Assessment of Trace Metal Concentrations in Feathers of White-chinned Petrels, Procellaria aequinoctialis, from the Patagonian Shelf

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Abstract

Cd, Cr, Cu, Fe, Ni, Pb, and Zn were measured in feathers from male and female White-chinned petrels Procelllaria aequinoctialis accidentally killed in longliners off Argentina in 2005. Cd, Pb, and Ni concentrations in adult feathers were below detection limits regardless of the birds' sex. Metal concentrations did not vary with sex for Cu, Fe and Zn in feathers of adult birds despite significant differences in body size between males and females. The Cu and Fe concentrations differed significantly between the type of feather within individual birds. The mean concentrations of metals determined here were within the range of concentrations reported in feather tissues of other related Procellariiformes species from different biogeographic areas. However, the concentrations of Cr were similar to those reported for other petrels from polluted areas of the North Pacific. Thus, it appears likely that levels of pollution in Patagonia may not be as negligible as previously thought for some trace metals.

Keywords: White-chinned Petrels, Procellaria aequinoctialis, Seabirds, Feather tissues, Trace metals, Pollution monitoring, Patagonian Shelf

1. Introduction

White-chinned petrels, Procellaria aequinoctialis, (WCP) are among the largest of all-dark petrels, only smaller than giant petrels (Onley & Scofield, 2007). White-chinned petrels breed in dense colonies on a number of widely spaced sub-Antarctic islands (Brooke, 2004). Georgias del Sur/South Georgia has long been considered to hold the largest breeding population of WCPs in the world, with an estimated two million pairs (Prince & Croxall, 1993). However, a recent survey of the whole archipelago indicates that the size of its breeding population is currently some 40-45 % of the former estimate (Martin et al., 2009). Even so, the area holds more WCPs than all other sites worldwide combined (ACAP, 2011). The majority of Georgias del Sur/South Georgia WCPs exploit waters of the Patagonian Shelf during the non-breeding, pre-laying exodus and incubation periods, and to a lesser extent during chick-rearing when they mainly forage over Georgias del Sur/South Georgia shelf and shelf-slope, around Orcadas del Sur/South Orkney (Berrow et al., 2000; Phillips et al., 2006), and occasionally in the Patagonian Shelf (Mackley et al., 2011).

The Patagonian Shelf is the marine ecosystem off the southern coast of Argentina, an important area of global concern, providing food for at least 700 species of marine vertebrates, some of which come from distant regions (e.g. Antarctica, Australia and New Zealand, Tristan da Cunha) (Arata et al., 2009; Croxall & Wood, 2002; Favero & Silva Rodriguez, 2005; Quintana et al., 2009). Although the Patagonian Shelf is often regarded as a relatively uncontaminated region (Barbieri et al., 2007; González-Solís et al., 2002; Stewart et al., 1999), the level of pollution in this vast marine area may not be as negligible as previously thought for some metals (González-Solís et al., 2002; Seco Pon et al., 2011). Additional robust information from the region is needed to understand contaminant concentrations and defining biological indicators in this important marine ecosystem of the Southern Hemisphere (www.lme.noaa.gov).

There have been several studies of contaminants in South Atlantic petrel species, including WCPs (Anderson et al., 2010; Becker et al., 2002; González-Solís et al., 2002; Muirhead & Furness, 1988; Thompson & Furness, 1989a, 1989b). White-chinned petrels are ideal indicators of contaminant accumulation in the pelagic marine environment, because they are apex predators, feeding primarily on krill and fish (Croxall et al., 1995) within defined feeding areas relatively close to their breeding grounds (Mackley et al., 2011; Phillips et al., 2006). It should be stressed that most of the studies were conducted in feather tissues and that for some metals (e.g. Pb and Hg) the concentrations in feathers are strongly correlated to the levels in blood (Burger & Gochfeld, 1990; Monteiro & Furness, 2001). Accordingly, feather samples have been used extensively for assessing trace metal exposure on several seabird species as a result of some methodological advantages they bring over other tissues (e.g. feathers provide easily obtainable and non-invasive matrices, they also provide retrospective time series analyses, and endangered species can be resample systematically and released without substantial harm) (Burger & Gochfeld, 2004, 2009; Burger, 1993; Monteiro et al., 1998; Pilastro et al., 1993). This is particularly relevant considering that half of all petrel species are globally threatened with extinction (IUCN BirdLife International, 2010).

In this baseline study, we assessed the concentrations of Cd, Cr, Cu, Fe, Pb, Ni, and Zn in feathers of male and female WCP coming from individuals accidentally captured by commercial longliners operating in the

Patagonian Shelf off Argentina. We focused on WCPs because: (1) this petrel has a strong interaction with commercial fisheries and as such is the species most often accidentally killed in Argentine waters (Favero, 2008; Seco Pon et al., 2007; among others), and (2) it is classified as "Vulnerable" by the International Union for the Conservation of Nature (IUCN BirdLife International, 2010) and included in Annex I of the Agreement on the Conservation of Albatrosses and Petrels (ACAP, 2011).

2. Materials and Methods

2.1 Sample Collection

All of the birds examined in this study were accidentally caught by demersal longliners targeting the fish, Kingclip Genypterus blacodes, in waters off the Patagonian Shelf, Argentina during summer 2005 chiefly between 42-47° S and 59-63° W. The fishing gear used by this industry has been described in previous studies (Gandini & Frere, 2006; Seco Pon et al., 2007). Overall, thirty five adult petrels, without evidence of predation while they had been immersed on the longline, were used in this study. Immediately after collection, petrels were given an identification number, bagged individually, and stored deep frozen and later transferred still frozen (-18 ℃) to the Centro de Investigaciones de Puerto Deseado, Santa Cruz Province, Argentina. Biometric measurements of these birds are presented in Appendix A. Sex was determined by visual inspection of gonads in the laboratory. The last grown primary feather (P10) (Warham, 1990) was systematically obtained from the right wing of each sampled individual and a random pinch of feathers was plucked from the right side of the breast of the same individual. Given the difficulty in handling and weighing single feathers, multiple breast feathers were grouped and placed in envelopes. Although there may be some variation in metal concentrations among breast feathers, by using several feathers the differences are averaged (Bond & Diamond, 2008). Primary feathers were stored apart from breast feathers.

2.2 Metal Analysis

Primary 10 and breast feathers were washed vigorously (at least three times) in deionized water alternated with acetone to remove loosely adherent external contamination (Burger et al., 1994) and then dried at 60 ℃. All materials associated with trace metal extraction were thoroughly acid-cleaned and rinsed with deionized water before use (Clesceri et al., 1998). Samples were digested in a mixture of concentrated acids, according to methods described by Marcovecchio and Ferrer (2005). About 250 mg were removed from the outermost (distal) segment of each feather and mineralized with a 1:3 perchloric-nitric acid mixture in a thermostatic bath (at 120 ± 10 ℃) up to minimum volume. Solutions were made up to 10 ml with 0.7 % nitric acid. Each feather segment was sectioned, and each section digested separately to ensure the reproducibility of the method. Element concentrations were determined using a Perkin-Elmer AA-2380 atomic absorption spectrophotometer with air/acetylene flame. Analytical grade reagents were used for the relevant blanks and calibration curves and the analytical quality (AQ) was tested against reference materials (mussel tissue flour, R.M.N°6) provided by the National Institute for Environmental Studies (NIES) from Tsukuba (Japan). All elements were analyzed in dry mass tissue. Percentage ranges of recovery in the analysis of reference materials to assess analytical quality were between 91-101 % for all the considered metals. The obtained values from the analysis of the reference materials were within the range of certified ones. The analytical precision expressed as coefficients of variance are < 10 % for all the metals based on replicate analysis. Instrumental detection limits ($\mu g\ g^{-1}$) were: Cd: 0.20, Cr: 0.29, Cu: 0.77, Fe: 2.73, Ni: 1.54, Pb: 1.50, and Zn: 0.88.

2.3 Statistical Analysis

Concentrations of Cd and Ni, which were below limits of detection (LOD), and concentrations of metals under the LOD in more than 40 % of overall samples were reported in the summary statistics but excluded from further considerations or statistical analyses (Anderson et al., 2010; Seco Pon et al., 2011). For the remaining metals, a few values were under the LOD for some metals (e.g. 22 % for Cr in breast feathers). To avoid missing values distorting the statistical outcomes, a value equal to one-half the LOD limit for the respective metal was assigned. To analyze the relationship of metal concentrations with type of feather and sex, we employed general linear mixed models (GLMM) with normal error structure and identity link function (Crawley, 2007). This analysis was performed using GLMM to consider the non-independence of the type of feather within an individual bird. The relationship between the metal concentrations and type of feather and sex was modeled with individual identity as a random effect and type of feather and sex as a fixed effect (Crawley, 2007). We also examined the covariation among elements in type of feathers of male and female birds using Pearson correlations. To run the GLMM and the Pearson, data were transformed using $\log_{10}(x)$ when necessary to accomplish assumptions of normality and variance homoscedasticity (Zar, 1999).

Metal concentrations (µg g^{-1} dry mass) are presented as means ± one SD. Both arithmetic and geometric means are given to facilitate comparisons with other studies in literature. Statistical analysis of the data was performed using R software, Version 2.5.1. (R Development Core Team, 2004). In all cases, we assumed the results were statistically significant when alpha was ≤ 0.05.

3. Results

The mean concentration of Cd, Cr, Cu, Fe, Ni, Pb, and Zn in the last grown primary and breast feathers of WCP is given in Table 1. Concentrations of Cd and Ni were below the LOD and concentrations of Cr and Pb were under the LOD in more than 40 % of samples.

Using GLMM, no significant interaction was found between feather type and sex for Cu, Fe, and Zn concentrations (GLMM, all $P > 0.10$). However, the Cu (GLMM: $F_{1,34} = 34.12$, $P < 0.001$), and Fe concentrations (GLMM: $F_{1,34} = 40.67$, $P < 0.001$) differed significantly among the type of feather within individual WCPs. Breast feathers had significantly higher concentrations of Cu than primary feathers, whilst primary feathers had significantly higher concentrations of Fe than breast feathers. Zn concentrations did not differ between individual feather tissues (GLMM: $F_{1,34} = 0.41$, $P = 0.52$).

Correlations among trace metal concentrations within feather type from birds of a particular sex were in general non significant (Pearson correlations, all $P > 0.10$). One exception was a significant positive correlation between Fe and Zn concentrations in primary feathers of female WCP (Pearson $r = 0.612$, $P = 0.03$, $n = 35$).

4. Discussion

Some caution should be taken when assessing statistical variability in the biophysical or chemical data, because sample numbers were small and the samples were not collected from the field to a rigorous protocol, but ad libitum from the longline by-catch. Statistically significant differences ($P < 0.001$) were observed for some metals among feather tissues within individual birds. Interestingly, most petrels undertake a simple descendent or variant molt of their primary or flight feathers (Bridge, 2006) but detailed studies of molt in WCPs are scarce. Given that birds are able to eliminate a substantial portion of their body burden of certain trace metals via feather moulting, the concentration of certain metals may vary during this period (Dauwe et al., 2003). Thus comparisons and conclusions in this study are based on breast feathers given that they are known to be the most representative of metal concentrations in plumage (Furness et al., 1986; Burger & Gochfeld, 2004).

No statistically significant differences were observed between feather type and sex for Cu, Fe, and Zn concentrations in petrels. These findings were similar to those of Burger (1993), who found no sex-related differences in metal concentrations in the feathers of seabirds in five out of eight species studied, that were hundreds of kilometers apart. For example, sex was not a significant variable for the same array of metals in feathers of North Pacific (Phoebastria immutabilis; Burger & Gochfeld, 2000a) and South Atlantic albatrosses (Thalassarche melanophrys; Seco Pon et al., 2011). Interestingly though, few statistically significant body-size differences were observed between WCP sexes (Appendix A). This finding was unexpected considering that metal concentrations were reported to vary in those species with body-size differences between sexes or different diets. Females also have additional routes of ridding the body of metals (i.e. by sequestering metals in the eggshell or transferring metals via the vitellus or albumen) (Bond & Diamond, 2009; Burger & Gochfeld, 2009; Burger, 1993; Furness, 1996, Lacoue-Labarthe et al., 2008).

The concentrations of metals determined in this study are, on average, within the range of concentrations reported in the few recent studies on metals in feather tissues of other related species from different biogeographic areas (Table 2). Moreover, the observed metal concentrations in WCPs are well below those considered to cause harmful effects in birds (see Furness, 1996; Lock et al., 1992). Interestingly, the concentrations of some metals, most notably Cr, are similar to those reported for other petrel species from polluted areas of the North Pacific (Burger & Gochfeld, 2000b). Cr is a non-essential metal that comes from a variety of anthropogenic sources (Eisler, 1986; Towill et al., 1978) and is considered to be potentially toxic to seabirds (Eisler, 1986). Thus, although this study provides essential baseline data for risk assessment modeling of the Patagonian Shelf, it appears likely that (in line with that reported for other species in the same area, Seco Pon et al., 2011) the level of pollution in Patagonia may not be as negligible as previously thought for some trace metals.

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Table 1. Mean trace metal concentrations (in μg g^{-1}, dry mass) ± SD in the last grown primary (P10) and breast feathers of adult White-chinned Petrel Procellaria aequinoctialis attending waters of the Patagonian Shelf, off Argentina in 2005. Geometric means are given in parentheses

	Female (*n* = 21)		Male (*n* = 14)	
	Primary feather	Breast feathers	Primary feather	Breast feathers
Cd	< LOD [a]	< LOD [a]	< LOD [a]	< LOD [a]
Cr	< LOD [a]	4.81 ± 4.47 (2.07)	< LOD [a]	7.03 ± 4.98 (3.99)
Cu	7.81 ± 3.94 (6.85)	10.40 ± 1.61 (10.28)	4.77 ± 2.30 (4.21)	10.78 ± 2.56 (10.55)
Fe	66.27 ± 31.46 (57.86)	35.50 ± 16.72 (31.48)	78.86 ± 29.56 (73.42)	31.08 ± 12.80 (28.94)
Pb	< LOD [a]	< LOD [a]	< LOD [a]	< LOD [a]
Ni	< LOD [a]	< LOD [a]	< LOD [a]	< LOD [a]
Zn	74.57 ± 18.32 (72.49)	84.19 ± 18.59 (82.41)	77.39 ± 17.63 (75.65)	70.67 ± 21.85 (67.91)

[a]<LOD below limit of detection.

Table 2. Average (arithmetic mean) trace metal concentrations (μg g^{-1}, dry mass) in petrels' feathers reported in other studies (post-1997 publications only)

Petrel species and location	Cd	Cr	Cu	Fe	Pb	Zn	Source
Other petrels from the Southern Ocean							
Northern Giant Petrel (*Macronectes halli*), GS/SG	0.083	Na	6.211	103.719	< LOD [a]	67.557	Anderson et al. (2010)
Southern Giant Petrel (*Macronectes giganteus*), GS/SG	0.289	Na	6.877	95.208	< LOD [a]	90.208	Anderson et al. (2010)
White-chinned Petrel (*Procellaria aequinoctialis*), GS/SG	0.138	Na	13.110	262.076	< LOD [a]	77.646	Anderson et al. (2010)
White-chinned Petrel (*Procellaria aequinoctialis*), SIO [b]	0.070	Na	10.400	Na	0.426	71.700	Kim et al. (1998)
White-chinned Petrel (*Procellaria aequinoctialis*), PS [c]	< LOD [a]	2.070	10.410	30.210	< LOD [a]	75.160	This study
Common-diving Petrel (*Pelecanoides urinatrix*), GS/SG	0.376	Na	31.254	130.911	< LOD [a]	301.098	Anderson et al. (2010)
South Georgia Diving-petrel (*Pelecanoides georgicus*), GS/SG	0.300	Na	20.176	791.151	< LOD [a]	22.283	Anderson et al. (2010)
Antarctic Prion (*Pachyptila desolata*), GS/SG	0.059	Na	20.176	1.010.868	< LOD	113.658	Anderson et al. (2010)
Blue Petrel (*Halobaena caerulea*), GS/SG	0.074	Na	8.745	986.705	< LOD [a]	6.953	Anderson et al. (2010)
Flesh-footed Shearwater (*Puffinus carneipes*), AUS [d]	0.188	Na	14.005	Na	0.493	50.416	Bond and Lavers (2010)
Flesh-footed Shearwater (*Puffinus carneipes*), NZ [d]	0.065	Na	14.063	Na	0.419	96.115	Bond and Lavers (2010)
Other Procellariidae globally							
Bonin Petrel (*Pterodroma hypoleuca*), NP	0.104	1.580	Na	Na	0.926	Na	Burger and Gochfeld (2000b)
Wedge-tailed Shearwater (*Puffinus pacificus*), NP	0.064	2.380	Na	Na	0.338	Na	Burger and Gochfeld (2000b)
Christmas Shearwater (*Puffinus nativitatis*), NP	0.573	2.050	Na	Na	1.680	Na	Burger and Gochfeld (2000b)

[a]<LOD below limit of detection; NA not analyzed.
[b]Black-browed Albatross, Grey-headed Albatross and White-chinned Petrel combined.
[c]Breast feathers of males and females combined.
[d]Values reported in fresh mass.
GS/SG Georgias del Sur/South Georgia; SIO Southern Indian Ocean; PS Patagonian Shelf; AUS Australia; NZ New Zealand; NP North Pacific.

	Sex	*n*	Mean	S.D.	Range	H (*P*)
Upper bill length (cm)	Female	18	0.470	0.07	0.46-0.54	6.327 (0.012)
	Male	14	0.508	0.02	0.47-0.54	
Minimum bill depth (cm)[a]	Female	20	0.156	0.01	0.11-0.20	1.609 (NS)
	Male	14	0.155	0.02	0.10-0.17	
Bill depth (cm)[b]	Female	20	0.202	0.01	0.16-0.22	7.366 (0.011)
	Male	14	0.215	0.01	0.19-0.25	
Head width (cm)	Female	20	0.416	0.01	0.40-0.43	2.009 (NS)
	Male	14	0.421	0.01	0.40-0.44	
Nape (cm)	Female	20	0.672	0.01	0.66-0.69	1.313 (NS)
	Male	14	0.662	0.02	0.61-0.70	
Wing length (cm)	Female	21	70.557	4.73	65.2-89	2.056 (NS)
	Male	14	68.371	3.04	63-73	
Tarsus lenght (cm)	Female	21	6.181	0.24	6-6.5	1.145 (NS)
	Male	14	6.271	0.25	6-6.5	
Lenght of middle toe (cm)	Female	21	9.123	0.26	8.5-9.6	1.444 (NS)
	Male	14	9.257	0.40	8.5-9.8	
Weight (g)	Female	21	1251.261	298.483	1150-1500	2.442 (NS)
	Male	14	1383.928	151.151	1125-1625	

NS not significant.
[a]Minimum depth at the mid length of the bill.
[b]Distance from the upper mandible to the lower mandible at the nostril.

Appendix A. Biometric data (mean ± SD) of adult White-chinned Petrel Procellaria aequinoctialis attending waters of the Patagonian Shelf, off Argentina. Kruskall-Wallis one-way ANOVA (*H*) and probabilities (*P*) are also given.