PERSISTENT ORGANIC POLLUTANTS AND STABLE ISOTOPES IN SEABIRDS OF THE ROCAS ATOLL, EQUATORIAL ATLANTIC, BRAZIL

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ABSTRACT

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Pollution is a key factor in efforts to conserve seabirds and their habitats. The Marine Biological Reserve of Rocas Atoll hosts the largest population of seabirds (~23 000 individuals) breeding in Brazilian waters. In the present study at Rocas Atoll, liver samples were collected from dead individuals of five species (adults and nestlings): Masked Booby *Sula dactylatra*, Brown Booby *Sula leucogaster*, Brown Noddy *Anous stolidus*, Black Noddy *Anous minutus*, and Sooty Tern *Onychoprion fuscatus*. They were analyzed for three persistent organic pollutants (POPs): polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers. Carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were also analyzed to assess feeding and foraging habits. PCBs (3.37–189 $ng \cdot g^{-1}$), OCPs (DDTs, 0.5–23.1 $ng \cdot g^{-1}$), and hexachlorobenzene (0.31–12.3 $ng \cdot g^{-1}$) were predominant overall, and results were generally consistent with previous studies. Despite the low levels of contaminants, intraspecific stratification was found, with lower concentrations in nestlings compared to adults, as expected. Stable isotopes revealed that differences in POP levels and PCB profiles were associated with diet and foraging patterns. Low levels of POPs at Rocas Atoll were consistent with patterns observed in studies of other low-latitude and high-latitude seabirds.

Key words: PCBs, organochlorine pesticides, trophic position, remote environments, long-range transport, stable isotopes, tropical seabirds

INTRODUCTION

Pollution by organic contaminants is an important issue in efforts to conserve seabirds and their habitats, as it has caused population declines in several species (Mallory & Braune 2012). Seabirds are relatively easy to study because of their colonial nesting habits and because they exhibit rapid responses to environmental threats (Furness & Camphuysen 1997). This sensitivity to changes in the environment is one of the main reasons why seabirds are commonly used to monitor ocean pollution (Guzzo *et al*. 2014).

Seabirds are vulnerable to a variety of persistent organic pollutants (POPs; Bourne 1976) such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs). POPs are high-molecular-weight, halogenated hydrocarbons that persist in the marine environment and accumulate in animal tissues (Clark 2001). The persistence of PCBs and OCPs is due to their low metabolism/excretion rates, which are directly linked to their molecular structure and lipophilic nature; together, this can lead to bioaccumulation and biomagnification (Tanabe *et al*. 1998). Seabirds occupy a high trophic level in the food web and, therefore, are subject to biomagnification of any persistent compounds that are ingested. POP concentrations in these organisms can be up to 106 times greater than in contaminated abiotic sources (Furness & Camphuysen 1997). The sublethal effects of POPs in seabirds include changes in enzyme activity, immune system deficiency, and deficient hormone levels; together, these can cause a delay in ovulation, reproductive failure, slow growth, impaired osmoregulation, and impaired calcium metabolism. Problems with calcium metabolism result in thinner egg shells and affect reproduction (Luzardo *et al*. 2014). For instance, the threshold for dioxin-like toxicity for PCBs can be as low as 103 $ng \cdot g^{-1}$ in gull *Larus* spp. eggs (Adelsbach *et al*. 2007) and even lower in tern *Sterna* spp. livers (Bosveld *et al*. 2000).

Analyzing stable isotopes of carbon ($\delta^{13}C$, which indicates mainly foraging habitat) and nitrogen ($\delta^{15}N$, which indicates mainly trophic position) can provide important ecological information, and these methods have been used in several studies on contamination by organic compounds (Forero & Hobson 2003, Cipro *et al*. 2012, Colabuono *et al*. 2014). Such analyses allow evaluation of seabird trophic structure, latitudinal differences in the distribution of foraging areas and wintering grounds (Quillfeldt *et al*. 2005), and the impact of human activities (Bearhop *et al*. 2002). Studies addressing a wide range of trophic positions suggest that POP concentrations in animals correlate with $\delta^{15}N$ (Elliott 2005) as an indicator of biomagnification. Conversely, other studies show that some POPs, especially PBDEs, do not exhibit a positive significant relationship with $\delta^{15}N$ (Mizukawa *et al*. 2009). This could be explained by chemical factors (e.g., variations in molecular mass or $\log_{octanol\text{-}water}$ partition coefficient), oceanographic factors (e.g., greater relative influence of marine or offshore inputs over trophic level), or biological factors (e.g., differences in temperature control mechanisms, excretion mechanisms, metabolism, or feeding rates) (Fisk *et al*. 2001, Elliott *et al*. 2009).

We studied pollutants in seabirds at Rocas Atoll, the only atoll in the southwestern Atlantic Ocean and the first marine biological reserve in Brazil (Schulz-Neto 2004). This site hosts the largest population of seabirds in the country, estimated to be about 23 000 individuals (Mancini *et al*. 2016). It includes the largest reproductive colonies of Masked Booby *Sula dactylatra*, Brown Noddy *Anous stolidus*,

and Sooty Tern *Onychoprion fuscatus* in the South Atlantic (Schulz-Neto 2004). The other two species included in this study, Brown Booby *Sula leucogaster* and Black Noddy *Anous minutus*, also breed at the reserve.

The Rocas Atoll is not exempt from the influence of POPs via atmospheric transport; this main route of entry into marine ecosystems (von Waldow *et al*. 2010) enables POPs to reach remote areas such as oceanic islands (Stefanelli *et al*. 2004, Dias *et al*. 2013). The aim of the present study is to determine the occurrence and distribution of POPs in seabirds at this site, and to characterize the influence of trophic level and ecological habits on the qualitative and quantitative distribution of POPs.

METHODS

Study area and sampling

Rocas Atoll (03°51′S, 033°49′W; Fig. 1) is a reef formation on a seamount in the South Atlantic Ocean. It is located 266 km northeast of Natal, Brazil and 150 km west of the Fernando de Noronha archipelago. With an area of 7.2 km^2, it is among the smallest atolls in the world (Kikuchi & Leão 1997). Rocas Atoll is bordered by the South Equatorial Current, which flows westward (Pereira *et al*. 2010). The prevailing winds are from the southeast and east during the summer, with an intensification of southeasterlies and a reduction of easterlies during the winter (Hoflich 1984).

Liver samples from 54 individuals found dead in or around nests were collected during four expeditions (January and June 2010, May and December 2011). The species sampled were Black Noddy (n = 3, all adults), Brown Noddy (n = 9: 4 adults, 5 juveniles), Sooty Tern (n = 33: 5 adults, 28 juveniles), Masked Booby (n = 8: 1 adult, 7 juveniles), and Brown Booby (n = 1, adult) (Table 1). Biometric data were collected, and life stage was determined by gonadal development according to Guioli *et al*. (2014) and by plumage according to Harrison (1985). Samples were wrapped in decontaminated aluminum foil (previously combusted for 4 h at 400 °C minimum), then kept frozen at −20 °C until analysis.

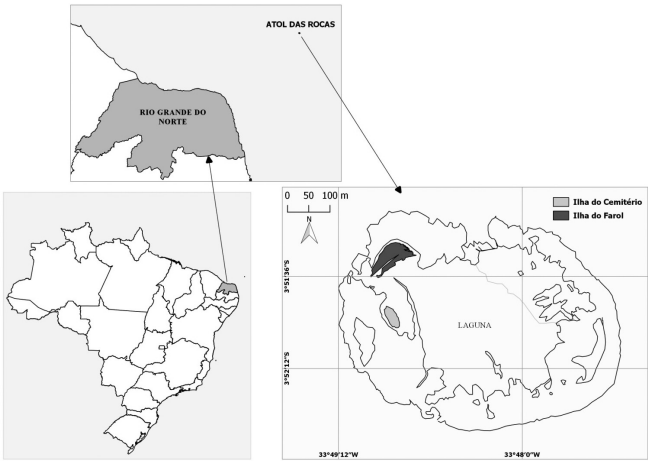

Fig. 1. Rocas Atoll and its location in the Atlantic Ocean.

TABLE 1
Persistent organic pollutants concentrations (in ng·g^{-1} of wet weight) in seabird species from Rocas Atoll, Atlantic Ocean

	Sooty Tern	Brown Noddy	Black Noddy	Masked Booby	Brown Booby
	***n* = 33**	***n* = 9**	***n* = 3**	***n* = 8**	***n* = 1**
Lipids (%)[a]	4.28 ± 2.09 (0–7.6)	5.56 ± 1.99 (2.8–8.4)	4.67 ± 1.40 (3.2–6.0)	5.05 ± 1.66 (3.2–8.4)	8.4
ΣHCHs	1.96 ± 1.63 (<0.43–7.60)	1.78 ± 0.73 (1.00–3.36)	4.44 ± 2.24 (2.81–6.99)	2.16 ± 0.77 (1.13–3.25)	2.76
HCB	2.65 ± 3.46 (0.31–12.33)	3.93 ± 3.62 (<0.30–8.95)	2.97 ± 2.65 (0.76–5.91)	3.18 ± 3.64 (0.68–11.91)	6.41
ΣDrins	<0.22	<0.22	<0.22	<0.22	<0.22
ΣChlordanes	<0.22	<0.22	<0.22	<0.22	<0.22
ΣDDTs[b]	2.58 ± 1.93 (<0.31–7.37)	5.93 ± 3.85 (0.50–14.36)	6.79 ± 4.00 (3.55–11.26)	7.40 ± 6.59 (2.74–23.06)	11.8
	a	***ab***	***ab***	***b***	
ΣEndosulfans	<0.27	<0.27	<0.27	<0.27	<0.27
Mirex	<0.41	<0.41	<0.41	<0.41	<0.41
ΣPCBs[b]	23.6 ± 21.7 (3.37–93.2)	65.2 ± 28.2 (15.7–123)	72.0 ± 8.91 (61.4–80.4)	60.9 ± 57.3 (12.3–189)	85.2
	a	***b***	***ab***	***b***	
Σ PBDEs	<0.76	<0.76	<0.76	<0.76	<0.76

[a] Mean ± SD, with ranges in parentheses of liver samples.
[b] Homogenous groups for each POP group indicated by letters in italics below respective concentration levels; α = 0.05 (ANOVA + unilateral Tukey's post hoc test)

Chemical analyses

The method used for analysis was adapted from MacLeod *et al*. (1985). Briefly, ~0.25 g of liver, ground with anhydrous Na_2SO_4 (Merck, Germany), was extracted with 80 mL of dichloromethane and hexane (1:1 v/v) in a Soxhlet extractor for 8 h. Prior to extraction, 2,2′,4,5′,6-pentachlorobiphenyl (PCB103) (AccuStandard, USA) and 2,2′,3,3′,4,5,5′,6-octachlorobiphenyl (PCB198) (AccuStandard, USA) were added to all samples and blanks, as surrogates for chlorinated pesticides, PCBs, and PBDEs. Extracts were initially purified using 5% deactivated silica-alumina (Merck, Germany; 8 g of SiO_2 to 16 g of Al_2O_3) column chromatography eluted with 80 mL of *n*-hexane:dichloromethane (1:1 v/v). The eluate was further purified using a high-performance liquid chromatography gel permeation column (HPLC/GPC binary pump, Perkin Elmer) to remove excess lipids. The internal standard 2,4,5,6-tetrachlorometaxylene (TCMX) was added, and the extract was concentrated to a volume of 0.9 mL in hexane prior to analysis by gas chromatography (GC). Chlorinated pesticides were analyzed using GC with an electron capture detector (ECD, Agilent Technologies 6890N). PCBs and PBDEs were quantitatively analyzed using a gas chromatograph coupled to a mass spectrometer (GC/MS, Agilent Technologies 5973N) in selected-ion monitoring (SIM) mode. Target POPs were quantified with analytical curves generated from nine standard solutions with concentrations of 1, 5, 10, 20, 50, 80, 100, 150, and 200 $ng \cdot L^{-1}$. Lipid weights were determined gravimetrically. The chlorinated pesticides analyzed were dichlorodiphenyltrichloroethanes (DDTs, in *o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, and *p,p′*-DDE configurations), chlordanes (α- and γ-chlordane, oxychlordane, heptachlor, and heptachlor epoxide), hexachlorocyclohexanes (HCHs, in α-, β-, γ-, and δ- configurations), drins (aldrin, dieldrin, isodrin, and endrin), hexachlorobenzene (HCB), and mirex. Also investigated were 48 isomers and congeners of PCBs (IUPAC # 8, 18, 28, 31, 33, 44, 49, 52, 56/60, 66, 70, 74, 87, 95, 97, 99, 101, 105, 110, 114, 118, 123, 126, 128, 132, 138, 141, 149, 151, 153, 156, 157, 158, 167, 170, 174, 177, 180, 183, 187, 189, 194, 195, 201, 203, 206, 209) and 7 congeners of PBDEs (IUPAC # 28, 47, 99, 100, 153, 154, 183).

Quality assurance and quality control were performed based on Wade & Cantillo (1994). Sample parameters were analyzed as procedural blanks (at least one for every 12 injections), matrix spikes, and precision tests with both matrix replicates and standard reference material (SRM1945 - Organics in Whale Blubber; National Institute of Standards and Technology); each was performed in triplicate before and after the whole dataset was injected.

Individual OCPs, PCBs, and PBDEs were identified based on GC retention times. For PCBs and PBDEs, the respective mass/charge ratio (m/z) of quantitation ions was also used. The method detection limit (MDL) was based on the standard deviation σ (student's *t*-value with 95% confidence σ) of ten replicates of a spiked fish liver sample containing target compounds at a concentration of 2.5 $ng \cdot g^{-1}$. The MDL was 0.87–2.12 $ng \cdot g^{-1}$ for pesticides, 1.36–4.89 $ng \cdot g^{-1}$ for PCBs, and 3.40–4.75 $ng \cdot g^{-1}$ for PBDEs. Samples below the MDL were excluded from statistical analysis. All solvents were organic-residue-analysis grade (J.T. Baker, USA), and blanks were checked under the same conditions as those used for the analyses. The samples were analyzed at the Marine Organic Chemistry Laboratory (LabQOM, University of São Paulo), which participates annually in inter-laboratory comparison exercises organized by the International Atomic Energy Agency's Marine Environmental Studies Laboratory. The LabQOM has obtained satisfactory results for OCP, PBDE, and PCB analyses in several matrices, including surrogate recovery ranging from 84.3% to 102% for PCB103 and from 94.2% to 97.7% for PCB198. In the present study, recoveries averaged 87.7% for OCPs and 90.5% for PCBs; concentrations are expressed as $ng \cdot g^{-1}$ of wet weight (ww).

Stable isotope analyses (SIA) were carried out in an elemental analyzer (Costec Instruments Elemental Combustion System) coupled to an isotope-ratio mass spectrometry detector (EA-IRMS, Thermo Scientific Delta V Advantage) at LabQOM. Prior to analysis, liver samples were freeze-dried and powdered. Subsamples (0.6–0.7 mg) were weighed and placed in tin capsules. Results are expressed in δ notation (‰). The certified calibration standards used for the isotopic analysis were USGS-40 (glutamic acid: $\delta^{13}C = -26.389‰$ vs. Pee Dee Belemnite (PDB); $\delta^{15}N = -4.5‰$ vs. atmospheric air) and IAEA-600 (caffeine: $\delta^{13}C = -27.771‰$ vs. PDB; $\delta^{15}N = +1.0‰$ vs. atmospheric air). According to Post *et al*. (2007), the influence of lipids on carbon isotopic analysis should be considered in tissues with high concentration of lipids and/or a C:N ratio greater than 3.5. The mean C:N ratio in the samples analyzed was 2.74 ± 0.21 and, moreover, liver lipid content was relatively low, probably due to the season of sampling. Evaluations of reproducibility and accuracy were performed based on the analysis of replicates ($n = 10$) of both the certified standards and the internal laboratory standards (homogenized albatross liver). Internal laboratory standards were analyzed for every 12 samples. The experimental precision (standard deviation, SD) for the analysis was ±0.12‰ for total carbon ($\delta^{13}C$) and ±0.15‰ for nitrogen ($\delta^{15}N$).

Statistical analysis

Parametric distribution was verified using the Shapiro-Wilk test and standardized using logarithmic transformation (for cases of asymmetry and positive heteroskedasticity). Analysis of variance (ANOVA with Tukey's unilateral HSD test) was performed to detect significant differences and the presence of homogeneous groups for each pollutant group, both among species and among life stages for each species. Spearman's rank correlation coefficient (*rs*) was applied to determine the correlations between POP concentrations and stable isotope values ($\delta^{15}N$) for each species; while it is normally used in non-parametrical cases, it was employed here as an indicator of monotonicity in the dataset. Statistical tests were performed using StatSoft Statistica (12.0), with the significance at $p < 0.05$. Comparisons are made with the whole dataset (adults and juveniles whenever available) unless stated otherwise.

RESULTS

Pollutant levels

The predominant POP compounds were PCBs (3.37–189.0 $ng \cdot g^{-1}$ ww), DDTs (0.5–23.1 $ng \cdot g^{-1}$ ww), and HCB (0.31–12.3 $ng \cdot g^{-1}$ ww; Table 1). None of the samples exhibited PBDEs above detection limits. Despite the low POP levels, we found intraspecific stratification. Overall, lower concentrations of pollutants were found in juveniles (mostly nestlings) compared to adults (for comparison of juveniles and adults, $p < 0.05$ for Brown Noddy, Sooty Tern, and Masked Booby; Fig. 2). The highest concentrations of pollutants

were found in an adult Masked Booby (∑PCB = 189 ng·g^{-1} ww; ∑DDTs = 23.1 ng·g^{-1} ww; HCB = 11.91 ng·g^{-1} ww).

Statistically significant differences in concentrations of DDTs and PCBs were found among juveniles of Sooty Tern, Brown Noddy, and Masked Booby; the same pattern was observed in the adults. For DDTs, a significant difference was found between Sooty Tern and Masked Booby (all age groups, p = 0.007; Table 1). For PCBs, Sooty Tern differed significantly from both Brown Noddy (p = 0.005) and Masked Booby (p = 0.023), with Sooty Tern exhibiting a lower concentration of both contaminants.

No significant differences among species were found in mean concentrations of HCB or HCH. The isomer γ-HCH, which is the predominant compound in the commercial insecticide Lindane, was the only HCH detected, and it was measured at the same order of magnitude in all samples analyzed. HCB was detected at the same order of magnitude in all species analyzed, but at lower levels than DDTs and PCBs, and with no significant variation among species.

PCBs were the predominant POP in the present study, with mean values one order of magnitude higher than the other classes of contaminants (Fig. 4). The general pattern found in the qualitative profiles (i.e., in the relative weight of PCB congeners) was consistent with previously presented data and with the ecological differences among species. Variation in the qualitative profile can

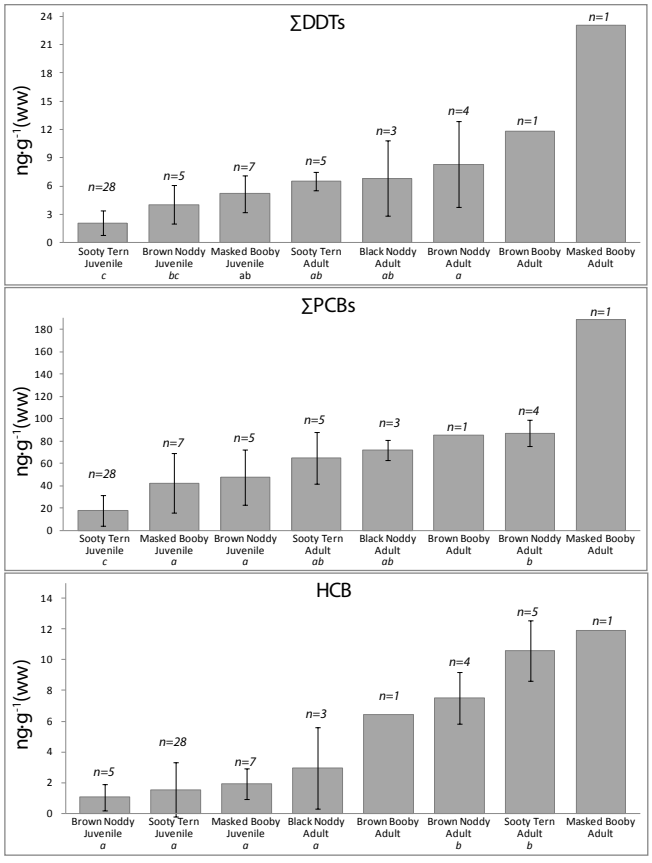

Fig. 2. Pollutant concentration levels (ng·g^{-1} ww) in five seabird species from Rocas Atoll. Homogeneous groups to α = 0.05 indicated by letters in italics

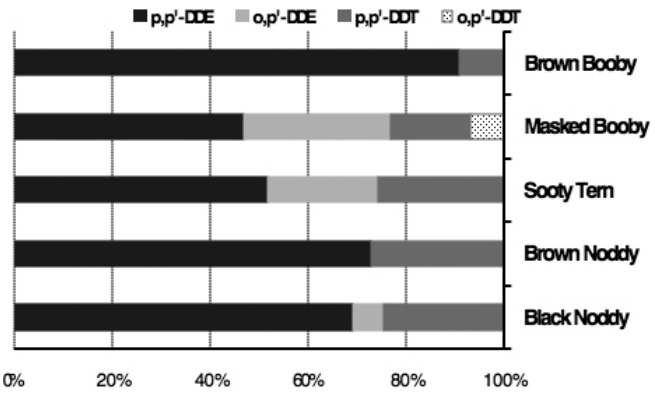

Fig. 3. Percentage distribution of DDT compounds in five seabird species from Rocas Atoll.

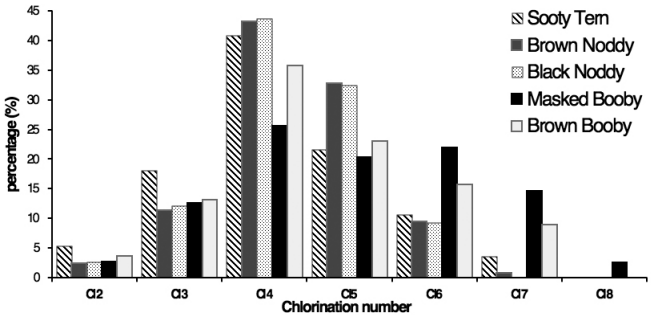

Fig. 4. Percentage distribution of PCBs by chlorination number in five seabird species from Rocas Atoll.

TABLE 2
Results of stable isotope analysis (in ‰) broken down by species and age class[a]

		n	$\delta^{13}C$	$\delta^{15}N$
Sooty Tern	adult	5	−14.96 ± 0.32 (−15.38 to −14.63)	9.27 ± 0.31 (9.01–9.75)
	juvenile	28	−16.09 ± 0.71 (−16.98 to −14.71)	8.13 ± 0.49 (7.45–9.26)
	total	33	−15.92 ± 0.78 (−16.98 to −14.63)	8.3 ± 0.62 (7.45–9.75)
Brown Noddy	adult	4	−15.51 ± 0.41 (−16.08 to −15.11)	9.5 ± 0.21 (9.19–9.63)
	juvenile	5	−15.72 ± 0.25 (−15.87 to −15.27)	8.84 ± 0.26 (8.50–9.20)
	total	9	−15.63 ± 0.33 (−16.08 to −15.11)	9.13 ± 0.41 (8.50–9.63)
Black Noddy	adult	3	−15.6 ± 0.05 (−15.65 to −15.55)	9.04 ± 0.11 (8.92–9.12)
Masked Booby	adult	1	−15.6	10.86
	juvenile	7	−15.52 ± 0.30 (−15.97 to −15.08)	10.64 ± 0.18 (10.38–10.87)
	total	8	−15.53 ± 0.28 (−15.97 to −15.08)	10.67 ± 0.19 (10.38–10.87)
Brown Booby	adult	1	−15.43	10.8

[a] Average ± SD, with range in parentheses

be linked to the different feeding habits, foraging areas, and trophic levels of the seabirds analyzed, but we could not statistically associate it with the life stage.

We found significant correlations between pollutant groups of similar volatility: *rs* = 0.67 for HCHs vs. HCB, and *rs* = 0.78 for HCB vs. bi- and tri-chlorinated PCBs (not included in Table 3 for editorial reasons). We also found significant correlations of DDTs and PCBs with HCB and HCHs that were not explained by the similarity in volatility, since DDTs and PCBs are generally less volatile.

Stable isotopes

In the present study, $\delta^{15}N$ and $\delta^{13}C$ values (Table 2; Fig. 5) showed a significant separation into three distinct homogeneous groups: Sooty Tern + Black Noddy, Black Noddy + Brown Noddy, and Masked Booby (Tukey's HSD). Black Noddy was likely included in both groups due to its small sample size (n = 3). Brown Booby (n = 1) was not included in the analyses. Our grouped data reflected similarities in diet and foraging areas for individuals within *Sula* and between individuals of *Anous* and *Onychoprion*; this was corroborated by the similar concentrations of contaminants.

TABLE 3
Spearman correlations among contaminants concentrations and nitrogen stable isotopes for four species of seabirds from Rocas Atoll, Atlantic Ocean and for the entire data set[a]

		∑PCBs	**HCB**	**∑HCHs**	**∑DDTs**	**$\delta^{15}N$**	**Cl2**	**Cl3**	**Cl4**	**Cl5**	**Cl6**	**Cl7**
Sooty Tern n = 33	∑PCBs	1.00										
	HCB	0.80	1.00									
	∑HCHs	0.66	0.66	1.00								
	∑DDTs	0.81	0.87	0.68	1.00							
	$\delta^{15}N$	0.71	0.68	0.47	0.61	1.00	0.59	0.73	0.70	0.62	0.42	0.61
	$\delta^{13}C$	0.43	0.54	0.26	0.42	0.71	0.23	0.41	0.45	0.36	0.25	0.46
Brown Noddy n = 9	∑PCBs	1.00										
	HCB	0.73	1.00									
	∑HCHs	0.18	0.68	1.00								
	∑DDTs	0.59	0.74	0.71	1.00							
	$\delta^{15}N$	0.71	0.46	0.31	0.18	1.00	0.28	0.52	0.17	0.50	0.44	0.24
	$\delta^{13}C$	0.42	0.26	–0.32	–0.09	0.56	–0.45	0.81	0.41	0.34	0.05	–0.24
Black Noddy n = 3	∑PCBs	1.00										
	HCB	0.95	1.00									
	∑HCHs	0.17	0.59	1.00								
	∑DDTs	0.65	0.86	0.20	1.00							
	$\delta^{15}N$	0.52	0.21	0.13	0.72	1.00	–0.20	0.81	–0.02	–0.66	–0.68	
	$\delta^{13}C$	–0.62	–0.34	–0.74	0.19	0.94	–0.52	0.56	–0.36	–0.88	–0.89	
Masked Booby n = 8	∑PCBs	1.00										
	HCB	0.97	1.00									
	∑HCHs	0.23	0.55	1.00								
	∑DDTs	0.95	0.98	0.34	1.00							
	$\delta^{15}N$	0.88	0.33	0.27	0.83	1.00	0.20	0.02	–0.25	0.02	0.34	0.38
	$\delta^{13}C$	0.09	–0.04	–0.35	–0.14	–0.22	0.50	0.30	0.29	0.21	–0.05	–0.05
All seabird samples	∑PCBs	1.00										
	HCB	0.71	1.00									
	∑HCHs	0.39	0.49	1.00								
	∑DDTs	0.86	0.71	0.34	1.00							
	$\delta^{15}N$	0.59	0.40	0.26	0.58	1.00	0.42	0.59	0.46	0.49	0.50	0.48
	$\delta^{13}C$	0.37	0.45	0.21	0.27	0.54	0.21	0.45	0.43	0.35	0.16	0.14

[a] Significant results ($p < 0.05$) shown in bold. Cl# refers to the PCB chlorination level.

We found significant correlations of $\delta^{15}N$ with HCB, DDTs, total PCBs, and PCB chlorination levels. This demonstrates its usefulness as a marker of trophic position and, consequently, an indicator of biomagnification. While we did not find significant correlations between $\delta^{15}N$ and HCHs in nearly all cases, significant correlations were found between $\delta^{15}N$ and PCB chlorination number groups when species were analyzed separately.

DISCUSSION

Persistent organic pollutants

Older individuals tend to have higher concentrations of POPs, which accumulate in tissues (Tanabe *et al*. 1998); our data provides further evidence of this. This can cause deleterious effects in seabirds, given their longevity. Members of the family Sternidae (Sooty Tern, Brown Noddy, and Black Noddy) generally reach sexual maturity at 2–3 years of age and live for an average of 20 years (Burger & Gochfeld 2001). Sulids (Masked Booby and Brown Booby) begin to breed at 3–6 years and live for ~20 years, with some individuals reaching 40 years (Carboneras 1992).

DDT concentrations in liver samples resulted from the presence of *p,p′*-DDE, *o,p′*-DDE, *p,p′*-DDT, and *o,p′*-DDT. DDEs (*p,p′* and *o,p′* orientations) represented more than 70% of DDT compounds in the seabirds analyzed. Moreover, *p,p′*-DDE was the most abundant compound in all species, accounting for over 40% of the total mean values in some cases (Fig. 3). This compound is a product of the oxidative biotransformation of *p,p′*-DDT and is commonly found in tissues and eggs of birds due to its high degree of chemical stability and persistence in the environment (Ohlendorf *et al*. 1978). Although DDTs were banned decades ago, high concentrations of *p,p′*-DDE are still found in top predatory birds due to bioaccumulation and biomagnification (Jones & de Voogt 1999, Cipro *et al*. 2013). Bouwman *et al*. (2012) detected DDT in eggs of Sooty Tern and Masked Booby on the Rodrigues Islands (western Indian Ocean), with values on the same order of magnitude as measured for the same birds in the present study. However, Bouwman found higher mean values for Sooty Tern compared to Masked Booby, which is different from our present findings. This might be because both species occupied the same trophic level in the Bouwman study, whereas in the present work, Masked Booby tended to be at a higher level than Sooty Tern; this is consistent with the findings of Mancini *et al*. (2014) and confirmed by our $\delta^{15}N$ results.

As for HCHs, the technical mixture (generally 60%–70% α, 5%–12% β, 10%–12% γ, and 6%–10% δ, with a α/γ ratio of 3–7; Saadati *et al.* 2012) marketed in the past has been replaced with pure Lindane (purified γ-HCH) in Europe and the Americas (Breivik *et al*. 1999). Thus, the absence of detectable levels of α-HCH (the predominant product in the technical mixture) and β-HCH (an isomer with more persistent, bioaccumulative, and toxic characteristics; Walker *et al*. 1999) may indicate that the technical mixture has not been used recently in regions affecting the foraging areas of the birds analyzed here. This class of contaminants is known to have high rates of elimination and is therefore considered less persistent in seabirds than other organochlorine contaminants, such as PCBs and DDTs (Moisey *et al*. 2001). According to Pandit *et al*. (2001), HCHs volatilize and degrade quickly in tropical areas. In combination with atmospheric transport, this may explain the low concentrations we observed.

We found no evidence for biomagnification of HCB in any species we studied. This contaminant is a relatively volatile compound that can be transported over long distances. Rocas Atoll, despite being located at low latitude, has nevertheless exhibited detectable concentrations, likely due to aerial transport. High concentrations are more commonly found in species occurring in polar regions (Van den Brink 1997, Cipro *et al*. 2012).

Due to the considerable environmental persistence of heavier PCB congeners, biomagnification leads to both higher values throughout the food chain as well as heavier qualitative profiles (Fuoco & Ceccarini 2001). Predominance of PCB compounds with light and intermediate mass, particularly tetra- and penta-chlorinated groups followed by the tri-chlorinated groups, indicates long-range atmospheric transport as the source of pollutants in the Rocas Atoll ecosystem. Compounds with a lower degree of chlorination have greater volatility, are less hydrophobic, and can disperse in the atmosphere by both wet and dry means (adsorbed onto particulate matter or as an aerosol; von Waldow *et al*. 2010).

Regarding the qualitative profile of PCBs, Masked and Brown Booby exhibited proportionally heavier profiles, followed by Brown and Black Noddy. Sooty Tern exhibited proportionally lighter profiles. This might be due to greater longevity among sulids (Carboneras 1992, Burger & Gochfeld 2001) when compared to the other species, and because the one adult Masked Booby in our study showed concentrations roughly four times higher and proportionally heavier (profiles concentrating in hexa- and hepta-chlorinated congeners) than those of the remaining juveniles.

Bouwman *et al*. (2012) found slightly different qualitative and quantitative PCB profiles in eggs of Brown Noddy and Sooty Tern than we did. They observed that heavier groups (penta- and hexa-chlorinated) predominated and mean concentrations in seabird livers were one order of magnitude smaller than the values we found. These authors stressed the direct influence of atmospheric transport as a source of contamination for their area of study, but also discussed the transference of the most hydrophobic compounds from females to eggs, which tends to alter PCB profiles.

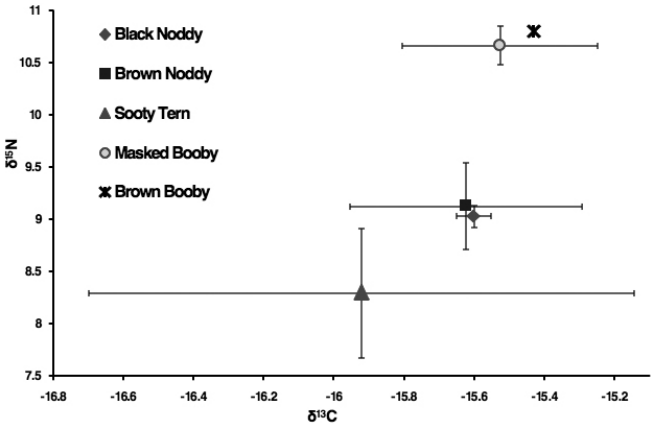

Fig. 5. Isotopic ratios of ^{13}C and ^{15}N (‰) in seabird species from Rocas Atoll.

As noted by Schulz-Neto (2004), the diet of Masked and Brown Booby studied at Rocas Atoll consists primarily of pelagic fish in significantly higher proportions compared to other seabird species of the Atoll. This places them in a higher trophic level than the other birds (confirmed by our $\delta^{15}N$ values), which primarily consume larval fish and small mollusks. This is consistent with the heavier qualitative PCB profile and with the larger absolute values of total PCBs in the present study. This conclusion is supported by the findings of Mancini *et al*. (2014), who used an SIA approach to establish this comparatively higher trophic level for these species.

Dias *et al*. (2013) found qualitative PCB profiles and concentrations in Brown Booby from the São Pedro and São Paulo Archipelago that are similar to those reported in the present study, demonstrating the ubiquitous nature and bioaccumulation of these contaminants in similar tropical oceanic environments.

Stable isotopes

In addition to our results for different homogenous groups of POPs, Mancini *et al*. (2014) used $\delta^{13}C$ to determine that the Rocas Atoll avifauna segregated into two groups (small and large birds) with significant differences in $\delta^{13}C$ and $\delta^{15}N$ for sympatric species. Our $\delta^{13}C$ data show a comparatively higher proportion of pelagic prey for the Sooty Tern and, to a lesser degree, the Masked Booby. This result is consistent with an earlier study by Schulz-Neto (2004), who inferred an overall segregation in the diet and foraging areas of these species, coupled with a significant difference in prey size. The larger digestive tract in Masked Booby allows them to capture significantly larger prey than can Sooty Tern (Schulz-Neto 2004, Mancini *et al*. 2014). This is important because body size is an excellent predictor of trophic position (as confirmed by $\delta^{15}N$) (Jennings *et al*. 2001) and because of biomagnification (discussed further below).

However, Marques *et al*. (2011) and Schulz-Neto (2004) claim that the Sooty Tern is the only breeding bird of Rocas Atoll with observable migratory habits. This is based on the great variation in $\delta^{13}C$ values from individual Sooty Terns in our study and in the literature (Marques *et al*. 2011), which is caused by the latitudinal gradient in its migration (Cherel *et al*. 2007). Marques *et al*. (2011) found similar isotope ratios to those reported here for the Sooty Tern. Using these, they were able to determine wintering areas by comparing isotopic values in feathers collected mainly in the oligotrophic Sargasso Sea, northwest of Rocas Atoll (Casey *et al*. 2007). These findings support the hypothesis of Schulz-Neto (2004) that Sooty Terns use foraging areas with relatively low productivity to reduce competition with boobies, for example. It also explains the relatively low $\delta^{15}N$ results and contaminant levels we found in Sooty Terns. In turn, $\delta^{15}N$ values for boobies were higher than for those of the other species, confirming their higher trophic level. Few significant correlations were found when the species were analyzed separately, particularly between $\delta^{15}N$ and PCBs chlorination levels, likely due to the small sampling size of some species (Table 3). Thus, despite the validity of having several samples of a single species for comparison, caution must be taken when grouping samples of different species.

Relationship between POPs and SIA

Since $\delta^{15}N$ presented no significant correlations to HCB, this may indicate a lack of biomagnification, or it may indicate $\delta^{15}N$ variations within the same trophic level due to baseline differences in primary producers (Cipro *et al*. 2017).

Some interesting $\delta^{13}C$ correlations were apparent, mostly for the Sooty Tern and for the whole dataset; these were likely affected by sample size. In both cases, $\delta^{13}C$ values positively correlated to ΣPCBs, HCB, ΣDDTs, and $\delta^{15}N$. This might be due to two factors: first, the weak trophic enrichment of $\delta^{13}C$ (DeNiro & Epstein 1978), and second, the migration and/or shift between inshore/offshore prey (Cipro *et al*. 2017). These results would indicate a comparatively higher exposure of birds consuming inshore prey, which are enriched in $\delta^{13}C$ (Corbisier *et al*. 2004, Cipro *et al*. 2017). With respect to the $\delta^{15}N$ correlations (significant for Sooty Tern and for the whole dataset), $\delta^{13}C$ and $\delta^{15}N$ values are typically positively correlated in marine systems, but this relationship can break down when birds forage across biomes (Hobson & Welch 1992) or when carbon is derived from lipids and carbohydrates in addition to protein (Blight *et al*. 2015).

Comparison with other ecosystems

Taking all the previously discussed limitations into account, our data assessed background contaminant levels in the avifauna of Rocas Atoll. We have compared these results to those from other remote locations (Table 4). Seabirds in Arctic ecosystems are largely affected by polar input of contaminants via "grasshopper effect/global distillation" (i.e., the evaporation and deposition cycles of volatile contaminants that transport them from warmer to colder regions) and by the comparative proximity with the most industrialized countries (von Waldow *et al*. 2010); they exhibit POP levels two to three orders of magnitude higher than seabirds of Rocas Atoll. On the other hand, seabirds from the Canary Islands, which are similar in latitude and remoteness to Rocas Atoll, mostly present POP concentrations on a similar order of magnitude. This suggests similar contaminant dispersion and accumulation processes on both the Canary Islands and Rocas Atoll. For example, cold deposition (by wet or dry deposition, or simply by condensation) may produce similar contaminant exposure to seabird prey in both locations. In summary, where comparisons were possible, our findings agree quantitatively and qualitatively with results from related studies. Seabirds from Rocas Atoll had concentrations and contaminant profiles in accordance with their trophic level, albeit at a lower level than in other remote locations in the Northern Hemisphere.

Finally, some considerations about toxicity in the levels quantified in our study. EROD activity is a suitable and sensitive biochemical marker for exposure to and biological activity of dioxin-like compounds in mammals and birds (Bosveld *et al*. 2000 and references therein). Bosveld *et al*. (2000) presented evidence that hepatic CYP1A (i.e., EROD) activity could be induced at PCB concentrations of approximately 25 ng TEQ/g liver lipid in fish-eating birds (Common Tern *Sterna hirundo*). Considering the congeners and the lipid-to-wet-weight conversion, one may conclude that average concentrations of absolute POPs (especially PCBs) for all species in the present work could surpass this threshold and therefore induce EROD activity. This means that these birds may have experienced the negative effects already reported for this biomarker, such as delayed egg-laying, prolonged incubation periods, and smaller eggs and chicks (Bosveld *et al*. 1995, Murk *et al*. 1996). However, to the best of our knowledge, no EROD studies have been done for the species we studied, so these considerations must be interpreted with caution.

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TABLE 4
POP levels (ng·g^{-1} ww, except when marked with **, then ng·g^{-1} lw (lipid weight)) reported in bird liver samples from selected studies

Species	Location	Year	∑DDTs	∑PCBs	∑HCHs	HCB	Reference
Brown Booby	Brazil (offshore archipelago)	1979	51	155	nd	nd	Weber 1983
Brown Booby	Brazil (offshore archipelago)	2009	8.13	85.81	2.31	1.88	Dias *et al.* 2013
Yellow-legged Gull *Larus michahellis* [a]	Turkey (Estuarine area)	2009	273–334	261–670	19–38	nd	Kocagöz *et al.* 2014
Glaucous-winged Gull *Larus glaucescens*	Alaska (Aleutian archipelago)	2000–2001	87–478	569–1812	37–142	nd	Ricca *et al.* 2008
Northern Fulmar *Fulmarus glacialis*	Alaska (Aleutian archipelago)	2000–2001	98–152	447–782	30–68	nd	Ricca *et al.* 2008
Tufted Puffin *Fratercula cirrhata*	Alaska (Aleutian archipelago)	2000–2001	25–51	139–552	12–53	nd	Ricca *et al.* 2008
Thick-billed Murre *Uria lomvia* [a]	Eastern Canadian Arctic	2007–2008	1175–3125	1454–2897	48.7–288	358–1645	Braune *et al.* 2014
Northern Fulmar [a]	Eastern Canadian Arctic	2007–2008	5759–6048	9184–11190	90.3–99.7	985–1440	Braune *et al.* 2014
White-tailed Eagle *Haliaeetus albicilla* [a]	Greenland (southwest coast)	1997–2009	7200	11000	220	780	Jaspers *et al.* 2013
Eurasian Sparrowhawk *Accipiter nisus*	Spain (Canary Islands)	2009–2012	1955.8	35.6	190.2	2.7	Luzardo *et al.* 2014
Long-eared Owl *Asio otus*	Spain (Canary Islands)	2009–2012	475.8	0.9	141.8	0.4	Luzardo *et al.* 2014
Common Buzzard *Buteo buteo*	Spain (Canary Islands)	2009–2012	403.9	0.8	135.4	0.1	Luzardo *et al.* 2014
Barbary Falcon *Falco pelegrinoides*	Spain (Canary Islands)	2009–2012	974.07	32.3	207.6	0.3	Luzardo *et al.* 2014
Common Kestrel *Falco tinnunculus*	Spain (Canary Islands)	2009–2012	699.6	16.8	210.1	1.2	Luzardo *et al.* 2014
Western Barn Owl *Tyto alba*	Spain (Canary Islands)	2009–2012	597.9	0.6	72.4	0.01	Luzardo *et al.* 2014
Glaucous Gull *Larus hyperboreus* [a]	Norway (Svalbard Island)	2003–2005	165.8**	769.8	0.67	13.2	Sagerup *et al.* 2009
Sooty Tern	Brazil (Rocas Atoll)	2010–2011	2.58	23.6	1.96	2.65	Present work
Brown Noddy	Brazil (Rocas Atoll)	2010–2011	5.93	65.2	1.78	3.93	Present work
Black Noddy	Brazil (Rocas Atoll)	2010–2011	6.79	72	4.44	2.97	Present work
Masked Booby	Brazil (Rocas Atoll)	2010–2011	7.4	60.9	2.16	3.18	Present work
Brown Booby	Brazil (Rocas Atoll)	2010–2011	11.8	85.2	276	6.41	Present work

[a] (*p*, *p*′-DDE only); nd: not determined

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