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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). $\delta^{13}C$ and $\delta^{15}N$ values, as well as OCs concentrations, exhibited a high degree of variability among individuals and overlap among species. $\delta^{13}C$ 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 $\delta^{15}N$). $\delta^{13}C$ and $\delta^{15}N$ 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.

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 ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) 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; Hoekstraa 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*),

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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 Albatrosses 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$_{plus}$ 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 δ notation as parts per thousand (‰) deviating from the international standards Pee Dee Belemnite 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,

Table 1
Carbon and nitrogen stable isotopes values (mean ± 1 SD, range) from liver and muscle of Procellariiformes seabirds from southern Brazil.

	N	Liver		Muscle	
		$\delta^{13}C$ (‰) Mean ± SD (range)	$\delta^{15}N$ (‰) Mean ± SD (range)	$\delta^{13}C$ (‰) Mean ± SD (range)	$\delta^{15}N$ (‰) Mean ± SD (range)
Wandering Albatross	5	−18.62 ± 1.10 (−19.81 to −17.03)	+14.58 ± 0.85 (+13.64 to +15.71)	−18.50 ± 1.10 (−19.74 to −17.32)	+13.57 ± 0.81 (+12.73 to +14.70)
Tristan Albatross	5	−18.24 ± 0.85 (−19.29 to −17.42)	+14.71 ± 0.97 (+13.08 to +15.49)	−17.83 ± 0.45 (−18.49 to −17.48)	+13.77 ± 0.83 (+12.32 to +14.43)
Black-browed Albatross	31	−16.67 ± 0.73 (−18.30 to −15.40)	+15.83 ± 1.02 (+14.05 to +18.00)	−16.70 ± 0.59 (−18.12 to −15.39)	±14.53 ± 0.85 (+12.46 to +16.06)
Atlantic Yellow-nosed Albatross	9	−17.17 ± 0.50 (−17.72 to −16.41)	+14.50 ± 0.74 (+13.24 to +16.41)	−16.96 ± 0.29 (−17.40 to −16.50)	+13.12 ± 0.40 (+12.55 to +13.90)
White-chinned Petrel	29	−17.22 ± 0.60 (−18.32 to −16.01)	+15.70 ± 1.13 (+13.70 to +18.24)	−17.07 ± 0.73 (−18.70 to −15.64)	+14.00 ± 1.52 (+11.96 to +16.79)