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Storm petrels as indicators of pelagic seabird exposure to chemical elements in the Antarctic marine ecosystem

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Abstract

Data on trace element bioavailability in the south-polar marine ecosystem is still scarce, compared to that relating to temperate zones. Seabirds can be used as indicators of ecosystem health and sentinels of environmental pollution, constituting a link between marine and terrestrial environments. Here, we analysed the concentration of 17 elements (with special emphasis on mercury, Hg) in feathers of adults and chicks of two pelagic seabirds – the Wilson's storm petrel *Oceanites oceanicus* and the black-bellied storm petrel *Fregetta tropica* – breeding sympatrically in the maritime Antarctic. Since adult feathers are formed during the non-breeding period away from the breeding grounds, but down and body feathers of chicks

grow at the breeding sites, we were able to evaluate the birds' exposure to contaminants at various stages of their annual life cycle and in various marine zones. We found that of the two studied species, adult black-bellied storm petrels had significantly higher mercury, selenium and copper levels (5.47±1.61; 5.19±1.18; 8.20±0.56 μg g^{-1} dw, respectively) than Wilson's storm petrels (2.38±1.47; 1.81±0.98; 2.52±2.35 μg g^{-1} dw, respectively). We found that Wilson's storm petrel chicks had a significantly different contaminant profile than adults. Arsenic, bismuth and antimony were detected exclusively in the chick feathers, and the Se:Hg molar ratio was higher in chicks than in adults. Our study also suggests considerable maternal transfer of Hg (to down feathers) in both species. As global contaminant emissions are expected to increase, birds inhabiting remote areas with sparse anthropogenic pollution can indicate the temporal trends in global contamination.

Keywords: contamination, feather, toxic metals, ICP-MS, mercury, Procellariiformes

1. Introduction

Organisms living in Antarctica are exposed to a number of environmental factors that may affect their health and survival. Of those, the most influential are harsh climate conditions, competition for food, and predation, but pollutants may also play an important role (Santos et al. 2006, Metcheva et al. 2006). Contaminants in the polar zone may originate from natural processes [i.e. volcanic activity, the input of sea-spray, mechanical and chemical rock weathering (Malandrino et al. 2009)] and biota [e.g. mammals or bird colonies can be a source of nutrients/organic matter (N, F) and several elements such as Cd, Hg, As, Se and Zn to the terrestrial and coastal ecosystem (Cipro et al. 2018)]. Anthropogenic sources of Antarctic ecosystem contamination are often located outside the region, e.g. lead from industrial emissions is transported from South America (Sañudo-Wilhelmy et al. 2002, Gaiero

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et al. 2003, Bargagli 2008). However, local sources, too, may contribute due to increasing research and tourism activities resulting in fuel combustion, accidental oil spills, waste disposal sites, sewage and paint residues (Bargagli 2008, Jerez et al. 2011, Mão de Ferro et al. 2013).

Pelagic seabirds living in the southern polar zone can be used in ecotoxicological studies to assess trace element pollution and marine ecosystem health (Carravieri et al. 2014). They constitute a valuable link between terrestrial and marine zones of the Antarctic (Santos et al. 2006). As they often cover vast distances in search of suitable foraging areas, they are exposed to pollutants in various geographical locations. They may also carry these contaminants between wintering and/or stop-over staging and breeding, due to migratory connectivity (Webster et al. 2002).

Seabirds' feathers are often used to evaluate their exposure to contaminants (e.g. Jerez et al. 2011, Bustamante et al. 2016, Philpot et al. 2019), providing a record of contaminant uptake at the time of feather growth and development (Bearhop et al. 2002, Jaspers et al. 2004). High metals affinity to sulfhydryl groups of the feather structural proteins are making them a suitable biomonitoring tool (Thompson et al. 1998). Elemental deposition in feather tissue is species-specific and depends on multiple factors, including diet, age, detoxification abilities and moulting pattern (Burger and Gochfeld 1997, Evers et al. 2008, Cipro et al. 2014, Pacyna et al. 2017). Knowledge of avian moulting sequences is essential to the reconstruction of the contamination period (Bustamante et al. 2016, Cherel et al. 2018). Adult feathers may provide a wider perspective on metal exposure over the annual cycle, but as seabirds may cover a vast area during the moulting period it is challenging to properly interpret their exposure over time. Also seasonal shifts in element concentrations can occur (Øverjordet et al. 2015). By contrast, chick feathers may provide information over a shorter period of exposure for a more defined area (Evers et al. 2005). Chick down is formed in the egg from

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maternal nutrients and as such represents female contamination during the pre-laying period (Ackermann et al. 2016). Thus, analysis of feathers collected at various life stages allows the elemental concentrations of various areas to be reconstructed, indicating temporal and spatial trends in pollution in the ecosystems being occupied at the time (Becker, 2003).

Despite the growing number of studies on Antarctic and sub-Antarctic food web contamination, still little is known about elemental concentrations in seabirds feeding at low trophic levels (i.e. preying on zooplankton and krill), likely due to their relatively lower exposure to contaminants compared to top predators. For instance, petrels (i.e. species from three families of Procellariiformes: Procellariidae, Oceanitidae, and Hydrobatidae) are still one of the most poorly examined seabird groups, mostly due to their small body size, their nesting predominantly on isolated and inaccessible islands, and their high mobility at sea (Rodríguez et al. 2019). However, even this group is exposed to a multitude of contaminants (e.g., Anderson et al. 2000, Bocher et al. 2003, Cipro et al. 2014, Fromant et al. 2016, Philpot et al. 2019).

In this study we determined levels of elements in feathers of two storm-petrel species breeding in the maritime Antarctica, the Wilson's storm petrel (*Oceanites oceanicus*, hereafter WSP) and the black-bellied storm petrel (*Fregetta tropica*, hereafter BBSP). We focused both on elements of wider ecotoxicological interest (i.e. arsenic [As], cadmium [Cd], chromium [Cr], copper [Cu], lead [Pb], mercury [Hg], selenium [Se], and zinc [Zn]) and on those rarely studied in avian tissues (i.e. antimony [Sb], bismuth [Bi], calcium [Ca], cobalt [Co], iron [Fe], nickel [Ni], magnesium [Mg], molybdenum [Mo], and strontium [Sr]). Gathering data about the concentration of various elements in tissues of living animals is crucial in order to properly assess ecosystem health and to comprehend pollutants' abilities for potential bioaccumulation and biomagnification. By studying rarely analysed elements, the results

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should provide background data for research detecting future inputs of elements in remote polar regions (Santos et al. 2006).

We aimed to:

1) present reference values for the concentrations of 17 elements that can be used in the future for monitoring contamination level in Antarctic marine predators;

2) compare elemental concentrations between feathers collected from different age groups representing various life-history stages (i.e. chick feathers representing the chick-growth period, chick down representing maternal input, and adult feathers representing part of the non-breeding period, when the feathers grew); by considering the spatial and temporal differences in feather growth between these groups, we expected to detect differences in elemental concentrations between the various types of feathers;

3) compare elemental concentrations in feathers grown during the non-breeding season between adults of the two species, with special emphasis on Hg and Se:Hg molar ratio (linked to protective action against Hg bioaccumulation and toxicity by creation of Hg-Se compounds [Nigro and Leonzio 1996, Khan and Wang 2009]). Considering inter-specific differences in trophic level (see Materials and Methods) and in the location of non-breeding areas (Fig. 1), we expected to detect differences in elemental concentrations between the species;

4) identify patterns in concentrations of elements, and thus identify possible common sources of contamination.

2. Materials and Methods

2.1. Studied species

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120 The two study species – the Wilson's storm petrel and the black-bellied storm petrel – are
121 small pelagic seabirds, with circumpolar breeding distributions including sub-Antarctic
122 islands and the maritime Antarctic. Both species breed sympatrically in the study area (see
123 below) during the austral summer (from December to March), with similar breeding biology:
124 single-egg clutch, incubation lasting 38–44 days, and chick rearing up to 71 days. Although
125 both species are among the smallest endotherms living in the Antarctic, they play an
126 important role as predators preying on Antarctic krill, myctophid fish and amphipods (Hahn
127 1998, Quillfeldt 2002, Quillfeldt et al. 2005, Wasilewski 1986). Preying on fish and
128 crustaceans in equal proportions (Hahn 1998), BBSP feeds at a higher trophic level than
129 WSP, which eats mainly crustaceans (80–90% of meals) (Quillfeldt 2002, Quillfeldt et al.
130 2017). After breeding, both species migrate northwards, where they spend the non-breeding
131 period at open sea and complete their moult (Beck and Brown 1972). They moult in the
132 Atlantic Ocean in a wide range of habitats: WSP from sub-Antarctic to subtropical waters and
133 BBSP primarily either in sub-Antarctic–subtropical waters or at the continental shelf (Phillips
134 et al. 2009) (Fig. 1).
135
136 *2.2. Sample collection*
137 We studied the two storm-petrel species in the breeding colonies located in the vicinity of
138 Henryk Arctowski Station in Admiralty Bay, King Gorge Island, South Shetlands, Antarctica
139 (62°02′S 58°21′W, Fig. 1) in 2017. King George Island is the largest island in the South
140 Shetlands Archipelago, 90% ice-covered, with rocks mainly formed by andesitic and basaltic
141 magma (Santos et al. 2006). We captured adult birds in the breeding colony (in their nests,
142 using mist-nets spread in the colony area) during the incubation period and collected 4–5
143 body feathers from the back. Back body feathers represent mostly trace element input from
144 food and water intake during part of the non-breeding period when the feathers grew, which

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takes place outside the colony in the Atlantic Ocean (Fig. 1). To sample chicks we caught them by hand in the nest and collected down (at the time they were starting to lose it, i.e. when their body feathers were well grown, thus minimising the risk of affecting thermoregulation), and 4–5 body feathers from the back (when the nestlings were about to fledge). Down feathers represent trace elements passed on by the female to the embryo, reflecting their input during the pre-laying period, probably from areas around the breeding colony (and likely predominantly reflecting the food intake). Chick body feathers represent the nesting period and input from marine environments (as food and water intake). We stored all the samples in individual plastic zip-lock bags until chemical analysis.

2.3. Analytical Procedure

Prior to chemical analysis, we cleaned all feather samples to remove external contamination, firstly with acetone (Sigma-Aldrich, USA) and then two times with deionised water (Mili-Q Gradient A10, Milipore, France) (procedure of Jaspers et al. 2004, modified). We air-dried the washed feathers for 24 h. If the total mass of the sample permitted, we used an aliquot of the collected material for the analysis of all trace elements, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

We determined concentrations of 17 trace elements by ICP-MS analytical technique in the following feather samples types: adult WSP (n=12), chick body WSP (n=4), chick down WSP (n=4) and adult BBSP (n=4). Mean feather mass was: for adults 10 mg (4–18 mg), for chick body feathers 6 mg (5–8 mg) and for chick down feathers 16 mg (6–39 mg). Due to insufficient amount of chick body BBSP and down BBSP feathers, we measured only Hg content in these samples using the cold vapour technique. In total, we determined Hg concentration in the following types of feather samples: adult WSP (n=35), chick body WSP

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(n=10), down WSP (n=16), adult BBSP (n=11), chick body BBSP (n=6) and down BBSP (n=6).

2.3.1. Trace element concentration

We homogenised dry feathers by cutting them up, then weighed them to the nearest 0.01 mg, and placed them in a clean Teflon vessel with 7 ml 65% HNO_3 (Merck, Suprapur). We carried out digestion using a high-pressure microwave emitter (Microwave Digestion System, Anton Paar). We increased the temperature from the ambient value to 90°C (approximately 6–8°C/min). We maintained these conditions for 25 minutes, after which we gradually lowered the temperature. Subsequently, we diluted the fully mineralised samples with deionised water to 25 ml in clean plastic flasks. To ensure quality control and check background contamination, we ran blank samples with every batch. To ensure accuracy of obtained results we ran certified reference material (CRM, Human hair ERM-DB001) in triplicate. We analysed samples using an ICP-MS 2030 (Shimadzu, Japan) (for measurement conditions and parameters see Table 1, Supplementary material)

2.3.2. Mercury concentration (cold vapour technique)

We weighed the dry samples to the nearest 0.01 mg in a ceramic boat, then we covered them with activated Al_2O_3 and analysed them using the thermal vaporisation atomic absorption method (MA-3000 Nippon Instruments Corporation). We analysed at least two feather aliquots (1–10 mg dry weight) for each individual. The details of the program used and the equipment specification are described in Pacyna et al. (2018). We determined total Hg concentration in duplicates or triplicates when possible, taking sub-samples of the homogenised feathers. We calculated the coefficient of variation (CV) based on these. If the CV was above 15%, we excluded samples from the analyses, deeming the estimation of Hg

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concentration unreliable. Thus, for statistical analysis we used: adult WSP (n=25), chick body WSP (n=5), down WSP (n=16), adult BBSP (n=8), chick body BBSP (n=5) and down BBSP (n=6). Mean CV was 8.64±4.65% for adults, 6.32±5.40% and 2.92±2.64% for chick body feathers and down, respectively. To check background contamination, we performed a quality control including blank samples every 5–6 subsamples. We analysed CRM every 10th subsample run.

2.4. Quality control

We found that results for CRM analysis were in agreement with the certified values (mass used for analysis: for trace elements 200 mg, for mercury 14-28 mg). Recoveries were high: As 98%, Cd 106%, Cu 104%, Hg 92%, Pb 96%, Se 92%, Zn 97%. To check accuracy and recoveries of other elements absent in this CRM we applied a treatment used before by Pacyna et al. (2019). We blank-corrected samples analysed on the ICP-MS (by a mean value of all blank samples). For Hg analysis, we found that background contamination was negligible and we did not perform blank correction. The limit of detection (LOD) and quantification (LOQ) values were calculated as the concentrations corresponding to signals equal to three and ten times the standard deviation of blank solution signal, respectively. For Hg LOD/LOQ were calculated based on the standard deviation of the response (s), and the slope of the calibration curve (b) according to the following formulas: LOD 3.3(s/b), LOQ 10(s/b). Method LOD/LOQ were in range of 0.004-0.92 and 0.013-3.07 ng/g respectively. We reported our results as $\mu g \cdot g^{-1}$ dry weight (dw). For statistical analysis results below the LOD we assigned half of the LOD value.

For calibration of the ICP-MS we used the ICP IV multi-element standard (Merck, USA) and As, Sb, Se, Mo and V (Sigma-Aldrich, USA), Hg (Merck, USA) as single standards. As internal standards we used: Sc, Rh, Tb and Ge in supra pure 1% HNO_3 (Merck,

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USA). For sample pre-treatment and sample dilution we used deionised water obtained from the Milli-Q Direct 8 Water Purification System.

2.5. Statistical analyses

To investigate variation in the qualitative and quantitative composition of trace elements in feathers, we firstly performed multivariate analyses for all elements to find general patterns and then we did univariate analyses for particular elements.

To compare the qualitative and quantitative compositions of all trace elements in feathers among the life-history stages and species, we applied the following multivariate methods:

1) a multivariate (for all trace elements together) PERMANOVA (non-parametric MANOVA based on the Bray–Curtis measure; Anderson 2001)) with concentrations of all elements as a response variable and birds' age (adult WSP, adult BBSP, chick down WSP, chick body WSP) as the explanatory variable;

2) A similarity percentage breakdown procedure (SIMPER) to assess the average percentage contribution of individual elements to the dissimilarity in all elements concentrations between age groups in a Bray–Curtis dissimilarity matrix (Clarke 1993).

To compare the qualitative and quantitative compositions of particular trace elements in feathers between the life-history stages and species, we used an unimodal Kruskal–Wallis test with a *U* Mann–Whitney test as a *post-hoc* test for all group pairs, excluding adult BBSP *vs* chick down WSP and adult BBSP *vs* chick body WSP. In a separate analysis, we compared Hg concentration among all categories for a larger sample size.

Then, to find the groups of elements with high degrees of association in feather elemental concentrations, we performed a Hierarchical Cluster Analysis (HCA). A high degree of association between element concentrations, expressed by clustering in one group, can be used to identify common sources of elements (e.g. Hashmi et al. 2013), but it does not

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require the formulation of any *a priori* hypothesis considering the nature of the relationships (Bianchi et al. 2008). We performed HCA with Euclidean distance as a distance measure, and the paired group method as the linkage method. For each cluster obtained, we calculated Bootstrap Probability (BP) using multiscale bootstrap resampling. BP of a cluster may take a value between 0 and 100, indicating how well the data supported the cluster, with a higher value indicating a better fit (Hammer et al. 2001). We only considered clusters with BP≥95. To determine how well the generated clusters represented dissimilarities between objects, we calculated the cophenetic correlation coefficient. Values close to 0 indicate poor clustering, and values close to 1 show strong clustering.

We performed PERMANOVA, SIMPER and HCA analyses on log(x+1) transformed data. We classified the strength of the correlation according to Hinkle et al. (2003): strong correlation with r=|0.90–1.00|, high correlation with r=|0.70–0.90|, moderate correlation with r=|0.50–0.70|, and low correlation with r=|0.30–0.50|.

We performed separate SIMPER and PERMANOVA analyses for three groups of elements:

1. all elements;
2. essential elements, i.e. As, Ca, Cr, Cu, Fe, Mg, Mo, Se and Zn; and
3. non-essential elements, i.e. Bi, Cd, Hg, Pb, Sb, and Sr.

We calculated Se:Hg molar ratios based on the mean Hg values and the mean Se values from our study. The Se:Hg molar ratio was obtained using the molecular weight (200.59 and 78.9 for Hg and Se, respectively) (Burger et al. 2013). We compared Se:Hg molar ratios between the studied age categories in both species using a chi-squared test.

We performed PERMANOVA, SIMPER, and HCA analyses in PAST software (Hammer et al. 2001) and the Kruskal–Wallis and Mann U Whitney test in R software (R Core Team 2018), using the ggpubr package (Kassambara 2018).

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3. Results

3.1. Element concentrations

Of all the metals examined, Ni and Co were below the limit of detection in all samples, and were thus excluded from further analysis. As, Bi and Sb were detected exclusively in chick feathers, both body and down. Concentrations of all elements (mean±SD) are presented in Table 1 and Table 4 (for Hg). Concentration chain for particular groups are:

a) For adult WSP Mg>Zn>Ca>Fe>Sr>Cu>Mo>Hg>Se>Cr>Pb
b) For adult BBSP Mg>Zn>Ca>Fe>Cu>Hg>Se>Sr>Cr>Mo>Pb
c) For chicks body feathers Mg>Ca>Fe>Zn>Bi>Sr>Mo>Cu>As>Se>Cr>Pb>Sb>Hg>Cd
d) For chicks down feathers Mg>Ca>Fe>Zn>Sr>Se>Mo>Pb=Hg=As>Bi=Cr=Cu>Cd>Sb

3.2. Inter-group differences in all elements concentration

All elements. The concentrations of all combined studied elements differed significantly between adult WSP and all other categories (PERMANOVA, Bonferroni-corrected $p<0.04$) (Table 2). SIMPER analysis showed that the overall average dissimilarity was 18.5%. Fe and Bi contributed most (14% and 11%, respectively) to the pattern of overall dissimilarity (Table 3). Bi, As and Fe contributed most (19%, 14% and 14%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and chick WSP body feathers. Fe and Se contributed most (14% and 12%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and chick WSP down. Cu, Fe, Hg and Se contributed most (16%, 15%, 14% and 12%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and BBSP.

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Essential elements. The concentrations of all combined studied elements differed significantly between adult WSP and all WSP chick categories (PERMANOVA, Bonferroni-corrected $p<0.006$). We found no differences between adult WSP and BBSP ($p=0.136$) (Table 2). The SIMPER analysis showed that the overall average dissimilarity was 14.2%. More than half of the pattern of overall dissimilarity observed in elemental concentrations was explained by Fe, As, Cu and Mo (22%, 15%, 13% and 13%, respectively) (Table 3). As, Fe and Mo together contributed over 50% (23%, 22% and 15%, respectively) to the pattern of dissimilarity observed in elemental concentrations between adult WSP and chick WSP body feathers. Fe, Ca, As, Zn and Mo together contributed over 50% (21%, 13%, 12%, 11% and 11%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and chick WSP down (Table 3). Cu, Fe, Se and Mo together contributed over 50% (23%, 21%, 18% and 13%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and BBSP (Table 3).

Non-essential elements. The concentrations of all combined studied elements differed significantly between adult WSP and all other categories (PERMANOVA, Bonferroni-corrected $p<0.05$; Table 2). The SIMPER analysis showed that the overall average dissimilarity was 39.3%. Bi, Sr, Hg and Pb together contributed over 50% (29%, 25%, 18% and 14%, respectively) to the pattern of overall dissimilarity observed in elemental concentrations (Table 3). Bi, Sb, Sr and Pb were responsible for 50%, 14%, 13% and 11%, respectively, of the dissimilarity pattern in elemental concentrations observed between adult WSP and chick WSP body feathers. Sr, Pb, Bi and Hg together produced the majority of dissimilarity in elemental concentrations observed between adult WSP and chick WSP down (36%, 19%, 19% and 14%, respectively). Hg, Sr and Pb together contributed over 50% (49%, 30% and 16%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and BBSP (Table 3).

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316 *3.3. Intergroup differences for particular elements*

317 Kruskal–Wallis inter-group tests comparing the concentration of particular elements revealed
318 significant differences for all elements ($p<0.05$) (Supplementary Materials2, Fig. ES1–ES7)
319 except Mg ($p=0.44$) and Mo ($p=0.12$). *Post-hoc* tests revealed the following pattern of
320 significant inter-group differences (Supplementary Materials2, Fig. ES1– ES7):
321 1. lower concentration of Cu, Hg and Se in adult WSP compared to adult BBSP;
322 2. lower concentration of As, Bi, Ca, Cr, Fe, Sb and Se in adult WSP compared to chick WSP
323 body feathers
324 3. higher concentration of Zn in adult WSP compared to chick WSP down
325 4. higher concentration of As, Bi, Ca, Cd, Fe, Hg, Pb, Sb, Se and Sr in chick WSP down
326 compared to adult WSP
327 5. higher concentration of As, Bi, Cr, Cu, Sb and Zn in chick WSP body feathers compared to
328 chick WSP down
329 6. lower concentration of Ca and Sr in chick WSP body feathers compared to WSP down
330 Other studied inter-group differences were not significant ($p>0.05$).
331
332 *3.4. Inter-group differences for Hg concentration determined by cold vapour technique*

333 The Kruskal–Wallis test revealed significant inter-group differences ($p < 0.05$) in the
334 concentration of Hg determined by cold vapour technique. *Post-hoc* tests results and pattern
335 of significant inter-group differences are presented in Fig. 2.
336
337 *3.5. Grouping of elements*

338 The Hierarchical Cluster Analysis for all studied groups combined (cophenetic correlation
339 0.902) recognised four main significant clusters grouping the trace elements (Fig. 3). The first
340 cluster included Ca and Zn (BP=100), while the second cluster contained Cd-Sb (BP=99).

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Then, the third was Bi-As (BP=96) and the fourth was Cu-Hg-Se (BP=96), with a subcluster of Hg-Se (BP=98).

4. Discussion

Our study provides reference values for concentration of 17 elements in feathers of two pelagic seabird species from the maritime Antarctic. We revealed several differences in elemental concentrations between the two species, as well as differences in exposure between life-cycle stages. We also identified some patterns in concentrations of particular elements.

4.1. Contaminant patterns of selected elements and comparisons with other seabirds from south polar areas

Although reference values of 17 elements are provided in our study, below, we discuss only the those considered most relevant in terms of possible effects on birds' health and survival.

4.1.1. Mercury

Hg is an endocrine disruptor associated with several adverse effects, including decreased body condition, immune responses and hormonal secretion (Wolfe et al. 1998, Scheuhammer et al. 2007, Tartu et al. 2014, 2015). As such, it affects birds reproduction and survival, and so may impact birds' population dynamics (Tartu et al. 2013, Goutte et al. 2014). Bird feathers are perceived as the main route for Hg excretion (Monteiro and Furness 1995, Santos et al. 2006), but its level would depend on multiple factors, including diet, excretion capacities in the feathers and moulting pattern (Becker et al. 2016, Bustamante et al. 2016). The Hg concentration reported in our results for BBSP adults (5.47±1.61 μg g^{-1} dw) are in a range of values reported previously by Carravieri et al. (2014; 4.22±2.53 μg g^{-1} dw). However, for

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adult WSP, our values (2.38±1.47 µg g^{-1} dw) were much higher compared to other studies (0.42±0.13 µg g^{-1} dw; Carravieri et al. 2014). Nevertheless, Hg levels in adult WSP from our study were comparable to mean levels reported for another low-trophic-level seabird, the Antarctic prion *Pachyptila desolata* (1.73–2.80 µg g^{-1} dw). In general, there is a high variability between petrel species (0.42–12.43 µg g^{-1} dw; Table 4). Here, the inter-species difference in Hg concentration is most likely associated with diet (Thompson and Furness 1989a, Bustamante et al. 2016, Blévin et al. 2013) as BBSP feeds at a higher trophic level than WSP (Quillfeldt et al. 2017). Such a dietary explanation was suggested in the study of Blévin et al. (2013), where chicks of 21 various species breeding in the Southern Ocean were been found to vary greatly in terms of Hg concentration (from 0.05±0.01 µg g^{-1} in the South Georgian diving petrel *Pelecanoides georgicus* to 5.31±1.12 µg g^{-1} in the northern giant petrel *Macronectes halli*).

Examining Hg concentrations in age groups, in both species we found that it was significantly higher in adults than in chicks of the same species (excluding WSP down; Fig. 2) probably due to the longer exposure time of adults. This is similar to white-chinned petrels *Procellaria aequinoctialis* (Carvalho et al. 2013), for which the same explanation has been suggested. In contrast, in the wandering albatross *Diomedea exulans*, Hg contamination was higher in immatures than adults, which may be associated with moulting intensity and detoxification capacities varying between adults and immatures (Bustamante et al. 2016).

4.1.2 *Selenium and its interaction with mercury*

Se is an essential trace element for proper organism functioning, including thyroid function (Burger et al. 2013), and it is known for its protective action against Hg bioaccumulation and

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toxicity through the creation of Hg-Se compounds (Nigro and Leonzio 1996, Khan and Wang 2009). However, excess Se may as well have toxic effects on vertebrates (Burger et al. 2013).

We found that Se levels significantly differ between the two studied species, with almost three times higher values found in adult BBSP compared to adult WSP (5.19±1.18 µg g^{-1} dw *vs* 1.81±0.98 µg g^{-1} dw). These values add to a wide range reported so far from other seabirds of the Southern Ocean (3.40–19.40 µg g^{-1} dw; Anderson et al. 2010, Fromant et al. 2016, Philpot et al. 2019). Interestingly, Se levels in *Pygoscelis* sp. penguins living in King George Island were similar to values found in our study (2.46–6.37 µg g^{-1} dw; Jerez et al. 2011), while for penguin *Pygoscelis* from other area Hg levels were lower, from <0.80 to 2.0 µg g^{-1} (Metcheva et al. 2006).

The studied WSP chicks had higher Se levels compared to adults (Table 1). This trend was not observed in gadfly petrels *Pterodroma spp*, where Se levels were significantly lower in chicks than in adults (Philpot et al. 2019). These differences are difficult to explain given the currently limited knowledge about Se distribution and metabolism.

Worldwide studies quantifying Hg-Se co-exposure and interaction in seabirds are still rare, but have increased in recent years, and show that seabirds' ability to deal with high mercury and selenium levels is still not fully understood, and may depend on age and species (e.g., Carvalho et al. 2013, Cipro et al. 2014, González-Solís et al. 2002, Carravieri et al. 2017, Philpot et al. 2019). Se-Hg molar ratios in our study differed between chicks and adults, being highest in WSP chick body feathers (Table 1). However, all ratios reported here were >1, suggesting activation of a defence mechanism against high Hg concentrations and a health impact associated with potential Se toxicity (Lucia et al. 2016).

4.1.3. Cadmium

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411 Cd is another toxic element that readily bioaccumulates in food webs (Cipro et al. 2014), and
412 Cd has been reported at even higher levels in Antarctic species including plankton, marine
413 benthic invertebrates, fishes, seabirds and marine mammals (see references in Jerez et al.
414 2011), than in their counterparts sampled in polluted coastal areas (Petri and Zauke 1993). In
415 our study, Cd levels in adults were mostly below the quantification limit (Table 1), but it is
416 not exceptional (e.g. penguin feathers were also generally low (<LOD–0.10 µg g^{-1} dw, Jerez
417 et al. [2011]; <0.15–0.21 µg g^{-1} dw [Metcheva et al. 2006]) and may be related to relatively
418 low deposition of Cd in feathers (Lucia et al. 2010, Cipro et al. 2014). Indeed, in Antarctic
419 prions, Cd levels in internal tissues (kidney 105±37 µg g^{-1} dw) were considerably higher than
420 in feathers (mean 0.06±0.03 µg g^{-1} dw) (Fromant et al. 2016). Thus, feathers would give only
421 partial information of bird exposure; i.e. only when it reaches high levels.

422

423 *4.1.4. Lead*

424 After Hg, Pb is another major contaminant of toxicological concern (Burger and Gochfeld
425 2009), affecting breeding success, migratory behaviour and survival of animals at various
426 trophic levels (Burger, 1995). It may affect food web dynamics e.g by decreasing the
427 abundance and availability of food prey, or by interfering with its natural hiding or escape
428 behaviour (Burger, 1995). Pb is accumulated in feathers at higher rate compared to Cd (Jerez
429 et al. 2011), but can be elevated due to exogenous contamination (Jaspers et al. 2004).
430 Adverse effects from lead toxicity might occur at levels of 4 µg g^{-1} in feathers (Burger and
431 Gochfeld 2000) but levels in adult seabirds are usually lower (0.51–1.68 µg g^{-1} dw, Mendes et
432 al. 2008, Burger and Gochfeld 2009). In our study the Pb concentration was generally low in
433 adults (<1.17 µg g^{-1} dw, with one outlier reaching 5.06 µg g^{-1} dw), and higher in chicks (0.36–
434 3.67 µg g^{-1} dw). Similarly low values of Pb concentration have been reported for other
435 seabirds from the Southern Ocean (Metcheva et al. 2006, Anderson et al. 2010, Jerez et al.

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2011, Fromant et al. 2016). However, for penguins breeding on King George Island, high Pb values have been also reported, which has been explained by local human activities (many scientific bases and a small airport in the study area; Jerez et al. 2011).

4.1.5. Zinc

Zn can be bioaccumulated in polar organisms, but most likely does not biomagnify (Santos et al. 2006). Variation of this element concentration in adult storm petrels was relatively low (WSP 109.20±18.50 µg g^{-1} dw, BBSP 99.95±13.01 µg g^{-1} dw), and in chicks was even lower, and with small inter-individual variability (WSP down and body feathers 48.30±7.08 and 93.00±11.3 µg g^{-1} dw, respectively). The reported Zn concentration falls well within the range reported from other seabirds (6.95–301 µg g^{-1} dw) (Anderson et al. 2010, Cipro et al. 2014, Fromant et al. 2016, Philpot et al. 2019, Metcheva et al. 2006, Jerez et al. 2011, Santos et al. 2006).

4.1.6. Copper

Cu, like Zn, is also an essential element, with concentrations in seabird tissues controlled mostly in homeostasis processes (Bocher et al. 2003). Variation in the concentration variation of Cu in the two species was much larger than for Zn (adults, WSP: 2.52±2.35 µg g^{-1} dw, BBSP: 8.12±0.56; chick body, WSP: 6.68±3.15, chick down WSP: 1.52±0.60 µg g^{-1} dw). These values also seem to fall well within the range reported so far for other seabirds (6.0–12.7 µg g^{-1} dw; Metcheva et al. 2006, Jerez et al. 2011).

4.2. Potential sources of elements

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458 The contamination of Antarctic biota may have both natural and anthropogenic sources (Jerez
459 et al. 2011, Lu et al. 2012, Deheyn et al. 2005). Our cluster analysis revealed some interesting
460 groupings of elements that suggested common source of contamination.

461 The Bi-As cluster suggests a volcanic origin of the two elements. Worldwide
462 emissions from volcanoes are deemed a considerable source of atmospheric Bi and As
463 (Candelone et al. 1995, Kabata-Pendias and Szteke 2015), and the soils on King George
464 Island are mostly composed of mineral and rock fragments with some volcanic ashes (Lee et
465 al. 2004). The ashes were blown from Deception Island, a volcanic island located ~130 km
466 south-west of King George Island (Jeong and Yoon 2001), where the most recent eruption
467 occurred in the late 1960s (Orheim 1972). Storm petrels may additionally gain As from food
468 sources, as low-trophic organisms (as petrels diet items) easily assimilate this element
469 (Rahman et al. 2012). Mão de Ferro et al. (2013) found As enrichment in several Antarctic
470 abiotic and biotic samples to probably be a result of past volcanic activity and sediment
471 petrologic characteristic, as well as As leaching processes. They also indicated that during a
472 high tide, leaching processes of As can occur to shore and semi-submerged areas, thus being
473 available to aquatic organisms (Mão de Ferro et al. 2013). All feathers were cleaned by the
474 exact same procedure, but we cannot exclude the possibility of external contamination by soil
475 particles, as both As and Bi were only detected in chicks feathers.

476 Both elements of the Ca-Zn cluster are necessary components in the synthesis of the
477 feather pigment melanin (McGraw et al. 2003). They also play an essential role in multiple
478 physiological body functions (Bogden & Klevay 2000). Thus, this cluster may reflect both co-
479 exposure from diet and the similar co-regulation mechanisms responsible for element
480 deposition. Both Ca and Zn accumulation in feathers may depend on melanin type and
481 content, as shown by element enrichment in pigmented feather parts (Niecke et al. 1999
482 2003).

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The Cd-Sb cluster may represent common food and/or water input. Cd may originate from anthropogenic pollution but also from rock weathering and/or natural sources, as it is more mobile in seawater than in other water bodies and is easily absorbed by aquatic biota (Kabata-Pendias and Szteke 2015). Natural sources (diffusive fluxes, upwelling and continental weathering) can be responsible for higher abundance of Cd in Antarctic water samples (Sañudo-Wilhelmy et al. 2002). High Cd concentrations were found in Antarctic krill *Euphasia superba*, which is the main dietary component for adults and chicks of both storm-petrel species (Wasilewski 1986, Petri and Zauke 1993, Hahn 1998, Nygård et al. 2001, Quillfeldt 2002). The natural sources of Sb and its compounds are volcanic eruptions, sea spray, forest fires and wind-blown dust, suggesting a non-anthropogenic source (Kabata-Pendias and Szteke 2015). Considering its clustering with Cd, we would suggest a natural source of both elements in the feathers of the studied birds.

The common clustering of Cu-Hg-Se may be explained by the properties of Se and the high concentration of all these elements in aquatic organisms, including fish. Marine aerosols are enriched in Se resulting from the formation of volatile Se-organic compounds. Volcanic emissions were suggested as a prevalent source of Hg in Deception Island (Mão de Ferro et al. 2014). Also, summer input from the Southern Ocean may be a net source for the gaseous element Hg in the marine boundary layer (Wang et al. 2017).

4.3. Species and age differences in elemental concentrations

Significant differences in concentration of various elements (i.e. Cu, Hg, and Se) between the WSP and BBSP found in our study are most likely to be associated with inter-species differences in foraging (different trophic levels with a different contribution of fish in their diet [Quillfeldt et al. 2017]).

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Significant differences in concentrations of various elements (Supplementary Materials2, Fig. ES1–ES7) between WSP age groups are also likely to be associated with diet, although here not with the difference in diet composition but more with the location of food resources exploited during the period of growth of relevant feathers. Chick feathers are more suitable for local exposure assessment, as levels are not affected by moulting patterns, because chicks receive food collected by parents in the vicinity of the breeding colony, and have a shorter exposure time. Thus, in cases when adult and offspring diet do not differ significantly, chick feathers may also be used to reconstruct adults' foraging ecology and adults' exposure to several pollutants during the chick-rearing period (Blévin et al. 2013).

Down feathers have been successfully used to estimate Hg concentrations in eggs (Santos et al. 2017), suggesting its potential as a suitable proxy for contaminant determination. A strong correlation between the levels of both Hg and Se in eggs and the liver of incubating females has been found in *Charadriiformes* (Ackermann et al. 2016). In our study, all examined elements except Ni and Co were detected in down feathers, enabling exposure assessment at the earliest phase of life. The highest Ca level was found in WSP down, probably because the developing embryo absorbs Ca and other elements, initially from the yolk and subsequently from the eggshell (Castilla et al. 2010). We found the lowest Zn level in down (48.30±7.08 µg/g dw), at almost two times lower than the level in chick body feathers (93.00±11.30 µg/g dw). Other elements, such as Pb, Hg, Se, Mg and Sr, were higher in down than in chick body feathers, suggesting that exposure changes over time. Maternal transfer of contaminants may be a reason for the increased levels of several metals in chick down feathers, as the maternal transfer is species- and element-specific (Ackermann et al. 2016).

5. Conclusions

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Our study provides a reference values for concentration of 17 elements in feathers of two pelagic seabird species from the maritime Antarctic. Such data may serve to monitor contaminant levels in marine systems and to evaluate variability in contaminant levels in tissues throughout birds' annual cycle (Rodríguez et al. 2019). We also revealed several differences in elemental concentrations between the two species, as well as differences in exposure between life-cycle stages. These inter-species and inter-age differences are attributed to the various diet compositions and geographic areas of feather growth. Finally, we identified some patterns in concentration of particular elements that suggest a primarily natural origin of most elements. We believe that our study contributes to understanding spatial and temporal patterns of contaminant accumulation in the Maritime Antarctic ecosystem. As emissions and global transport of elements such as Hg, Pb and Cd are expected to increase in the future, monitoring studies on seabirds breeding in the Southern Hemisphere may be a warning system for global changes and the consequences of elevated emissions into the marine food web. Despite the limitations of our study, such as: the relatively small sample size; sample collection being restricted to one site and one season; and the lack of data on elemental concentrations in prey items, our study delivered important reference values for elemental concentrations in various age groups of the two study species. The Antarctic Treaty members urge long-term monitoring and sustained observations of the Antarctic environment and the associated data management, to detect, understand and forecast the impacts of climate-change-driven environmental variability (ATCM 2007).

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Table
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Table 1. Elemental concentrations of the studied elements in feathers of storm-petrels, mean ± SD (min–max) µg g^{-1} dw, N = the number of individuals sampled, LOD = detection limit, LOQ= quantification limit, Se:Hg – Se:Hg molar ratio

Element	Adult WSP (N=12)	Adult BBSP (N=4)	Chick down WSP (N=4)	Chick body WSP (N=4)
As	75% <LOD	100%<LOQ	1.76 ± 1.10 (0.45–3.16)	6.11 ± 1.43 (4.77–8.38)
Bi	92%<LOD	100%<LOD	1.57 ± 1.78 (<LOD–3.92)	14.16 ± 5.92 (6.50–20.50)
Ca	96.0 ± 21.0 (77.7–156.0)	80.0 ± 11.2 (72.8–99.3)	242.8 ± 60.4 (165.0–326.0)	136.50 ± 11.79 (124.9–156.0)
Cd	<LOD (<LOD–0.45)	<LOQ	0.45 ± 0.22 (<LOQ–0.68)	0.51 ± 0.24 (<LOD– 0.70)
Cr	0.67 ± 0.45 (0.11–4.36)*	0.71 ± 0.45 (0.09–1.22)	1.54 ± 0.48 (0.88–2.08)	3.46 ± 0.78 (2.73–4.78)
Cu	2.52 ± 2.35* (<LOD–13.9)	8.12 ± 0.56 (7.52–9.06)	1.52 ± 0.60 (0.67–2.26)	6.68 ± 3.15 (2.49–10.96)
Fe	20.40 ± 18.00 (<LOD–263.0)*	10.73 ± 5.04 (<LOD–16.16)	74.30 ± 19.30 (50.80–102.4)	131.7 ± 81.8 (63.1–270.0)
Mg	478 ± 130 (315–773)	429 ± 101 (306–529)	538 ± 354 (316–1152)	378 ± 91 (285–513)
Mo	2.41 ± 4.09 (<LOD–14.6)	0.56 ± 0.27 (0.16–0.84)	1.92 ± 1.77 (0.36–4.90)	7.13 ± 7.42 (1.52–19.79)
Pb	0.33 ± 0.37* (<LOD–5.06)	0.36 ± 0.24 (0.11–0.74)	1.77 ± 0.91 (0.75–2.76)	1.43 ± 1.32 (0.36–3.67)
Sb	100%<LOD	75% <LOD	0.22 ± 0.15 (<LOD–0.43)	1.17 ± 0.53 (0.58–1.92)

Element	Adult WSP (N=12)	Adult BBSP (N=4)	Chick down WSP (N=4)	Chick body WSP (N=4)
Se	1.81 ± 0.98 (<LOD–4.65)	5.19 ± 1.18 (3.74–6.62)	4.06 ± 0.50 (3.29–4.69)	3.63 ± 1.01 (2.39–5.13)
Sr	5.77 ± 3.62 (2.47–13.53)	2.79 ± 1.21 (1.35–4.58)	23.19 ± 8.75 (13.4–37.1)	9.57 ± 1.020 (8.63–11.27)
Zn	109.20 ± 18.50 (70.3–141)	99.95 ± 13.01 (85.5–120)	48.30 ± 7.08 (40.30–59.60)	93.00 ± 11.30 (79.4–110.8)
Se:Hg	1.92	2.75	6.00	13.90

* for Cr, Cu, Fe and Pb one outlier was excluded from the mean calculation, but it is shown as the maximal value

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Table
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Table 2 Intergroup differences (one-way PERMANOVA, Bonferroni-corrected p values) of elemental concentration, log(x + 1) transformed, in the feathers of the four studied groups of storm-petrels: body feathers of adult Wilson's (WSP_Ad) and back-bellied (BBSP_Ad) storm-petrels, down from Wilson's storm-petrel chicks (WSP_down) and body feathers from Wilson's storm petrel fledglings (WSP_CHF)

PERMANOVA, F = 11.48, p = 0.0001				
All elements	WSP_CHF	WSP_down	BBSP_Ad	WSP_Ad
WSP_CHF	-	0.182	0.176	0.007
WSP_down		-	0.157	0.004
BBSP_Ad			-	0.037
WSP_Ad				-
PERMANOVA, F = 9.26, p = 0.0001				
Essential	WSP_CHF	WSP_down	BBSP_Ad	WSP_Ad
WSP_CHF	-	0.160	0.181	0.003
WSP_down		-	0.166	0.005
BBSP_Ad			-	0.136
WSP_Ad				-
PERMANOVA, F = 12.93, p = 0.0001				
Non-essential	WSP_CHF	WSP_down	BBSP_Ad	WSP_Ad
WSP_CHF	-	0.160	0.181	0.003
WSP_down		-	0.166	0.012
BBSP_Ad			-	0.043
WSP_Ad				-

Table

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Table 3 Sources of variability (average percentage dissimilarity) in the elemental concentrations (log(x+1) transformed) in: body feathers of adult Wilson's (WSP_Ad) and back-bellied (BBSP_Ad) storm-petrels, down from Wilson's storm-petrel chicks (WSP_down) and body feathers from Wilson's storm petrel fledglings (WSP_CHF), according to the SIMPER analysis. Only elements with a contribution > 10% are shown. ADis - Average Dissimilarity, Contr. (%) – percentage contribution, Overall - overall average similarity

	Overall dissimilarity		WSP_Ad vs WSP_CHF			WSP_Ad vs WSP_down			WSP_Ad vs BBSP_Ad	
	ADis	Contr.		ADis	Contr.		ADiss	Contr.		ADis	Contr.
All elements											
Fe	2.57	13.9	Bi	4.18	19.1	Fe	2.67	14.0	Cu	2.12	16.1
Bi	2.07	11.2	As	3.06	14.0	Sr	2.36	12.4	Fe	1.94	14.7
			Fe	3.02	13.8				Hg	1.89	14.3
									Se	1.63	12.3
Overall	18.46			21.87			19.30			13.23	
Essential											
Fe	3.10	21.9	As	3.72	22.6	Fe	3.24	21.5	Cu	2.43	22.8
As	2.06	14.5	Fe	3.68	22.4	Ca	1.94	12.9	Fe	2.22	20.8
Cu	1.83	12.9	Mo	2.40	14.6	As	1.85	12.3	Se	1.87	17.6
Mo	1.83	12.9	Cr	1.87	11.3	Zn	1.71	11.3	Mo	1.41	13.3
			Cu	1.82	11.0	Mo	1.66	11.0			
Overall	14.16			16.46			15.07			10.65	
Non-Essential											
Bi	11.47	29.2	Bi	23.67	50.3	Sr	13.96	36.4	Hg	14.42	49.1

	Overall dissimilarity		WSP_Ad vs WSP_CHF			WSP_Ad vs WSP_down			WSP_Ad vs BBSP_Ad		
	ADis	Contr.		ADis	Contr.		ADiss	Contr.		ADis	Contr.
Sr	10.01	25.5	Sb	6.70	14.2	Pb	7.43	19.4	Sr	8.68	29.5
Hg	7.02	17.9	Sr	6.26	13.3	Bi	7.27	19.0	Pb	4.74	16.1
Pb	5.59	14.2	Pb	5.38	11.4	Hg	5.25	13.7			
Overall	39.31			47.09			38.33			29.41	

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Table
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Table 4 Variability of mercury (Hg) levels in feathers of *Procellariiformes*. N – number of individuals

Species	Study area	Tissue	N	Age	Concentrat. mean± SD µg g^{-1} dw	Reference
Antarctic prion (*Pachyptila desolata*)	Kerguelen archipelago	body feathers	10	unknown	2.8±1.2	Fromant et al. 2016
			10	adult	1.73±0.50	Carravieri et al. 2014
White-headed petrel (*Pterodroma lessonii*)	Kerguelen archipelago	body feathers	10	adult	12.43 ± 2.01	Carravieri et al. 2014
			10	chicks	1.54 ± 0.34	Blévin et al. 2013
spectacled petrel (*Procellaria conspicillata*)	southwestern Atlantic Ocean off the Brazilian coast	contour feathers	38	unknown	11.17 ± 3.78	Carvalho et al. 2013
white-chinned petrel (*Procellaria aequinoctialis*)	Kerguelen archipelago	body feathers	22	unknown	7.63 ± 3.87	Cipro et al. 2014
	southwestern Atlantic Ocean off the Brazilian coast	contour feathers	9	adults	3.45 ± 2.84	Carvalho et al. 2013
			21	juveniles	1.14 ± 2	
	Kerguelen archipelago	body feathers	14	chicks	1.82 ± 0.51	Blévin et al. 2013
Leach's storm-petrel	Machias Seal Island, New Brunswick,	Breast feathers	15	adult	7.01*	Bond and Diamond,
			20	chicks	1.42*	

(*Oceanodroma leucorhoa*)	Canada					2009
Wilson's storm-petrel (*Oceanites oceanicus*)	Kerguelen Islands	body feathers	12	adult	0.42 ± 0.13	Carravieri et al. 2014
	King Gorge Island, South Shetlands, Antarctica	body feathers	25	adult	2.38 ± 1.47	present study
		body feathers	5	chick	0.67 ± 0.27	
		down	16	chick	1.72 ± 0.65	
Black-bellied storm-petrel (*Fregetta tropica*)	Kerguelen Islands	body feathers	10	adult	4.22 ± 2.53	Carravieri et al. 2014
	King Gorge Island, South Shetlands, Antarctica	body feathers	8	adult	5.47 ± 1.61	present study
		body feathers	5	chick	1.87 ± 0.29	
		down	6	chick	3.99 ± 1.07	

** Estimated marginal mean*

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Figure
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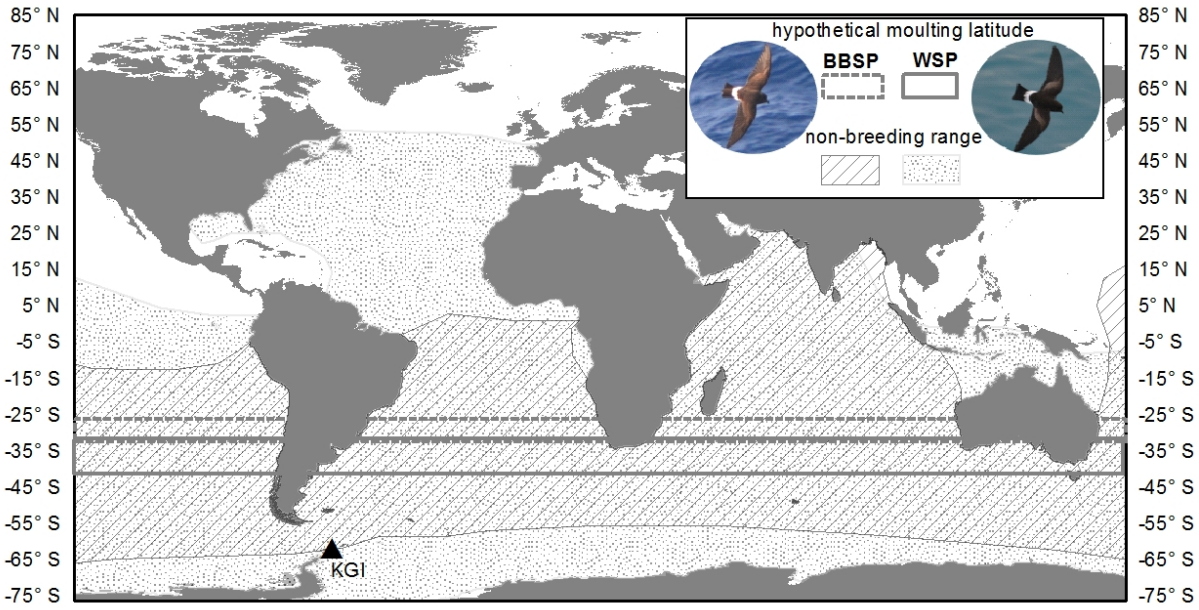

Fig. 1 Range of the studied species and possible areas of elemental input: triangle – study area, King George Island (KGI), grey rectangles – moulting latitudes for adult storm-petrels (dotted for black-bellied storm-petrel (BBSP) and solid for Wilson's storm-petrel (WSP); according to isotopic data from Quillfeldt et al. 2005 and Phillips et al. 2009 calculated based on equation proposed by Quillfeldt et al. 2005). Storm-petrels non-breeding range map source: BirdLife International and Handbook of the Birds of the World 2018. Photos by DJ

Figure
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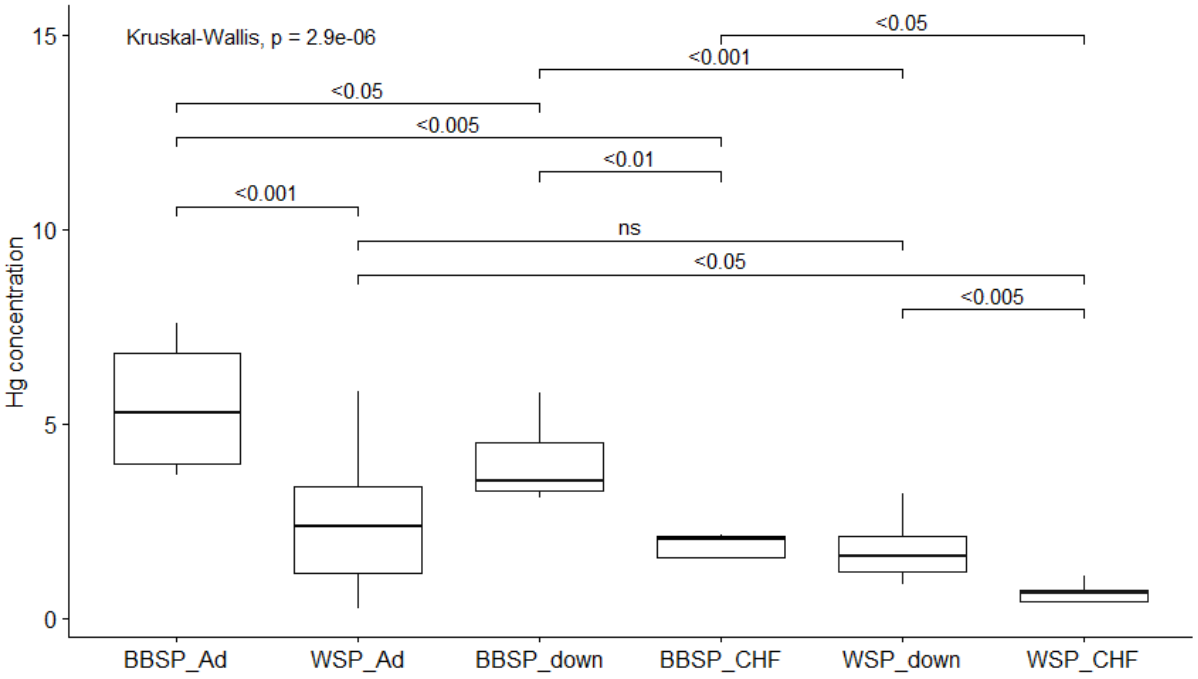

Fig. 2 Concentration of Hg (µg·g^{-1} dw) in feathers of the six studied groups of storm-petrels: body feathers of adult Wilson's (WSP_Ad) and back-bellied (BBSP_Ad) storm-petrels, down from Wilson's (WSP_down) and black-bellied (BBSP_down) storm-petrel chicks and body feathers from Wilson's (WSP_CHF) and black-bellied (BBSP_CHF) storm-petrel chicks. Boxplots show the median (band inside the box), the first (25%) and third (75%) quartile (box), and the lowest and the highest values within 1.5 interquartile range (whiskers)

Figure
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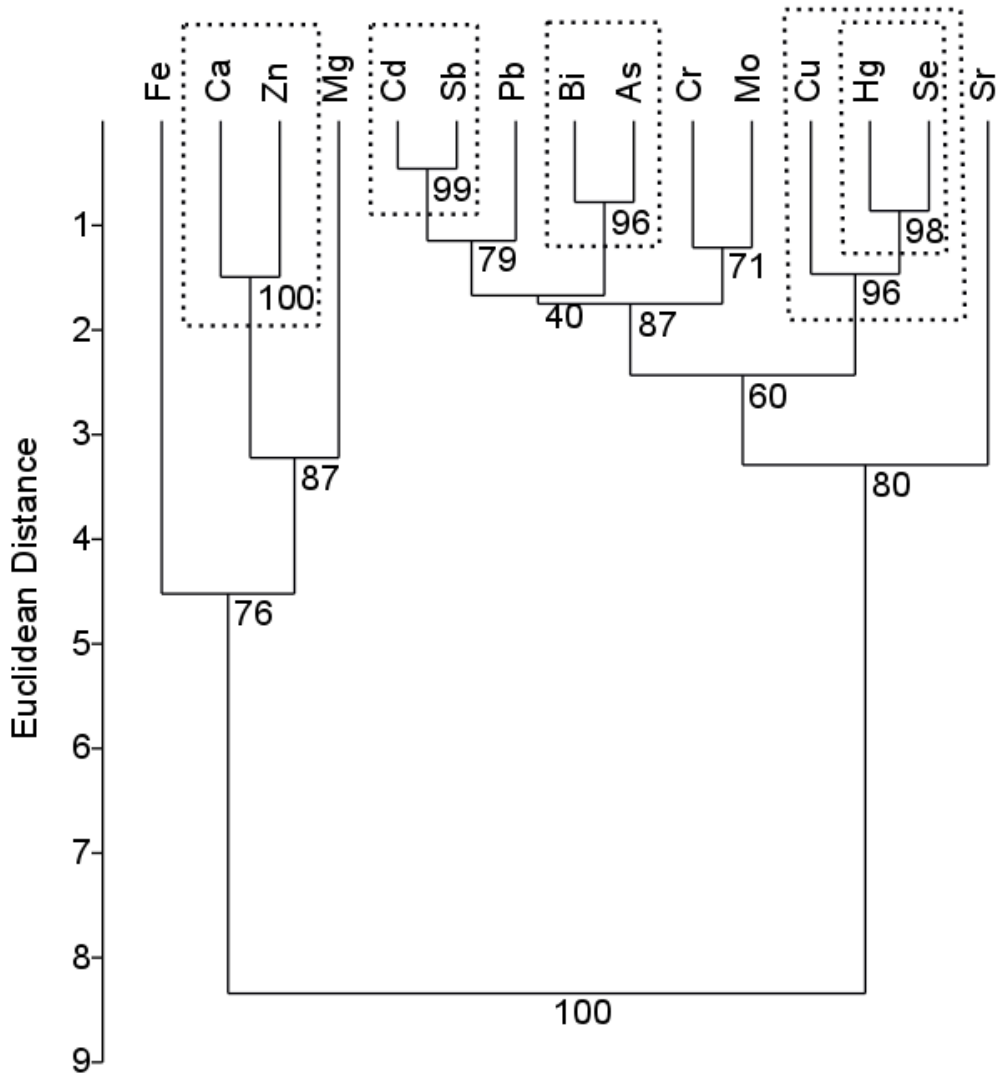

Fig. 3 Hierarchical dendrogram of the studied elements in the feathers of the studied storm-petrels (all age and feather type groups combined), obtained using a paired group method and Euclidean distance matrix (the distance reflects degree of association between different elements). Numbers below branches indicate bootstrap probability values (bootstrap n = 1000). Clusters with bootstrap support ≥ 95 denoted with a dotted rectangle.

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