers. *P. papua* from Livingston and King George islands differed in only 36.6% of model solutions.

4. Discussion

To the best of our knowledge, this research analyzed for the first time multivariables in order to understand which factors may influence the exposure of *Pygoscelis* penguins to TEs through the analysis of feather and blood. These matrices did not show significant correlations in TEs and stable isotope values. Significant differences for TEs and stable isotopes values were found among species within the studied breeding localities. TEs interspecific differences are related to diet, foraging zone ($\delta^{13}C$ and $\delta^{34}S$) and geographical location, but poorly by the trophic position ($\delta^{15}N$). This finding on $\delta^{15}N$ may be a consequence of the fact that the penguin species, despite their interspecific variations, share a similar trophic position. The intraspecific variations in TEs levels are influenced by sex (feeding and egg laying) and maturity stage of penguins (feeding habits and bioaccumulation).

4.1 *Correlations between blood and feather values*

No significant correlation was found in the present study between blood and feather TE concentrations or isotope values, except for a negative correlation for Rb, and a positive correlation for Cu. Fenstad et al. (2017) found significant positive correlations between blood and feather concentrations for Se and Cr; however, for the remaining elements (Hg, Pb, Cd, As, Zn, and Cu), blood and feather concentrations did not correlate. Taking into account stable isotopes in *Pygoscelis* penguins, Polito et al. (2016) observed significant positive correlations between blood and feather stable isotope values for $\delta^{15}N$, but not for $\delta^{13}C$. The literature has not shown a clear pattern for the correlation between feather and blood TE concentrations and stable isotopes values. The trace element concentrations in blood represent a short-term dietary exposure to and / or remobilization of circulating contaminants (Evers et al., 2008), while feathers constitute a metabolically inert matrix, whose values correspond to a longer time period than blood (Burger, 1993). Additionally, feathers are generally enriched at ^{13}C and ^{15}N in relation to blood, and the comparison of the raw isotopic data of these two matrices is blurred by specific factors related to the isotopic discrimination of each tissue (Kelly, 2011; Vanderklift and

Ponsard, 2003). This difference in time between both matrices, added to seasonal variation in environmental parameters in Antarctica, and the variations in the ecology of the penguins can influence TE concentrations (Burger, 1993; Polito et al., 2016) and help explaining the absence of a clear pattern for correlations between feather and blood values.

4.2 *Comparison to the TE concentrations found in literature*

Essential element concentrations (Mg, Ca, Fe, Mn, Cu, Zn, Se) were within the range earlier reported for Southern Ocean *Pygoscelis* penguins, suggesting that these essential elements levels represent either background or normal physiological and ecological levels (Celis et al., 2014; Espejo et al., 2017; Jerez et al., 2013a, 2013b, 2011; Metcheva et al., 2006). Such consistency is expected, since essential elements are under homeostatic control, with the nutritional requirements of the individual regulating their absorption (Walsh, 1990). Few studies report the toxic levels of TEs in feathers; however, the literature has shown that levels starting at 200 µg. g^{-1} (dw) for Zn and at 26 µg. g^{-1} (dw) for Se may be harmful for birds growth and reproduction (Einoder et al., 2018). Levels reported in the present study are below these limits.

However, it is worth noting the increase in essential elements over the years, which may reflect the increase in human activities in the region. Our results suggest a certain increase in Mn levels in Antarctica compared to previous work on *Pygoscelis* penguins by Jerez et al. (2011; Mn 1.17 ± 1.05 µg. g^{-1}, mean ± SD, dw) and Metcheva et al. (2006; Mn 1.5 ± 0.73 µg. g^{-1}, mean ± SD, dw) in feathers of *P. papua* from Livingston Island. Additionally, Mn levels found in this study were similar to those found in birds from the Northern Hemisphere (1.63 - 2.33 µg. g^{-1}, dw; Burger and Gochfeld, 2000), which may be an indicative of anthropogenic influence on Mn concentrations in Antarctica.

Concentrations of the non-essential elements Li, Be, Cr, Rb, Sr, Sn, Ba, and Pb were in a similar range to those found in *Pygoscelis* spp and other penguins worldwide (Espejo et al., 2017; Finger et al., 2017; Jerez et al., 2013b, 2011; Metcheva et al., 2006). The present study showed higher Cd concentrations than those determined by previous studies in the same species (Espejo et al., 2017; Jerez et al., 2011; Metcheva et al., 2006), and higher than values reported for feathers of the Procellariiforme Antarctic prion (*Pachyptila desolata*) (Fromant et al., 2016). This could indicate an anthropogenic influence in environmental concentrations of this metal in King George Island, since this region has the highest number of multinational facilities in Antarctica (nine permanent stations and a runway), being also one of the favourite destinations for tourist cruises in the continent.

Chromium and Pb were lower than those observed by Jerez et al. (2011) in feathers of *Pygoscelis* penguins: 1.15 - 8.08 for Cr and 0.14 - 1.76 for Pb μg. g^{-1} dry weight (dw). However, Pb values were similar to those observed by Finger et al. (2017) in blood of little penguin (*Eudyptula minor*) (0.04 - 0.07 μg. g^{-1} dw) from Australian Coast, a more polluted area. Chromium and Pb concentrations are associated to major human presence and activities in the Antarctic Peninsula (Jerez et al., 2011). Temporal studies on Antarctic snow have shown that elements such as Cr and Pb have increased their levels over the years (Hur et al., 2007; Planchon et al., 2002). This fact is probably due to the transport of elements from anthropic activities, such as mining and smelting of non-ferrous metals, carried out in southern hemisphere countries (Hur et al., 2007; Planchon et al., 2002).

4.3 *Geographical differences*

Volcanic activities increase the concentrations of several TEs in marine and continental ecosystems and Livingston is near to Deception Island, an active submarine volcano (Almendros et al., 1997). The local geothermal activity generates higher concentrations of Mn, Zn, and Fe in environmental matrices and biota (Deheyn et al., 2005). This fact may explain the higher concentrations of these three metals in *P. papua* from Livingston compared to King George Island. However, it is worth mentioning that there was an absence of geographically-related differences for the remaining measured elements, despite the distinct geothermal and prey availability in the different locations. This may be related to the proximity of the two islands, where the collection points are less than 100 km apart. Celis et al. (2014) collected soil samples from different locations in Antarctica, and observed great variations among the studied locations, most of them decreasing along the latitudinal gradient, related to the decrease of human presence and activities from North to South. Additionally, penguins often cover huge distances when feeding, making foraging trips that exceed 100 km (Davis and Darby, 2012), despite breeding in different locations, they feed in much wider areas, which can also contribute to the dilution of the effect of chemical inputs.

Another factor that can also influence the geographical differences in trace element levels is the availability of prey in different locations, corroborated by our SEA_b data, in which variations in the trophic niche of *Pygoscelis* penguins were observed between King George and Livingston. The availability of prey is a determining factor in the feeding plasticity of the penguins, and such availability can change not only in different locations, but also over the years (Miller et al., 2010).

4.4 *Interspecific differences*

Interspecific patterns of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ indicate that the three species have differences in foraging habits. The ratio of stable nitrogen isotopes is typically used as a tracer of the trophic level occupied by the species, and carbon and sulphur stable isotope ratios are commonly used to identify the sources of organic matter that sustain food webs (Connolly et al., 2004; Pizzochero et al., 2017; Polito et al., 2016). Most correlations between trace element concentrations and stable isotope values were negative and observed for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$. TE levels are, in general, found in close relationship with the foraging habitat, in a way that lower TE levels are usually found in habitats with very negative $\delta^{13}C$ and low $\delta^{34}S$ (*i.e.*, more pelagic /open areas), and higher concentrations are verified in coastal habitats. The correlations between TE and $\delta^{34}S$ 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 $\delta^{34}S$ values compared to coastal benthic sediment-associated producers (Connolly et al., 2004).

Our SEA_b results suggest that the niches of the three adult species have a similar size, however suggests a greater differentiation of *P. antarcticus* in relation to other species. Our results show that the diet plays an important role in the exposure of *Pygoscelis* penguins to TEs. Although krill is the main dietary component of *Pygoscelis* penguins in the Antarctic peninsula region, variations in the proportion of fish consumed (Polito et al., 2016; Volkman et al., 1980), as well as the foraging area (Herman et al., 2017) might explain our findings. Previous studies at King George Island have indicated a greater use of offshore foraging habitats by *P. adeliae* and *P. antarcticus* relative to *P. papua* (Miller et al., 2010; Polito et al., 2016), and our data corroborate those findings.

Herman et al. (2017) observed that *P. antarcticus* have a specialized diet, which feeds more on krill, compared to generalist strategy presented by *P. papua* and the intermediary one presented by *P. adeliae.* These findings may help explaining the significantly lower values of $\delta^{15}N$, and higher concentrations of Cu, Cd and Se found for *P. antarcticus* in the present study.

The Antarctic krill contains high amounts of Cu as a component of hemocyanin, their blood pigment (Nygård et al., 2001). This would explain the greatest Cu concentrations in *P. antarcticus,* which also exhibited the lowest $\delta^{15}N$ values. These stable isotope values are coherent with the lower trophic position occupied by krill in comparison to the fish consumed by the penguins (Polito et al., 2016). Cadmium is another element also found in high concentrations in krill. Nygård et al. (2001) associated the deep ocean upwelling to the high Cd concentrations in Antarctic krill, which may explain high Cd levels in *P. antarcticus*.

4.5 *Intraspecific differences*

Regarding the investigation of possible sex-related variations, the results on $\delta^{13}C$ and $\delta^{15}N$ values in *P. papua* indicate differences in diet and/or foraging areas between males and females. Xavier et al. (2017) observed that males of *P. papua* feed at a higher trophic level than females. The present study showed the same pattern in blood samples, since males were ^{15}N-enriched compared to females. Previous studies have shown that males rely more on fish than females and this feeding pattern is observed in both adults and chicks (Jennings et al., 2016; Miller et al., 2010; Xavier et al., 2017). Differences in diet between males and females have been also reported for *P. adeliae* (Jennings et al., 2016), as male chicks were fed a greater proportion of fish than female chicks due to differences in the pattern of parental feeding. The literature shows sex-related differences in diet and foraging habitat for *P. papua* (Bearhop et al., 2006), but no gender-related differences were observed for *P. adeliae* or *P. antarcticus* (Miller et al. 2010). Polito et al. (2015) found little to no dietary differences between sexes for *P. antarcticus* and *P. papua*. Likewise, Gorman et al. (2014) found sex-related differences in $\delta^{15}N$ values for *P. antarcticus* and *P. papua* in the same magnitude as analytical measurement error and no sex-related differences in $\delta^{13}C$ values.

Regarding ontogenetic differences, our SEA_b data showed the isotopic niches of chicks were markedly separated from that of adults, which suggests both age classes have different ecological niches, reflecting also in their trace element concentrations. The scientific literature on stable isotope data shows that diet composition can differ between adults and chick (Tierney et al., 2008). The fact that adults preferentially fed the chicks with fish rather than with invertebrates (Jerez et al., 2013; Tierney et al., 2008) may help explaining the results. In addition, the higher concentrations of Se, Cd, and Fe found in adults compared to chicks seems to be a consequence of the bioaccumulation process, which is the increase in pollutants throughout life (Wang, 2016), since the literature has been observed an increase of Cd (Burger and Gochfeld, 2000), and Se (Padilha et al., 2018) with age in seabirds.

Ontogenetic and gender-related differences were found for Ca concentrations. In blood samples, negative correlations were found between Ca levels and stable isotope data ($\delta^{13}C$ and $\delta^{15}N$). Still regarding this alkaline-earth metal, our results were similar to the concentrations found by Janssens et al. (2001; 904 - 1160 $\mu g. g^{-1}$ dw) while analyzing feathers of birds from Belgium. Newman et al. (1997) observed that plasma calcium concentrations differed between male and female seabirds from Alaska. These differences occur due to egg laying, which alters Ca concentrations in females (Newman et al., 1997).

Rubidium varied between locality, species, gender, and negative correlations were observed between this alkali metal and stable isotope values ($\delta^{13}C$ and $\delta^{34}S$), indicating that many factors are influencing the distribution of this element within *Pygoscelis spp*. Campbell et al. (2005) observed biomagnification of Rb in Arctic and temperate aquatic food webs. We observed significantly positive correlation between Rb concentrations and $\delta^{15}N$ values in *P. adeliae* feathers which can support the tendency of Rb to biomagnify.

5. Conclusions

Our results reinforce the value of environmental studies engaged in sampling efforts using different species, age class, and gender at different geographic areas. The use of a single species of the same age and sex, in the same location limits the comprehension of all the factors that may influence the exposure of that population to a particular contaminant. Our approach demonstrated the combined influence of several factors on the exposure to TEs and therefore, better reflects general trends. We confirm that geographical location, foraging zone ($\delta^{13}C$ and $\delta^{34}S$) and diet influence the interspecific differences among *Pygoscelis* penguins. In addition, intraspecific variations in TE levels are influenced by sex (feeding and egg laying) and maturity stage of penguins (feeding habits).

Our results also showed that some of the TEs concentrations were similar to those measured in birds from the Northern Hemisphere (Mn, Cr, Pb, Cd), where there is greater anthropogenic pressure. The apparent increase in Mn and Cd concentrations compared to previous studies reinforces the importance of monitoring polar birds in future investigations, since the increase in human activities at a local and global scale may lead to the exposure of these animals to pollutants.

This study presents essential baseline data that will assist in future investigations seeking to use *Pygoscelis* penguins as sentinels for TEs availability in the Antarctic marine environments. For TEs trophodynamics studies, it is recommended to incorporate species that compose penguin diet in the sampling design. Further investigations should also aim to understand in depth the role of sex and age in TEs trophodynamics in *Pygoscelis* penguins. Furthermore, additional studies should aim to provide further clarification of the factors that influence TEs concentrations in different penguin populations.

6. Limitations

Antarctica is a remote location difficult to access; therefore, sampling in some cases is limited and incomplete. In the present study, logistical limitations for moving to different collection sites made it impossible to collect an adequate number of chick samples as well as blood for the molecular determination of sex in the species of the two sampled locations. Although not ideal, as those were rare, difficult to access and therefore valuable samples, the study was carried out with a reduced sample size in some cases. However, this fact does not reduce the scientific relevance of the results obtained or change how this study can help future investigations to understand the factors that influence the exposure of *Pygoscelis* penguins to TEs.

Acknowledgements

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Tables and Figures

Table 1. Sampling data (tissue, species, state of maturity, and number of individuals - *n*) from King George Island (Point Hennequin, Penguin Island and Turret Point) and Livingston Island (Hannah Point) in the Antarctic Peninsula during 2012-2013 and 2013-2014 austral summers.

Tissue	Species	State of maturity	*n*	Location
Blood	*Pygoscelis antarcticus*	Adult	35	King George
			5	Livingston
	Pygoscelis adeliae	Adult	17	King George
		Chicks	9	King George
	Pygoscelis papua	Adult	31	King George
			17	Livingston
		Chicks	8	Livingston
Feathers	*Pygoscelis antarcticus*	Adult	21	King George
			4	Livingston
	Pygoscelis adeliae	Adult	17	King George
	Pygoscelis papua	Adult	22	King George
			6	Livingston

Table 2**.** Blood and feather trace elements concentrations (μg.g^{-1}, dry weight) in penguins from King George (KG) and Livingston (L) Islands, Antarctic Peninsula. Mean concentration ± SD and (number of individuals below detection limit). <LD: below detection limit.

Tissue	Species	Place	*n*	Li	Be	Mg	Ca	Cr	Fe	Mn	Ni	Cu	Zn	Se	Rb	Sr	Cd	Sn	Ba	Pb
Blood	***Pygoscelis papua***	A KG	28	0.12± 0.05 (4)	<LD	387.4± 50.40	255.3± 35.72	0.06± 0.02	2399± 192.2	1.89± 0.24	0.05± 0.02 (13)	1.97± 0.20	20.6± 2.54	9.77± 2.48	3.18± 0.38	0.62± 0.39	0.03± 0.05	0.21± 0.18 (26)	0.13± 0.24 (18)	0,09± 0,02 (24)
		AM KG	6	0.13± 0.08 (4)	<LD	368.3± 33.16	246.8± 32.42	0.05± 0.005 (2)	2380± 198.5	1.82± 0.14	0.05± 0.01 (2)	1.88± 0.26	20.1± 2.09	8.03± 2.53	3.48± 0.44	0.57± 0.23	0.02± 0.02	<LD	0.02± 0.001	0.09 (5)
		AF KG	11	0.12± 0.03 (3)	<LD	372.7± 43.74	244.4± 31.42	0.06± 0.02 (1)	2393.6± 2019.2	1.93± 0.33	0.05± 0.01 (6)	1.92± 0.16	21.4± 1.52	10.9± 1.40	2.97± 0.35	0.50± 0.10	0.03± 0.03	<LD	0.06± 0.05	0.09± 0.03
		A L	17	0.08± 0.03 (6)	<LD	383.9± 50.99	273.9± 69.72	0.07± 0.04 (2)	2382.2± 158.65	2.34± 1.91	0.09± 0.10 (12)	2.20± 0.36	22.1± 3.67	9.20± 11.06	3.62± 0.46	0.63± 0.57	0.03± 0.03	0.03± 0.01 (13)	0.19± 0.35 (9)	0.07± 0.01
		C L	8	0.07± 0.01	<LD	679.4± 111.1	416.4± 42.32	0.08± 0.05	1958.8± 198.38	1.69± 0.24	<LD	2.39± 0.21	35.0± 3.79	6.04± 0.38	3.73± 0.65	1.75± 0.20	0.01± 0.02	<LD	0.06± 0.07 (3)	<LD
	Pygoscelis adeliae	A KG	10	0.07± 0.006 (6)	<LD	387.4± 33.79	212± 23.31	0.07± 0.04	2404± 200.1	1.83± 0.17	0.06 (5)	1.82± 0.26	18.1± 0.84	19.9± 7.98	3.63± 0.38	0.50± 0.12	0.01± 0.003	0.07 (5)	0.04± 0.02	<LD
		AF KG	4	0.07± 0.006 (1)	<LD	391.5± 12.83	225± 36.18	0.06± 0.01	2475± 270.6	1.91± 0.23	<LD	2.07± 0.19	18.0± 0.96	17.2± 3.59	3.64± 0.41	0.50± 0.15	0.01± 0.004	<LD	0.02 (5)	<LD
		C L	9	<LD	<LD	631.8± 119.3	508.7± 88.46	0.09± 0.12	2021± 180.8	1.67± 0.13	<LD	2.09± 0.43	29.8± 2.13	6.07± 1.25	3.77± 0.36	3.65± 1.69	0.02± 0.03	<LD	0.04± 0.02	<LD
	Pygoscelis antarcticus	A KG	35	0.08± 0.03 (21)	<LD	406.4± 51.24	278.9± 71.09	0.07± 0.04	2415± 209.4	1.93± 0.27	0.07± 0.04 (14)	2.25± 0.49	20.5± 2.24	50.13± 19.87	3.86± 0.43	0.96± 0.62	0.04± 0.02	0.04± 0.002 (33)	0.06± 0.05 (17)	0,08± 0,01 (32)
		AM KG	8	0.08± 0.04 (4)	<LD	388.1± 26.62	208.6± 75.08	0.07± 0.03 (2)	2508.7± 289.4	1.95± 0.20	0.05± 0.02 (2)	2.06± 0.26	20.3± 1.60	63.1± 13.2	3.97± 0.67	0.65± 0.29	0.03± 0.02	0.04 (7)	0.02± 0.03	<LD
		AF KG	6	0.06± 0.01 (3)	<LD	404.3± 24.62	313.3± 26.99	0.07± 0.02 (2)	2438.3± 161.2	1.91± 0.16	0.07± 0.01 (1)	2.12± 0.34	23.5± 1.55	63.2± 14.5	3.73± 0.36	0.83± 0.23	0.04± 0.01	<LD	0.04± 0.03	<LD
		A L	12	0.07± 0.02 (6)	<LD	400± 94.5	317± 103	0.07± 0.01 (3)	2274± 374.5	1.93± 0.43	0.06± 0.01 (8)	2.18± 0.40	20.4± 2.99	60.9± 23.4	3.83± 0.39	1.20± 0.73	0.06± 0.04	0.03 (11)	0.07± 0.07	<LD

*A= adult; AM= adult male; AF= adult female; C= chick

Table 2. (Continued)

Tissue	Species	Place	*n*	Li	Be	Mg	Ca	Cr	Fe	Mn	Ni	Cu	Zn	Se	Rb	Sr	Cd	Sn	Ba	Pb
Feather	***Pygoscelis papua***	A KG	25	1.07± 0.61	<LD	1314± 468.0	1214± 386.8	0.18± 0.09	11.15± 9.66	0.29± 0.19	0.27± 0.12	17.71± 6.12	80.2± 28.2	1.8± 0.05	0.11± 0.05	14.2± 4.85	0.14± 0.07	<LD	0.42± 0.82	<LD
		AM KG	9	1.09± 0.06	<LD	1423± 727.4	1296± 589.9	0.18± 0.11	9.29± 5.25	0.28± 0.14	0.29± 0.18	17.64± 6.13	81.1± 34.7	1.98± 0.80	0.10± 0.03	15.3± 7.38	0.15± 0.09	<LD	0.55± 1.19	<LD
		AF KG	15	1.08± 0.06	<LD	1273± 163.4	1183± 141.4	0.17± 0.08	8.44± 10.6	0.22± 0.21	0.26± 0.04	16.77± 6.10	71.0± 15.4	1.66± 0.59	0.09± 0.06	13.9± 1.69	0.14± 0.03	0.13 (14)	0.22± 0.25	<LD
		AL	6	0.09± 0.02	<LD	1019± 212.2	1433± 348.3	0.19± 0.02	95.07± 72.87	1.93± 1.14	0.20± 0.05	18.4± 2.26	103± 10.5	1.94± 0.24	0.17± 0.04	15.7± 4.45	0.14± 0.07	<LD	1.34± 1.91	<LD
	Pygoscelis adeliae	A KG	17	0.16± 0.06	<LD	1264± 185.7	1185± 175.7	0.20± 0.21	8.40± 3.52	0.43± 0.93	0.24± 0.08	17.45± 2.49	70.5± 10.5	4.32± 1.08	0.09± 0.03	14.7± 2.34	0.14± 0.06	0.07± 0.04 (14)	0.32± 0.47	<LD
		AM KG	5	0.18± 0.78	<LD	1420± 164.6	1344± 131.2	0.18± 0.05	10.9± 4.71	0.24± 0.07	0.25± 0.08	17.36± 2.04	74.2± 9.5	4.12± 0.80	0.10± 0.02	17.1± 1.94	0.18± 0.05	<LD	0.24± 0.25	<LD
		AF KG	7	0.14± 0.06	<LD	1202± 177.9	1112± 161.2	0.13± 0.04	6.69± 1.42	0.69± 1.42	0.22± 0.04	17.03± 2.50	67.71± 9.50	4.35± 0.76	0.08± 0.03	13.7± 1.64	0.13± 0.04	0.07± 0.04 (5)	0.48± 0.66	<LD
	Pygoscelis antarcticus	A KG	20	2.02± 2.78	<LD	1090± 472.7	1070± 361.3	0.17± 0.06	17.31± 23.47	0.49± 0.79	0.28± 0.15	25.72± 4.85	88.9± 19.9	2.85± 0.61	0.13± 0.05	12.1± 4.66	0.21± 0.14	0.09± 0.07 (17)	0.15± 0.09	<LD
		AM KG	7	2.08± 2.28	<LD	1079± 374.2	1042± 279.2	0.16± 0.04	10.36± 2.98	0.26± 0.06	0.24± 0.08	22.74± 5.09	78.0± 12.6	2.48± 0.46	0.11± 0.04	11.6± 3.75	0.18± 0.08	0.07 (6)	0.09± 0.02	<LD
		AF KG	5	2.08± 1.44	<LD	1242± 175.8	1194± 237.3	0.22± 0.07	41.22± 35.82	1.17± 1.32	0.38± 0.09	24.38± 2.02	85.4± 16.9	3.06± 0.71	0.12± 0.03	13.8± 2.10	0.24± 0.08	0.04± 0.005 (3)	0.24± 0.12	<LD
		A L	4	0.11± 0.06	<LD	927± 598	975± 670	0.19± 0.10	24.55± 33.13	0.59± 0.71	0.16± 0.07	29.4± 6.22	116.5± 32.15	3.63± 0.65	0.20± 0.11	10.3± 7.91	0.14± 0.11	<LD	0.72± 1.15	<LD

*A= adult; AM= adult male; AF= adult female; C= chick

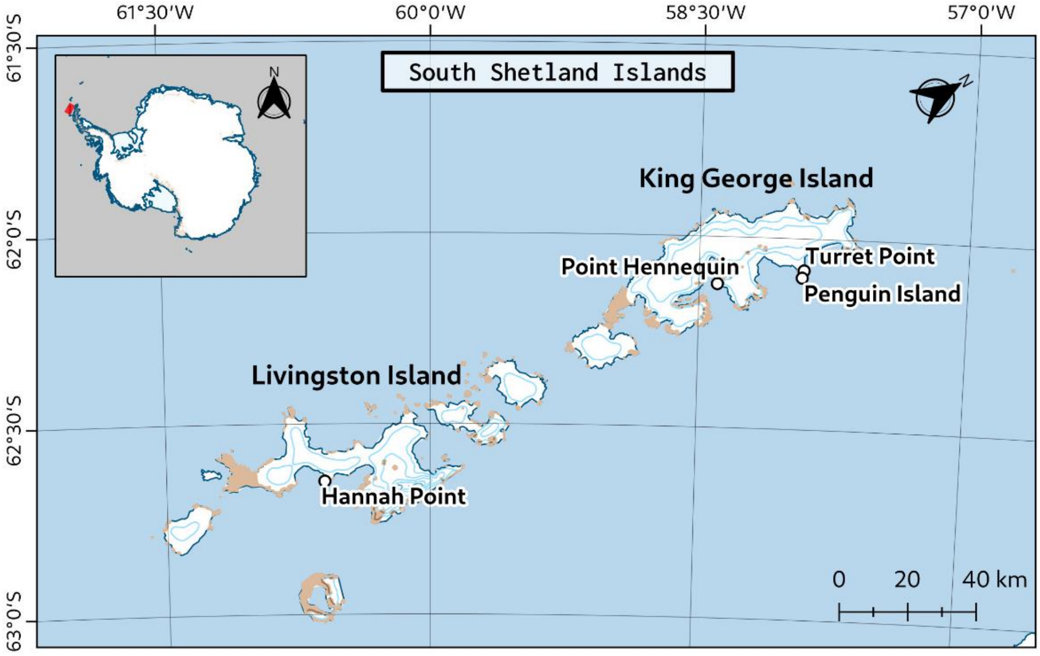


Figure 1. Map of the Antarctic Peninsula, highlighting the South Shetland Islands, as well as King George and Livingston Islands. The sampling points, *i.e.*, Hannah Point, as well as Point Hennequin, Penguin Island, and Turret Point are additionally stressed.

Table 3. The values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ in blood and feathers of gentoo, Adélie and chinstrap penguins from King George and Livingston Islands.

Tissue	Specie	Place	Age	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)
Blood	*Pygoscelis papua*	King George	Adult	29	-26.47 ±0.46	7.88 ±0.35	-
			Male	10	-26.40 ±0.34	7.99 ±0.29	-
			Female	16	-26.50 ±0.47	7.77 ±0.39	-
		Livingston	Adult	19	-26.06 ±0.21	8.38 ±0.33	-
			Chicks	7	-27.61 ±0.16	7.96 ±0.26	-
	Pygoscelis adeliae	King George	Adult	5	-26.13 ±0.28	8.09 ±0.29	-
			Female	3	-26.36 ±0.25	7.95 ±0.29	-
			Chicks	9	-27.37 ±1.51	8.08 ±1.01	-
	Pygoscelis antarcticus	King George	Adult	16	-26.15 ±0.62	8.21 ±0.31	-
			Male	8	-25.94 ±0.62	8.17 ±0.24	-
			Female	6	-26.28 ±0.45	8.02 ±0.29	-
		Livingston	Adult	4	-26.62 ±0.61	8.47± 0.53	-
Feather	*Pygoscelis papua*	King George	Adult	26	-24.76 ±0.69	9.61 ±0.46	15.28 ±0.97
			Male	8	-24.36 ±0.50	9.89 ±0.47	15.56 ±0.65
			Female	14	-25.04 ±0.73	9.54 ±0.28	15.39 ±1.04
		Livingston	Adult	6	-24.89 ±0.78	9.67 ±0.69	13.33 ±1.11
	Pygoscelis adeliae	King George	Adult	20	-24.48 ±0.80	9.72 ±0.47	14.91 ±1.07
			Male	6	-24.64 ±1.03	9.98 ±0.46	14.70 ±1.18
			Female	7	-24.45 ±0.63	9.70 ±0.39	15.32 ±1.07
	Pygoscelis antarcticus	King George	Adult	20	-25.39 ±0.74	9.25 ±0.50	14.39 ±1.07
			Male	5	-25.51 ±0.90	9.13 ±0.71	14.03 ±1.03
			Female	6	-25.33 ±0.91	8.93 ±0.67	14.99 ±1.42

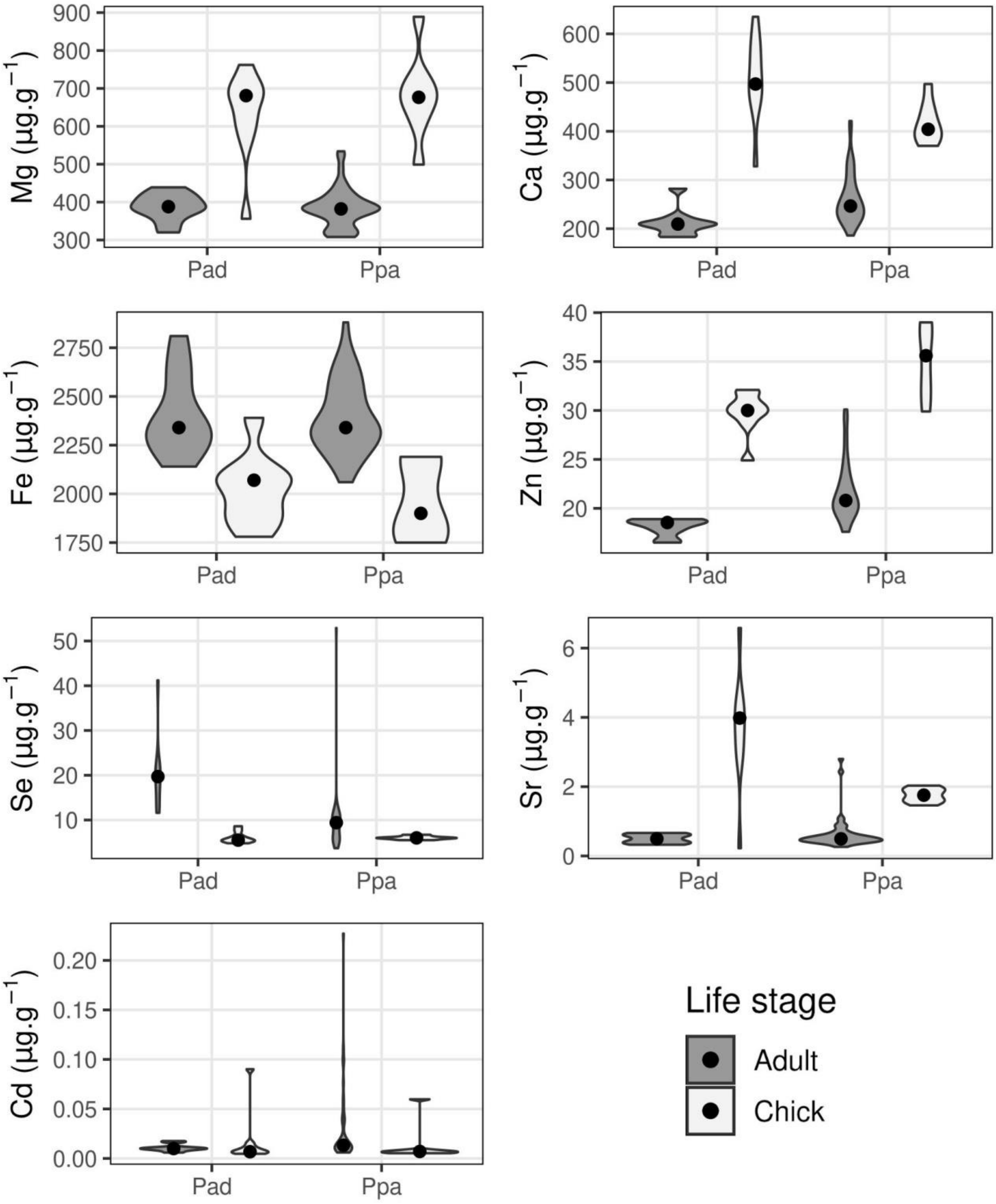
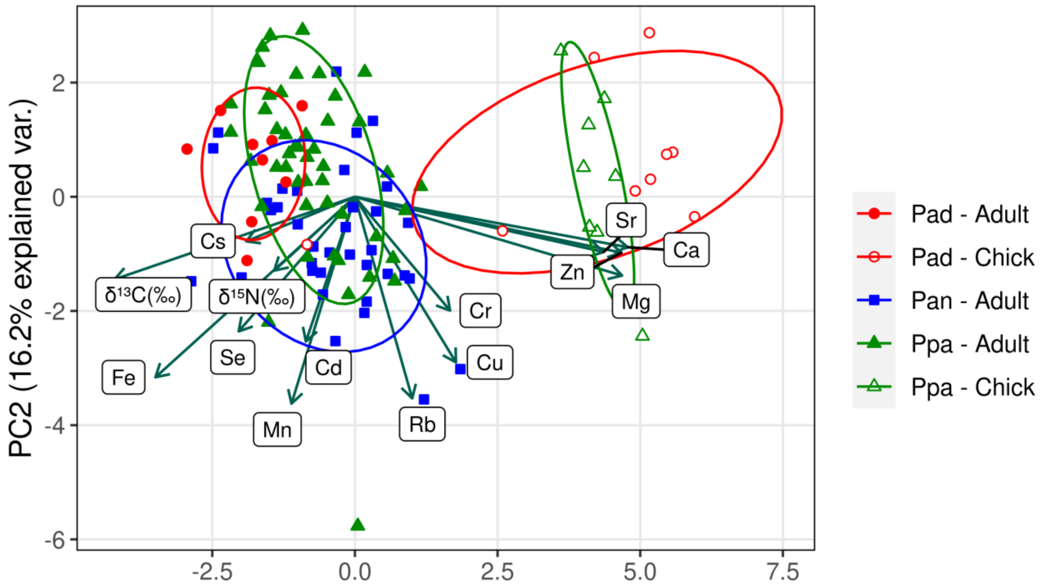


Figure 2. Violin plots showing differences in blood levels of iron, cadmium, magnesium, calcium, selenium, zinc and strontium (µg. g^{-1}, dw; $p < 0.01$) between adults and chicks regarding *Pygoscelis papua* - Ppa from Livingston Island, and *Pygoscelis adeliae* - Pad from King George Island, Antarctic Peninsula.

A)



B)

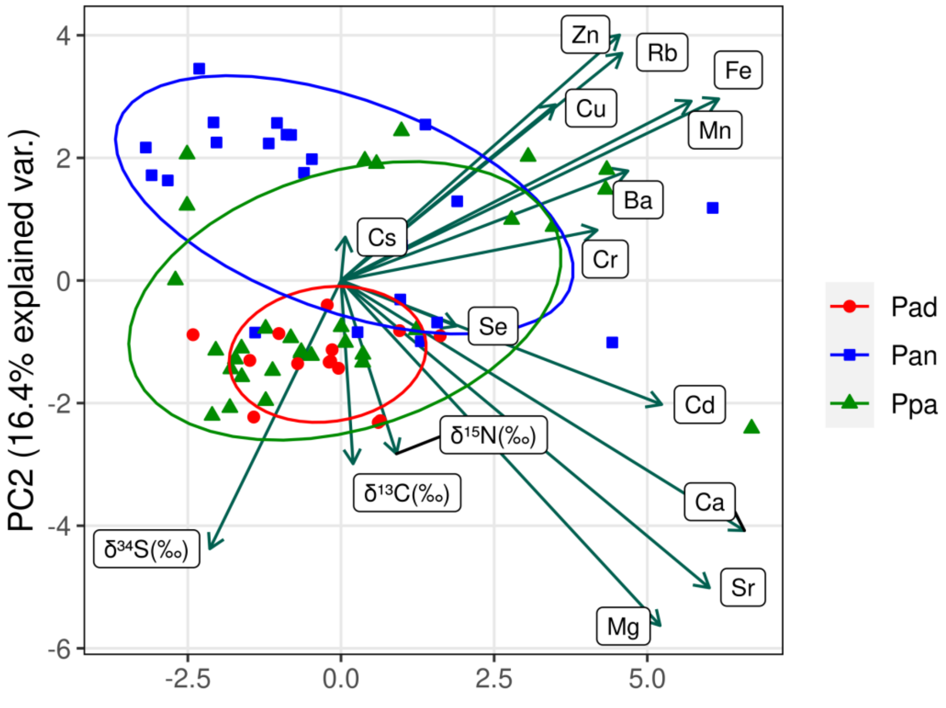


Figure 3. PCA blood (A) and feathers (B) of *Pygoscelis papua* - Ppa, *Pygoscelis antarcticus* - Pan and *Pygoscelis adeliae* - Pad from Antarctic Peninsula. The length of the vector's projection reflects its contribution to the principal component. The angle between two vectors gives the correlation between the corresponding variables, as well as between variables and principal components. Acute or obtuse angles indicate positive or negative correlations, respectively. A right angle indicates no correlation.

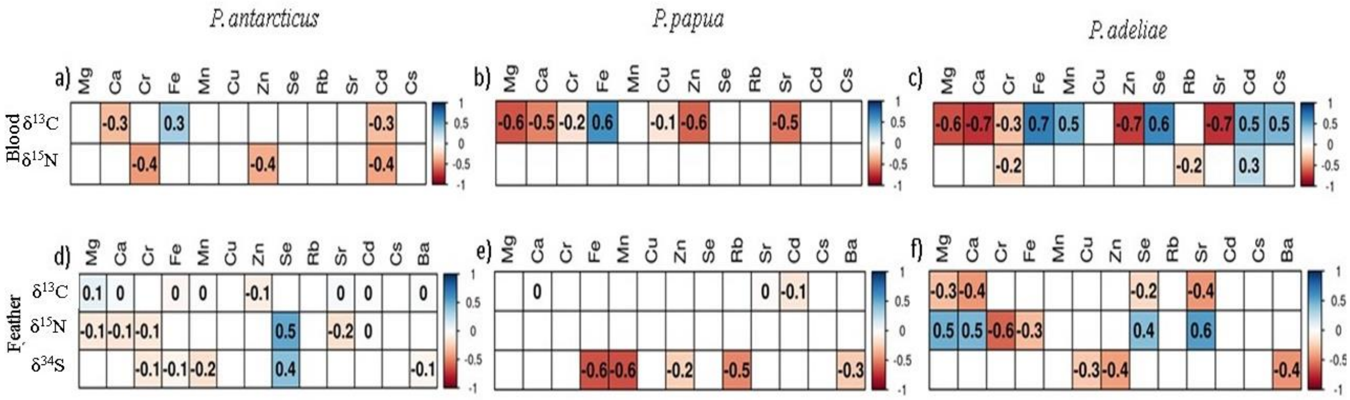


Figure 4. Spearman rank correlation matrix between trace elements and stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) in blood (a, b, c) and feathers (d, e, f) and sulphur ($\delta^{34}S$) in feather (d, e, f) samples of *P. antarcticus, P. papua* and *P. adeliae* from Antarctic Peninsula. Statistically significant spearman rank correlations (r_S, $p < 0.05$) are shown in blue (positive correlation) and red (negative correlation) colour scale (colour intensity related to r_S value), while non-significant correlations are left blank.

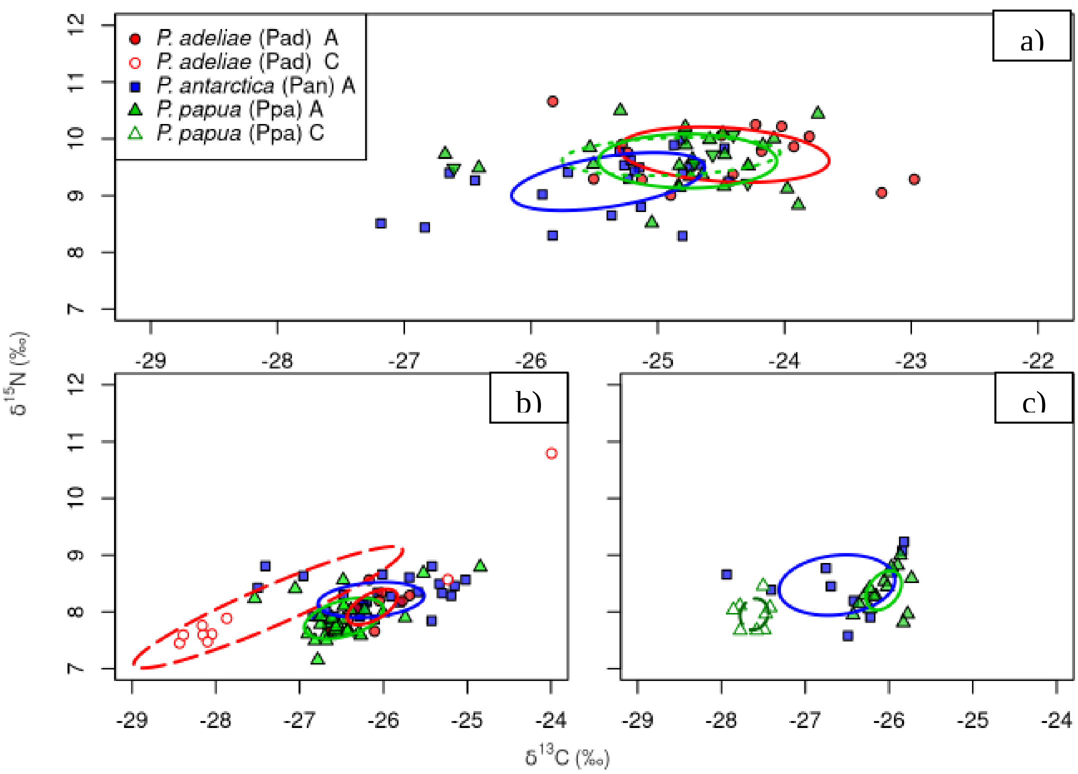


Figure 5. Isotopic niche sizes for feathers (a) and blood (b,c) of adults (A) and chicks (C) of gentoo (Ppa), chinstrap (Pan) and adélie (Pad) penguins, with their respective small sample-size corrected standard ellipses (SEAc). The feather graph (a) has data from King George populations, plus data for gentoo from Livingstone (filled triangle point down green), and the blood graph has data from Livingston (b) and King George (c) Islands.

A)

B)

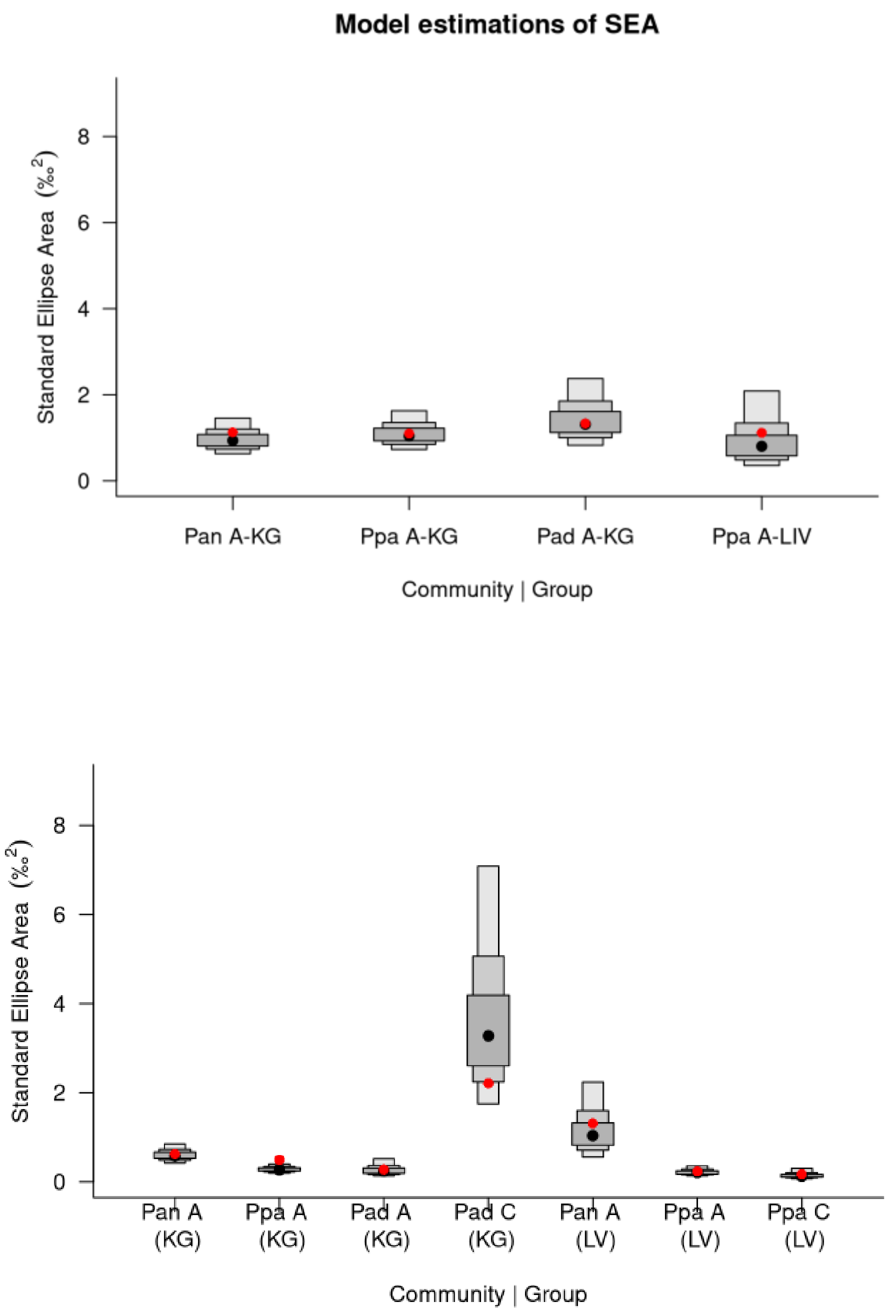


Figure 6. Standard ellipse areas (SEAb) for the groups observed in feathers (A) and blood (B). The boxes represent the 95, 75 and 50% credible intervals, with the mode indicated by the black circles. The maximum likelihood estimate for the corresponding SEAc is indicated by the red circle.

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