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METAL AND SELENIUM CONCENTRATIONS IN BLOOD AND FEATHERS OF PETRELS OF THE GENUS *PROCELLARIA*

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Abstract: Concentrations of copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), mercury (Hg), and selenium (Se) were determined in blood and feathers of spectacled (*Procellaria conspicillata*) and white-chinned (*Procellaria aequinoctialis*) petrels, species that are phylogenetically related, but with distinct ecological niches. In winter, they feed on similar foods, indicated by an overlapping range of whole-blood stable isotopes values ($\delta^{15}N$; $\delta^{13}C$). No relation was found between blood metal concentration and stable isotope values. In spectacled petrels, metal concentrations appeared lower in blood (Cu = 0.79–20.77 μg/g; Zn = 10.95–28.02 μg/g; Cd = 1.73–10.11 μg/g; Pb = 5.02–26.03 μg/g; Hg = 0.84–9.86 μg/g) than in feathers (Cu = 1.05–21.57 μg/g; Zn = 45.30–81.49 μg/g; Cd = 3.76–10.44 μg/g; Pb = 16.53–59.00 μg/g; Hg = 4.24–24.03 μg/g). In white-chinned petrels, metal concentrations also appeared lower in blood (Cu = 0.62–10.4 μg/g; Zn = 10.73–24.69 μg/g; Cd = 2.00–6.31 μg/g; Pb = 5.72–24.03 μg/g) than in feathers (Cu = 2.68–23.92 μg/g; Zn = 48.96–93.54 μg/g; Cd = 5.72–24.03 μg/g; Pb = 18.62–55.51 μg/g), except for Hg (blood = 0.20–15.82 μg/g; feathers = 0.19–8.91 μg/g). Selenium (0.24–14.18 μg/g) and Hg (0.22–1.44 μg/g) concentrations showed a positive correlation in growing feathers of spectacled petrels. Blood and feather Hg levels were higher in spectacled petrels while feathers Cu and Zn concentrations were greater in white-chinned petrels. Juvenile white-chinned petrels exhibited greater blood Hg concentrations than adults. In the south Atlantic Ocean, discards from commercial fishing operations consumed by spectacled petrels year-round and by white-chinned petrels during the wintering period have elevated Hg concentrations. Because Hg toxicity is associated with behavioral and reproductive changes in birds, it could potentially have impacts on breeding of these seabirds, as both species are listed as threatened by extinction. *Environ Toxicol Chem* 2013;32:1641–1648. © 2013 SETAC

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INTRODUCTION

Seabirds are generally top predators with long life spans and extended breeding cycles; thus, they tend to accumulate pollutants. Therefore, they are employed as sentinels to monitor the occurrence and impact of marine pollutants [1,2]. Egg, feather, and blood samples are collected from birds to evaluate local and regional contamination. These biological materials are usually selected to minimize any impact on the species, and avoid the necessity of killing the animal to collect internal tissues. This is especially important when threatened species are under consideration [1].

Some metals preferentially accumulate in bird feathers, where they bind to proteins during the process of feather formation. For example, Hg accumulation in feathers generally represents a long-term contamination process, while its concentrations in blood represent a recent contamination directly associated with feeding [3].

Metals can be considered as essential when necessary to the organism's metabolism (e.g., Cu, Zn). These metals can cause toxicity when present at elevated concentrations in the environment, although adverse effects are rarely reported in birds. Selenium is also an essential element, with a protective role against Hg toxicity [4]. In contrast, metals like Cd, Pb, and Hg have no metabolic functions, and are thus considered nonessential. These metals can cause toxicity even at low concentrations in the environment. Adverse effects of Hg in birds are associated with changes in behavior and reproduction, such as lower hatchability, lower clutch and egg size, and increased nestling mortality [5]. Lead also affects reproductive success by causing decreased testis weight, lower sperm count, altered egg shell thickness, and a deleterious effect on developing embryos [6]. Cadmium is known as primarily causing kidney toxicity, but reproductive effects (reduced egg production and thin egg shells) and behavioral changes (decreased food intake) induced by exposure to this metal have also been reported [7].

Contamination with metals can occur through different pathways, including respiration and skin contact, but most often via food ingestion [1]. Carbon and nitrogen stable isotopes are widely employed in feeding studies and evaluation of food webs involving birds. Nitrogen isotopes ($\delta^{15}N$) are employed to indicate changes in trophic levels, while carbon ($\delta^{13}C$) can be related to food sources. In the marine environment, the latter can be used to discriminate coastal and oceanic areas, high- and low-latitude regions, and pelagic and demersal areas [8]. In ecotoxicological studies, isotope values can be used to identify contamination sources. Carbon and nitrogen stable isotope values from species feeding at different trophic levels within the food web can identify relationships between trophic level and pollutant concentrations (e.g., Hg, which becomes concentrated by a process of biomagnification, affecting top predators the most [9]).

Procellariiformes (albatrosses and petrels) can have relatively high concentrations of metals compared with coastal and terrestrial bird species [10]; therefore, a combined study of stable

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isotopes and tissue metal levels in these species could increase our understanding of the processes involved in accumulation and toxicity of metals in seabirds. The white-chinned petrel *Procellaria aequinoctialis* and the spectacled petrel *Procellaria conspicillata* are phylogenetically closely related, but have distinct ecological niches. The white-chinned petrel is distributed in the sub-Antarctic region, tends to occur on colder waters over the continental shelf, and is more active during the nighttime [11]. The spectacled petrel is endemic to Inaccessible Island (Tristan da Cunha group), has a preference for deeper and warmer waters, and does not show differences in diurnal and nocturnal activities [12]. These 2 species of *Procellaria* petrels are abundant off the southern Atlantic coast (Rio Grande do Sul State, southern Brazil), behave as fishing-boat followers, and interact with the longline fisheries. Increased mortality occurs due to accidental capture in fishing gear [13]. Even so, the spectacled petrel population is increasing at an annual rate of 7% [14], but it is still listed as vulnerable to extinction due to its endemism, and thus falls in the same category as the white-chinned petrel, which is listed as vulnerable due to population declines [15].

White-chinned and spectacled petrels feed intensively on discards from pelagic longline fishing during the nonbreeding period in southern Brazil [16]. This behavior may have health implications for these species, as consumption of demersal fishing discards has been shown to increase the Hg burden in seabirds of the Mediterranean Sea [17]. However, the full impacts of chemical contamination, absorbed during the nonreproductive phase, are unknown.

The aim of the present study was to evaluate and compare the tissue metal and Se concentrations in 2 congener species of *Procellaria* that show distinct areas of distribution at sea and in the breeding season, but that are sympatric during the wintering period. We predict that spectacled petrels should have higher concentrations of elements due to longer exposure to metals during winter and breeding periods, especially Hg, which accumulates in tissues. We also predict that Cd concentrations would be higher in white-chinned petrels, which feeds on Antarctic krill with high concentrations of Cd during the breeding period. Blood and feathers were taken nondestructively from both species to determine element concentrations (Cu, Zn, Cd, Pb, Hg, and Se) and carbon and nitrogen stable isotope values. To our knowledge, the data presented in the present study on the metal tissue burden in spectacled petrels are the first to be published for this species.

MATERIALS AND METHODS

Sample collection

Seabirds were captured at sea onboard pelagic longline and handline fishing vessels. They were attracted to the vessels with fish and shark viscera and captured using cast nets. Captures were performed in the southwestern Atlantic Ocean off the Brazilian coast (Figure 1) during the chick-rearing period (February–June 2006) to the wintering period (August–September 2007). White-chinned petrels were in the area only during wintering periods.

A total of 38 spectacled petrels (20 males and 18 females) and 30 white-chinned petrels (20 males and 10 females) petrels were captured. Biometric data of birds analyzed were previously reported by Bugoni and Furness [18]. Blood samples (~1 mL) were collected by puncture of the tarsal vein, using a disposable syringe and needle, and stored in absolute ethanol (Merck). This procedure was previously used to assess metal concentrations in blood samples of seabirds [19], and assumed not to affect concentrations of elements measured. Contour feathers (fully grown) from different body areas were collected and stored dry in plastic bags. Growing feathers were also collected when specimens were molting.

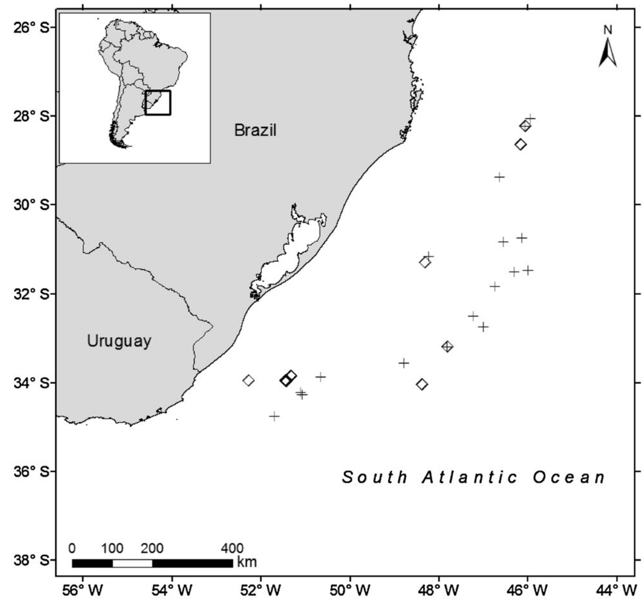

Figure 1. Sites where white-chinned petrels *Procellaria aequinoctialis* (+) and spectacled petrels *Procellaria conspicillata* (◇) were sampled from February to July 2006 and from August to September 2007.

Sex and age determination

The sex of each individual was determined from blood samples after DNA extraction and polymerase chain reaction analysis of the chromo-helicase DNA-binding genes [20]. Age classes of seabirds analyzed were determined through the molting pattern of the species studied [18].

Stable isotope analysis

Blood samples were lyophilized, ground, and homogenized for stable isotope analyses. Approximately 0.7 mg (0.65–0.75 mg) of sample was weighed into a tin capsule for combustion in a Costech Elemental Analyser coupled to a Thermo Finnigan Delta Plus XP Mass Spectrometer, at the Scottish Universities Environmental Research Centre (UK), as described by Bugoni et al. [16]. Growing feather samples were washed 5 times with distilled water, oven-dried at 70 °C for 3 h, cut in small pieces (0.9–1.1 mg) with scissors, inserted in tin capsules, and analyzed at the University of Georgia (Athens, GA, USA) using a Thermo Finnigan Delta Plus XP Mass Spectrometer. Because samples analyzed in different laboratories might not be directly comparable, $\delta^{15}N$ and $\delta^{13}C$ values of growing feathers ($n = 10$) of the yellow-nosed albatross *Thalassarche chlororhynchos* were analyzed in both laboratories. A paired t test showed no significant difference between the results obtained for $\delta^{13}C$ ($t = -1.61$; $p = 0.14$) but did show a difference for $\delta^{13}N$ ($t = 2.49$; $p = 0.03$). Thus, $\delta^{13}C$values obtained in the 2 laboratories could be compared directly, but $\delta^{13}N$ values were corrected through a linear regression equation, making values comparable between laboratories.

Element analysis

In all cases, 5 or 6 complete contour feathers were taken from each individual for metal concentration analysis. Each feather was washed 3 times with acetone and then rinsed with Milli-Q water to remove external contamination. Blood samples and feathers were dried at 60 °C for 72 h, weighed, and completely digested with concentrated nitric acid (65% HNO_3, SupraPur, Merck). After complete digestion, samples were diluted (1:1) with Milli-Q water.

Element (Cu, Zn, Cd, Pb, Hg, and Se) concentrations in blood and feathers were determined using an atomic absorption spectrophotometer (AAS-932 Plus, GBC). For Hg, samples were analyzed by cold vapor atomic absorption spectrometry using a hydride generator (HG 3000, GBC) coupled to the atomic absorption spectrophotometer. In this case, 1 mL of sample was previously diluted with 25 mL of Milli-Q water. Quality assurance controls were also performed.

Because tissue Hg concentration was clearly different in the 2 petrel species (see *Results* section), it was also determined in samples of growing feathers in white-chinned ($n = 9$) and spectacled petrel ($n = 21$). In this case, Se concentration was also measured because this element shows a potential protective effect against Hg effects. From each individual, 3 to 5 growing feathers were processed as described above.

Measurement accuracy and standard curves were obtained using standard Cd, Cu, Pb, and Zn solutions (Standard Reference Material 3114) from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Percentages of metal recovery based on standard reference material (European Reference Material ERM-CE278) prepared as described for tissue samples were 98.9%, 94.2%, 103.8%, and 102.9% for Cd, Cu, Pb and Zn, respectively. Reference material for Hg and Se was unfortunately not available in our laboratory at the time of analysis. Tissue metal concentration was expressed as μg/g dry weight. For blood, the detection limits for Cu, Zn, Cd, Pb, Hg, and Se were 0.69 μg/g, 0.46 μg/g, 0.008 μg/g, 0.38 μg/g, 0.005 μg/g, and 0.008 μg/g, respectively. For feathers, they were 1.22 μg/g, 0.82 μg/g, 0.014 μg/g, 0.68 μg/g, 0.010 μg/g, and 0.014 μg/g, respectively.

Statistical analysis

Data were expressed as mean ± standard deviation. Mean values of tissue metal concentrations between species were compared using the *t* test. Data were mathematically (log) transformed when assumptions of the parametric tests (data normality and homogeneity of variances) were not met. The nonparametric Mann–Whitney test was used to analyze the Hg data due to lack of normality or homogeneity of variances observed after application of different mathematical transformations. A similar situation was observed for the isotopic data of both species. Therefore, the Mann–Whitney test was also employed to compare values between species. However, values of $\delta^{15}N$ and $\delta^{13}C$ in the blood and feathers of the same specimens were compared using the paired *t* test. Differences in metal concentrations between sex and age classes were compared using the Mann–Whitney test.

The relations between tissue metal concentrations were tested using the Spearman correlation coefficient (r_s) for Hg (nonparametric data) and the Pearson correlation coefficient (r) for other metals analyzed (parametric data). In both cases, data were log-transformed prior to the analysis.

In all cases, values were considered statistically different when $p < 0.05$.

RESULTS

Stable isotopes

The 2 petrel species showed similar mean blood values of $\delta^{15}N$ (white-chinned = 15.06 ± 1.92%; spectacled = 14.41 ± 0.76%; $U = 482.5$; $Z = -1.08$; $p = 0.28$) and $\delta^{13}C$ (white-chinned = −17.97 ± 1.80%; spectacled = −17.23 ± 0.46%; $U = 551.0$; $Z = 0.23$; $p = 0.81$; Figure 2). However, white-chinned petrel showed higher values of both carbon and nitrogen isotope ratios in the growing feathers: $\delta^{15}N$ (white-chinned = 17.38 ± 2.67%; spectacled = 15.23 ± 0.42%; $U = 21$; $Z = 3.33$;

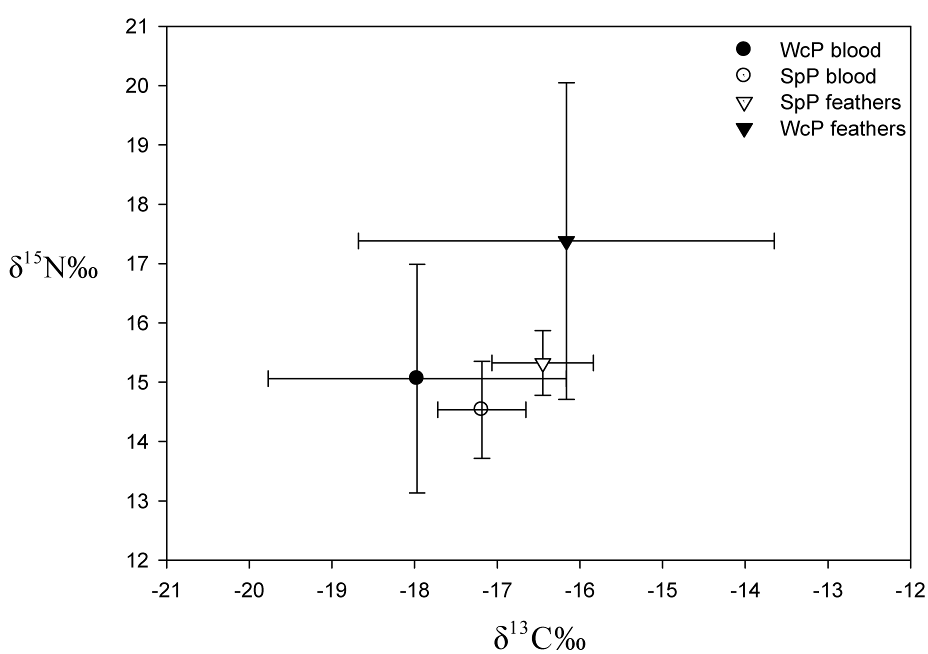

Figure 2. Mean values and standard deviation of $\delta^{15}N$ and $\delta^{13}C$ in the blood (circles) and growing feathers (triangles) of white-chinned petrels (WcP) *Procellaria aequinoctialis* (closed symbol), and spectacled petrels (SpP) *Procellaria conspicillata* (open symbol).

Table 1. Metal concentration (μg/g dry wt) in the blood of 2 species of *Procellaria* petrels[a]

	Cu	Zn	Cd	Pb	Hg
White-chinned petrel *P. aequinoctialis* $n = 30$	3.49 ± 1.82 3.28 (0.62–10.40)	13.64 ± 2.76 13.16 (10.73–24.69)	2.93 ± 0.98 2.68 (2.00–6.31)	8.21 ± 3.53 7.34 (5.72–24.03)	3.20 ± 3.67* 1.78 (0.20–15.82)
Spectacled petrel *P. conspicillata* $n = 38$	4.77 ± 4.46 3.23 (0.79–20.77)	14.44 ± 3.03 13.89 (10.95–28.02)	3.31 ± 1.58 2.85 (1.73–10.11)	9.30 ± 4.33 7.81 (5.02–26.03)	3.41 ± 2.14* 2.59 (0.84–9.86)

[a]Data are expressed as mean ± standard deviation and median value. Values in parenthesis represent the minimum and maximum values found for each metal.
*$p < 0.05$.

$p < 0.01$); $\delta^{13}C$ (white-chinned = −16.16 ± 2.52%; spectacled = −16.45 ± 0.61%; $U = 31$; $Z = 2.87$; $p < 0.01$).

The $\delta^{15}N$ value was higher in growing feathers than in blood for paired samples from the same bird (blood = 15.27 ± 1.54%; growing feathers = 15.87 ± 1.78%; $n = 30$: $t = -3.88$; $p < 0.01$). The same was observed for the $\delta^{13}C$ values (blood = −17.20 ± 1.04%; growing feathers = −16.36 ± 1.42%; $n = 30$; $t = -7.88$; $p < 0.001$).

Element concentrations

For all elements, concentrations appeared lower in blood than in feathers (Tables 1 and 2), except for Hg in white-chinned petrel (Figure 3). There was no difference in the blood concentrations of Cd, Pb, Cu, and Zn between the 2 species. However, Hg concentration was greater in spectacled petrel ($U = 387$; $Z = -2.26$; $p = 0.02$). In feathers, Cu ($t = 2.15$; $p = 0.04$) and Zn ($t = 2.20$; $p = 0.03$) concentrations were greater in white-chinned petrel, while Hg concentration was again higher in spectacled petrel ($U = 26$; $Z = -6.72$; $p < 0.001$).

In the blood, significant positive correlations ($p < 0.01$) were found between the concentrations of Cd, Pb, Cu, and Zn (Table 3), while Hg concentration showed a significant correlation only with Cd concentration ($r_s = 0.36$; $p < 0.01$). In feathers, the only significant relation was observed between Cd and Pb concentrations ($r = 0.68$; $p < 0.01$; Table 4). In growing feathers of spectacled petrels ($n = 15$), there was a significant and positive relation ($r = 0.62$; $p = 0.01$) between Se (4.60 ± 4.08 μg/g) and Hg (0.69 ± 0.33 μg/g) concentrations. Selenium and Hg concentrations in white-chinned petrels were below the detection limit of the technique employed.

There was no correlation between the stable isotope values ($\delta^{15}N$ or $\delta^{13}C$) and metal (Cu, Zn, Cd, Pb, and Hg) concentrations in blood or in the growing feathers ($p > 0.05$).

Age and sex

White-chinned petrels could be separated into 2 age classes (21 juveniles and 9 adults), based on molting patterns. In spectacled petrels, age could not be determined. When white-chinned adults ($n = 9$) and juveniles ($n = 21$) were compared, juveniles showed a greater Hg concentration in blood (juveniles = 4.27 ± 3.94 μg/g; adults = 0.70 ± 0.36 μg/g; $U = 4$; $Z = 4.10$; $p < 0.01$) and a lower Hg concentration in feathers (juveniles = 1.14 ± 2 μg/g; adults = 3.45 ± 2.84 μg/g; $U = 24$; $Z = -3.19$; $p < 0.01$; Figure 4). No difference between juveniles and adults was found for the other elements analyzed. Because age could not be determined for most spectacled petrels, comparison was not possible.

For blood and feathers, no significant difference ($p > 0.05$) was observed in metal and Se concentrations between male white-chinned ($n = 20$) and spectacled petrels ($n = 20$) or female white-chinned ($n = 10$) and spectacled petrels ($n = 18$).

DISCUSSION

Stable isotopes

Data obtained in the present study for $\delta^{15}N$ and $\delta^{13}C$ in the blood of spectacled and white-chinned petrels indicate that these seabird species share the same trophic level [16] and have similar diets during the wintering period even though they occupy distinct ecological niches. However, higher individual variation was observed in white-chinned petrels, measured as standard deviation of the mean values. This increased variation reflects dietary and physiological variability in the sample population, which included recent arrivals from high-latitude breeding grounds, and first-year juveniles, which generally feed on krill [16]. Greater values of $\delta^{15}N$ and $\delta^{13}C$ were found in growing feathers compared with those found in blood and can be explained by the 2 tissues having a similar dietary source, but a different isotopic fractionation between food–blood and food–feathers. Differences in carbon and nitrogen isotope values between blood and feathers (grown simultaneously) have been demonstrated in other seabird species, and the values obtained from spectacled petrels in the present study are of similar magnitude to those reported in previous studies [21].

Table 2. Metal concentration (μg/g dry wt) in feathers of 2 species of *Procellaria*[a]

	Cu	Zn	Cd	Pb	Hg
White-chinned petrel *P. aequinoctialis* $n = 30$	10.74 ± 5.56* 9.88 (2.68–23.92)	67.48 ± 11.64* 64.58 (48.96–93.54)	7.34 ± 1.70 6.97 (5.72–24.03)	33.05 ± 8.48 33.16 (18.62–55.51)	1.84 ± 2.48** 0.70 (0.19–8.91)
Spectacled petrel *P. conspicillata* $n = 38$	7.97 ± 5.05* 6.65 (1.05–21.57)	62.05 ± 7.58* 61.23 (45.30–81.49)	7.33 ± 1.57 7.27 (3.76–10.44)	32.26 ± 8.71 31.66 (16.53–59.00)	11.17 ± 3.78** 11.28 (4.24–24.03)

[a]Data are expressed as mean ± standard deviation and median value. Values in parentheses represent the minimum and maximum values found for each metal.
*$p < 0.05$.
**$p < 0.01$.

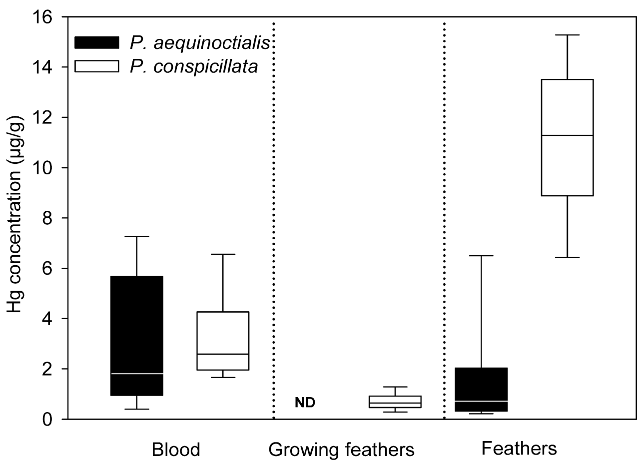

Figure 3. Hg concentration (µg/g dry wt) in the blood (n = 30 and 38), growing feathers (n = 9 and 21), and feathers (n = 30 and 38) in 2 species of *Procellaria* petrels. Data are shown as median values and the corresponding 75% to 25% and 95% to 5% quartiles. ND = not detected.

The values of $\delta^{15}N$ (15.06 ± 1.92%) and $\delta^{13}C$ (−17.97 ± 1.80%) found in the blood of white-chinned petrel were similar but slightly greater than those reported by Anderson et al. [9] for specimens sampled in the breeding period ($\delta^{15}N$ = 14.22 ± 0.66%; $\delta^{13}C$ = −18.13 ± 0.33%). This may indicate a change in the feeding behavior of white-chinned petrels during the wintering period, when they may take more prey of higher trophic status while foraging at lower latitudes, as indicated by the values of $\delta^{15}N$ and $\delta^{13}C$, respectively.

Element concentrations

There was marked individual variation in the metal concentrations in tissues (blood and feathers) of the 2 petrel species analyzed. This fact is likely associated with an individual feeding specialization or with specific physiological processes of metal detoxification in seabirds.

Blood metal concentrations were not different between the 2 petrel species as a whole, except for Hg, which exhibited greater concentrations in spectacled than white-chinned petrel. This may be due to overlap in the foraging area of the 2 species in the nonreproductive period, which is consistent with at sea census data [13], and the fact that birds were sampled in areas where the 2 petrel species co-occur. Blood distributes nutrients to the different body regions and organs. Rapid distribution of metals throughout body results in a more stable metal concentration in blood than in those tissues in which metals accumulate (liver), metabolize (liver), and are excreted (kidneys and digestive tract) [2,3].

Blood Cd concentration was greater in specimens of white-chinned petrels from the breeding areas, which were identified by their lower $\delta^{15}N$ and $\delta^{13}C$ values. Higher metal concentrations were expected in blood from this group, because the Antarctic/sub-Antarctic ecosystems show high levels of Cd associated with natural sources [22]. However, elevated dietary Cd could also derive from cephalopods inhabiting the southern Brazilian waters, especially the squid *Illex argentinus* [23]. Squid is common bait for the pelagic longline fishery and is frequently consumed by both spectacled and white-chinned petrels [24]. Seabirds feeding on cephalopods and crustaceans (krill) generally show greater Cd levels than those feeding on fish, although the latter may result in higher Hg concentration [25].

In feathers, Cu and Zn concentrations were greater in white-chinned petrel than spectacled petrel. This result could be explained by the occurrence of white-chinned petrel over inshore waters compared with the more pelagic spectacled petrel [11,12]. Feeding on the continental shelf, white-chinned petrels would be exposed to higher discharges of contaminants and able to feed on demersal organisms from discards, which generally accumulate higher concentrations of metals [17]. Copper and Zn concentrations in the Antarctic krill are not elevated despite the occurrence of marked annual variations [26].

Copper and Zn concentrations in feathers of white-chinned petrel are quite similar to those previously reported by Anderson et al. [9] for specimens collected on breeding grounds (13.11 ± 17.79 µg/g dry wt and 77.65 ± 17.98 µg/g dry wt, respectively). However, values found for Pb (not detected) and Cd (0.14 ± 0.13 µg/g dry wt) by Anderson et al. [9] were lower than those observed in the present study, suggesting the presence of local sources of pollution. The specimens sampled by Anderson et al. [9] do not necessarily forage over southern Brazilian waters during the wintering period, unlike the birds sampled in the present study. During the incubation period, white-chinned petrels breeding at South Georgia generally forage on the Patagonian continental shelf until chicks are hatched, when they shift their foraging to regions closer to the colony [11,27].

Concentrations of Pb and Hg found in the blood of petrels analyzed in the present study are similar to those reported in the same tissue of the northern *Macronectes halli* and southern *Macronectes giganteus* giant petrels [19]. However, blood Cd concentrations were lower in seabirds from the present study. Copper and Zn concentrations measured in feathers of petrels from the present study were similar to those reported for feathers of petrels and albatrosses from the Antarctic region (10.4 µg/g dry wt and 71.7 µg/g dry wt, respectively). However, Cd and Pb concentrations were greater than those reported for the Antarctic petrels and albatrosses (0.07 µg/g dry wt and 0.42 µg/g dry wt, respectively) [25].

Mercury concentration showed a 6-fold increase in feathers of spectacled petrels than in those of white-chinned petrels. Higher Hg concentration was also observed in blood of

Table 3. Pearson (r) and Spearman (r_s) correlations between metal concentrations (log-transformed values) in the blood of white-chinned petrel *Procellaria aequinoctialis* and spectacled petrel *Procellaria conspicillata*

	Cu (r)	Zn (r)	Cd (r)	Pb (r)	Hg (r_s)
Cu (r)		0.52*	0.55*	0.63*	0.03
Zn (r)	0.52*		0.82*	0.71*	0.12
Cd (r)	0.55*	0.82*		0.90*	0.36*
Pb (r)	0.63*	0.71*	0.90*		0.24
Hg (r_s)	0.03	0.12	0.36*	0.24	

*$p < 0.01$.

Table 4. Pearson (r) and Spearman (r_s) correlations between metal concentrations (log-transformed values) in feathers of white-chinned petrel *Procellaria aequinoctialis* and spectacled petrel *Procellaria conspicillata*

	Cu (r)	Zn (r)	Cd (r)	Pb (r)	Hg (r_s)
Cu (r)		0.10	0.14	0.08	−0.17
Zn (r)	0.10		−0.17	−0.09	−0.19
Cd (r)	0.14	−0.17		0.68*	0.04
Pb (r)	0.08	−0.09	0.68*		0.03
Hg (r_s)	−0.17	−0.19	0.04	0.03	

*$p < 0.01$.

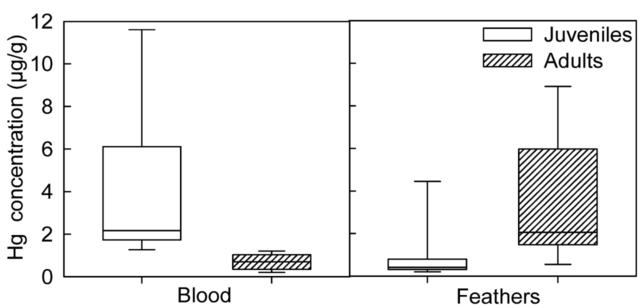

Figure 4. Hg concentration (µg/g dry wt) in the blood and feathers of juveniles and adults of white-chinned petrels *Procellaria aequinoctialis*. Data are shown as median values and the corresponding 75% to 25% and 95% to 5% quartiles.

spectacled petrels, but the difference between species was less dramatic. These findings seem to reflect the high Hg concentration observed in waters off the southern Brazilian coast, as found in swordfish *Xiphias gladius*, blue shark *Prionace glauca*, and hammerhead sharks *Sphyrna* spp. captured by the longline fishery in southeast and southern Brazil [28,29], and whose discards are a major food of both petrels [16]. These high Hg values are in contrast to much lower concentrations found in the Antarctic region [30], where white-chinned petrels breed. In turn, spectacled petrels are found at lower latitudes (Inaccessible Island, Tristan da Cunha, and subtropical/temperate waters), even during the reproductive period, when it interacts with fishing vessels and feeds in southern Brazilian waters [31]. Anderson et al. [9] and Becker et al. [32] analyzed the Hg concentration in white-chinned petrels and found greater levels in feathers from individuals collected during the breeding period (3.79 ± 1.72 µg/g dry wt and 7.43 ± 1.97 µg/g dry wt, respectively). However, these feathers were grown during the nonreproductive period, and the numbers are lower than those found for feathers of spectacled petrels in the present study. This fact can be explained by the wider wintering range of white-chinned petrel [11].

An Hg concentration of 5 µg/g dry weight in feathers has been suggested to be the adverse effect level in birds. The threshold concentration for Pb and Cd is 4 and 2 µg/g, respectively [33]. All individuals of both species had Pb and Cd concentration in feathers above the adverse effect level. For Hg, spectacled petrels and some white-chinned petrels had feather concentrations above the adverse effect level. Despite such elevated metal concentrations, previous studies have demonstrated that Procellariiformes can have higher concentrations of metals without presenting adverse effects [10].

The trade-off between positive and negative aspects of fishing discards as an alternative food source for seabirds has been studied and debated [16]. Results from the present study, as previously shown by Arcos et al. [17], indicate a negative impact from this behavior, exposing animals to higher concentrations of metals than under natural conditions. This is important when considering the conservation status of petrel and other threatened seabird species that depend on fishing discards.

Relation between metals

A relation was found among various metals in blood of petrels in the present study. Other studies on Procellariiformes also reported positive correlations between essential and nonessential metals in other tissues like liver, muscle, and kidney [19,25]. Selenium concentration is generally correlated with Hg, relating to the formation of an Hg–Se complex that binds to specific plasma proteins, generating a highly stable complex [4]. This renders the Hg nonreactive and ameliorates its toxicity. Cadmium and Zn correlations have been reported for other species of seabirds. High levels of Cd induce metallothionein synthesis, leading to greater binding of Zn [34].

It is well known that feathers are an important route of Hg excretion, especially as methylmercury [35]. For other metals, this may not be the case. The present study found an almost complete lack of correlation among metals in feathers, with only Cd and Pb showing a correlation.

In the growing feathers of spectacled petrels, where the levels of Hg and Se could be simultaneously determined, a positive correlation was observed, consistent with the protective effect of Se over Hg. Kim et al. [36] and Scheuhammer et al. [37] also reported an association between Hg and Se in liver of albatrosses and petrels (Procellariiformes), and eggs of the common loon *Gavia immer*.

Becker et al. [32] found a negative correlation between Hg concentration and the proportion of krill in the bird diet and concluded that the trophic level of Antarctic birds is the major factor that explains the Hg concentrations. No stable isotope data were obtained to determine the trophic level [32]; however, we found no relation between tissue metal concentrations and the stable isotope ($\delta^{15}N$ and $\delta^{13}C$) values in the present study. Similarly, Anderson et al. [9] found no relation between values of nitrogen and carbon isotopes and metal concentration, except for Hg, in several Procellariiformes species.

Age and sex

Juvenile white-chinned petrels showed greater blood Hg concentration than adults, and it is possible they not had the chance to eliminate Hg from the body through molt, an important route of excretion. Hindell et al. [38] reported greater Hg concentrations in kidney and liver of adults than in immatures for wandering albatross (*Diomedea exulans*), but it is not clear whether the immature birds had molted. The greater Hg concentrations found in feathers of adult white-chinned petrels, whose feathers had grown in both breeding and wintering grounds, confirm that low levels of the metal are present in the

Antarctic environment [26,30], where all feathers of juveniles had grown. Feathers from juveniles grow before they leave the nest. Adult white-chinned petrels had greater Hg concentrations in fully grown feathers than in blood, and this may be the result of metal excretion during the molting processes.

Sexual size dimorphism in albatrosses and petrels frequently results in sexual segregation in wintering areas [39]. When it occurs and if areas have different metal concentrations in the environment/food resources, as is the case between southern Brazil (high Hg [28,29] and low Cd) and Antarctica (low Hg [30] and high Cd [22]), metals could serve as tracers of the origin of birds. For white-chinned and spectacled petrels analyzed in the present study, sex was not a factor found to affect tissue metal concentration, similarly to the Procellariiformes species studied previously [32], which can probably be explained by the limited sexual dimorphism in both species [18], thus suggesting the nonexistence of sexual segregation in wintering grounds at sea. González-Solís et al. [19], however, have reported a difference in metal concentrations, especially Hg, between male and female giant petrels, species with marked sexual dimorphism and sexual segregation in foraging areas and food sources.

Eggs are also considered a route of some metal excretion in female birds [40]. Because no significant difference in tissue metal concentrations was observed between male and female petrels, it is suggested that metal excretion via eggs was not significant for the white-chinned and spectacled petrel specimens analyzed in the present study, although this effect may be obscured by sampling of individuals that includes juveniles and immature birds.

CONCLUSIONS

Spectacled and white-chinned petrels show similar concentrations of most metals analyzed in blood and feathers even though they forage in waters of different temperature and depth and breed at distinctly different locations. However, differences in blood Hg concentrations observed between the 2 species appear to indicate a more recent dietary source of Hg contamination. Furthermore, metal concentrations in fully grown feathers of these 2 species show differential long-term accumulation patterns for essential and nonessential metals.

Stable isotope values for blood and feathers are similar for spectacled and white-chinned petrels, indicating that they feed at a similar trophic level in winter, and consistent with the use of discards from pelagic longline fisheries [16]. The present study adds to the body of data suggesting that the use of longline fishing discards is harmful to petrels. Sharks and swordfish, which contain high levels of some metals (especially Hg), are targeted by fisheries; the offal is discarded and eaten by seabirds [28,29]. Similarly, the squid bait used in longline fisheries, and also consumed by petrels, has high levels of Cd [23]. High levels of those metals were found in the blood and feathers of both petrels analyzed. For nonessential metals, these levels were considered toxic (above threshold concentration recognized to cause health effects on some avian species), despite previous studies demonstrating that Procellariiformes cope quite well with high doses of metals [10]. Under natural conditions (without the availability of discards), high levels of metals, especially Hg, would not be found in these species. Despite the finding of elevated concentrations of some metals (e.g., Hg) in petrel tissues, especially in spectacled petrels, there seems to be no impact on the reproductive success of this species, whose population has grown over recent years.

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