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CONCENTRAÇÃO DE METAIS NO SANGUE E EM PENAS DE PETRÉIS DO GÊNERO *Procellaria*

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RESUMO

No presente estudo, indivíduos de duas espécies irmãs de petréis do gênero *Procellaria* (*P. conspicillata* e *P. aequinoctialis*) foram avaliados para verificar possíveis diferenças nas concentrações de metais essenciais (Cu e Zn) e não-essenciais (Cd, Pb e Hg) em espécies filogeneticamente próximas, porém com nichos ecológicos distintos. Na invernagem, estas espécies são simpátricas em algumas áreas, com valores semelhantes de isótopos estáveis de nitrogênio ($\delta^{15}N$) e carbono ($\delta^{13}C$) no sangue, indicando que estas se encontram em um mesmo nível trófico e que possuem alimentação semelhante durante este período. No entanto, *P. aequinoctialis* apresentou maior variação individual, com valores isotópicos menores em indivíduos oriundos da região Antártica. Não houve correlações entre os valores de isótopos estáveis e a concentração de metais no sangue das duas espécies analisadas, porém, foi detectada diferença na concentração de Hg no sangue e nas penas, bem como de Cu e Zn nas penas, entre as duas espécies. Para ambas as espécies, todos os metais apresentaram menores concentrações no sangue que nas penas, exceto o Hg em *P. aequinoctialis.* Nas penas, a concentração de Hg foi dez vezes maior em *P. conspicillata* do que em *P. aequinoctialis.* E, além de distinguir as duas espécies, a concentração de Hg também foi diferente entre indivíduos juvenis e adultos de *P. aequinoctialis.* Todos esses resultados indicam que o alimento utilizado no Oceano Atlântico Sul Ocidental, onde *P. conspicillata* se distribui durante todo o ano e *P. aequinoctialis* forrageia durante o inverno, apresenta elevadas concentrações de Hg.

Palavras-chave: aves marinhas, metais, mercúrio, Procellariiformes, poluição

ABSTRACT

In the present study, specimens of two sister species from the genus *Procellaria* (spectacled petrel *P. conspicillata* and white-chinned petrel *P. aequinoctialis*) were evaluated to verify potential differences in the concentrations of essential (Cu and Zn) and non-essential (Cd, Pb and Hg) metals in phylogenetically closely-related species, but with distinct ecological niches. In wintering period, these species are sympatric in some areas, showing similar blood values of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$ stable isotopes. This finding indicates that they are in the same trophic level and are feeding on similar food resources during this period. However, white-chinned petrel showed higher individual variation, with lower isotopic values in individuals from the Antarctic region. For both species, there were no correlations between blood values of stable isotopes and metal concentrations. However, there was a significant difference between species in Hg concentration in blood and feathers, as well as in Cu and Zn concentrations in feathers. For both species, all metals analyzed showed lower concentrations in blood than in feathers, except for Hg in white-chinned petrel. In feathers, Hg concentration was 10-fold higher in spectacled petrel than in white-chinned petrel. Besides to distinguish the two species, Hg was also different between juveniles and adults white-chinned petrels. Taken altogether, these findings indicate that food consumed in the South Atlantic Ocean, where spectacled petrels occurs throughout the year and where white-chinned petrel forages in wintering period, has elevated concentrations of Hg.

Keywords: seabirds, metals, mercury, Procellariiformes, pollution

INTRODUÇÃO

A poluição marinha vem crescendo ao longo dos anos e está diretamente associada a descargas de efluentes domésticos e industriais, bem como de resíduos de atividades agrícolas em áreas estuarinas (Clark 2001). Animais que sirvam como biondicadores destes processos nestes ambientes podem ser utilizados para monitorar os avanços e as consequências da poluição.

Os metais são poluentes conservativos, pois não são degradáveis e permanecem no ambiente, enquanto a ação antrópica acaba concentrando estes metais e contaminando uma determinada área (Clark 2001). Por não serem facilmente excretados, estes elementos se acumulam nos organismos e acabam se biomagnificando ao longo da cadeia trófica, até atingirem maiores concentrações nos predadores de topo da cadeia trófica.

As aves marinhas, por estarem em sua maioria no topo da cadeia alimentar e possuírem vida longa tendem a acumular contaminantes e, deste modo, são importantes ferramentas para o monitoramento de poluentes no ambiente marinho (Furness 1993, Burger & Gochfeld 2002). Essas aves também costumam se deslocar por grandes áreas, além de possuírem padrões de migração em sua maioria já conhecidos, reproduzindo-se periodicamente nos mesmos locais (Hamer *et al.* 2001).

Os metais podem ser classificados como sendo essenciais e não essenciais. Os metais essenciais como cobre (Cu), zinco (Zn) e selênio (Se), que são necessários ao metabolismo do organismo, podem causar reações adversas quando presentes em altas concentrações. Por outro lado, os metais não-essenciais como cádmio (Cd), chumbo (Pb) e mercúrio (Hg), que não possuem funções metabólicas conhecidas, causam toxicidade ao organismo mesmo em baixas concentrações. Alguns metais essenciais têm

efeito protetor contra os metais não essenciais, como é o caso do Se que atua como protetor na contaminação por Hg (Cuvin-Aralar & Furness 1991). Esses metais formam um complexo Hg-Se que se liga a uma proteína específica do plasma gerando um complexo altamente estável (Yoneda & Suzuki 1997), o que torna o Hg inerte.

Diferentemente de contaminantes orgânicos, os metais tendem a ser acumulados também nas penas das aves, onde se ligam às proteínas durante o período de formação das mesmas (Bearhop *et al.* 2000a, Burger & Gochfeld 2002), indicando uma contaminação mais antiga, referente à última muda da plumagem. Por sua vez, o sangue apresenta uma contaminação recente associada diretamente à alimentação (Kahle & Becker 1999). A concentração de metais em penas em crescimento está diretamente relacionada com a concentração de metais no sangue, visto que as penas absorvem estes elementos no momento de sua formação, quando ainda são irrigadas por sangue. Por isso, amostras de ovos, penas e sangue, coletadas de forma não destrutiva, têm sido utilizadas para estudar a contaminação em aves sem causar maiores problemas para sua conservação, ao evitar que indivíduos sejam mortos para a coleta de tecidos internos, sendo isto particularmente importante para as espécies ameaçadas de extinção (Goede & Bruin 1986, Thompson *et al.* 1998, Burger & Gochfeld 2002).

A contaminação por metais pode ocorrer por várias vias, como, por exemplo, respiração, contato com a pele e mais comumente através da alimentação (Burger & Gochfeld 2002). Neste contexto, isótopos estáveis têm sido utilizados há algumas décadas para estudos de alimentação e avaliação de cadeias tróficas envolvendo as aves (Thompson *et al.* 2005, Hobson 2011). Os isótopos de nitrogênio ($\delta^{15}N$) são utilizados para indicar variação nos níveis tróficos, enquanto os de carbono ($\delta^{13}C$) podem ser relacionados às fontes de alimentação, separando no ambiente marinho as regiões

costeiras e pelágicas, pelágicas e demersais, e as altas e baixas latitudes (Quillfeldt *et al.* 2005, Cherel & Hobson 2007). Além disso, os isótopos estáveis vêm sendo empregados em estudos de ecotoxicologia, visando identificar a origem da contaminação (Atwell *et al.* 1998, Bearhop *et al.* 2000b, Blais *et al.* 2005, Anderson *et al.* 2009), além de comparar espécies de diferentes níveis tróficos e sua correlação com poluentes, como o Hg (Atwell *et al.* 1998, Anderson *et al.* 2009). Neste sentido, é importante salientar que Stewart *et al.* (1999) observaram que espécies filogeneticamente próximas possuem níveis semelhantes de contaminantes. Porém, outros estudos têm demonstrado que o local e o tipo de alimentação podem determinar diferentes níveis de contaminação entre espécies próximas (Gochfeld *et al.* 1999).

Considerando que vários estudos com Procellariiformes (albatrozes e petréis) mostram que essas aves tendem a ter altas concentrações de metais quando comparadas com aves costeiras e terrestres (Lock *et al.* 1992, Thompson *et al.* 1993, Bearhop *et al.* 2000a, b, Anderson *et al.* 2009, 2010), os resultados de estudos combinando o uso de isótopos estáveis associado à análise dos níveis de contaminação por metais neste grupo de aves parecem ser de fundamental importância para um melhor entendimento dos processos de acumulação e contaminação de metais nestes organismos.

A pardela-preta (*Procellaria aequinoctialis*) distribui-se na região Sub-Antártica, reproduzindo-se em várias ilhas, dentre as quais a Geórgia do Sul possui o maior número de pares reprodutivos (Ryan *et al.* 2006, ACAP 2009a, BirdLife International, 2011a). No período reprodutivo, esta ave possui uma alimentação baseada no krill, *Euphausia superba* (Berrow & Croxall 1999), mas também pode se alimentar de peixes, cefalópodes e outros crustáceos. Por sua vez a pardela-de-óculos (*P. conspicillata*), considerada anteriormente como uma subespécie de *P. aequinoctialis*,

foi reconhecida como espécie em 1998 (Ryan 1998, Techow *et al.* 2009). Esta ave é endêmica da Ilha Inacessível (Tristão da Cunha) e possui uma alimentação semelhante àquela de *P. aequinoctialis*, ou seja, à base de peixes, cefalópodes e crustáceos (ACAP 2009b, BirdLife International 2011b).

Apesar de serem filogeneticamente próximas, *P. aequinoctialis* e *P. conspicillata* possuem nichos ecológicos distintos. *Procellaria conspicillata* tem preferência por águas mais profundas e quentes, e não apresenta diferença de atividade entre dia e noite (Bugoni *et al*. 2009), enquanto *P. aequinoctialis* tem tendência a ocorrer sobre águas mais frias, sobre a plataforma continental e possui maior atividade no período noturno (Weimerskirch *et al.* 1999, Phillips *et al.* 2006).

Estas duas espécies de *Procellaria* são abundantes na costa do Rio Grande do Sul e possuem comportamento de aves seguidoras de embarcações pesqueiras, interagindo com o espinhel, arte de pesca na qual muitas vezes são acidentalmente capturadas (Olmos 1997, Neves *et al.* 2006, Bugoni *et al.* 2008a, Jiménez *et al*. 2011). Cabe ressaltar que estas duas espécies de *Procellaria* estão classificadas como vulneráveis a extinção (IUCN 2011). Apesar das mortalidades associadas à pesca, a população de *P. conspicillata* está aumentando (7% ao ano), porém esta se encontra classificada como espécie vulnerável desde 2008, devido ao seu endemismo (Ryan & Ronconi 2011).

Estudos realizados com *P. aequinoctialis* e *P. conspicillata* demonstraram o intenso uso de descarte provenientes da pesca de espinhel pelágico na alimentação de ambas as espécies durante o período não reprodutivo no sul do Brasil (Bugoni *et al.* 2010). Arcos *et al.* (2002) demonstraram que o consumo de descarte de pesca demersal aumenta a concentração de Hg nas aves. Outras ameaças às espécies são a poluição

através da ingestão de plásticos (Colabuono *et al.* 2009) e as altas capturas incidentais, principalmente na pesca de espinhel (Laich & Favero 2007, Bugoni *et al.* 2008a, Jiménez *et al.* 2011), mas também em outras pescarias (Bugoni *et al.* 2008c). No entanto, os efeitos de outros fatores durante o período não reprodutivo, como a contaminação que elas absorvem nessa fase, são ainda desconhecidos.

Neste contexto, o objetivo do presente estudo foi avaliar e comparar a concentração de metais nas duas espécies do gênero *Procellaria* com áreas de reprodução e distribuição distintas, avaliando dois tipos de amostras não destrutivas (sangue e pena). Cabe ressaltar que, até o presente momento, não há dados disponíveis sobre os níveis de poluentes em *P. conspicillata*.

MATERIAL E MÉTODOS

Coleta das amostras

As aves foram capturadas no Oceano Atlântico Sudoeste ao largo da costa brasileira, a bordo de navios de pesca de espinhel pelágico e linha de mão (Bugoni *et al.* 2008c) (Anexo: Fig. 1). As espécies foram atraídas com o uso de vísceras de peixes e capturadas com o auxílio de tarrafa (Bugoni *et al*. 2008b), no período de fevereiro a junho de 2006 e em agosto e setembro de 2007.

Foram capturados 38 indivíduos de *P. conspicillata* e 30 de *P. aequinoctialis.* Amostras de sangue (~1 ml) foram coletadas com seringa e agulha por punção da veia do tarso e armazenadas em frascos plásticos com álcool absoluto (Merck®). Também foram coletadas cinco a seis penas de contorno de diferentes regiões do corpo, as quais foram armazenadas a seco em sacos plásticos etiquetados. Quando encontradas foram também coletadas penas de contorno em crescimento.

Análise de isótopos estáveis

Os valores de isótopos estáveis de nitrogênio ($\delta^{15}N$) e carbono ($\delta^{13}C$) foram obtidos a partir de amostras do sangue analisadas através de espectrometria de massa de razão isotópica com fluxo-contínuo (CF-IRMS) no *Scottish Universities Environmental Research Centre*, Reino Unido da Grã-Bretanha, conforme descrito por Bugoni *et al.* (2010). Amostras de penas de contorno em crescimento (0,09-0,12 mg) também foram analisadas na Universidade de Georgia (Estados Unidos).

Determinação sexual e etária

O sexo dos indivíduos foi determinado molecularmente a partir de amostras de sangue com extração de DNA e técnica de PCR (*Polymerase Chain Reaction*) dos genes CHD (*Chromo-Helicase-DNA-binding)* (Bugoni *et al.* 2011). A idade estimada dos indivíduos foi determinada através dos padrões de muda das espécies (Bugoni & Furness 2009).

Análise dos metais

Cinco ou seis penas de contorno totalmente crescidas de cada indivíduo foram utilizadas para a análise da concentração de metais. Também foram analisadas três a cinco penas de contorno em crescimento. As penas foram previamente lavadas três vezes com acetona seguida de enxágue com água tipo Milli-Q® para remover possível contaminação externa (Burger *et al.* 2009). As amostras de sangue e pena foram secas em estufa a 60^{o}C por 72 h. As amostras foram então pesadas e digeridas com ácido

nítrico (HNO_3, 65%, SupraPur®, Merck®). Após completa digestão das amostras, a diluição das mesmas foi realizada com água tipo Milli-Q® (1:1).

As análises das concentrações dos metais (Cd, Pb, Cu, Zn e Hg) nas amostras foram realizadas através de espectrofotometria de absorção atômica (AAS-932 Plus, GBC) no Instituto de Ciências Biológicas da Universidade Federal do Rio Grande (ICB/FURG). No caso do Hg, as amostras foram analisadas através da técnica de espectrofotometria de absorção atômica com geração de vapor frio (CVASS) utilizando-se um gerador de hidretos (HG 3000, GBC) acoplado ao espectrofotômetro de absorção atômica (AAS-932 Plus, GBC).

Uma vez que foi observada uma segregação das espécies de acordo com os valores de concentração de Hg nas penas e sangue dos indivíduos (vide Resultados), foram também analisadas as concentrações de Hg e Se nas penas em crescimento de *P. aequinoctialis* (N = 9) e *P. conspicillata* (N = 21). As penas em crescimento (3 a 5 por indivíduo) foram submetidas ao mesmo processamento para penas descrito acima, e a análise dos metais foi realizada no Laboratório da ISATEC (Rio Grande, RS, Brasil).

Em todos os casos, as concentrações dos metais foram expressas em $\mu g.g^{-1}$ de peso seco do tecido.

Análise estatística

Os dados foram expressos como média ± desvio padrão da média. Para a comparação das concentrações médias da concentração dos metais no sangue e penas entre as duas espécies de petréis foi utilizado o teste *t*. Quando os dados não atenderam aos pré-requisitos dos testes paramétricos (normalidade e homocedasticidade), os mesmos foram matematicamente transformados (log) (Zar 1984, Sokal & Rohlf 1995).

Os valores das concentrações de Hg e também de isótopos estáveis das duas espécies de petréis não apresentaram normalidade e homogeneidade de variâncias, mesmo após diversas transformações matemáticas. Portanto, nestes casos, foi utilizado o teste não paramétrico Mann-Whitney para comparar as concentrações de Hg e valores isotópicos entre as espécies.

A correlação entre as concentrações dos metais foi testada usando o índice de correlação de Spearman (não paramétrico) para o Hg e o índice de correlação de Pearson (paramétrico) para os demais metais. Em ambos os casos, foram utilizados os dados das concentrações após transformação matemática (log).

Para todas as análises estatísticas, os valores foram considerados significativamente diferentes quando $p<0,05$.

RESULTADOS

As duas espécies analisadas não diferiram entre si quanto ao valor de $\delta^{15}N$ e $\delta^{13}C$ no sangue, mas diferiram quanto aos valores observados nas penas em crescimento. Os valores isotópicos nas penas foram maiores quando comparados aos valores no sangue do mesmo indivíduo (Anexo: Fig. 2), sendo que *Procellaria aequinoctialis* apresentou maior variação individual que *P. conspicillata.*

Para todos os metais analisados, as concentrações sanguíneas foram menores que as das penas (Anexo: Tabelas 1 e 2; Fig. 3), exceto para Hg em *P. aequinoctialis* (Anexo: Fig. 4). Não houve diferença na concentração sanguínea de Cd, Pb, Cu e Zn entre as duas espécies. No entanto, a concentração de Hg foi maior em *P. conspicillata*. Nas penas houve diferença nas concentrações de Cu, Zn e Hg, e as concentrações de Cu

e Zn foram maiores em *P. aequinoctialis*, enquanto a concentração de Hg foi maior em *P. conspicillata*.

Foram observadas correlações positivas e significativas entre as concentrações sanguíneas de Cd, Pb, Cu e Zn (Anexo: Tabela 3). O Hg somente apresentou uma correlação positiva e significativa com o Cd. Nas penas, somente o Cd e o Pb apresentaram correlação positiva e significativa entre si (Anexo: Tabela 4). Nas penas em crescimento, foi observada uma correlação positiva entre Se e Hg nos indivíduos de *P. conspicillata*. Já em *P. aequinoctialis* não foi detectado Se e Hg nas amostras de penas em crescimento.

Não houve correlação entre os isótopos estáveis $\delta^{15}N$ e $\delta^{13}C$ e as concentrações de nenhum dos metais analisados no sangue e penas em crescimento dos petréis.

Para as duas espécies, machos e fêmeas não apresentaram diferença significativa na concentração de nenhum dos metais analisados, em ambos os tecidos.

A partir da separação de *P. aequinoctialis* em duas classes etárias (21 juvenis e 9 adultos) através dos padrões de muda da plumagem, verificou-se que houve diferença na concentração de Hg no sangue e penas entre as duas classes. Os juvenis tiveram maior concentração de Hg no sangue e menor nível deste nas penas quando comparados aos adultos (Anexo: Fig. 5).

CONCLUSÕES

- Através das análises de isótopos estáveis no sangue pode-se concluir que *Procellaria aequinoctialis e P. conspicillata* compartilham do mesmo alimento durante o período de invernagem.

- A maior concentração de Hg nas penas de *P. conspicillata* do que em *P. aequinoctialis* indica que o alimento disponível no Oceano Atlântico Sul Ocidental, onde *P. conspicillata* se distribui durante todo o ano e *P. aequinoctialis* forrageia durante o inverno, apresenta elevadas concentrações desse metal.
- A diferença da concentração de Hg em indivíduos adultos e juvenis de *P. aequinoctialis* também indica a maior concentração deste metal no Oceano Atlântico Sul Ocidental, visto que as penas dos indivíduos juvenis cresceram quando estes ainda se encontravam na colônia, onde recebiam alimentos dos pais oriundos da região Antártica.
- As altas concentrações de metais, especialmente de Hg, em *P. conspicillata* não parecem estar afetando o sucesso reprodutivo da espécie, visto que as populações desta se encontram em crescimento.

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ANEXO

Concentração de metais no sangue e em penas de petréis do gênero *Procellaria*

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Environmental Pollution.

Metal concentrations in blood and feathers of petrels from the genus *Procellaria*

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Capsule: The highest Hg concentration in feathers of spectacled petrels is probably associated with its occurrence throughout the year over contaminated SW Atlantic waters.

Abstract

Metal (Cu, Zn, Cd, Pb and Hg) concentrations were determined in blood and feathers of spectacled (*Procellaria conspicillata*) and white-chinned (*P. aequinoctialis*) petrels, species phylogenetically close with distinct ecological niches. In wintering, they showed similar values of whole-blood stable isotopes ($\delta^{15}N$ and $\delta^{13}C$), indicating that they were feeding on similar preys. However, individual variation was higher in white-chinned petrels with lower values for specimens recently arrived from sub-Antarctic breeding grounds. There were no correlations between blood metal and stable isotopes values. Metal concentrations were lower in blood than in feathers, except for Hg in white-chinned petrels. Blood Hg and feather Hg, Cu and Zn concentrations were higher in spectacled petrels. Hg concentration was higher in juvenile than in adult white-chinned petrels. Findings indicate that food consumed in the South Atlantic Ocean, where spectacled petrels are present over the year and white-chinned petrels feed during wintering period, has elevated Hg concentrations.

Keywords: seabirds, metals, mercury, Procellariiformes, pollution

Introduction

Seabirds are generally top predators and have a long lifespan and extended breeding cycles, thus showing tendency to accumulate pollutants. Therefore, they are being employed as biological tools to monitor the occurrence and impact of marine pollutants (Burger and Gochfeld, 2002; Furness, 1993).

Egg, feathers and blood samples are being collected to evaluate the degree of contamination by chemical substances in birds. These biological materials are usually selected to avoid any impact on the species, thus avoiding the need of killing the animal to collect internal tissues. This is specially important when considering threatened species (Burger and Gochfeld, 2002; Goede and Bruin, 1986; Thompson et al., 1998).

Differently from the organic pollutants, metals tend to be accumulated also in bird feathers, where they bind to proteins during the feather formation process (Bearhop et al., 2000a; Burger and Gochfeld, 2002). Therefore, metal accumulation in feathers generally represents a long-term contamination process, while metal blood accumulation represents a recent contamination directly associated with feeding (Kahle and Becker, 1999).

Metals can be considered as essential when necessary to the organism metabolism, such as Cu, Zn and Se. Despite their essentiality, these metals can cause toxicity when present at elevated environmental concentrations. On the other hand, metals like Cd, Pb and Hg are not involved in metabolic functions, thus being considered as non-essentials. These metals can cause toxicity even when at low concentrations in the environment. It is important to note that the essential metal Se can have a protective role against Hg toxicity (Cuvin-Aralar and Furness, 1991). These

67 metals complex each other forming a Hg-Se complex, which binds to an specific plasma
68 protein generating a highly stable complex (Yoneda and Suzuki, 1997) and
69 consequently turning the Hg non reactive.
70 Contamination with metals can occur through different pathways like
71 respiration, skin contact and more often via food ingestion (Burger and Gochfeld,
72 2002). In this context, stable isotopes have been employed for decades in feeding
73 studies and evaluation of food webs involving birds (Hobson, 2011; Thompson et al.,
74 2005). Isotopes of nitrogen ($\delta^{15}N$) are employed to indicate changes in trophic levels,
75 while those of carbon ($\delta^{13}C$) can be related to food sources. In the marine environment,
76 the latter can be used to discriminate coastal and pelagic areas, high and low latitude
77 regions, and pelagic and demersal areas (Cherel and Hobson, 2007; Quillfeldt et al.,
78 2005). Therefore, stable isotopes have been employed in ecotoxicological studies to
79 identify contamination sources (Anderson et al., 2009; Atwell et al., 1998; Bearhop et
80 al., 2000b; Blais et al., 2005). They can also be useful to compare species from different
81 levels in the food web, as well as their correlations with pollutants, such as Hg
82 (Anderson et al., 2009; Atwell et al., 1998).
83 Since several studies with Procellariiformes (albatrosses and petrels) show that
84 these birds tend to have high concentrations of metals when compared to coastal and
85 terrestrial bird species (Anderson et al., 2009, 2010; Bearhop et al., 2000ab; Lock et al.,
86 1992; Thompson et al., 1993), results from studies combining the analysis of stable
87 isotopes and tissue metal levels are of great importance for a better understanding of the
88 processes involved in accumulation and toxicity of metals in seabirds.
89 The white-chinned petrel *Procellaria aequinoctialis* and the spectacled petrel
90 *Procellaria conspicillata* are phylogenetically closely-related, but have distinct

ecological niches. White-chinned petrel is distributed in the Sub-Antarctic region (BirdLife International, 2011a; Ryan et al., 2006), have the tendency to occur on colder waters over the continental shelf, and is more active during the nighttime (Phillips et al., 2006; Weimerskirch et al., 1999). On the other hand, spectacled petrel is endemic from the Inaccessible Island (Tristão da Cunha group) (BirdLife International, 2011b), has preference for deeper and hotter waters, and do not show differences in diurnal and nocturnal activities (Bugoni et al., 2009).

These two 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 fishing, being often accidentally captured by this fishing gear (Bugoni et al., 2008a; Jiménez et al., 2011; Neves et al., 2006; Olmos, 1997). Despite the fishing-related mortality, the spectacled petrel population is increasing at an annual rate of 7% (Ryan and Ronconi, 2011). However, it is listed as 'vulnerable' to the extinction due to its endemism, same category of white-chinned petrel, listed as such due to population declines (IUCN, 2011).

Studies performed with white-chinned petrel and spectacled petrel showed that these petrel species feed intensively on discards from the pelagic longline fishing during the non-breeding period in Southern Brazil (Bugoni et al., 2010). In the Mediterranean sea Arcos et al. (2002) showed that consumption of demersal fishing discards increases the Hg burden in seabirds. Other threats to seabirds health are the pollution associated with plastic ingestion (Colabuono et al., 2009) and the elevated rates of incidental captures, especially by the longline fishing (Bugoni et al., 2008a; Jiménez et al., 2011; Laich and Favero, 2007), but also in other hook-and-line fisheries (Bugoni et al.,

2008b). However, the impacts of other factors like the chemical contamination absorbed during the non-reproductive phase are still unknown.

In light of the above, the aim of the present study was to evaluate and compare the tissue metal concentrations in two congener species of *Procellaria*, showing distinct areas of distribution at sea and breeding grounds, but which are sympatric during the wintering period. Non-destructive samples of two tissues (blood and feathers) were used. Currently, no data on metal tissue burden is actually available for spectacled petrels.

Material and methods

Sampling collection

Seabirds were captured at sea onboard of pelagic longline and handline fishing vessels. Firstly, they were attracted close to the vessel using fish and shark viscera. Afterwards they were captured using castnets (Bugoni et al., 2008c). Captures were performed in the Southwestern Atlantic Ocean along the Brazilian coast, from February to June 2006 and from August to September 2007 (Fig. 1).

Thirty eight and thirty specimens of spectacled and white-chinned petrels were captured, respectively. Blood samples (~1 ml) were collected by puncture of the tarsus vein, using disposable syringe and needle, and stored in absolute ethanol (Merck®). Contour feathers from different body areas were also collected and stored dry in plastic bags. Growing feathers were also collected when specimens were molting.

Stable isotopes analysis

Values of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) were obtained from blood analysis. Blood samples were lyophilized, grinded and homogenized. Approximately 0.7 mg (0.65-0.75 mg) of each sample was inserted in tin capsules for posterior analysis by continuous-flow isotope ratio mass spectrometry (CF-IRMS) at the Scottish Universities Environmental Research Centre (UK), as described by Bugoni et al. (2010). Growing feathers samples (0.9-1.1 mg) were washed five times with distilled water, dried in oven at 70ºC for 3 h, cut in small pieces with scissors, inserted in tin capsules and analyzed at the University of Georgia (USA). Since sample analyzed in different laboratories could not be directly comparable (Mill et al., 2008), $\delta^{15}N$ and $\delta^{13}C$ values of growing feathers (N = 10) of the yellow-nosed albatross *Thalassarche chlororhynchos* were previously analyzed in the two laboratories. A paired-*t* test showed no significant difference between the results obtained ($\delta^{15}N$: $t = 1.64$, $P = 0.14$; $\delta^{13}C$: $t = 1.41$, $P = 0.19$), indicating that values obtained in the two laboratories would be thus pooled together.

Sex and age determination

Sex of each individual was determined from blood samples after DNA extraction and PCR (Polymerase Chain Reaction) analysis of the CHD (Chromo-Helicase-DNA-binding) genes (Bugoni et al., 2011). Age of seabirds analyzed was determined through the molting pattern of the species studied (Bugoni and Furness, 2009).

Metal concentration analysis

161 Five or six contour feathers completely formed from each individual were
162 employed for metal concentration analysis. They were previously washed three times
163 with acetone and then rinsed with Milli-Q® water to remove any possible external
164 contamination (Burger et al., 2009). Blood samples and feathers were dried at 60°C for
165 72 h, weighed, and completely digested with concentrated nitric acid (65% HNO_3,
166 SupraPur®, Merck). After complete digestion, samples were diluted (1:1) with Milli-Q®
167 water.
168 Metal (Cd, Pb, Cu, Zn and Hg) concentrations in blood and feathers were
169 determined using an atomic absorption spectrophotometer (AAS-932 Plus, GBC). For
170 Hg, samples were analyzed by the cold vapor technique (CVASS) using a hydride
171 generator (HG 3000, GBC) coupled to the atomic absorption spectrophotometer (AAS-
172 932 Plus, GBC). In this case, 1 ml of sample was previously diluted with 25 ml of Milli-
173 Q® water. Quality assurance controls were also performed. Measurements accuracy and
174 standard curves were built employing standard Cd, Cu, Pb, and Zn solutions (Standard
175 Reference Material® 3114) from the National Institute of Standards & Technology
176 (Gaithersburg, MD, USA). Percentages of metal recovery based on standard reference
177 material (European Reference Material ERM®-CE278, Geel, Belgium) prepared as
178 described for tissue samples were 98.9, 94.2, 103.8 and 102.9% for Cd, Cu, Pb and Zn,
179 respectively. Reference material for Hg was unfortunately not available. Tissue metal
180 concentration was expressed as $\mu g.g^{-1}$ dry weight.
181 Since tissue Hg concentration was clearly different in the two petrel species (see
182 Results section), only Hg and Se concentrations were further determined and correlated
183 in blood and growing feathers of white-chinned petrel (N = 9) and spectacled petrel (N
184 = 21). Three to five growing feathers from each individual were processed as described

above. Hg was determined by CVAAS technique as described above, while Se was analyzed by AAS as described for Cu, Zn, Pb and Cd. Hg and Se tissue concentration were also expressed as $\mu g.g^{-1}$ dry weight.

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 parametrical tests (data normality and homogeneity of variances) were not met. The non-parametric test of Mann-Whitney was used to analyze the Hg data since a lack of normality and/or homogeneity of variances was observed even after the application of different mathematical transformations (Sokal and Rohlf, 1995; Zar, 1984). 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 species were compared using the paired-*t* test.

Correlations between tissue metal concentrations were tested using the Spearman correlation index for Hg (non-parametric data) and the Pearson correlation index for other metals analyzed (parametric data). In both cases, data were analyzed after the adequate mathematical (*log*) transformation.

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

Results

209

210 *Stable isotopes*

211 The two petrel species showed similar mean blood values of $\delta^{15}N$ (U = 482.5; Z
212 = -1.08; P = 0.28; white-chinned: 15.06 ± 1.92; spectacled: 14.41 ± 0.76) and $\delta^{13}C$ (U =
213 551.0; Z = 0.23; P = 0.81; white-chinned*:* -17.97 ± 1.80; spectacled*:* -17.23 ± 0.46)
214 (Fig. 2). However, white-chinned petrel showed higher values of both stable isotopes in
215 the growing feathers than spectacled petrels: $\delta^{15}N$ (U = 21; Z = 3.33; P <0.01; white-
216 chinned: 17.38 ± 2.67; spectacled: 15.32 ± 0.55); $\delta^{13}C$ (U = 31; Z = 2.87; P < 0.01;
217 white-chinned: -16.16 ± 2.52; spectacled: -16.45 ± 0.61).

218 The $\delta^{15}N$ value for the same specimen was higher in growing feathers than in
219 blood (*t* = 3.36; P < 0.01; blood: 15.27 ± 1.54; growing feathers: 15.94 ± 1.77; N = 30).
220 The same was observed for the $\delta^{13}C$ values (*t* = 5.34; P < 0.001; blood: -16.98 ± 1.19;
221 growing feathers: -16.36 ± 1.42; N = 30).

222

223 *Tissue metal concentrations*

224 For all metals, concentrations were lower in blood than in feathers (Tables 1 and
225 2; Fig. 3), except for Hg in white-chinned petrel (Fig. 4). There was no difference in the
226 blood concentration of Cd, Pb, Cu and Zn between the two species. However, Hg
227 concentration was higher in spectacled petrel (U = 387; Z = -2.26; P = 0.02). In feathers,
228 Cu (*t* = 2.15; P = 0.04) and Zn (*t* = 2.20; P = 0.03) concentrations were higher in white-
229 chinned petrel, while Hg concentration was higher in spectacled petrel (U = 26; Z = -
230 6.72; P < 0.001).

231 In the blood, significant positive correlations (P < 0.01) were found between the
232 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 correlation 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 correlation ($R = 0.62$; $P = 0.01$) between Se (4.60 ± 4.08 $\mu g.g^{-1}$) and Hg (0.69 ± 0.33 $\mu g.g^{-1}$) concentrations. For white-chinned petrels, Se and Hg concentrations were below the detection limit of the technique employed.

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

For the two petrel species, males and females did not show any significant difference in the metal concentrations in both blood and feathers ($P > 0.05$). When white-chinned adults ($N = 9$) and juveniles ($N = 21$) were compared, juveniles showed a higher Hg concentration in blood (juveniles: 4.27 ± 3.94 $\mu g.g^{-1}$; adults: 0.70 ± 0.36 $\mu g.g^{-1}$, $U = 4$; $Z = 4.10$; $P < 0.01$) and a lower Hg concentration in feathers (juveniles: 1.14 ± 2.00 $\mu g.g^{-1}$; adults: 3.45 ± 2.84 $\mu g.g^{-1}$ $U = 24$; $Z = -3.19$; $P < 0.01$) (Fig. 5).

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 and have similar feeding conditions during the wintering period. However, the higher individual variation, measured as standard deviation of the mean values, observed in white-chinned petrel can be explained considering the presence of samples

257 collected from specimens recently arrived from high latitudes, which were returning
258 from the reproductive period, or from first-year juveniles, when they generally feed on
259 krill (Bugoni et al., 2010). The higher values of $\delta^{15}N$ and $\delta^{13}C$ found in growing feathers
260 when compared to those in the blood can be explained considering a similar turnover
261 and a differential isotopic fractioning between food-blood and food-feathers, as already
262 demonstrated for other seabird species (Quillfeldt et al., 2008).
263 The values of $\delta^{15}N$ (15.06 ± 1.92) and $\delta^{13}C$ (-17.97 ± 1.80) found in the blood of
264 white-chinned petrel in the present study were higher than those reported by Anderson
265 et al. (2009) for specimens in the breeding period ($\delta^{15}N$ = 14.22 ± 0.66; $\delta^{13}C$ = -18.13 ±
266 0.33). This finding indicates a substantial change in feeding of white-chinned petrels
267 during the wintering period, when it feeds on preys of higher trophic levels at lower
268 latitudes, as indicated by the levels of $\delta^{15}N$ and $\delta^{13}C$, respectively.
269
270 *Tissue metal concentrations*
271 It was observed a marked individual variation in the metal concentrations in both
272 tissues (blood and feathers) of the two petrel species analyzed. This fact is likely
273 associated with an individual feeding specialization or with specific physiological
274 processes of metal detoxification in seabirds.
275 Blood metal concentrations were not different between the two petrel species,
276 except for Hg, which concentration was higher in spectacled than white-chinned petrel.
277 These findings can be associated with the overlapping in the foraging area of the two
278 species in the non-reproductive period, which is consistent with at sea census performed
279 onboard fishing boats (Bugoni et al., 2008a; Jiménez et al., 2011; Neves et al., 2006).
280 Another possible explanation is that birds were sampled in areas where the two petrel

281 species occurred simultaneously. Furthermore, it must be considered that blood is a
282 tissue involved in the nutrient distribution to the different body regions. Therefore, it
283 can also play an important role in the inter-organ distribution of metals. In this case, the
284 fast distribution of metals among body tissues makes the concentrations of metals to be
285 more stable in blood than in those tissues mainly involved in accumulation (liver),
286 metabolism (liver) and excretion (kidneys and digestive tract) of trace metals.
287 Blood Cd concentration was higher in specimens of white-chinned petrels from
288 the reproductive areas, which were identified by the lower $\delta^{15}N$ and $\delta^{13}C$ values
289 measured. Higher metal concentrations in blood were in fact expected in these
290 specimens, since the Antarctic/sub-Antarctic ecosystems generally show high levels of
291 Cd associated with natural sources (Bargagli et al., 1996; Honda et al., 1987), especially
292 krill (*Euphausia superba*) and other crustaceans (Petri and Zauke, 1993; Rainbow,
293 1989; Yamamoto et al., 1987). However, this finding can also be explained by the high
294 levels of Cd found in cephalopods inhabiting the Southern Brazilian waters, especially
295 the squid *Illex argentinus* (Dorneles et al., 2007; Gerpe et al., 2000), which is common
296 bait for the pelagic longline fishery and frequently consumed by both *spectacled* and
297 white-chinned petrels (Colabuono and Vooren, 2007). In fact, seabirds feeding on
298 cephalopods and crustaceans (krill) generally show higher Cd levels than those preying
299 more on fish, which in turn present higher Hg concentration (Kim et al., 1998).
300 In feathers, Cu and Zn concentrations were higher in white-chinned petrel than
301 spectacled petrel. This result could be explained as white-chinned petrel occurs mostly
302 over inshore waters than the more pelagic spectacled petrel (Phillips et al., 2006;
303 Bugoni et al., 2009). Feeding on the continental shelf, white-chinned petrels would be
304 exposed to higher discharges of contaminants and able to feed on demersal organisms,

305 which generally accumulate higher concentrations of metals (Arcos et al., 2002).
306 Furthermore, it is important to note that Cu and Zn concentrations in the Antarctic krill
307 are not elevated in spite of the occurrence of marked annual variations (Yamamoto et
308 al., 1987).
309 Cu and Zn concentrations found in feathers of white-chinned petrel in the
310 present study are quite similar to those previously reported by Anderson et al. (2009) for
311 specimens collected on breeding grounds (13.11 ± 17.79 and 77.65 ± 17.98 $\mu g.g^{-1}$ dry
312 weight, respectively). However, values found for Pb (not detected) and Cd (0.14 ± 0.13
313 $\mu g.g^{-1}$ dry weight) by Anderson et al. (2009) were lower than those observed in the
314 present study, suggesting the presence of local sources of pollution. This statement is
315 based on the fact that specimens sampled by Anderson et al. (2009) do not necessarily
316 forage over Southern Brazilian waters during the wintering period. During the
317 incubation period, white-chinned petrels breeding at South Georgia generally forage on
318 the Patagonian continental shelf until chicks are hatched, when they shift their foraging
319 area to regions closer to the colony (Berrow et al., 2000; Phillips et al., 2006).
320 Concentrations of Pb and Hg found in the blood of petrels analyzed in the
321 present study are similar to those reported in the same tissue of the Northern
322 *Macronectes halli* and southern *M. giganteus* giant petrels (González-Solís et al. 2002).
323 However, blood Cd concentrations were lower in seabirds from the present study. Cu
324 and Zn concentrations measured in feathers of petrels from the present study were
325 similar to those reported for feathers of petrels and albatrosses from the Antarctic
326 region (10.4 and 71.7 $\mu g.g^{-1}$ dry weight, respectively). However, Cd and Pb
327 concentrations were higher than those reported for the Antarctic petrels and albatrosses
328 (0.07 and 0.42 $\mu g.g^{-1}$ dry weight, respectively) (Kim et al. 1998).

329 Regarding Hg, its concentration was 10-fold higher in feathers of *spectacled*
330 *petrels* than in those of giant petrels. Higher Hg concentration was also observed in
331 blood of spectacled petrels, but the difference between species was not greatly marked.
332 These findings seems to reflect the high Hg concentration found in waters off the
333 Southern Brazilian coast, as demonstrated by some studies with sediments (Marins et
334 al., 2004), swordfish *Xiphias gladius*, blue shark *Prionace glauca* and hammerhead
335 sharks *Sphyrna* spp. captured by the longline fishery in Southeast and Southern Brazil
336 (Dias et al., 2008; Mársico et al., 2007). These high values clearly contrast with the low
337 Hg concentrations found in the Antarctic region (Honda et al., 1987; Yamamoto et al.,
338 1987), where white-chinned petrels breed and feed during the reproductive period. In
339 turn, spectacled petrel is found at lower latitudes (Inaccessible Island, Tristan da Cunha
340 and subtropical/temperate waters), even during the reproductive period, when it also
341 interacts with fishing vessels and feeds on Southern Brazilian waters (Bugoni et al.,
342 2008b). Furthermore, Anderson et al. (2010) and Becker et al. (2002) analyzed the Hg
343 concentration in white-chinned petrels and found higher levels of this metal in feathers
344 of individuals collected during the breeding period. However, it is worth to note that
345 these feathers grew up during the non-reproductive period (3.79 ± 1.72 and 7.43 ± 1.97
346 $\mu g.g^{-1}$ dry weight, respectively). Despite that these values are still lower than those
347 found in feathers of spectacled petrels in the present study. This fact can be explained
348 considering the wider wintering area of white-chinned petrel.
349 The trade-off between positive and negative aspects of using fishing discards as
350 an alternative food source for seabirds has been studied and debated (Bugoni et al.,
351 2010; Furness, 2003). Results from the present study, as previously shown by Arcos et
352 al. (2002), clearly indicate the negative impact caused by seabird feeding on fishing

discards, a situation that expose animals to high concentrations of metals, which normally would not be available under natural conditions. This fact can be important when considering the conservation status of petrel species evaluated in the present study and other seabird species feeding on fishing discards, especially because several are threatened species. In addition to the high contamination levels, it is also important to stress that despite fishing discards are easily accessible, they can be calorically poor. If the ideal food for hatchlings, which normally requires highly caloric food, is replaced by fishing discards, a retarded development of hatchlings and sometimes even a lower survival rate can be observed (Grémillet et al., 2008).

Correlation between metals

The levels of metals observed in blood of petrels analyzed in the present study showed positive correlations among them. Other studies on Procellariiformes also report positive correlations between essential and non-essential metals in other tissues like liver, muscle and kidney (Gonzáles-Solís et al., 2002; Kim et al., 1998; Stewart et al., 1999).

Regarding feathers, it is well known that they are an important route of Hg excretion, especially as methylmercury (Monteiro and Furness, 1995, 2001). However, it is not confirmed that they also serve as a major route for other metals. This could thus explain the almost complete lack of correlation among metals in feathers in the present study, since the only correlation observed was between Cd and Pb.

Hg concentrations in blood and feathers of white-chinned petrels were lower than those found in spectacled petrels. Furthermore, the Hg concentration was not detected in growing feathers of white-chinned petrels. Also, the concentration of Se,

377 which is generally associated with the concentration of Hg (Cuvin-Aralar and Furness,
378 1991), was not detected in growing feathers of white-chinned petrel. The association
379 between Hg and Se is basically related to the protective effect of Se against the toxic
380 effect of Hg. Together, these metals form a Hg-Se complex which binds to specific
381 plasma proteins generating a highly stable complex (Yoneda and Suzuki, 1997),
382 detoxifying the Hg. For spectacled petrels, which it was possible to determine the levels
383 of Hg and Se, a positive correlation was observed between the concentrations of these
384 two metals in growing feathers. Kim et al. (1996) and Scheuhammer et al. (2001) also
385 reported a positive correlation between Hg and Se in liver of albatrosses and petrels
386 (Procellariiformes), and eggs of the common loon *Gavia immer*, respectively.
387 According to Becker et al. (2002), the trophic level of Antarctic birds is the
388 major factor that explains the observed Hg concentrations in these animals, since a
389 negative correlation between the Hg concentration and the proportion of krill in the bird
390 diet was seen. Unfortunately, stable isotopes data were not evaluated to determine the
391 trophic level in that study. In the present study, no correlation was observed between
392 tissue metal concentrations and the stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) levels in the present
393 study. In addition, Anderson et al. (2009) did not find any correlation between the levels
394 of nitrogen and carbon isotopes and the concentration of some metals, except Hg, in
395 several species of Procellariiformes breeding in the Sub-Antarctic region.
396
397 *Age and sex*
398 In the present study, no difference in the concentration of most metals (Cd, Pb,
399 Cu and Zn) analyzed were observed between juveniles and adults. However, juveniles
400 of white-chinned petrels showed higher blood Hg concentration than adults. Hindell et

401 al. (1999) also reported a higher Hg concentration in adults than in juveniles in three
402 species of albatrosses. This finding indicates that Hg is more accumulated in petrel
403 species that did not performed the first plumage molting, and consequently had not the
404 chance to eliminate the metal from the body through this route of excretion. The fact
405 that lower Hg concentrations were found in feathers of white-chinned petrel also
406 support the idea that low levels of the metal is present in the Antarctic environment
407 (Honda et al., 1987; Yamamoto et al., 1987). This is based on the fact that feathers from
408 juveniles had grown during their development when they were still on the nest. On the
409 other hand, adult white-chinned petrels showed higher Hg concentration in feathers
410 completely formed than in the blood, likely because they have already had the chance to
411 excrete this metal through the previous molting processes.
412 Some species of large albatrosses and petrels showing marked sexual
413 dimorphism present sex segregation in wintering areas (Phillips et al., 2011). In this
414 case, they could show differences in metal concentrations related to sex, since males
415 and females are foraging in distinct sites. For white-chinned and spectacled petrels
416 analyzed in the present study, sex was not a factor determining the tissue metal
417 concentration. Stewart et al. (1999) and Becker et al. (2002) also did not observe
418 significant difference in metal concentrations between males and females in
419 Procellariiformes. On the other hand, González-Solís et al. (2002) have reported a
420 significant difference in metal concentrations, especially Hg, between males and
421 females giant petrels. However, it is well known that these birds show a marked sexual
422 segregation in foraging areas and food sources. Furthermore, the sexual dimorphism in
423 giant petrels is well marked, while the *Procellaria* species studied in Southern Brazil
424 did not show a marked sexual size dimorphism (Bugoni and Furness, 2009). Therefore,

425 the sexual segregation of these species in the non reproductive period is not evident like
426 that observed in some other species of Procellariiformes (Phillips et al., 2009, 2011).
427 However, some studies on fishing and incidental capture of white-chinned petrels show
428 a tendency of more females being captured during the non-reproductive period (Bugoni
429 et al., 2011), while males are more captured around the colonies (Delord et al., 2005).
430 Finally, some studies suggest that eggs are a significant route of metal excretion
431 in female birds (Burger et al., 2008; Burger and Gochfeld, 1991). Since no significant
432 difference in tissue metal concentrations was observed between male and female petrels,
433 it is suggested that metal excretion via eggs was not significant for the white-chinned
434 and spectacled petrel specimens analyzed in the present study. Also, it is possible that
435 the response observed could be explained if we consider that sampling of both species
436 included juveniles and immature individuals.
437
438 **Conclusions**
439 Based on findings reported in the present study, we can conclude that spectacled
440 and white-chinned petrels show similar concentrations of most metals analyzed in blood
441 and feathers in spite of the fact that they forage on waters of different temperature and
442 depth and breed at distinct locations. However, it was possible to discriminate these two
443 petrel species based on the marked differences in the recent contamination with non-
444 essential metals, indicated by the differential blood Hg concentrations observed between
445 them. In addition, the two seabird species also showed differential long-term
446 accumulation of essential and non-essential metals, which was indicated by the different
447 concentrations of some metals found in completely grown feathers of the two studied
448 species.

449 Regarding data on levels of stable isotopes in the blood, petrels analyzed showed
450 a similar isotopic signature, indicating that they are sharing the same food items during
451 wintering, which is in turn likely related to the use of discards from pelagic longline
452 fisheries. Findings reported in the present study suggest that the use of this food source
453 is harmful to petrels, since elevated levels of metals, especially Hg, were found in the
454 blood and feathers of the specimens analyzed. However, such high levels of metals,
455 especially Hg, would not be bioavailable under natural conditions. This fact is important
456 for the conservation of the species analyzed in the present study, but also for other
457 seabird species feeding on discards from fishing activities in Southern Brazil. Despite
458 the possible negative effects associated with the elevated concentrations of metals found
459 in petrels tissues, especially in spectacled petrel specimens, this fact seems not still
460 affect the reproductive success of this species, which population has grown over the last
461 years.
462
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464
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472

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679 Table 1. Metal concentration (μg.g^{-1} dry weight) in the blood of two species of *Procellaria* petrels. Data are expressed as mean ± standard

680 deviation and median value. Values into brackets represent the minimum and maximum values found for each metal. * $P < 0.05$.

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

681

682

683 Table 2. Metal concentration ($\mu g.g^{-1}$ dry weight) in feathers of two species of *Procellaria.* Data are expressed as mean ± standard deviation

684 and median value. Values into brackets represent the minimum and maximum values found for each metal. * $P < 0.05$; ** $P < 0.01$.

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

685 Table 3. Pearson (R) and Spearman (R_s) correlation indices between metal
686 concentrations (*log* transformed values) in the blood of white-chinned petrel *Procellaria*
687 *aequinoctialis* and spectacled petrel *P. conspicillata.* * $P < 0.01$.

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

688

689

690 Table 4. Pearson (R) and Spearman (R_s) correlation indices between metal
691 concentrations (*log* transformed values) in feathers of white-chinned petrel *Procellaria*
692 *aequinoctialis* and spectacled petrel *P. conspicillata*. * $P < 0.01$.

	Cd	Pb	Cu	Zn	Hg
Pb	0.68*				
Cu	0.14	0.08			
Zn	-0.17	-0.09	0.10		
Hg (R_s)	0.04	0.03	-0.17	-0.19	

693

694

695 **Figure captions**

696

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

700

701 **Figure 2**. Mean and standard deviation of $\delta^{15}N$ and $\delta^{13}C$ in the blood (circle) and
702 growing feathers (triangle) of white-chinned petrels *Procellaria aequinoctialis* (closed)
703 and spectacled petrels *P. conspicillata* (open).

704

705 **Figure 3**. Concentration of Cd, Pb, Cu and Zn ($\mu g.g^{-1}$ dry weight) in the blood and
706 feathers of two species of *Procellaria* petrels. Data are shown as median values and the
707 corresponding 75-25% and 95-5% quartiles.

708

709 **Figure 4**. Hg concentration ($\mu g.g^{-1}$ dry weight) in the blood (N = 30 and 38), growing
710 feathers (N = 9 and 21) and feathers (N = 30 and 38) in two species of *Procellaria*
711 petrels. Data are shown as median values and the corresponding 75-25% and 95-5%
712 quartiles. ND = not detected.

713

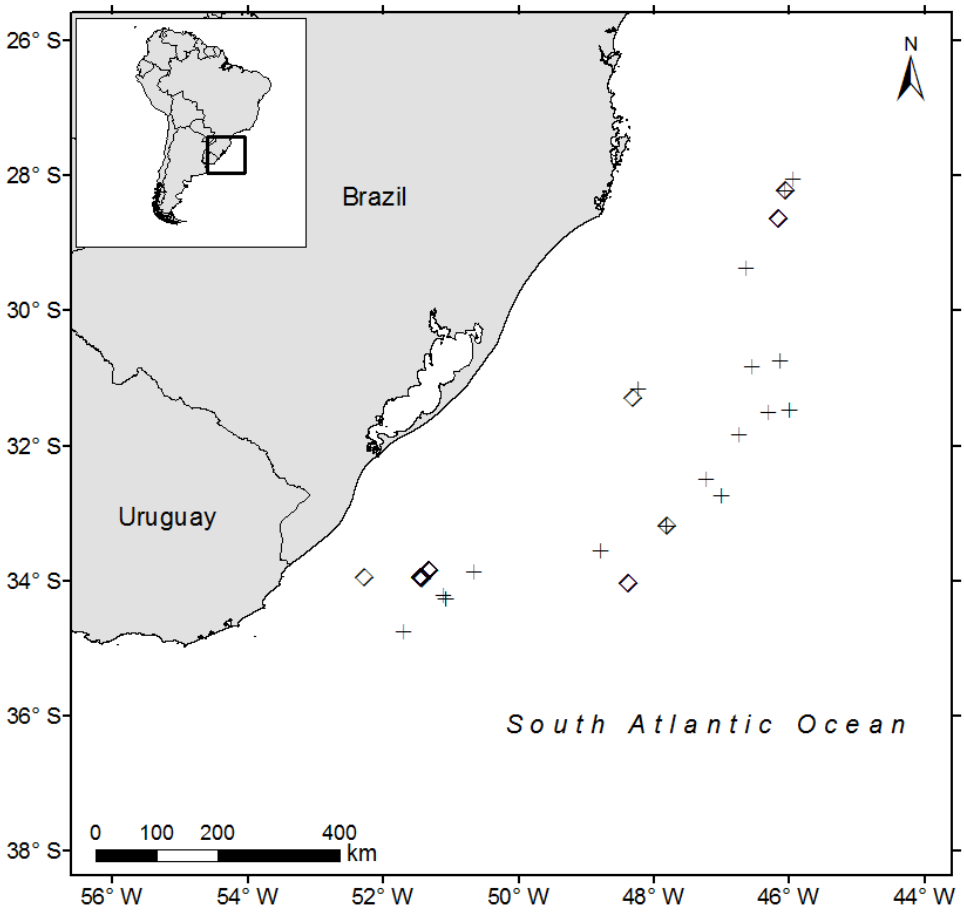
714 **Figure 5**. Hg concentration ($\mu g.g^{-1}$ dry weight) in the blood and feathers of juveniles
715 and adults of white-chinned petrels *Procellaria aequinoctialis*. Data are shown as
716 median values and the corresponding 75-25% and 95-5% quartiles.

717

718 Figure 1

719

720



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723 Figure 2

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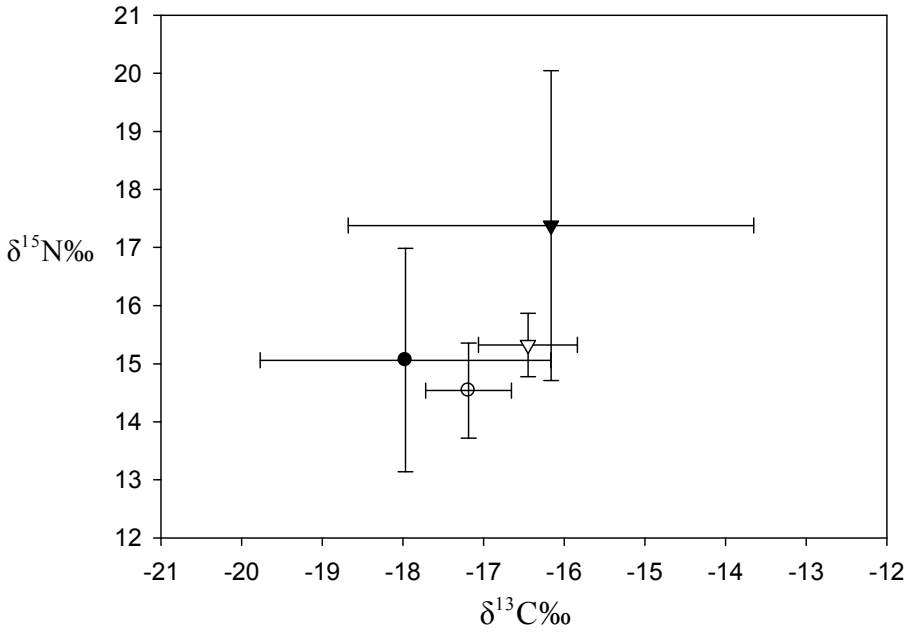

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727

728 Figure 3

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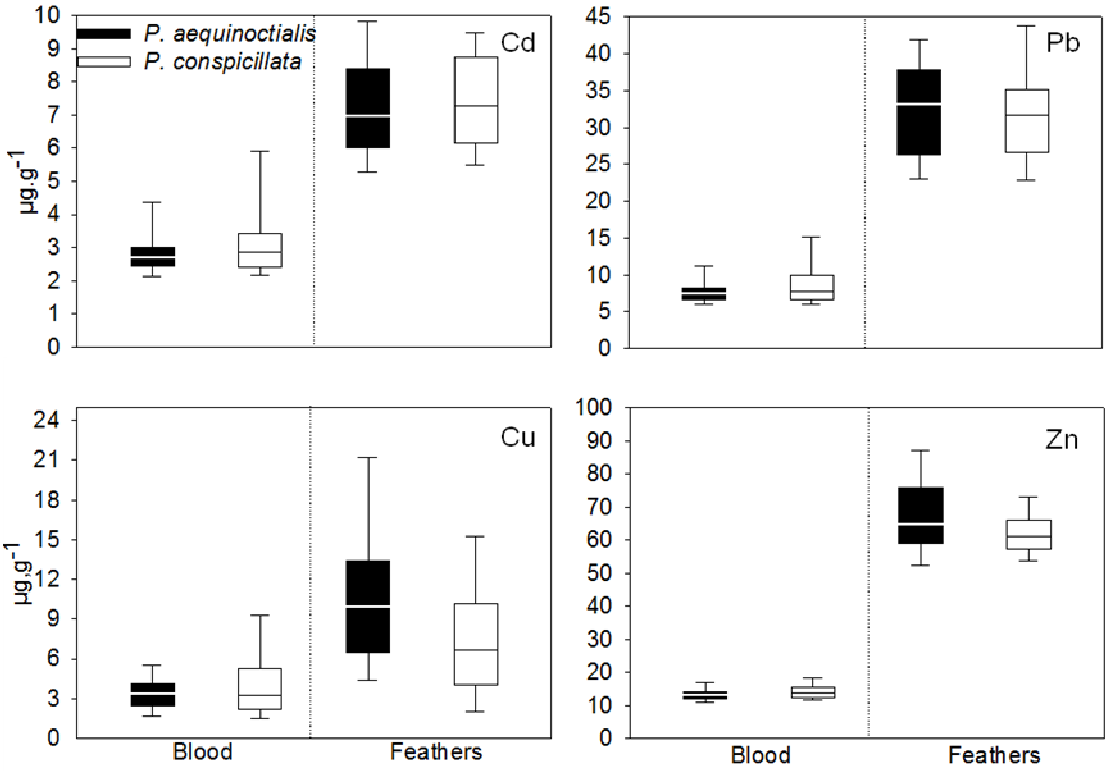

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733 Figure 4

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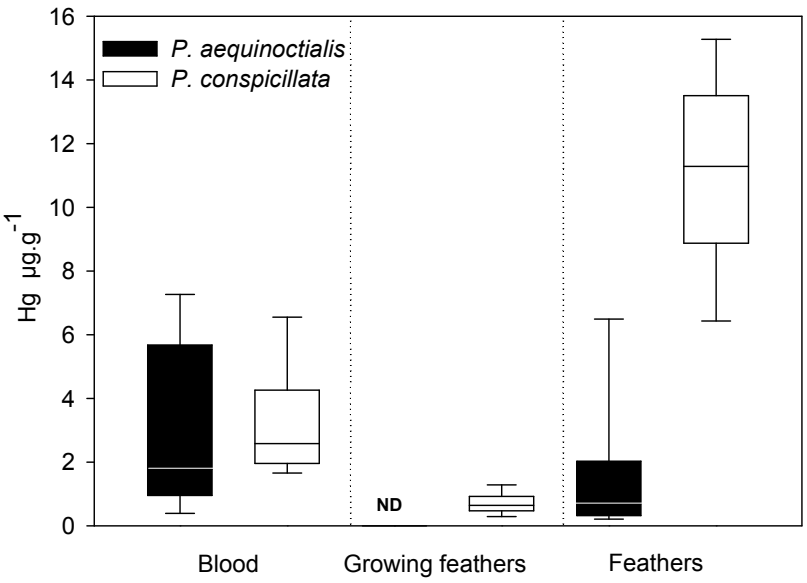

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737

738 Figure 5

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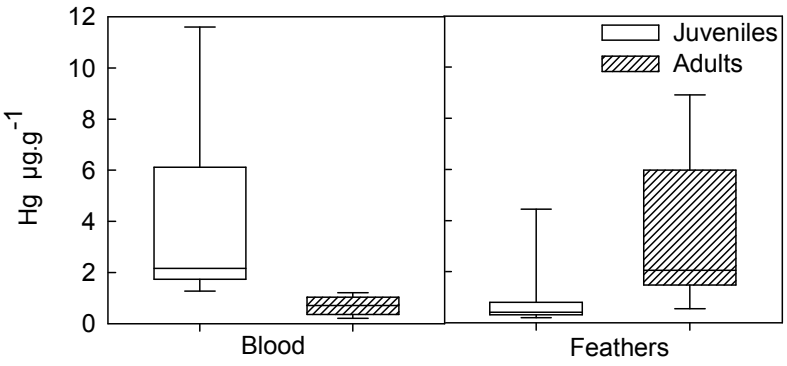

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