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Stable isotopes of carbon and nitrogen in the study of organochlorine contaminants in albatrosses and petrels

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Abstract
Carbon and nitrogen stable isotopes in albatrosses and petrels collected off southern Brazil were compared with concentrations of organochlorine contaminants (OCs). δ13C and δ15N values, as well as OCs concentrations, exhibited a high degree of variability among individuals and overlap among species. δ13C values reflected latitudinal differences among species, with lower values found in Wandering and Tristan Albatrosses and higher values found in Black-browed and Atlantic Yellow-nosed Albatrosses and White-chinned Petrels. Some relationships were found between OCs and stable isotopes, but in general a partial ‘uncoupling’ was observed between OCs concentrations and stable isotopes ratios (especially for δ15N). δ13C and δ15N values in Procellariiformes tissues during the non-breeding season appear to be a better indicator of foraging habitats than of trophic relationships, which may partially explain the high degree of variability between concentrations of OCs and stable isotopes ratios in birds with a diversified diet and wide foraging range.

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1. Introduction

Persistent organic pollutants such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) have been reported in seabird tissues worldwide. These compounds are long-lived in the environment and tend to accumulate at higher trophic levels through biomagnification (Tanabe, 2002). Previous studies showed a high degree of intraspecific variability in concentrations of PCBs and OCPs in seabirds (e.g. Elliott, 2005; Colabuono et al., 2012), which can be attributed to several factors, such as diet, distribution and bioaccumulation over time (Elliott, 2005). However, high individual variability in pollutant levels often complicates the interpretation of contamination patterns, toxicology and exposure to these pollutants.

Relative differences in isotopic ratios of carbon (δ13C) and nitrogen (δ15N) provide useful information on the biology and ecology of migratory seabirds, which can assist in understanding variation in contamination by organic contaminants (Hobson et al., 2002; Hoekstra et al., 2003; Elliott, 2005). Isotope studies have been widely used to assess the trophic position of seabirds, to infer the latitudinal distribution of their feeding areas, and to determine the long-term impacts of human activities on marine foodwebs (e.g. Fisk et al., 2001; Forero and Hobson, 2003; Quillfeldt et al., 2008; Phillips et al., 2009; Bugoni et al., 2010).

In this study, we use carbon and nitrogen stable isotopes to elucidate ecological factors (e.g. diet, occurrence and distribution) of albatrosses and petrels (Procellariiformes) collected off southern Brazil. We compared these data with concentrations of organochlorine contaminants (OCs) previously reported for these birds (Colabuono et al., 2012) to better understand the high variability in OC levels found in their tissues. The analysis of carbon and nitrogen stable isotopes in albatross and petrel tissues during their migration to Brazilian waters may provide information on ecological connections during this poorly studied period (Bugoni et al., 2010). This information, together with the determination of contamination levels of organic pollutants, may allow a better understanding of the influence of different aspects related to exposure to pollutants.

2. Materials and methods

2.1. Sampling

Abdominal fat, pectoral muscle and liver samples were collected from 79 birds of five species (Table 1): Wandering Albatross (Diomedea exulans), Tristan Albatross (Diomedea dabbenena),
Black-browed Albatross (Thalassarche melanophris), Atlantic Yellow-nosed Albatross (Thalassarche chlororhynchos) and White-chinned Petrel (Procellaria aequinoctialis). Birds sampled were killed accidentally by pelagic longline fisheries operating off southern Brazil (Fig. 1). All Atlantic Yellow-nosed Albatross samples were from adults, whereas all Black-browed Albatross were juveniles. Eighty percent of Wandering Albatross samples were from adults, while juveniles represented the same proportion in Tristan Albatross samples. In White-chinned Petrels, age is not easy to confirm such as in albatrosses (e.g. Bugoni and Furness, 2009), and could not be determined.

2.2. Stable isotope analyses

Muscle and liver samples were freeze-dried and powdered. As lipids are depleted in $^{13}C$ compared to whole tissues (Post et al., 2007), separate subsamples were run for estimates $\delta^{13}C$ and $\delta^{15}N$ (Sotiropoulos et al., 2004). For carbon analyses, lipids were extracted from subsamples of each tissue (~50 mg) individually placed in filter paper envelopes and extracted in 400 ml chloroform and methanol (2:1, v/v) by ultrasound (Branson 2210, Branson Ultrasonics Corporation) for one hour, with 50 samples in each batch. This extraction procedure was repeated twice. After extraction, the subsamples were oven dried at 40 °C for 24 h.

Subsamples (0.6 to 0.7 mg) of both raw and lipid-extracted material were placed in tin capsules and analyzed by continuous-flow isotope ratio mass spectrometry using an Elemental Analyzer (Finnigan Flash EA 1112) coupled to a Thermo Finnigan Delta XP Mass Spectrometer. Stable isotope analysis was performed in the Stable Light Isotope Laboratory, Department of Archeology, University of Cape Town, South Africa. Results were expressed in $\delta$ notation as parts per thousand (‰) deviating from the international standards Pee Dee Belemnitite limestone (carbon) and atmospheric air (nitrogen). Internal laboratory standards (sucrose from Australian National University (ANU) and DL valine from Sigma and Merck gel from Merck) were analyzed for every 16 samples to correct any instrument drift. All the in-house standards had been calibrated against IAEA (International Atomic Energy Agency) standards. Analytical precision was estimated as ±0.05‰.

2.3. Analysis of polychlorinated biphenyls and organic pesticides

The analytical procedure followed that described by MacLeod et al. (1986), with minor modifications (see Colabuono et al., 2012). Briefly, wet tissue (0.25 g of fat and liver tissues and 2.5 g of muscle tissue) was extracted in a Soxhlet apparatus for 8 h using 80 ml of n-hexane and methylene chloride (1:1, v/v). Before extraction, surrogates for OCPs and PCBs were added to all samples, blanks and reference material. After the determination of the extractable lipids through gravimetric analysis in one aliquot, the extracts were cleaned up using column chromatography with silica and alumina and eluted with methylene chloride. The fraction was further purified using high-performance liquid chromatography. The extract was concentrated to a volume of 0.9 ml in n-hexane and an internal standard was added prior to the gas chromatographic analysis. A procedural blank was run for every eight samples. The identification and quantification analyses regarding OCPs were performed using an Agilent Technologies 6890 N gas chromatograph with an electron capture detector and PCBs were analyzed using a 5973 N Agilent Technologies gas chromatograph coupled to a mass spectrometer in a selected ion mode (SIM 70 eV).

The analytical methodology was validated using a standard reference (SRM 1945 – organics in whale blubber) purchased from the National Institute of Standards and Technology (NS&T, USA). SRM 1945 was analyzed in duplicate and the average recovery of analytes was within the range accepted by NS&T (Wade and Cantillo, 1945). The analytical methodology was validated using a standard reference (SRM 1945 – organics in whale blubber) purchased from the National Institute of Standards and Technology (NS&T, USA). SRM 1945 was analyzed in duplicate and the average recovery of analytes was within the range accepted by NS&T (Wade and Cantillo, 1945).
The recovery of analytes in spiked blanks and matrices produced satisfactory results (67%–115%). Analytes in the laboratory blanks were subtracted from the samples. Method quantification limits (QL) ranged from <1.9 ng g\(^{-1}\) to 8.5 ng g\(^{-1}\) wet weight (ww). Analyte quantification was performed using a nine-level analytical curve and followed the internal standard procedure. Surrogate recoveries were acceptable and presented a mean ± standard deviation value of 97% ± 7.

### 2.4. Statistical analysis

Inter-species differences among \(\delta^{13}C\) and \(\delta^{15}N\) values were compared by analysis of variance (ANOVA). When significant differences were found, Tukey HSD tests were used for post hoc comparisons between pairs. Spearman's rank correlations \((r_s)\) were used to determine correlations between \(\delta^{13}C\) and \(\delta^{15}N\) values in different tissues.

Principal Components Analysis (PCA) with Varimax rotation (eigenvalues > 1) was used to examine correlations and reduce the number of contaminant variables (PCA 1). Component scores from PCA 1 were then used in a second PCA (PCA 2) to examine the relationships between OCs and stable isotope values, using species as a grouping variable. Data reduction allowed a better representation of the importance of the variables in each factor. Contaminants with concentrations below the QL for more than 25% of samples (drins, chlordanes, Mirex and HCB in liver tissue and PCBs, drins, chlordanes and Mirex in muscle tissue) were excluded from statistical analyses. OCs wet weight concentrations were log transformed in order to obtain a normal distribution. Normality assumption was checked with Kolmogorovi–Smirnov tests. All statistical analyses were performed using Statistica 12 (Statsoft, 2013) with the significance level set at 5% \((p < 0.05)\).

### 3. Results

#### 3.1. Stables isotope ratios

Stable isotope values exhibited a high degree of variability among individuals, with considerable overlap among species, especially for \(\delta^{15}N\) (Table 1; Fig. 2; Supplementary Data Tables S1 and S2). In general the mean \(\delta^{15}N\) values from liver were higher than muscle for all species. The mean \(\delta^{15}N\) value in Black-browed
Albatrosses and White-chinned Petrels was 1% higher than in Atlantic Yellow-nosed Albatrosses (Tukey’s HSD, \( p < 0.05 \)), which were similar to the two Diomedea albatrosses (Table 1).

Wandering and Tristan Albatrosses had lower \( \delta^{13}C \) values than the other species, and these differences were significant between Diomedea and Thalassarche species for both liver and muscle (Tukey’s HSD, \( p < 0.05 \)). Black-browed and Atlantic Yellow-nosed Albatrosses had similar \( \delta^{13}C \) values (Table 1), which are typical of bird samples from subtropical waters (Jaeger et al., 2010).

White-chinned Petrels showed segregation in two distinct groups for \( \delta^{13}C \): in liver, eight individuals (3 males; 5 females) had \( \delta^{13}C > -17\% \), whereas 20 individuals (11 males; 9 females) had \( \delta^{13}C < -17\% \), while in muscle tissue 14 individuals (7 males; 7 females) had \( \delta^{13}C > -17\% \), and 15 (7 males; 8 females) had \( \delta^{13}C < -17\% \). Segregation in \( \delta^{15}N \) values was observed only in muscle tissue, in which 15 individuals had \( \delta^{15}N > +14\% \) (7 males; 8 females) and 14 had \( \delta^{15}N < +14\% \) (7 males; 7 females). Liver appears to be more enriched in \( ^{15}N \) than muscle and all but one bird had \( \delta^{15}N \) greater than +14.0% (exception +13.7% ). The groups were not linked to sex, and they were not related to seasonality, because 94% of petrel samples were collected in mid-winter (July and August).

There is an overlap of 95% of the individuals between the groups observed in muscle tissue for \( \delta^{15}N \) and \( \delta^{13}C \), which means that, in general, the birds more depleted in \( ^{13}C \) are also depleted in \( ^{15}N \) and vice versa. Strong significant correlations were found to individual \( \delta^{15}N \) (\( r_s = 0.83, p < 0.05 \)), and \( \delta^{13}C \) values (\( r_s = 0.68, p < 0.05 \)) between liver and muscle.

3.2. Stable isotopes and organochlorine contaminants

The organochlorine concentrations in fat, liver and muscle of the birds have already been reported by Colabuono et al. (2012). Overall, the organochlorine concentrations were highly variable among individuals. PCBs and DDTs (mainly \( p,p' - DDE \)) were predominant in all tissues of the five species sampled. Other organochlorine compounds found included chlordanes, drins, HCB and Mirex (Fig. 3: Supplementary Data Tables S3 and S4).

The first two principal components (PC) of PCA 1 captured 76% of the variance among the 10 contaminant variables and revealed strong correlations among contaminants (loadings > 0.7). PCBs, Mirex, DDTs and chlordanes were loaded significantly on PC1 (variance explained = 62%) and HCB and drins on PC2 (variance explained = 14%).

The first three PCs of PCA 2 retained 91.2% of the total variance (loadings > 0.8). \( \delta^{15}N \) and \( \delta^{13}C \) were loaded in PC1 (variance explained = 56.8%), while contaminant groups represented the two other factors (PC2 – HCB and drins, variance

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![Fig. 3.](image-url) Median values (ng g\(^{-1}\) wet weight) of PCBs and OCPs in fat, liver and muscle of Wandering Albatrosses (WA), Tristan Albatrosses (TA), Atlantic Yellow-nosed Albatrosses (AYNA), Black-browed Albatrosses (BBA) and White-chinned Petrels (WCP). Data drawn from Colabuono et al. (2012).
explained = 19.0%; PC3 – PCBs, DDTs, Mirex and chlordanes, variance explained = 15.3%).

By comparing PC scores and loadings we can identify the relationships between samples and variables. For example, Wandering and Tristan Albatrosses presented some of the highest scores for PC2, where loadings were influenced by HCB and drins and were grouped in the opposite quadrant to where \( \delta^{13}C \) values had greater importance (Fig. 4). This means that birds with high concentrations of HCB and drins tended to have low \( \delta^{13}C \) values (Figs. 2 and 3), suggesting a negative relationship among these contaminants and \( \delta^{13}C \) values (Fig. 4). Wandering and Tristan Albatrosses had also a good association with PC3, once these species showed the highest concentrations in all contaminants groups.

A few White-chinned Petrels showed the same pattern as Wandering and Tristan Albatrosses (Fig. 4), but most White-chinned Petrels grouped with Black-browed Albatrosses, exhibiting high (but variable) \( \delta^{15}N \) and \( \delta^{13}C \) values and low OC concentrations. Atlantic Yellow-nosed Albatrosses comprise a third group, with higher levels of contaminants but lower \( \delta^{15}N \) values than, for example, Black-browed Albatrosses.

4. Discussion

High variability in \( \delta^{13}C \) and \( \delta^{15}N \) is typical of species that feed on a wide range of trophic levels (Bird et al., 2008) and have extensive foraging ranges that span regions with different nutrient regimes, and thus variable \( \delta^{13}C \) and \( \delta^{15}N \) baselines (Post, 2002; Cherel and Hobson, 2007; Graham et al., 2010). The overlap in \( \delta^{13}C \) and \( \delta^{15}N \) values among the five seabird species may also be associated with a non-specific diet and/or competition for the same food sources. For instance, previous studies have reported the importance of fishery discards in the diet of these species as well as intra-species and inter-species competition for discards, which constitute an abundant food source for these birds (Colabuono and Vooren, 2007; Bugoni et al., 2010).

The low \( \delta^{13}C \) values found in Wandering and Tristan Albatrosses are normally expected for birds from high latitudes (Cherel et al., 2006; 2013), \( \delta^{13}C \) and \( \delta^{15}N \) values reflect the distribution and diet of birds during the time of tissue formation, which varies depending on the isotopic turnover time in the tissue analysed (approximately 30 days for muscle and only a few days for liver) (Bearhop et al. 2002; Hobson and Clark, 1992; Hobson et al., 1994). Thus, it is likely that some individuals of Wandering Albatross still reflect \( \delta \) values from their sub-Antarctic breeding grounds, especially for muscle tissue. The same is true for Tristan Albatross, which almost all breed on Gough Island (40°S, 10°W), where they are exposed to both sub-Antarctic and subtropical waters (Peterson and Stramma, 1991; Cuthbert et al., 2005; Phillips et al., 2009). Possible explanations for finding \( \delta^{13}C \) values typical of sub-Antarctic regions in some of the birds collected in Brazilian waters include the presence of individuals that use the waters off southern Brazil as feeding grounds even when breeding, as reported for Wandering (Bugoni et al., 2010) and Tristan Albatrosses (Cuthbert et al., 2005), or individuals that use areas near the breeding colonies for foraging outside their breeding season. According to Mackley et al. (2010), Wandering Albatrosses return to areas near the colony during the non-breeding period and also forage in areas further to the south, performing circumpolar migrations. With both hypotheses, the \( \delta^{13}C \) in samples from individuals collected at lower latitudes, such as southern Brazil, may reflect both diet in breeding areas (Antarctic and sub-Antarctic regions) and non-breeding areas (subtropical regions). In our study, one Wandering Albatross with sub-Antarctic \( \delta^{13}C \) values in both the liver and muscle tissues was identified as an adult female (18 years of age) outside its breeding season (information obtained from tagging) and was likely using regions further to the south for feeding.

**Fig. 4.** Scores plot of the two main factors extracted in the Principal Component Analysis used to examine the relation between contaminants and stable isotopes in the Wandering Albatrosses (WA), Tristan Albatrosses (TA), Atlantic Yellow-nosed Albatrosses (AYNA), Black-browed Albatrosses (BBA) and White-chinned Petrels (WCP). Variables influencing the loadings are shown in the quadrants.

<table>
<thead>
<tr>
<th>( \delta^{13}C ) (liver)</th>
<th>( \delta^{13}C ) (muscle)</th>
<th>( \delta^{15}N ) (liver)</th>
<th>( \delta^{15}N ) (muscle)</th>
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<tr>
<td>PCBs</td>
<td>DDTs</td>
<td>Mirex</td>
<td>Chlordanes</td>
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<tr>
<td>WA</td>
<td>TA</td>
<td>AYNA</td>
<td>BBA</td>
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</table>
The presence of two distinct isotopic groups of White-chinned Petrels off Brazil previously has been attributed to individuals collected in different seasons (Bugoni et al., 2010). However, seasonality does not explain the differences in $\delta^{13}$C and $\delta^{15}$N values detected in our study, because most White-chinned Petrels were sampled in winter. Another possibility is sex-linked differences in foraging areas, as reported for other species of Procellariiformes (González-Solís et al., 2000; Phillips et al., 2004; Phillips et al., 2009), but there was no predominance of males or females in the groups identified. The differences probably reflect juveniles versus older birds (Barquete, 2012), but unfortunately the birds were not aged.

Values of $\delta^{13}$C and $\delta^{15}$N are primarily used to infer a consumer’s feeding habitat and trophic position, respectively (Jaeger et al., 2010). However, differences in marine $\delta^{15}$N baselines may overshadow trophic effects, providing instead further evidence of foraging habitats (Anderson et al., 2010; Cherel et al., 2013). Enriched isotopic signatures usually correspond to a high trophic position, but also characterize organisms feeding in neritic areas (Phillips et al., 2009; Jaeger et al., 2010). Black-browed Albatrosses and White-chinned Petrels forage mainly in productive inshore waters during the non-breeding period, which may explain their high $\delta^{15}$N values compared to other Procellariiformes species, such as Wandering and Tristan Albatrosses that usually feed in oceanic waters (Phillips et al., 2009; Bugoni et al., 2010; Cherel et al., 2013).

High OC concentrations should be associated with high $\delta^{15}$N values, because persistent compounds biomagnify through the food web, and/or in species foraging in inshore waters that generally present higher OC concentrations than oceanic waters (Tanabe et al., 2004). However, Black-browed Albatrosses presented the opposite pattern, with low OC concentrations associated with high $\delta^{15}$N signatures. This unexpected result may be influenced by age, once all Black-browed Albatrosses sampled were juveniles (<1 year old) and so had little chance to accumulate large OC loads (Warham, 1996; Donaldson et al., 1997). Most White-chinned Petrel samples exhibited the same pattern, and so also might be expected to be juveniles.

Seasonal diet shifts (e.g. breeding versus non-breeding seasons) may also result in a partial ‘uncoupling’ between contaminant levels and stable isotope ratios, especially for $\delta^{15}$N (Elliott, 2005; Anderson et al., 2009; Thompson et al., 1998). Muscle and liver isotope ratios represent diet in a relative short period of time (from few days to a month) whereas OCs accumulate in tissues over a longer period (Bearhop et al., 2002; Hobson et al., 1994; Donaldson et al., 1997).

The high OC concentrations found in Wandering and Tristan Albatrosses presumably occur because both species occupy high trophic levels (Anderson et al., 2010; Bugoni et al., 2010), and diet is the main exposure route for contamination by persistent pollutants (Borga et al., 2004). As a result, the relatively low $\delta^{15}$N values found in muscle and liver of Wandering and Tristan Albatrosses were surprising, and probably reflect the oceanic foraging range rather than trophic position (Cherel et al., 2013). The inverse relationship between HCB concentrations and $\delta^{13}$C in these albatrosses may also be related to their distribution. Lower $\delta^{13}$C values are associated with high latitudes (Cherel et al., 2006), where HCB is concentrated due to atmospheric transport (Simonich and Hites, 1995; van den Brink, 1997).

The partial ‘uncoupling’ between OC concentrations and $\delta^{13}$C and $\delta^{15}$N may relate to several factors influencing the exposure and accumulation of contaminants. However, relative differences of carbon and nitrogen isotopic ratios can be useful tools for providing information on the biology and ecology of migratory birds and can assist in studies on contamination by persistent organic compounds. All the Procellariiformes species studied here have a diversified diet and wide foraging range. It appears that nitrogen and carbon stable isotopes elucidate better their foraging habitats than trophic relationships, at least during the non-breeding period, which may partially explain the high degree of variability regarding concentrations of organochlorines and stable isotopes ratios.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2014.03.046.

References


