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Element patterns in albatrosses and petrels: influence of trophic position, foraging range, and prey type

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Trophic position, foraging range, and prey type were found to influence element compositions and concentrations in Procellariiformes from South Georgia.

Abstract

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We investigated the concentrations of 22 essential and non-essential elements among a community of Procellariiformes (and their prey) to identify the extent to which trophic position and foraging range governed element accumulation. Stable isotope analysis (SIA) was used to characterise trophic ($\delta^{15}N$) and spatial patterns ($\delta^{13}C$) among species. Few consistent patterns were observed in element distributions among species and diet appeared to be highly influential in some instances. Arsenic levels in seabird red blood cells correlated with $\delta^{15}N$ and $\delta^{13}C$, demonstrating the importance of trophic position and foraging range for arsenic distribution. Arsenic concentrations in prey varied significantly across taxa, and in the strength of association with $\delta^{15}N$ values (trophic level). In most instances, element patterns in Procellariiformes showed the clearest separation among species, indicating that a combination of prey selection and other complex species-specific characteristics (e.g. moult patterns) were generally more important determining factors than trophic level *per se*.

Key words: Elements; Procellariiformes; Trophic Position; Diet; South Georgia

1. Introduction

Some metals and metalloids are essential elements (EE) involved in physiological and biochemical processes (Abdulla and Chmielnicka, 1990), but can be toxic when exposure and assimilation is excessive. Others, such as lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg), are non-essential elements (NEE) that are also potentially toxic.

Marine organisms can be exposed to elements from anthropogenic and natural sources, and exposure is governed by various factors, including foraging location and trophic position.

Although the Southern Ocean is a remote environment, it is subject to anthropogenic metal inputs due to global transport of elements in the atmosphere and through oceanic circulation (Gaiero et al., 2003). Seabirds provide an ideal model with which to investigate contaminant dynamics in marine food webs (see Gilbertson et al., 1987; Furness, 1993; Furness and Camphuysen, 1997) and the Procellariiformes exploit a wide range of ecological niches in the Southern Ocean. Many species occupy high trophic positions and so can act as sentinels of exposure, highlighting potential threats to other apex predators. Previous studies have examined mercury (Hg) and other elements in lower order species in the Southern Ocean (e.g. Bargagli et al., 1998; Negri et al., 2006) but, apart from Hg, concentrations of other elements in biota at higher trophic levels have received much less attention (although see Lock et al., 1992; Stewart et al., 1999; González-Solís et al., 2002; Bocher et al., 2003).

Conventional approaches for quantifying dietary composition and assessing metal intake, such as analysis of gut content or regurgitate, have a number of biases (see Votier et al., 2003) and samples are often difficult to obtain over long periods. In this study, we analysed stable isotopes to elucidate broad-scale, inter- and intra-specific dietary patterns in Procellariiformes (Hobson et al., 1994), and so determine whether differences in foraging strategy explained variation in elemental uptake. Use of stable isotopes for this purpose is based on the predictable increase in the ratio of $^{15}N:^{14}N$ (by ca. 3.4‰) with each trophic level,, and so $\delta^{15}N$ can be used as a proxy for trophic position (e.g. DeNiro and Epstein, 1981; Hobson and Welch, 1992; Bearhop et al., 1999). $\delta^{13}C$ can vary depending on whether animals feed inshore or offshore, or on benthic or pelagic prey (e.g. Chisholm et al., 1982; Hobson et al., 1994); $\delta^{13}C$ values also vary with latitude (Cherel and Hobson, 2007) and, as such, provide a coarse-scale proxy for foraging location in wide-ranging organisms.

The overarching aim of the present study was to investigate the distributions and dynamics of toxic heavy metals, essential metals and metalloids in Southern Ocean seabirds, and assess the extent to which they may be bioaccumulated or biomagnified through foodchains (see Bargagli et al., 1996; McIntyre and Beauchamp, 2007). We analysed red blood cells (hereafter blood) and feathers collected during the breeding season to relate element assimilation to trophic position. The residence times of dietary isotopic signatures are dependent on the regenerative time of the tissue analysed (Hobson and Clarke, 1992a, 1992b; Hobson 1999). Blood cells are short-term integrators of dietary isotope signatures and are derived from prey consumed within the past 2-3 weeks. Blood metal concentrations also reflect recent exposure, concentrations equilibrating between the blood and the body organs (Burger and Gochfeld, 1997). Hence, the isotopic signature and element concentrations in blood are temporally matched and reflect foraging in the breeding season. In contrast, most Procellariiformes grow feathers during the non-breeding period and so feathers provide a means for characterising diet and element burdens acquired at this time. Feathers, once grown, are metabolically inert. They therefore provide a dietary isotope and element exposure ‘snap-shot’ for the period of feather growth (Mizutani et al., 1990, 1992, Bearhop et al., 2002). By randomly sampling body feathers, the measured isotope signature and element concentration is averaged over the moult period (Bearhop et al., 2000). The temporal matching of the feather isotope signature and element concentrations is unlikely to be as good as that for blood because feather data are based on averaged values obtained over a relatively long time period. Overall, however, it is possible to gather information on trophic level and foraging range for the breeding (blood stable isotopes) and non-breeding season (feather stable isotopes), although it is also important to determine isotope values for likely prey items (as determined from previous conventional dietary studies) so that the extent to which certain prey types may affect metal accumulation in birds can also be evaluated.

In this study, our specific objectives were to investigate: (1) current levels of exposure of Procellariiformes to a range of EE and NEE by measuring concentrations in blood and feathers; (2) the extent to which assimilation varies between species; and, (3) the extent to which foraging area, diet, and trophic level explains intra- and inter-individual variation in metal accumulation in Procellariiformes.

2. Materials and methods

2.1 Sample collection and preparation

Fieldwork was undertaken on Bird Island, South Georgia (54º 00'S, 38º 03'W) from December 2001 for a period of four months. Ten species were sampled in total: Antarctic prion (*Pachyptila desolata*), black-browed albatross (*Thalassarche melanophrys*), blue petrel (*Halobaena caerulea*), common diving petrel (*Pelecanoides urinatrix*), South Georgian diving petrel (*Pelecanoides georgicus*), grey-headed albatross (*T. chrysostoma*), northern giant petrel (*Macronectes halli*), southern giant petrel (*M. giganteus*), white-chinned petrel (*Procellaria aequinoctialis*) and wandering albatross (*Diomedea exulans*). Blood and feathers from adults of each species were taken from surface-nesting birds at the end of the brood-guard period and from burrowing birds whilst mist-netting adjacent to breeding colonies. Whole blood (0.2-1.0ml) (from the tarsal vein) was spun to separate cells and plasma, immediately frozen, subsequently freeze-dried to a constant mass and then again frozen until analysis. Only red blood cells were analysed for isotopes and trace elements. Six to 8 feathers sampled at random were taken from the mantle region of each bird. Feathers were stored in plastic bags at room temperature until analysed. Fresh prey samples (muscle tissue) were obtained from seabird regurgitates, or from fisheries vessels operating in waters surrounding South Georgia, and frozen at -20°C after identification. Cephalopods were identified from beaks according to Clarke (1986) and Rodhouse et al. (1992), fish from external morphology according to Gon and Heemstra (1990), or from otloliths according to Reid (1996), and crustaceans from Kirkwood (1984). Identifications were checked against BAS reference collections. Prey were of unknown size and age.

2.2 Stable isotope analyses

All blood and feather samples were homogenised in a freezer mill prior to analysis and oven-dried for >24hrs at 50ºC to a constant mass. In the case of prey samples, lipids were extracted prior to SIA over a 4 hour period using Soxhlet apparatus containing 1:1 methanol to chloroform solvent mixture. Stable carbon and nitrogen isotope analyses were performed by continuous flow isotope ratio mass spectrometry (CF-IRMS) on 0.7mg sub-samples of material loaded into tin cups and combusted in a Costech ECS 4010 elemental analyser coupled to a Thermo Finnigan Delta Plus XP mass spectrometer. Every 8-10 samples were followed by two laboratory standards allowing correction for drift. Isotope ratios are expressed in standard δ notation against international reference standards, vPDB (virtual Pee Dee Belemnite) for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$ according to the equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio ^{15}N:^{14}N or ^{13}C:^{12}C. Precision for both $\delta^{13}C$ and $\delta^{15}N$ was routinely estimated to be ≤ 0.2‰.

2.3 Element analyses

Samples were oven-dried for 24hrs at 50ºC to a constant mass. Between 0.05 and 0.1g dry weight of sample (mean weights for each tissue type; 0.6g for prey, 0.09g for blood, and 0.05g for feathers) was weighed accurately and added to 2ml nitric acid and left to cold digest for 24hrs. Samples were then hot digested for 50 minutes at 120ºC, after which 0.5ml of hydrogen peroxide was added and left for a final 15 minutes. The samples were made up to 5ml with deionised water. Total absolute concentrations for 22 essential and non-essential elements were measured using an ELAN 6100 dynamic reaction cell inductively coupled plasma mass spectrometer (DRC ICP-MS) (PerkinElmer, Connecticut, US) with a reference material and internal standard run with every 10 unknowns. Spiked samples and blanks were also run. Limits of detection (LoD) for particular elements are in

Table 1. Precision and accuracy (Table 2) were measured using replicate samples and certified reference material (TORT-2 lobster hepatopancreas, NRCC, Canada). Element concentrations are expressed throughout as mean ± SD in $ng.g^{-1}$ on a dry weight basis, unless otherwise specified.

2.4 Data analyses

Elements with mean concentrations below LoD (beryllium (Be) for prey; aluminium (Al), scandium (Sc), cobalt (Co), zinc (Zn), antimony (Sb), tungsten (W), and Be for blood; and Sc, caesium (Cs), lithium (Li), Pb, and Be for feathers) are reported in the summary statistics but are excluded from further consideration or statistical analyses. Among the remaining elements, concentrations in some samples in some species were below the LoD (maximum 13% of samples for rubidium (Rb) in feathers, 1-3% of samples for vanadium (V), iron (Fe), molybdenum (Mo), nickel (Ni), Cs, Hg, manganese (Mn), and selenium (Se) in various tissue types) and were assigned a value equal to half the LoD for the particular tissue. Where element concentrations were below the LoD in >30% of samples overall for a particular tissue type (V, barium (Ba), uranium (U), Li, and Pb for blood; V, Sb, and W for feathers), those elements were also included in summary statistics but excluded from subsequent statistical analyses, as in other studies (Borgå et al., 2006). Fe was excluded from all statistical analyses of blood data because of its physiological function in this tissue.

For elements that were included in the statistical analysis, we first used principal component analyses (PCA) in Canoco 4.5 for Windows® to identify co-variance among elements, and between elements and isotope ratios (the latter entered as passive variables, following Borgå et al., 2006) in seabird tissues. PCA produces ordination axes that reduce the total residual sum of squares among all the variables included (i.e. all elements, $\delta^{15}N$, and $\delta^{13}C$). The primary axis (PC1) represents the greatest proportion of component variation among the samples. The secondary axis (PC2) explains the greatest proportion of any remaining variation that is uncorrelated with PC1. Variables are shown as lines, with the length of each line demonstrating relative contribution of a particular variable to the separation of samples in ordination space. Angles between lines represent a greater or lesser degree of co-variance between elements; with acute angles representing the greatest degree of co-variance, a 180º angle demonstrating a negative correlation, and a 90º angle represent elements that are completely un-correlated. All PCA were performed on standardised values, so that those elements with vastly different ranges could be analysed together. We then used univariate general linear models (GLMs) to test the significance of relationships between species, $\delta^{15}N$, $\delta^{13}C$, and elements of high factor loadings that were identified through PCA, (elements with ≥ 0.50 factor loadings for PC1 and/or PC2). These analyses were applied to both blood and feather data separately and the most parsimonious univariate models were determined using Akaike Information Criterion (AIC) (Akaike 1973). Absolute element concentrations were logarithmically transformed ($\log_{10}$) in some instances to reduce skewness and heterogeneity. Model residuals were also examined by way of Q-Q plots to assess goodness of fit. Statistical tests were performed in statistical packages R ver. 2.6.2 and SPSS® ver. 14.

3. Results

In total, we analysed samples for 22 essential and non-essential elements. Mercury was detected in all three tissue types but, because of the particular biomagnification properties and risks associated with this element, the results have been examined in greater detail and reported elsewhere; essentially, mercury levels were found to increase with trophic level, indicating strong biomagnification among Procellariiformes (Anderson et al. 2009). Fourteen elements were quantifiable in blood (Table 3) and seventeen in feathers (Table 4). Species varied in the extent to which they accumulated different elements and no single species accumulated the highest concentrations of all elements (Tables 3-5). Of all the elements, Fe and Se had the highest concentrations in blood (Table 3), and Fe and Zn the highest concentrations in feathers (Table 4).

In prey samples, 21 elements were detected, only Be was below detection limits. Iron and Zn had the highest concentrations of all elements tested for (Table 5). Aluminium concentrations were generally an order of magnitude higher in crustaceans than in fish and cephalopods, and Cd concentrations were likewise some 10-fold higher in several squid species and in *Themisto gaudichaudii*, compared with other species (Table 5). Benthic-feeding fish, such as Patagonian toothfish (*Dissostichus eleginoides*) (García de la Rosa et al., 1997), often had the highest As concentrations. Isotopic analyses indicated a high degree of variation in trophic position ($\delta^{15}N$) and foraging range ($\delta^{13}C$) between seabird species (Fig. 1), and considerable trophic overlap between fish and cephalopod prey. The greatest degree of spatial variation (largest range in $\delta^{13}C$ ratios) in prey was between fish species (Fig. 1).

Initial PCA analyses identified strong associations between As concentrations and both $\delta^{15}N$ and $\delta^{13}C$ in blood (Fig. 2a). There was a similar but weaker association for caesium (Cs), as indicated by the shorter line on the PCA plot (Fig. 2a), but no similar associations for other elements in blood. The distribution of As in ordination space was predominantly governed by white-chinned petrel, suggesting that blood As concentrations were elevated in this species during the breeding season; this association did not occur in feathers (Fig. 2b). There were other similar associations between specific elements and seabird species. The positions of Rb and Cd in ordination space were largely governed by grey-headed albatross, indicating that blood concentrations of these elements were highest in this species during the breeding season (Fig. 2a); again there were no such associations for feathers (Fig. 2b). The ordinal positions of white-chinned petrel and wandering albatross were influenced by $\delta^{15}N$ and $\delta^{13}C$ in both blood and feathers. Overall, As and Cd (NEE) contributed most to PC1 in blood, while Se (EE), Rb (NEE), and Mo (EE) contributed most to PC2 (Table 2, supplementary material). In contrast, Mn (EE), Fe (EE), Co (EE), As (NEE), and Mo (EE) contributed most to PC1 in feathers, while Se and U (NEE) contributed most to PC2.

With the exception of blood As, all elements contributing $\geq$0.50 to factor loadings in the PCA were found to differ significantly between seabird species (Table 6). Univariate GLM analyses identified significant differences between species for a number of elements. Blood concentrations of Cd, Se, Rb, and Mo all differed significantly between species (Table 6), and in feathers, significant differences between species were found in the levels of Mn, Fe, Co, As, Mo, Se, and U.

Univariate GLMs demonstrated that there was a significant and positive association between As and $\delta^{15}N$ in blood, although the pattern of this relationship varied between species, as demonstrated by the significant interaction term in the model (Table 6). Blood As concentrations were negatively associated with $\delta^{13}C$ across all species (i.e., at community level, Table 6) but the exact nature of this relationship may also have varied between species, the interaction term in the model approaching significance (Table 6). There was a significant relationship between Se and $\delta^{13}C$ in feathers (Table 6). There were no other significant relationships between element concentrations and trophic position and/or foraging location in either tissue type (Table 6).

The positive association between blood As concentrations and trophic position ($\delta^{15}N$) was consistent with the concept that As is bioconcentrated along food chains. To investigate this further, we examined how As concentrations in prey species varied with $\delta^{15}N$ and $\delta^{13}C$ values (Table 7). Arsenic concentrations differed significantly between taxonomic groups but also varied with $\delta^{15}N$ (Table 7). However, associations between $\delta^{15}N$ and As concentrations were not consistent between prey groups (Table 7), indicating that associations between the trophic position of species and As are complex (Fig. 3).

4. Discussion

The large number of elements analysed in this study required us to focus the discussion only on those elements for which a significant relationship was identified among the parameters in the univariate analyses. We have also focused particularly on those elements for which a significant relationship occurred in blood as blood is the better tissue for characterising trophic and spatial patterns in relation to element composition within these birds. This is because feathers have the potential to acquire element burdens both directly through diet and from remobilisation of elements from internal tissues (Lewis and Furness, 1991; Monteiro, 1996). This may be why the relationships identified between stable isotope ratios and elements in blood in the present study were not always mirrored with feathers.

4.1 Arsenic

Of the elements analysed in seabird blood, only As showed significant interactions with $\delta^{15}N$ and $\delta^{13}C$ at the community level (Table 6), suggesting that trophic position and/or foraging range affect As assimilation by Procellariiformes during the breeding season. The significant and positive association between blood As and $\delta^{15}N$ may be due to bioconcentration or reflect changes in isotopic baseline that coincide with variation in environmental As concentrations. We found no evidence of bioconcentration of As through the foodchain. Although As concentrations varied significantly between prey groups (crustacea, cephalopods, and fish), there was no consistent relationship between As concentrations and trophic position in prey (Table 7, Fig. 3).

Given the lack of clear evidence of bioconcentration, it is possible that foraging location is the prime factor affecting As assimilation in Procellariiformes during the breeding season, and any associations with $\delta^{15}N$ may be coincidental. In this respect, As strongly influenced the position of white-chinned petrel in the PCA ordination space (Fig.

2a), reflecting their high blood As concentrations (2500 ng g^{-1} dry weight) relative both to other species in this study and to other species elsewhere; reference values for blood As in birds from uncontaminated areas is 20 ng g^{-1} wet weight (Burger and Gochfeld, 1997), approximately equivalent to a dry weight red blood cell concentration of 400 ng g^{-1}. White-chinned petrels have amongst the greatest foraging distances of any seabird and breeding birds from South Georgia regularly forage on the northern Patagonian shelf, >2000km from the colony (Phillips et al., 2006). Prey food webs on the Patagonian shelf have greater trophic complexity than those in waters around South Georgia (Forero et al., 2004, 2005), and so $\delta^{15}N$ levels in birds feeding in that region may be elevated, reflecting longer food chain lengths. The Patagonian shelf region is also more likely to be subject to greater anthropogenic inputs from riverine influxes, mineral activities, and industry than the more remote regions of the Southern Ocean (González-Solís et al., 2002), which could explain the elevated arsenic levels in birds that feed there. It is also possible, however, that enhanced environmental exposure to As may stem from natural processes. For example, disruption in the phosphorus/nitrogen ratio could lead to greater As accumulation by phytoplankton in a particular region and subsequently enhance As levels in the food chain.

In our study, there was no relationship between feather As concentrations and $\delta^{15}N$. Feather As concentrations were highest in Antarctic prion (APR), but only about half the concentrations measured in black-footed albatross (*Phoebastria nigripes*) feathers from the North Pacific (Fujihara et al., 2004). Low feather As concentrations in Antarctic Procellariiformes may reflect exposure during the non-breeding season to relatively low-level natural levels of As. In our study, As concentrations in prey tended to be highest in benthic/demersal species (Table 5). This suggests that a major source of As in the prey of

Antarctic Procellariiformes around South Georgia may stem from metallic mineralisation of the seabed rather than from atmospheric transport of particles. Indeed, marine sediments are thought to be the largest geochemical reservoir of arsenic with residence times of ca. 100 million years (Maher and Butler 1988). Furthermore, manganese (Mn) concentrations were also elevated in benthic species, again suggesting a natural source of As; elevated As levels *without* matching high levels of Mn are thought to be indicative of anthropogenic As input (Peterson and Carpenter, 1986).

Although there was a significant relationship between $\delta^{13}C$ and blood As concentrations in the Procellariiformes in our study, no such relationship occurred among prey species. This may be because prey were collected from a small geographic area, relative to the foraging areas of the birds. However, there was still considerable variation in $\delta^{13}C$ ratios between prey species (Fig. 1) and this probably reflects the influence of depth on prey $\delta^{13}C$ ratios. The lack of association between $\delta^{13}C$ and As in prey indicates that As uptake by seabirds is unlikely to be influenced by the proportion of mesopelagic prey in the diet, unlike patterns of Hg uptake which are heavily influenced by prey type (see Monteiro et al., 1996; Thompson et al., 1998). The association observed between $\delta^{13}C$ and As in Procellariiformes is most likely due to geographical variation in background As concentrations within the marine environment.

4.2 Cadmium

Blood cadmium concentrations in seabirds varied significantly between species but not with isotopic signatures, indicating that neither trophic position nor foraging location dictated Cd burdens during the breeding season. Our results match those of Gonzalez-Solis et al. (2002) in that Cd concentrations were greater in northern than southern giant petrels (Table 3). Gonzalez-Solis et al. (2002) postulated that the former are exposed to higher levels through foraging over the Patagonian shelf, as the region is thought to be more exposed to heavy metal contamination than the waters immediately adjacent to South Georgia. However, if latitudinal variation in foraging location was the major factor affecting Cd assimilation in Antarctic Procellariiformes, we would anticipate a correlation between Cd concentrations in birds and $\delta^{13}C$. It may be that foraging location does dictate Cd uptake, but that any such influence reflects the existence of Cd 'hot spots' rather than a latitudinal gradient *per se*. In fact, Nygård et al. (2001) linked deep ocean upwelling, which is often naturally Cd-rich (Holm-Hansen, 1985), to high Cd concentrations in Antarctic krill *Euphausia superba* and other prey of Procellariiform seabirds (Petri and Zauke, 1993).

Previous studies have attributed high Cd concentrations in Procellariiformes to consumption of squid (Muirhead and Furness, 1988; Honda and Marcovecchio, 1990; Elliott, 2005; Stewart et al., 1999). Our results support this, in that higher Cd levels were found in the blood of grey-headed albatrosses and white-chinned petrels (Table 3). Both white-chinned petrels and grey-headed albatrosses from South Georgia consume large amounts of squid, the latter feeding in particular on *Martialia hyadesi*, and, also on lamprey *Geotria australis* (Berrow and Croxall, 1999, Xavier et al., 2003). In our study, both these prey types had relatively elevated Cd concentrations (Table 5). Hence, high Cd burdens in Procellariiformes do not necessarily indicate anthropogenic contamination. Levels of cadmium in feathers, for example, were largely similar to those in similar species, e.g.

black-footed and Laysan albatrosses (*Phoebastria immutabilis*) in the more contaminated North Pacific (Burger and Gochfeld, 2000).

Squid are thought to accumulate high Cd concentrations naturally (Kurihara et al., 1993; Gerpe et al., 2000). Elevated burdens in Procellariiformes can also result from amphipod consumption (Cheng et al., 1984; Elliott and Scheuhammer, 1997), and in our study, it was notable that we recorded the highest Cd levels of any prey item in *Themisto gaudichaudii* (Table 5). Rainbow (1989) concluded that *Themisto* species are a significant source of Cd in the diets of some seabirds, and our findings are consistent with this as the highest blood Cd levels in birds in our study were in blue petrels, a known predator of *T. gaudichaudii* (Prince, 1980). Moreover, hyperiid amphipods may also contribute to the diet of some squid species (Croxall and Prince, 1980) which can then be eaten by seabirds. Thus, dietary preferences, rather than foraging area, may comprise the dominant influence on Cd burdens in Procellariiformes.

4.3 Selenium

Blood (hence breeding season) concentrations of Se, an essential element, varied significantly between seabird species, but did not show a significant relationship with trophic position ($\delta^{15}N$) and foraging location ($\delta^{13}C$) (Table 6). Therefore, it seems probable that Se does not biomagnify among Procellariiformes. Additionally, our data suggest that foraging location does not dictate Se burdens in Southern Ocean Procellariiformes, although this may in fact reflect the constrained foraging ranges of many species during the breeding season rather than a true absence of association between the two parameters.

Selenium concentrations were the highest of any element in Procellariiformes blood except Fe. Moreover, blood Se levels in both giant petrel species were consistent with those previously recorded by González-Solís et al. (2002). Blue petrels and Antarctic prions had the highest blood Se concentrations of the birds we analysed and both species feed predominantly on crustacea, including Antarctic krill (Prince, 1980; Croxall et al., 1997). Of the prey species we analysed, crustacea, especially Antarctic krill and *T. gaudichaudii*, had high Se concentrations relative to other prey species and groups. The physico-chemical form in which Se is sequestered by different prey may also vary and affect subsequent bioavailability. Hence, it would appear that inter-specific variation in blood Se concentrations in seabirds most likely reflects species differences in prey selection.

Selenium concentrations in feathers varied considerably between species and were also significantly and negatively related to foraging location (i.e. $\delta^{13}C$) (Table 6). This was unexpected as concentrations of Se might be expected to increase with decreasing latitude, as birds move into potentially more contaminated waters north of the sub-Antarctic polar front. The negative relationship in this instance is difficult to explain, but could potentially stem from natural sources of Se being more elevated in specific regions closer to the Antarctic continent. In other words, Se burdens accumulated by Procellariiformes in the non-breeding period may be dictated by naturally occurring Se in the marine environment, potentially stemming from Antarctic intermediate and bottom waters which are sporadically rich in organic Se (Cutter and Cutter, 2001). Evidence to support this hypothesis, comes from the comparable levels of Se in the species covered by this study, to those of other studies. Burger and Gochfeld (2000) examined Se in feathers from black-footed and Laysan albatrosses in the North Pacific, and identified levels comparable to those found in this study.

4.4 Rubidium and Molybdenum

Two other elements, Rb and Mo, also varied between seabird species, but not with either $\delta^{15}N$ or $\delta^{13}C$, indicating that trophic position and foraging location were not key determinants in the distribution of these two elements within Procellariiformes. Rb is known to bioaccumulate through the foodchain (Nyholm and Tyler, 2000), and in fact, Campbell et al. (2005) reported a significant correlation between Rb and $\delta^{15}N$ in marine organisms, potentially suggesting biomagnification. While our results were not significant in the presence of other parameters, there was a significant, albeit weak, positive relationship between Rb and $\delta^{15}N$ in blood (linear regression, $F_{1,140} = 9.9$, $p = 0.002$, $r^2 = 0.07$). However, whether this is evidence of biomagnification of Rb within this particular system is doubtful given there was no significant relationship between Rb concentrations and $\delta^{15}N$ in prey (linear regression, $F_{1,82} = 1.5$, $p = 0.229$, $r^2 = 0.02$). Given that the accumulated evidence concerning Rb bioconcentration through foodchains is unclear, further investigations into Rb distributions and bioaccumulation in the marine environment appear warranted.

5. Conclusions

This study presents new data on the distributions and dynamics of heavy metals, essential metals and metalloids in Southern Ocean seabirds. Our results indicate that there are considerable differences in residues accumulated by different species. For arsenic, residues in Procellariiformes appear to be partially explained by changes in foraging range and trophic positioning, although these patterns were not always consistent across species. Arsenic was accumulated at higher concentrations in Procellariiformes feeding at higher trophic levels, although this may be confounded to some extent by individuals foraging across different food webs with varying $\delta^{15}N$ baselines. There was little or no evidence that trophic level was important in the accumulation of other elements. Our data suggest that differences between species in feeding preferences for prey and the location of the foraging sites are the likely major factors determining element uptake by seabirds in the South Atlantic.

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Fig. 1. Range plots for $\delta^{15}N$ (a) and $\delta^{13}C$ (b) values for all Procellariiformes (blood) and prey species. Species are ordered by increasing $\delta^{15}N$ values within each of the four groupings. Data is shown for Procellariiform blood samples and prey muscle samples.

Fig. 2. Biplot of Procellariiform species mean scores extracted by principal component analyses (PCA) and element loadings on the two principal axes (PC1 and PC2), with standardised element and isotope values in (a) blood and (b) feathers from ten species. APR=Antarctic prion, BBA=Black-browed albatross, BLP=Blue petrel, CDP=Common diving petrel, GDP=South Georgian diving petrel, GHA=Grey-headed albatross, NGP=Northern giant petrel, SGP=Southern giant petrel, WCP=White-chinned petrel, WNA=Wandering albatross.

Fig. 3. Patterns in $\log_{10}$ arsenic concentrations in muscle tissue samples from three Procellariiform prey groups in relation to (a) $\delta^{15}N$ values and (b) $\delta^{13}C$ values from the same tissue samples.

Table 1. Limits of Detection (LoD) for prey, blood, and feather samples (ng g^{-1}).

Element	Prey LoD	Blood LoD	Feather LoD
Al	199	1357	2365
As	3	18	32
Ba	20	136	237
Be	1	7	12
Cd	1	5	8
Co	2	14	24
Cs	1	5	8
Cu	7	45	79
Fe	332	2261	3942
Li	1	7	12
Mn	2	14	24
Mo	10	68	118
Ni	3	23	39
Pb	20	136	237
Rb	1	5	8
Sb	3	23	39
Sc	17	113	197
Se	10	68	118
U	1	5	8
V	7	45	79
W	3	23	39
Zn	332	2261	3942

Table 2. TORT-2 lobster hepatopancreas reference material for elements; expected and obtained CRM values (μg g^{-1} dry wt.). All elements, where possible, were CRM checked against an expected value. However, not all elements tested had a reported certified concentration in the CRM. For these elements only the obtain CRM value is reported.

Element	Expected CRM Value	± 95% CI	Obtained CRM Value	± 95% CI
Al			17.0	0.9
As	21.6	1.8	26.4	0.8
Ba			2.0	0.1
Be			0.01	0.0
Cd	26.7	0.6	28.8	0.8
Co	0.5	0.1	0.5	0.1
Cs			0.0.2	0.0
Cu	106.0	10.0	93.3	3.0
Fe	105.0	13.0	126.0	10.8
Li			0.3	0.0
Mn	13.6	1.2	12.8	0.4
Mo	0.9	0.1	1.1	0.1
Ni	2.5	0.2	2.4	0.1
Pb			0.2	0.0
Rb			2.7	0.1
Sb			0.1	0.0
Sc			0.1	0.0
Se	5.6	0.7	7.5	0.3
U			0.1	0.0
V	1.6	0.2	1.9	0.1
W			0.01	0.0
Zn	180.0	6.0	191.7	12.2

Table 3. Mean concentrations of elements (ng g^{-1} dry wt.) ± SD in blood from 10 species of Procellariiformes.

Species	*n*	Al	As	Ba	Be	Cd	Co	Cs	Cu	Fe	Li	Mn	Mo	Ni	Pb	Rb	Sb	Sc	Se	U	V	W	Zn
Antarctic prion	16	<LoD	431 ± 186	345 ± 289	<LoD	48 ± 58	<LoD	5 ± 4	414 ± 366	2604580 ± 168540	1990 ± 7849	132 ± 66	226 ± 172	2140 ± 723	1052 ± 3203	4161 ± 523	<LoD	<LoD	198425 ± 59904	8 ± 5	85 ± 130	<LoD	<LoD
Black-browed albatross	15	<LoD	373 ± 267	192 ± 234	<LoD	86 ± 177	<LoD	15 ± 10	815 ± 579	4330039 ± 6571356	6 ± 10	150 ± 128	84 ± 113	1728 ± 1286	140 ± 172	4185 ± 1898	<LoD	<LoD	102335 ± 85049	11 ± 15	140 ± 126	<LoD	<LoD
Blue petrel	2	<LoD	388 ± 47	191 ± 174	<LoD	346 ± 27	<LoD	<LoD	71 ± 68	2440536 ± 75575	3 ± 0	91 ± 3	225 ± 46	1652 ± 276	68 ± 0	4368 ± 178	<LoD	<LoD	261757 ± 131638	12 ± 3	<LoD	<LoD	<LoD
Common diving petrel	15	<LoD	389 ± 129	187 ± 170	<LoD	19± 29	<LoD	<LoD	639 ± 1010	2265035 ± 355674	3 ± 0	128 ± 78	217 ± 131	1442 ± 589	322 ± 718	3612 ± 625	<LoD	<LoD	84842 ± 29419	9 ± 6	162 ± 285	<LoD	<LoD
S. Georgian diving petrel	15	<LoD	476 ± 197	310 ± 324	<LoD	27 ± 32	<LoD	14 ± 6	196 ± 186	2570513 ± 582271	29 ± 20	190 ± 76	88 ± 68	2007 ± 553	281 ± 446	3824 ± 967	<LoD	<LoD	118897 ± 60846	10 ± 5	60 ± 97	<LoD	<LoD
Grey-headed albatross	15	<LoD	896 ± 380	303 ± 198	<LoD	228 ± 81	<LoD	15 ± 10	670 ± 905	2483096 ± 239518	106 ± 46	252 ± 430	68 ± 80	1936 ± 530	235 ± 648	4526 ± 777	<LoD	<LoD	194381 ± 43946	6 ± 5	<LoD	<LoD	<LoD
Northern giant petrel	16	<LoD	417 ± 787	<LoD	<LoD	88 ± 213	<LoD	7 ± 4	638 ± 246	2333908 ± 659977	60 ± 23	97 ± 38	94 ± 26	1131 ± 966	<LoD	4991 ± 819	<LoD	<LoD	151170 ± 39472	<LoD	75 ± 188	<LoD	<LoD
Southern giant petrel	16	<LoD	184 ± 156	<LoD	<LoD	37 ± 105	<LoD	5 ± 3	690 ± 109	2447292 ± 698505	111 ± 70	202 ± 494	90 ± 13	1480 ± 990	<LoD	5052 ± 712	<LoD	<LoD	151432 ± 77353	<LoD	<LoD	<LoD	<LoD
White-chinned petrel	16	<LoD	2492 ± 1375	<LoD	<LoD	179 ± 60	<LoD	10 ± 5	241 ± 250	2482217 ± 219636	46 ± 31	192 ± 69	127 ± 71	1866 ± 561	<LoD	4532 ± 904	<LoD	<LoD	97951 ± 40289	<LoD	<LoD	<LoD	<LoD
Wandering albatross	15	<LoD	686 ± 377	<LoD	<LoD	50 ± 60	<LoD	17 ± 6	889 ± 455	2398513 ± 681723	98 ± 47	86 ± 17	92 ± 50	1312 ± 970	<LoD	3913 ± 653	<LoD	<LoD	80187 ± 21270	<LoD	<LoD	<LoD	<LoD

Table 4. Mean concentrations of elements (ng g^{-1} dry wt.) ± SD in feathers from 10 species of Procellariiformes.

Species	*n*	Al	As	Ba	Be	Cd	Co	Cs	Cu	Fe	Li	Mn	Mo	Ni	Pb	Rb	Sb	Sc	Se	U	V	W	Zn
Antarctic prion	16	28396 ± 25222	411 ± 227	1654 ± 1930	<LoD	59 ± 110	605 ± 1295	<LoD	185292 ± 644966	1010868 ± 3457595	<LoD	5254 ± 16921	3411 ± 12163	36011 ± 68146	<LoD	153 ± 79	120 ± 116	<LoD	7319 ± 2192	19 ± 9	610 ± 2060	285 ± 571	113658 ± 364227
Black-browed albatross	16	17924 ± 13028	121 ± 39	1481 ± 1802	<LoD	578 ± 246	270 ± 481	<LoD	8613 ± 11995	610216 ± 1657667	<LoD	2704 ± 6669	1686 ± 4057	3284 ± 6669	<LoD	194 ± 27	84 ± 43	<LoD	3381 ± 923	17 ± 3	321 ± 782	128 ± 160	39592 ± 25311
Blue petrel	5	18704 ± 22291	164 ± 88	1065 ± 291	<LoD	74 ± 90	498 ± 329	<LoD	8745 ± 1953	968705 ± 1285689	<LoD	4972 ± 5981	3462 ± 4877	7862 ± 9365	<LoD	123 ± 110	121 ± 82	<LoD	6597 ± 2029	21 ± 7	545 ± 799	47 ± 40	6953 ± 10470
Common diving petrel	16	37468 ± 70275	177 ± 126	1226 ± 1108	<LoD	376 ± 349	140 ± 115	<LoD	31254 ± 58194	130911 ± 119824	<LoD	954 ± 837	581 ± 437	6534 ± 15075	<LoD	140 ± 157	192 ± 283	<LoD	5050 ± 3878	19 ± 14	207 ± 525	21 ± 4	301098 ± 481053
S. Georgian diving petrel	16	65277 ± 34649	261 ± 163	1402 ± 981	<LoD	300 ± 353	419 ± 241	<LoD	20176 ± 15166	791151 ± 572700	<LoD	5077 ± 3490	2979 ± 2133	25442 ± 55886	<LoD	162 ± 115	80 ± 84	<LoD	9797 ± 4917	42 ± 15	865 ± 504	95 ± 94	22283 ± 37587
Grey-headed albatross	16	21372 ± 27043	125 ± 29	769 ± 605	<LoD	196 ± 163	161 ± 101	<LoD	5661 ± 1820	229219 ± 190078	<LoD	1291 ± 940	893 ± 730	12683 ± 14224	<LoD	220 ± 46	61 ± 59	<LoD	5395 ± 1467	17 ± 5	163 ± 113	56 ± 46	50115 ± 20426
Northern giant petrel	16	11944 ± 8969	145 ± 72	641 ± 454	<LoD	83 ± 51	90 ± 31	<LoD	6211 ± 1559	103719 ± 54442	<LoD	608 ± 244	552 ± 229	3811 ± 10126	<LoD	243 ± 55	59 ± 45	<LoD	8377 ± 2346	16 ± 4	124 ± 71	25 ± 14	67557 ± 28067
Southern giant petrel	17	13939 ± 14005	112 ± 36	815 ± 631	<LoD	289 ± 93	101 ± 60	<LoD	6877 ± 3788	95208 ± 42181	<LoD	587 ± 184	509 ± 142	930 ± 1118	<LoD	246 ± 35	<LoD	<LoD	9757 ± 3539	17 ± 6	111 ± 65	25 ± 10	90208 ± 23541
White-chinned petrel	16	13355 ± 15522	127 ± 69	502 ± 407	<LoD	138 ± 132	101 ± 56	<LoD	13110 ± 17785	262076 ± 195334	<LoD	1399 ± 992	1007 ± 646	19449 ± 25581	<LoD	116 ± 21	<LoD	<LoD	4819 ± 1202	8 ± 2	177 ± 120	50 ± 38	77646 ± 17983
Wandering albatross	16	7126 ± 7482	93 ± 30	547 ± 344	<LoD	317 ± 478	114 ± 123	<LoD	6032 ± 2175	166858 ± 276706	<LoD	991 ± 1514	772 ± 1275	1720 ± 2043	<LoD	151 ± 66	<LoD	<LoD	4574 ± 878	<LoD	149 ± 176	40 ± 30	58160 ± 52039

Table 5. Mean concentrations of elements (ng g^{-1} dry wt.) ± SD in muscle tissue of 17 prey species.

Species	*n*	Al	As	Ba	Be	Cd	Co	Cs	Cu	Fe	Li	Mn	Mo	Ni	Pb	Rb	Sb	Sc	Se	U	V	W	Zn
Crustaceans																							
Euphausia superba	10	240691 ± 194587	2344 ± 251	46063 ± 27443	<LoD	176 ± 40	155 ± 83	33 ± 16	22123 ± 5644	372020 ± 230259	463 ± 284	7797 ± 4172	142 ± 49	1192 ± 605	167 ± 123	1960 ± 515	14 ± 14	299 ± 75	5486 ± 1141	61 ± 20	1178 ± 761	37 ± 101	49485 ± 18771
Themisto gaudichaudii	7	162821 ± 56608	4119 ± 465	10519 ± 1519	<LoD	19229 ± 2453	4269 ± 10614	10 ± 2	18279 ± 4106	395128 ± 152043	389 ± 154	3927 ± 992	692 ± 919	1397 ± 1362	255 ± 391	724 ± 116	6 ± 10	516 ± 169	5142 ± 711	145 ± 34	971 ± 314	2 ± 0	51472 ± 25824
Fish																							
Champsocephalus gunnari	16	8046 ± 7507	3795 ± 2288	1257 ± 1266	<LoD	29 ± 16	38 ± 28	65 ± 40	2053 ± 1734	82261 ± 50544	284 ± 221	1108 ± 796	41 ± 45	281 ± 360	33 ± 88	1830 ± 874	7 ± 5	65 ± 19	3043 ± 518	10 ± 9	189 ± 141	4 ± 5	43330 ± 11102
Dissostichus eleginoides	2	1036 ± 118	9226 ± 2770	168 ± 31	<LoD	1 ± 0	14 ± 1	117 ± 21	924 ± 495	47900 ± 6075	113 ± 17	923 ± 180	8 ± 2	137 ± 110	14 ± 6	1716 ± 224	5 ± 2	63 ± 3	2224 ± 258	2 ± 0	11 ± 2	2 ± 0	11550 ± 2978
Geotria australis	11	5787 ± 3719	1698 ± 822	1060 ± 856	<LoD	1948 ± 1151	30 ± 31	4 ± 3	4241 ± 1370	43360 ± 21458	39 ± 31	558 ± 242	49 ± 24	86 ± 78	19 ± 15	486 ± 184	5 ± 2	40 ± 21	1815 ± 337	4 ± 4	207 ± 146	3 ± 3	26612 ± 12891
Parachaenichthys georgianus	1	4348[a]	4161[a]	2241[a]	<LoD	426[a]	77[a]	103[a]	3089[a]	92029[a]	241[a]	2641[a]	34[a]	212[a]	10[a]	2081[a]	7[a]	48[a]	2801[a]	6[a]	168[a]	4[a]	41845[a]
Patagonotothen guntheri	8	10750 ± 11364	5968 ± 6388	2965 ± 2770	<LoD	168 ± 148	91 ± 115	79 ± 88	2480 ± 3288	285291 ± 369982	930 ± 1170	7854 ± 9008	42 ± 43	476 ± 705	38 ± 47	2791 ± 3064	14 ± 19	131 ± 158	4100 ± 4509	33 ± 41	442 ± 564	11 ± 10	63892 ± 68792
Pseudochaenichthys georgianus	5	4151 ± 1720	5152 ± 882	426 ± 226	<LoD	14 ± 14	31 ± 18	155 ± 37	1011 ± 161	104317 ± 75579	190 ± 67	449 ± 181	9 ± 3	118 ± 67	20 ± 12	2065 ± 144	8 ± 2	69 ± 23	2622 ± 203	4 ± 3	128 ± 180	2 ± 1	33603 ± 12931
Cephalopods																							
Galiteuthis glacialis	3	19409 ± 8627	1434 ± 509	14951 ± 10737	<LoD	2105 ± 1745	44 ± 3	8 ± 3	61979 ± 31048	66581 ± 11678	121 ± 102	1161 ± 213	793 ± 1109	704 ± 446	682 ± 547	578 ± 230	55 ± 11	117 ± 100	3703 ± 346	231 ± 109	379 ± 374	17 ± 2	85928 ± 34899
Gonatus antarcticus	2	6378 ± 165	2065 ± 596	1378 ± 835	<LoD	901 ± 676	15 ± 1	6 ± 0	16853 ± 13774	19097 ± 10696	18 ± 23	1058 ± 471	1423 ± 1894	549 ± 329	10 ± 0	353 ± 39	39 ± 33	26 ± 25	3162 ± 271	75 ± 48	401 ± 409	2 ± 0	94001 ± 111988
Kondakovia longimana	2	7472 ± 3945	1706 ± 113	2133 ± 1636	<LoD	9216 ± 6413	30 ± 4	23 ± 14	13216 ± 1778	46122 ± 11729	122 ± 28	1021 ± 117	181 ± 176	473 ± 262	24 ± 20	1212 ± 52	13 ± 10	<LoD	4734 ± 381	118 ± 129	246 ± 184	2 ± 0	91585 ± 35517
Martialia hyadesi	2	4056 ± 1167	4828 ± 1290	82 ± 14	<LoD	14524 ± 1805	46 ± 5	18 ± 2	28745 ± 10952	19018 ± 400	139 ± 25	1083 ± 2	68 ± 3	353 ± 14	51 ± 31	4997 ± 464	1 ± 0	55 ± 8	3498 ± 347	8 ± 2	109 ± 34	2 ± 0	69918 ± 10882
Moroteuthis knipovitchi	4	4830 ± 3376	2944 ± 1689	976 ± 844	<LoD	10009 ± 10296	32 ± 17	12 ± 5	17517 ± 16023	46102 ± 40016	129 ± 106	1652 ± 231	345 ± 551	328 ± 142	90 ± 148	1888 ± 1203	22 ± 37	46 ± 26	4709 ± 1817	47 ± 63	222 ± 183	17 ± 31	95067 ± 36352
Psychroteuthis glacialis	2	9130 ± 5520	1397 ± 94	4598 ± 4137	<LoD	2248 ± 460	37 ± 13	2 ± 2	16654 ± 5875	27134 ± 2526	93 ± 130	774 ± 383	1176 ± 1447	297 ± 107	61 ± 72	513 ± 339	10 ± 12	51 ± 62	4024 ± 617	153 ± 152	577 ± 166	2 ± 0	24226 ± 34026

[a] No SD available due to low sample size (included in table for completeness but excluded in statistical analyses).

Table 6. Univariate GLM results for element concentrations in blood and feathers of 10 Procellariiform species. Model components were selected by AIC from all possible model sub-sets with two factorial levels. Parameter estimates (β) shown as Na if non-significant or from multi-level parameters. Significance values represent values for the parameter within the overall model.

Tissue	Element	Parameter	df	F	*p*	β	s.e.
Blood	LogAs[a]	$\delta^{15}N$	1	13.8	<0.001***	4.2	1.1
		$\delta^{13}C$	1	13.9	<0.001***	-2.8	0.7
		Species	8	1.9	0.061	Na	Na
		Species* $\delta^{13}C$	8	1.9	0.067	Na	Na
		Species* $\delta^{15}N$	8	2.9	0.006**	Na	Na
		$\delta^{13}C$* $\delta^{15}N$	1	13.4	<0.001***	0.2	0.1
	LogCd[b]	Species	9	13.1	<0.001***	Na	Na
	Se[c]	$\delta^{13}C$	1	2.8	0.103	-19944.2	23857.4
		Species	9	2.4	0.014*	Na	Na
		Species* $\delta^{13}C$	1	2.5	0.011*	Na	Na
	LogRb[d]	Species	9	4.9	<0.001***	Na	Na
	LogMo[e]	$\delta^{13}C$	1	3.9	0.052	-0.1	0.03
		Species	9	7.5	<0.001***	Na	Na
Feathers	LogMn[f]	Species	9	4.6	<0.001***	Na	Na
	LogFe[g]	Species	9	5.0	<0.001***	Na	Na
	LogCo[h]	Species	9	7.45	<0.001***	Na	Na
	LogAs[i]	Species	9	5.8	<0.001***	Na	Na
		$\delta^{15}N$	1	2.3	0.129	0.03	0.02
	LogMo[j]	Species	9	2.2	0.029*	Na	Na
		$\delta^{15}N$	1	2.6	0.112	0.2	0.1
		Species* $\delta^{15}N$	9	2.0	0.043*	Na	Na
	Se[k]	$\delta^{13}C$	1	5.1	0.025*	-551.4	243.2
		Species	9	6.9	<0.001***	Na	Na
	LogU[l]	Species	9	14.2	<0.001***	Na	Na

[a] $R^2 = 0.69$ [g] $R^2 = 0.24$
[b] $R^2 = 0.48$ [h] $R^2 = 0.32$
[c] $R^2 = 0.51$ [i] $R^2 = 0.30$
[d] $R^2 = 0.25$ [j] $R^2 = 0.34$
[e] $R^2 = 0.37$ [k] $R^2 = 0.44$
[f] $R^2 = 0.23$ [l] $R^2 = 0.48$

Table 7. Univariate GLM results for $\log_{10}$ transformed arsenic concentrations of tissue samples from prey groups (see Table 5 for groups). Model components specified in table as selected by AIC from all possible model sub-sets with two factorial levels. Parameter estimates (β) shown as Na for multi-level parameters.

Parameter	df	F	*p*	β	s.e.
Group	2	8.9	<0.001***	Na	Na
$\delta^{15}N$	1	5.6	0.021*	-0.2	0.1
$\delta^{13}C$	1	3.8	0.054	0.2	0.1
Group* $\delta^{15}N$	2	9.4	<0.001***	Na	Na
Cephalopod* $\delta^{15}N$	1	Na	<0.001***	-0.3	0.1
Crustacea* $\delta^{15}N$	1	Na	0.010*	-0.3	0.1
Fish* $\delta^{15}N$	1	0^b	0^b	0^b	0^b
$\delta^{13}C$* $\delta^{15}N$	1	5.6	0.021*	-0.02	0.01

[a] $R^2 = 0.43$
[b] This parameter is set to zero because it is redundant.