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Storm petrels as indicators of pelagic seabird exposure to chemical elements

3	in the	Antarctic	marine	ecosystem
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15	
16	Abstract
17	Data on trace element bioavailability in the south-polar marine ecosystem is still scarce,
18	compared to that relating to temperate zones. Seabirds can be used as indicators of ecosystem
19	health and sentinels of environmental pollution, constituting a link between marine and
20	terrestrial environments. Here, we analysed the concentration of 17 elements (with special
21	emphasis on mercury, Hg) in feathers of adults and chicks of two pelagic seabirds - the
22	Wilson's storm petrel Oceanites oceanicus and the black-bellied storm petrel Fregetta tropica

24 non-breeding period away from the breeding grounds, but down and body feathers of chicks

- breeding sympatrically in the maritime Antarctic. Since adult feathers are formed during the

grow at the breeding sites, we were able to evaluate the birds' exposure to contaminants at 25 various stages of their annual life cycle and in various marine zones. We found that of the two 26 studied species, adult black-bellied storm petrels had significantly higher mercury, selenium 27 and copper levels $(5.47\pm1.61; 5.19\pm1.18; 8.20\pm0.56 \text{ µg g}^{-1} \text{ dw}$, respectively) than Wilson's 28 storm petrels (2.38 \pm 1.47; 1.81 \pm 0.98; 2.52 \pm 2.35 µg g⁻¹ dw, respectively). We found that 29 Wilson's storm petrel chicks had a significantly different contaminant profile than adults. 30 31 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 32 transfer of Hg (to down feathers) in both species. As global contaminant emissions are 33 expected to increase, birds inhabiting remote areas with sparse anthropogenic pollution can 34 indicate the temporal trends in global contamination. 35

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

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38 1. Introduction

Organisms living in Antarctica are exposed to a number of environmental factors that may 39 affect their health and survival. Of those, the most influential are harsh climate conditions, 40 41 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 42 processes [i.e. volcanic activity, the input of sea-spray, mechanical and chemical rock 43 weathering (Malandrino et al. 2009)] and biota [e.g. mammals or bird colonies can be a 44 source of nutrients/organic matter (N, F) and several elements such as Cd, Hg, As, Se and Zn 45 to the terrestrial and coastal ecosystem (Cipro et al. 2018)]. Anthropogenic sources of 46 47 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 48

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 60 61 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. 62 2004). High metals affinity to sulfhydryl groups of the feather structural proteins are making 63 them a suitable biomonitoring tool (Thompson et al. 1998). Elemental deposition in feather 64 tissue is species-specific and depends on multiple factors, including diet, age, detoxification 65 66 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 67 of the contamination period (Bustamante et al. 2016, Cherel et al. 2018). Adult feathers may 68 69 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 70 exposure over time. Also seasonal shifts in element concentrations can occur (Øverjordet et 71 72 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 73

74 maternal nutrients and as such represents female contamination during the pre-laying period 75 (Ackermann et al. 2016). Thus, analysis of feathers collected at various life stages allows the 76 elemental concentrations of various areas to be reconstructed, indicating temporal and spatial 77 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 78 contamination, still little is known about elemental concentrations in seabirds feeding at low 79 80 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 81 three families of Procellariiformes: Procellariidae, Oceanitidae, and Hydrobatidae) are still 82 one of the most poorly examined seabird groups, mostly due to their small body size, their 83 nesting predominantly on isolated and inaccessible islands, and their high mobility at sea 84 (Rodríguez et al. 2019). However, even this group is exposed to a multitude of contaminants 85 86 (e.g., Anderson et al. 2000, Bocher et al. 2003, Cipro et al. 2014, Fromant et al. 2016, Philpot et al. 2019). 87

In this study we determined levels of elements in feathers of two storm-petrel species 88 breeding in the maritime Antarctica, the Wilson's storm petrel (Oceanites oceanicus, hereafter 89 WSP) and the black-bellied storm petrel (Fregetta tropica, hereafter BBSP). We focused both 90 91 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 92 studied in avian tissues (i.e. antimony [Sb], bismuth [Bi], calcium [Ca], cobalt [Co], iron [Fe], 93 94 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 95 properly assess ecosystem health and to comprehend pollutants' abilities for potential 96 97 bioaccumulation and biomagnification. By studying rarely analysed elements, the results

98 should provide background data for research detecting future inputs of elements in remote99 polar regions (Santos et al. 2006).

100

We aimed to:

101 1) present reference values for the concentrations of 17 elements that can be used in the future102 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 sourcesof contamination.

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118 2. Materials and Methods

119 2.1. Studied species

The two study species - the Wilson's storm petrel and the black-bellied storm petrel - are 120 121 small pelagic seabirds, with circumpolar breeding distributions including sub-Antarctic islands and the maritime Antarctic. Both species breed sympatrically in the study area (see 122 below) during the austral summer (from December to March), with similar breeding biology: 123 single-egg clutch, incubation lasting 38–44 days, and chick rearing up to 71 days. Although 124 both species are among the smallest endotherms living in the Antarctic, they play an 125 important role as predators preving on Antarctic krill, myctophid fish and amphipods (Hahn 126 1998, Quillfeldt 2002, Quillfeldt et al. 2005, Wasilewski 1986). Preying on fish and 127 crustaceans in equal proportions (Hahn 1998), BBSP feeds at a higher trophic level than 128 129 WSP, which eats mainly crustaceans (80-90% of meals) (Quillfeldt 2002, Quillfeldt et al. 2017). After breeding, both species migrate northwards, where they spend the non-breeding 130 period at open sea and complete their moult (Beck and Brown 1972). They moult in the 131 132 Atlantic Ocean in a wide range of habitats: WSP from sub-Antarctic to subtropical waters and BBSP primarily either in sub-Antarctic-subtropical waters or at the continental shelf (Phillips 133 et al. 2009) (Fig. 1). 134

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136 2.2. Sample collection

We studied the two storm-petrel species in the breeding colonies located in the vicinity of 137 Henryk Arctowski Station in Admiralty Bay, King Gorge Island, South Shetlands, Antarctica 138 (62°02'S 58°21'W, Fig. 1) in 2017. King George Island is the largest island in the South 139 Shetlands Archipelago, 90% ice-covered, with rocks mainly formed by andesitic and basaltic 140 magma (Santos et al. 2006). We captured adult birds in the breeding colony (in their nests, 141 142 using mist-nets spread in the colony area) during the incubation period and collected 4-5 body feathers from the back. Back body feathers represent mostly trace element input from 143 144 food and water intake during part of the non-breeding period when the feathers grew, which

takes place outside the colony in the Atlantic Ocean (Fig. 1). To sample chicks we caught 145 146 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 147 thermoregulation), and 4–5 body feathers from the back (when the nestlings were about to 148 fledge). Down feathers represent trace elements passed on by the female to the embryo, 149 reflecting their input during the pre-laying period, probably from areas around the breeding 150 colony (and likely predominantly reflecting the food intake). Chick body feathers represent 151 the nesting period and input from marine environments (as food and water intake). We stored 152 all the samples in individual plastic zip-lock bags until chemical analysis. 153

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155 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).

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

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172 2.3.1. Trace element concentration

We homogenised dry feathers by cutting them up, then weighed them to the nearest 0.01 mg, 173 and placed them in a clean Teflon vessel with 7 ml 65% HNO₃ (Merck, Suprapur). We carried 174 175 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 6-176 177 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 178 to 25 ml in clean plastic flasks. To ensure quality control and check background 179 180 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 181 analysed samples using an ICP-MS 2030 (Shimadzu, Japan) (for measurement conditions and 182 parameters see Table 1, Supplementary material) 183

184 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 185 with activated Al₂O₃ and analysed them using the thermal vaporisation atomic absorption 186 187 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 188 equipment specification are described in Pacyna et al. (2018). We determined total Hg 189 190 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 191 CV was above 15%, we excluded samples from the analyses, deeming the estimation of Hg 192

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 $8.64\pm4.65\%$ for adults, $6.32\pm5.40\%$ and $2.92\pm2.64\%$ 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.

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200 2.4. Quality control

We found that results for CRM analysis were in agreement with the certified values (mass 201 used for analysis: for trace elements 200 mg, for mercury 14-28 mg). Recoveries were high: 202 As 98%, Cd 106%, Cu 104%, Hg 92%, Pb 96%, Se 92%, Zn 97%. To check accuracy and 203 recoveries of other elements absent in this CRM we applied a treatment used before by 204 205 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 206 207 negligible and we did not perform blank correction. The limit of detection (LOD) and 208 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 209 Hg LOD/LOQ were calculated based on the standard deviation of the response (s), and the 210 slope of the calibration curve (b) according to the following formulas: LOD 3.3(s/b), LOQ 211 10(s/b). Method LOD/LOQ were in range of 0.004-0.92 and 0.013-3.07 ng/g respectively. We 212 reported our results as $\mu g \cdot g^{-1}$ dry weight (dw). For statistical analysis results below the LOD 213 we assigned half of the LOD value. 214

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% HNO₃ (Merck, USA). For sample pre-treatment and sample dilution we used deionised water obtained fromthe Milli-Q Direct 8 Water Purification System.

220 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

require the formulation of any *a priori* hypothesis considering the nature of the relationships 243 244 (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 245 Bootstrap Probability (BP) using multiscale bootstrap resampling. BP of a cluster may take a 246 value between 0 and 100, indicating how well the data supported the cluster, with a higher 247 value indicating a better fit (Hammer et al. 2001). We only considered clusters with $BP \ge 95$. 248 To determine how well the generated clusters represented dissimilarities between objects, we 249 calculated the cophenetic correlation coefficient. Values close to 0 indicate poor clustering, 250 and values close to 1 show strong clustering. 251

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:

258 1. all elements;

259 2. essential elements, i.e. As, Ca, Cr, Cu, Fe, Mg, Mo, Se and Zn; and

260 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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269	3. Results
270	3.1. Element concentrations
271	Of all the metals examined, Ni and Co were below the limit of detection in all samples, and
272	were thus excluded from further analysis. As, Bi and Sb were detected exclusively in chick
273	feathers, both body and down. Concentrations of all elements (mean±SD) are presented in
274	Table 1 and Table 4 (for Hg). Concentration chain for particular groups are:
275	a) For adult WSP Mg>Zn>Ca>Fe>Sr>Cu>Mo>Hg>Se>Cr>Pb
276	b) For adult BBSP Mg>Zn>Ca>Fe>Cu>Hg>Se>Sr>Cr>Mo>Pb
277	c) For chicks body feathers Mg>Ca>Fe>Zn>Bi>Sr>Mo>Cu>As>Se>Cr>Pb>Sb>Hg>Cd
278	d) For chicks down feathers Mg>Ca>Fe>Zn>Sr>Se>Mo>Pb=Hg=As>Bi=Cr=Cu>Cd>Sb

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280 *3.2. Inter-group differences in all elements concentration*

All elements. The concentrations of all combined studied elements differed significantly 281 between adult WSP and all other categories (PERMANOVA, Bonferroni-corrected p<0.04) 282 (Table 2). SIMPER analysis showed that the overall average dissimilarity was 18.5%. Fe and 283 Bi contributed most (14% and 11%, respectively) to the pattern of overall dissimilarity (Table 284 3). Bi, As and Fe contributed most (19%, 14% and 14%, respectively) to the pattern of 285 dissimilarity in elemental concentrations observed between adult WSP and chick WSP body 286 287 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. 288 Cu, Fe, Hg and Se contributed most (16%, 15%, 14% and 12%, respectively) to the pattern of 289 290 dissimilarity in elemental concentrations observed between adult WSP and BBSP.

Essential elements. The concentrations of all combined studied elements differed 291 significantly between adult WSP and all WSP chick categories (PERMANOVA, Bonferroni-292 corrected p<0.006). We found no differences between adult WSP and BBSP (p=0.136) (Table 293 2). The SIMPER analysis showed that the overall average dissimilarity was 14.2%. More than 294 half of the pattern of overall dissimilarity observed in elemental concentrations was explained 295 by Fe, As, Cu and Mo (22%, 15%, 13% and 13%, respectively) (Table 3). As, Fe and Mo 296 together contributed over 50% (23%, 22% and 15%, respectively) to the pattern of 297 dissimilarity observed in elemental concentrations between adult WSP and chick WSP body 298 feathers. Fe, Ca, As, Zn and Mo together contributed over 50% (21%, 13%, 12%, 11% and 299 300 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 301 302 over 50% (23%, 21%, 18% and 13%, respectively) to the pattern of dissimilarity in elemental concentrations observed between adult WSP and BBSP (Table 3). 303

Non-essential elements. The concentrations of all combined studied elements differed 304 significantly between adult WSP and all other categories (PERMANOVA, Bonferroni-305 corrected p<0.05; Table 2). The SIMPER analysis showed that the overall average 306 dissimilarity was 39.3%. Bi, Sr, Hg and Pb together contributed over 50% (29%, 25%, 18% 307 308 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%, 309 respectively, of the dissimilarity pattern in elemental concentrations observed between adult 310 WSP and chick WSP body feathers. Sr, Pb, Bi and Hg together produced the majority of 311 312 dissimilarity in elemental concentrations observed between adult WSP and chick WSP down 313 (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 314 observed between adult WSP and BBSP (Table 3). 315

316 *3.3. Intergroup differences for particular elements*

317 Kruskal–Wallis inter-group tests comparing the concentration of particular elements revealed

significant differences for all elements (p<0.05) (Supplementary Materials2, Fig. ES1–ES7)

steps 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):
- 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
- 4. higher concentration of As, Bi, Ca, Cd, Fe, Hg, Pb, Sb, Se and Sr in chick WSP downcompared to adult WSP
- 5. higher concentration of As, Bi, Cr, Cu, Sb and Zn in chick WSP body feathers compared tochick WSP down
- 6. lower concentration of Ca and Sr in chick WSP body feathers compared to WSP down
 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

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

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3.5. Grouping of elements

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

343

344 **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.

349

4.1. Contaminant patterns of selected elements and comparisons with other seabirdsfrom 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.

354 4.1.1. Mercury

Hg is an endocrine disruptor associated with several adverse effects, including decreased body 355 condition, immune responses and hormonal secretion (Wolfe et al. 1998, Scheuhammer et al. 356 2007, Tartu et al. 2014, 2015). As such, it affects birds reproduction and survival, and so may 357 impact birds' population dynamics (Tartu et al. 2013, Goutte et al. 2014). Bird feathers are 358 359 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 360 361 feathers and moulting pattern (Becker et al. 2016, Bustamante et al. 2016). The Hg concentration reported in our results for BBSP adults (5.47 \pm 1.61 µg g⁻¹ dw) are in a range of 362 values reported previously by Carravieri et al. (2014; 4.22 \pm 2.53 µg g⁻¹ dw). However, for 363

adult WSP, our values (2.38 \pm 1.47 µg g⁻¹ dw) were much higher compared to other studies 364 $(0.42\pm0.13 \ \mu g \ g^{-1} \ dw; \ Carravieri et al. 2014)$. Nevertheless, Hg levels in adult WSP from our 365 study were comparable to mean levels reported for another low-trophic-level seabird, the 366 Antarctic prion *Pachyptila desolata* (1.73–2.80 μ g g⁻¹ dw). In general, there is a high 367 variability between petrel species (0.42–12.43 μ g g⁻¹ dw; Table 4). Here, the inter-species 368 difference in Hg concentration is most likely associated with diet (Thompson and Furness 369 1989a, Bustamante et al. 2016, Blévin et al. 2013) as BBSP feeds at a higher trophic level 370 than WSP (Quillfeldt et al. 2017). Such a dietary explanation was suggested in the study of 371 Blévin et al. (2013), where chicks of 21 various species breeding in the Southern Ocean were 372 been found to vary greatly in terms of Hg concentration (from $0.05\pm0.01 \ \mu g \ g^{-1}$ in the South 373 Georgian diving petrel *Pelecanoides georgicus* to $5.31\pm1.12 \ \mu g \ g^{-1}$ in the northern giant petrel 374 Macronectes halli). 375

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).

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384 4.1.2 Selenium and its interaction with mercury

385 Se is an essential trace element for proper organism functioning, including thyroid function 386 (Burger et al. 2013), and it is known for its protective action against Hg bioaccumulation and 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 389 almost three times higher values found in adult BBSP compared to adult WSP (5.19±1.18 µg 390 g^{-1} dw vs 1.81±0.98 µg g^{-1} dw). These values add to a wide range reported so far from other 391 seabirds of the Southern Ocean (3.40–19.40 μ g g⁻¹ dw; Anderson et al. 2010, Fromant et al. 392 2016, Philpot et al. 2019). Interestingly, Se levels in Pygoscelis sp. penguins living in King 393 George Island were similar to values found in our study (2.46–6.37 μ g g⁻¹ dw; Jerez et al. 394 2011), while for penguin *Pygoscelis* from other area Hg levels were lower, from <0.80 to 2.0 395 $\mu g g^{-1}$ (Metcheva et al. 2006). 396

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.

401 Worldwide studies quantifying Hg-Se co-exposure and interaction in seabirds are still 402 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 403 (e.g., Carvalho et al. 2013, Cipro et al. 2014, González-Solís et al. 2002, Carravieri et al. 404 405 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 406 >1, suggesting activation of a defence mechanism against high Hg concentrations and a health 407 408 impact associated with potential Se toxicity (Lucia et al. 2016).

409

410 *4.1.3. Cadmium*

Cd is another toxic element that readily bioaccumulates in food webs (Cipro et al. 2014), and 411 Cd has been reported at even higher levels in Antarctic species including plankton, marine 412 benthic invertebrates, fishes, seabirds and marine mammals (see references in Jerez et al. 413 2011), than in their counterparts sampled in polluted coastal areas (Petri and Zauke 1993). In 414 our study, Cd levels in adults were mostly below the quantification limit (Table 1), but it is 415 not exceptional (e.g. penguin feathers were also generally low (<LOD–0.10 µg g⁻¹ dw, Jerez 416 et al. [2011]; <0.15–0.21 µg g⁻¹ dw [Metcheva et al. 2006]) and may be related to relatively 417 low deposition of Cd in feathers (Lucia et al. 2010, Cipro et al. 2014). Indeed, in Antarctic 418 prions, Cd levels in internal tissues (kidney $105\pm37 \ \mu g \ g^{-1} \ dw$) were considerably higher than 419 in feathers (mean $0.06\pm0.03 \ \mu g \ g^{-1} \ dw$) (Fromant et al. 2016). Thus, feathers would give only 420 partial information of bird exposure; i.e. only when it reaches high levels. 421

422

423 *4.1.4. Lead*

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

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).

439

440 *4.1.5. Zinc*

Zn can be bioaccumulated in polar organisms, but most likely does not biomagnify (Santos et 441 al. 2006). Variation of this element concentration in adult storm petrels was relatively low 442 (WSP 109.20 \pm 18.50 µg g⁻¹ dw, BBSP 99.95 \pm 13.01 µg g⁻¹ dw), and in chicks was even lower, 443 and with small inter-individual variability (WSP down and body feathers 48.30±7.08 and 444 93.00 \pm 11.3 µg g⁻¹ dw, respectively). The reported Zn concentration falls well within the range 445 reported from other seabirds (6.95–301 μ g g⁻¹ dw) (Anderson et al. 2010, Cipro et al. 2014, 446 447 Fromant et al. 2016, Philpot et al. 2019, Metcheva et al. 2006, Jerez et al. 2011, Santos et al. 448 2006).

449 *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: $2.52\pm2.35 \ \mu g \ g^{-1} \ dw$, BBSP: 8.12 ± 0.56 ; chick body, WSP: 6.68 ± 3.15 , chick down WSP: $1.52\pm0.60 \ \mu g \ g^{-1} \ dw$). These values also seem to fall well within the range reported so far for other seabirds (6.0– $12.7 \ \mu g \ g^{-1} \ dw$; Metcheva et al. 2006, Jerez et al. 2011).

456

457 **4.2. Potential sources of elements**

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

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

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

The Cd-Sb cluster may represent common food and/or water input. Cd may originate 483 484 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 485 (Kabata-Pendias and Szteke 2015). Natural sources (diffusive fluxes, upwelling and 486 continental weathering) can be responsible for higher abundance of Cd in Antarctic water 487 samples (Sañudo-Wilhelmy et al. 2002). High Cd concentrations were found in Antarctic 488 489 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, 490 Quillfeldt 2002). The natural sources of Sb and its compounds are volcanic eruptions, sea 491 spray, forest fires and wind-blown dust, suggesting a non-anthropogenic source (Kabata-492 Pendias and Szteke 2015). Considering its clustering with Cd, we would suggest a natural 493 source of both elements in the feathers of the studied birds. 494

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).

501

502 **4.3. Species and age differences in elemental concentrations**

503 Significant differences in concentration of various elements (i.e. Cu, Hg, and Se) between the 504 WSP and BBSP found in our study are most likely to be associated with inter-species 505 differences in foraging (different trophic levels with a different contribution of fish in their 506 diet [Quillfeldt et al. 2017]).

Significant differences in concentrations of various elements (Supplementary 507 Materials2, Fig. ES1–ES7) between WSP age groups are also likely to be associated with diet, 508 although here not with the difference in diet composition but more with the location of food 509 510 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, 511 512 because chicks receive food collected by parents in the vicinity of the breeding colony, and 513 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 514 adults' exposure to several pollutants during the chick-rearing period (Blévin et al. 2013). 515

516 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 517 determination. A strong correlation between the levels of both Hg and Se in eggs and the liver 518 of incubating females has been found in Charadriiformes (Ackermann et al. 2016). In our 519 study, all examined elements except Ni and Co were detected in down feathers, enabling 520 521 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 522 523 the yolk and subsequently from the eggshell (Castilla et al. 2010). We found the lowest Zn 524 level in down (48.30 \pm 7.08 µg/g dw), at almost two times lower than the level in chick body feathers (93.00 \pm 11.30 µg/g dw). Other elements, such as Pb, Hg, Se, Mg and Sr, were higher 525 526 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 527 528 down feathers, as the maternal transfer is species- and element-specific (Ackermann et al. 529 2016).

530

531 **5. Conclusions**

Our study provides a reference values for concentration of 17 elements in feathers of two 532 533 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 534 tissues throughout birds' annual cycle (Rodríguez et al. 2019). We also revealed several 535 differences in elemental concentrations between the two species, as well as differences in 536 537 exposure between life-cycle stages. These inter-species and inter-age differences are 538 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 539 natural origin of most elements. We believe that our study contributes to understanding spatial 540 541 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 542 the future, monitoring studies on seabirds breeding in the Southern Hemisphere may be a 543 544 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 545 546 size; sample collection being restricted to one site and one season; and the lack of data on 547 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 548 549 members urge long-term monitoring and sustained observations of the Antarctic environment 550 and the associated data management, to detect, understand and forecast the impacts of climate-change-driven environmental variability (ATCM 2007). 551

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

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

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

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 = 0.00	001		
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 = 0.000)1		
,_	,, F	-		
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 = 0.00	001		
Non-essential	WSP_CHF	WSP_down	BBSP_Ad	WSP_Ad
		0.160	0 101	0.002
WSP_CHF	-	0.160	0.181	0.003
WSP_down		-	0.166	0.012
BBSP_Ad			-	0.043
WSP_Ad				-

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		WSP_Ad vs		WSP_Ad vs			WSP_Ad vs				
	dissimila	rity		WSP_CHF		WSP_down			BBSP_Ad		
	ADis	Contr.		ADis	Contr.		ADiss	Contr.		ADis	Contr.
All elemen	nts										
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	Мо	2.40	14.6	As	1.85	12.3	Se	1.87	17.6
Мо	1.83	12.9	Cr	1.87	11.3	Zn	1.71	11.3	Mo	1.41	13.3
			Cu	1.82	11.0	Мо	1.66	11.0			
Overall	14.16			16.46			15.07			10.65	
Non-Esser	ntial										
Bi	11.47	29.2	Bi	23.67	50.3	Sr	13.96	36.4	Hg	14.42	49.1

Overall		WSP_Ad vs		WSP_Ad vs			WSP_Ad vs				
dissimilarity		WSP_CHF		WSP_dow	WSP_down			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	

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

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

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

* Estimated marginal mean



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



Fig. 2 Concentration of Hg (μ g·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)



Fig. 3 Hierarchical dendrogram of the studied elements in the feathers of the studied stormpetrels (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 \geq 95 denoted with a dotted rectangle.

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