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Trophic ecology drives contaminant concentrations within a tropical seabird community☆

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A B S T R A C T

To support environmental management programs, there is an urgent need to know about the presence and understand the dynamics of major contaminants in seabird communities of key marine ecosystems. In this study, we investigated the concentrations and trophodynamics of trace elements in six seabird species and persistent organic pollutants (POPs) in three seabird species breeding on Grand Connétable Island (French Guiana), an area where the increase in human population and mining activities has raised concerns in recent years. Red blood cell Hg concentrations in adults were the highest in Magnificent frigatebirds *Fregata magnificens* (median: 5.6 μg g^{-1} dw; range: 3.8–7.8 μg g^{-1} dw) and lowest in Sooty terns *Onychoprion fuscatus* (median: 0.9 μg g^{-1} dw; range: 0.6–1.1 μg g^{-1} dw). Among POPs, dichlorodiphenyldichloroethylene (*p,p'*-DDE) was the most abundant compound in plasma of Cayenne terns *Thalasseus sandvicensis* (median: 1100 pg g^{-1} ww; range: 160 ± 5100 pg g^{-1} ww), while polychlorinated biphenyls (PCBs) were the most abundant compound class in plasma of Magnificent frigatebirds (median: 640 pg g^{-1} ww; range 330 ± 2700 pg g^{-1} ww). While low intensity of POP exposure does not appear to pose a health threat to this seabird community, Hg concentration in several adults Laughing gulls *Leucophaeus atricilla* and Royal terns *Thalasseus maximus*, and in all Magnificent frigatebirds was similar or higher than that of high contaminated seabird populations. Furthermore, nestling red blood cells also contained Hg concentrations of concern, and further studies should investigate its potential health impact in this seabird community. Differences in adult trophic ecology of the six species explained interspecific variation in exposure to trace element and POPs, while nestling trophic ecology provides indications about the diverse feeding strategies adopted by the six species, with the consequent variation in exposure to contaminants.

1. Introduction

Exposure to persistent toxicants may have detrimental effects on reproductive success, immunity, regulation of oxidative balance, endocrine system and survival perspectives of wildlife, even years after these toxicants have been banned (Burger and Gochfeld, 2001; Costantini et al., 2014; Erikstad et al., 2013; Goutte et al., 2015; Tartu et al., 2015a, 2015b). Persistent organic pollutants (POPs) are among the major contaminants currently detected in wildlife, of which polychlorinated biphenyls (PCBs) remain the most dominant chemical class despite that they have been banned more than 30 years ago (Tartu et al., 2015c). Among trace elements there has been growing interest in mercury (Hg), lead (Pb), and cadmium (Cd) because of their well-known detrimental effects on vertebrates

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(Beyer et al., 2011). For example, after Hg is deposited in aquatic ecosystems, it is rapidly transformed by microorganisms into methyl-Hg, its most toxic form that bioaccumulates in organisms and biomagnifies in food webs (Fitzgerald et al., 2007). Because seabirds are apex long-lived predators, they are particularly exposed to these major environmental contaminants (Rowe, 2008), and are therefore utilized as sentinel species for environmental monitoring (Furness and Camphuysen, 1997; Moreno et al., 2011).

While considerable attention has been paid to the occurrence and health effects of these contaminants in sub-polar and polar regions due to their potential to act as final sink (Blévin et al., 2016; Tartu et al., 2015b, 2016), comparatively less attention has been given to wildlife from other geographical regions (Bastos et al., 2015; Costantini et al., 2017; De Andres et al., 2016; Frery et al., 2001), especially in South America (De Andres et al., 2016; Guirlet et al., 2010; Sebastiano et al., 2016). This is surprising because local releases of contaminants (e.g. Hg) from major mining activities in the Amazon area may be considerable (Fujimura et al., 2012; Lodenius and Malm, 1998). Although to the best of our knowledge there are no sources of organic pollutants, the long-range transport of these contaminants and the bioaccumulation and biomagnification processes they undergo, might pose a threat to top predators. The Grand Connétable Island, a small rocky island located off the coast of French Guiana and close to the Brazilian border, with its strategic position for wildlife and the presence of six breeding seabird species, offers a unique opportunity to assess the presence and quantify the concentrations of both organic and inorganic pollutants in a tropical seabird community. Moreover, given the expected high variation in trophic ecology of the seabirds breeding on the Grand Connétable Island, this also enabled us to assess the importance of feeding ecology in driving inter- and intraspecific variation in exposure to contaminants.

To this end, we quantified the concentrations of POPs and trace elements in plasma and red blood cells, respectively, in the seabird community on Grand Connétable Island. We also measured the stable nitrogen and carbon isotope composition of red blood cells to test whether trophodynamics explain among and within species variation in contaminant burden. Of the trace elements, we focused particularly on Hg given the growing concern about the impact of this element on the health of South-American ecosystems.

2. Materials and methods

2.1. Sample collection

In 2013 we performed sample collection on the Grand Connétable Nature Reserve, a small island located 18 km off Cayenne (French Guiana, 4°49′30 N; 51°56′00 W). The seabird community of Grand Connétable Island typically includes six species: the Laughing gull *(Leucophaeus atricilla)*, the Brown noddy *(Anous stolidus)*, the Royal tern *(Thalasseus maximus)*, the Cayenne tern *(Thalasseus sandvicensis)*, the Magnificent frigatebird *(Fregata magnificens;* hereafter Frigatebird*)*, and the Sooty tern *(Onychoprion fuscatus)* (Dujardin and Tostain, 1990). These six seabird species differ in both feeding style (pelagic versus benthic) and foraging area (inshore versus offshore). For instance, Frigatebirds feed on both pelagic and benthic fish by surface dipping, kleptoparasitism and opportunistic feeding (mostly on shrimp trawler discards), and although most foraging occurs in coastal waters, some foraging trips can exceed 200 km away from the breeding colony (Weimerskirch et al., 2003). Finally, they are also seen to follow tuna school formations because tuna and other marine predators push other small fish toward the surface, making them accessible to frigatebirds. The Laughing gull feeds on coastal pelagic fish, marine invertebrates and fishery discards, while the Brown noddy feeds on fish and squid in offshore waters by dipping the surface, and may show kleptoparasitism. Terns diet consists predominantly of small fish, squids and crustaceous, obtained by dipping the surface and occasionally diving (del Hoyo et al., 1996; Dujardin and Tostain, 1990).

Adult seabirds were sampled during the incubation or early chick rearing (27th to 30th of May) while nestlings were sampled within a few weeks after adult sampling (24th to 26th of June). A total of 101 adults and 102 nestlings were captured. Since all nestlings were captured by hand on their nests while adults were captured by mist nets (or, in case of frigatebirds, were captured with a noose attached to a fishing rod), adults and nestlings are likely unrelated to each other. For the Laughing gull, Royal tern, Cayenne tern, Sooty tern and Brown noddy, the egg laying period usually begins around mid-April and ends around the end of April (Dujardin and Tostain, 1990), and the incubation period lasts 25–30 days (except for the Brown noddy that can take a few more days; Dujardin and Tostain, 1990). Therefore, nestlings of these species had approximately the same age. Frigatebird, instead, were a few weeks older than the nestlings of the other species, with an approximate age of three to four months (the age of nestling frigatebirds was incorrectly reported in Sebastiano et al., 2016). Blood samples (around 2 mL) were collected from the brachial vein using a heparinized syringe (25 G needle) within a few minutes after capture, and samples were immediately put on ice. Blood was then centrifuged within one hour to separate plasma and red blood cells. Both fractions were kept at −20 °C until laboratory analyses.

2.2. Stable isotope analysis

The analysis of the carbon and nitrogen stable isotopes is considered an important tool for the interpretation of both the foraging area and the trophic level of the species. In marine ecosystems, higher nitrogen values are associated with higher trophic level prey, e.g. bigger prey (Overman and Parrish, 2001), while the carbon stable isotopes can decrease with decreasing latitudes (Kelly, 2000) and seem to decrease from the coast to the open sea in the Southern Indian Ocean (Cherel and Hobson, 2007), even if proofs of such stratification in the Southern Atlantic Ocean are not available. In this study, the stable carbon and nitrogen values were measured in red blood cells, therefore providing trophic information integrated over a few weeks prior to sampling (Hobson and Clark, 1993; Newsome et al., 2007). The composition of the carbon and nitrogen isotopes of the species, which provides information on the isotopic niches of the birds, was used as a proxy of their ecological niche (Jackson et al., 2011). Analyses were carried out following a previous protocol (Sebastiano et al., 2016), and results are expressed as δ (‰) for δ^{13}C and δ^{15}N, respectively, calibrated against the international isotopic references (atmospheric nitrogen for δ^{15}N and Pee Dee Belemnite for δ^{13}C). The experimental imprecision, based on secondary isotopic reference material, did not exceed ± 0.15 and ± 0.20‰ for δ^{13}C and δ^{15}N, respectively.

2.3. Contaminant analysis

Trace element concentration analyses (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn) were performed by the Littoral Environnement et Sociétés (LIENSs) laboratory on lyophilized red blood cells as previously described (Sebastiano et al., 2016). In brief, an Altec Advanced Mercury Analyzer AMA 254 spectrophotometer was used for the quantification of total Hg, while the other trace elements were quantified using a Varian Vista-Pro ICP-OES or a Series II Thermo Fisher Scientific ICP-MS (aliquots mass: 3–8 mg for AMA and 50–200 mg dw for ICP). Analyses on blanks and Certified Reference Materials (CRM) from NRCC (dogfish liver DOLT-4 and lobster hepatopancreas TORT-2) were carried out as for the samples. Since results showed low standard deviations and were in good agreement with the certified values, the methodology showed good repeatability. Quantification limits and mean recovery rates were, respectively, equal to 0.1 μg L^{-1} and 79% for Ag, 1 μg L^{-1} and 94% for As, 0.1 μg L^{-1} and 99% for Cd, 0.1 μg L^{-1} and 97% for Co, 0.1 μg L^{-1} and 95% for Cr, 0.5 μg L^{-1} and 96% for Cu, 20 μg L^{-1} and 92% for Fe, 0.5 μg L^{-1} and 94% for Mn, 0.2 μg L^{-1} and 99% for Ni, 0.1 μg L^{-1} and 89% for Pb, 0.5 μg L^{-1} and 118% for Se, 2 μg L^{-1} and 98% for V, and 20 μg L^{-1} and 105% for Zn. All trace element concentrations are expressed as μg g^{-1} dry weight (dw).

Persistent organic pollutant analyses were carried out at the University of Antwerp (Toxicological Centre), following a previous protocol (Sebastiano et al., 2016). The protocol allowed the detection of 26 PCB congeners (CB 28, 49, 52, 74, 99, 101, 105, 118, 128, 138, 146, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196, 199, 203, 206, and 209), organochlorine pesticides (OCPs), amongst which dichlorodiphenyltrichloroethane (*p,p′*-DDT) and its metabolite dichlorodiphenyldichloroethylene (*p,p′*-DDE), hexachlorobenzene (HCB), α, β- and γ-hexachlorocyclohexanes (HCHs), the Chlordanes (CHLs) *cis*-nonachlor (CN), *trans*-nonachlor (TN), and oxychlordane (OxC), and 7 polybrominated diphenyl ethers (PBDEs: BDE 28, 47, 99, 100, 153, 154, and 183). Plasma samples (around 1 mL) were analysed for POPs. Solid-phase extraction (SPE) on OASIS HLB cartridges was used followed by fractionation on SPE cartridges topped with 1.5 g of acidified silica (44% H_2SO_4, w/w) and eluted with 10 mL hexane: dichloromethane (1:1). The cleaned extract was evaporated to incipient dryness and re-dissolved in 100 μL isooctane. POPs were further analysed by gas chromatography coupled to mass spectrometry operated either in electron capture negative chemical ionization (GC-ECNI-MS) or electron ionization (GC-EI-MS) depending on the analyses' sensitivity. All plasma POP concentrations are expressed as pg g^{-1} wet weight (ww). POPs were analysed in the Cayenne tern, Frigatebird, and Brown noddy. These species were chosen because they differ in the feeding strategies and they are representative of the region.

2.4. Quality assurance/quality control

The extraction, clean-up, and fractionation steps were evaluated following a previous protocol (Dimitriadou et al., 2016). Mean ± SD recoveries of the internal standards PCB 143, ε-HCH and BDE 77 were 86± 6%, 98± 8% and 93± 10%, respectively. The quality control was performed by regular analyses of procedural blanks, sample replicates, by random injection of standards, spiked samples and solvent blanks. The quality control scheme was also assessed through regular participation to inter-laboratory comparison exercises (POPs in serum) organized three times per year by the arctic monitoring and assessment program (AMAP, 2015). The obtained values were deviating with less than 20% from the consensus values.

2.5. Statistical analysis

Concentrations of trace elements and POPs below the limit of quantification (LOQ) were replaced with a value equal to ½*LOQ when the detection frequency was greater than 50%. For Ag, Co, Cr, and V, concentrations were below the LOQ for all individuals (both adults and nestlings), and were therefore excluded from the statistical analyses. Furthermore, since the concentration of Se is important in detoxifying Hg (Ralston and Raymond, 2010), the molar ratio Hg:Se was also calculated using the formula "[concentration Hg (μg g^{-1})/atomic weight Hg (g mol^{-1})]/[concentration Se (μg g^{-1})/atomic weight Se (g mol^{-1})]".

Linear models were used to test trace element and POP concentration variations across the six species and both age classes. When the interaction between *species* and *age* was significant, statistical testing was performed for adults and nestlings separately in order to increase the statistical power by excluding biologically meaningless post-hoc comparisons (e.g. adults of one species versus nestlings of another species). Then, a principal component analysis (PCA) based on the correlation matrix was used to reduce contaminant data to fewer uncorrelated variables. This approach (which has been used only on trace elements since POP concentrations were very low in all species), enabled us to investigate if the contaminant pattern was similar between age classes. The Kaiser-Mayer-Olkin measure of sampling adequacy (KMO = 0.58 in nestlings and KMO = 0.67 in adults) and the Bartlett's test of sphericity ($P < 0.01$ in both groups), confirmed the appropriate use of the PCA. After the scree plot was examined, components with an eigenvalue >1 were selected. Parametric correlations were used to test associations between stable isotope values in the different species and age classes, which is important to describe the association between the trophic level of the species and the location of the food source. Any data transformation to achieve normality or any violation of models assumptions is reported in the manuscript when necessary. The graphic representation of the isotopic niche of the different species has been performed using Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al., 2011). In order to graphically compare individual groups within the community with each other, we used the Standard Ellipse Area (SEA) method (Jackson et al., 2011). In this package, the stable carbon and nitrogen isotopes are used to calculate the isotopic niche of each species in the community, allowing a comparison among species. Furthermore, the calculation of the ellipse area of each species is not influenced by the sample size, allowing a graphical and statistical comparison among different species or diverse studies with different samples sizes (Jackson et al., 2011). Furthermore, linear models estimating Hg or *log*-transformed POP class concentrations based on stable isotope values were also carried out on both the pooled data and separately for each species. For pooled data (overall models), linear mixed models using species as a random effect were applied in order to control for the non-independence of data points. All statistical analyses were performed using R (3.1.1 version).

3. Results

3.1. Stable isotopes

The overall correlation between $\delta^{13}C$ and $\delta^{15}N$ was significant both in adults ($n = 101$; $r = 0.54$; $P < 0.01$; Fig. 1, Table 1) and nestlings ($n = 102$; $r = 0.88$; $P < 0.01$; Fig. 1, Table 1), and density plots of the Standard Ellipse Area (SEA) representing the niche of the six species are shown in Fig. 2. Species-specific correlations between isotopes, which explain the relationship between the trophic level and the foraging area of each species, are shown in Table 1. $\delta^{13}C$ and $\delta^{15}N$ levels significantly differed among species and also within the same species between adults and nestlings ($\delta^{13}C$: $F = 36.40$, $P < 0.01$ and $\delta^{15}N$: $F = 21.50$, $P < 0.01$, respectively), and interspecific differences in the isotope values between adults and nestlings are shown in Fig. S1 and Table S1.

3.2. Trace elements

Cd was detected only in the Brown noddy (both adults and nestlings) and in Sooty tern adults, while Ni was detected in Laughing gull and Brown noddy nestlings only. Linear models for Cu and Pb did not show a significant interaction between species and age, and post-hoc analysis was not carried out. For the other

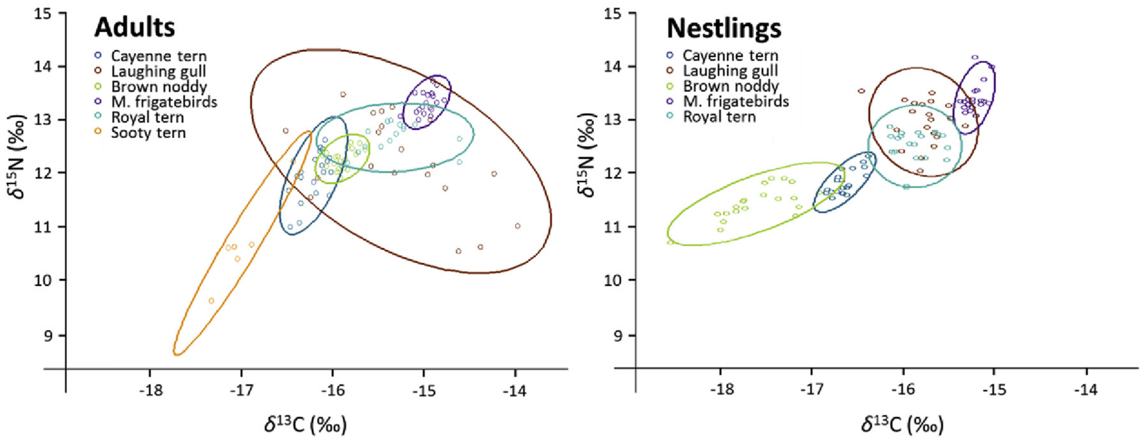

Fig. 1. Individual δ^{15}N and δ^{13}C values (‰) in adults (left) and nestlings (right) of the six seabird species from the Grand Connétable Island, French Guiana.

Table 1
Pearson's correlations between δ^{13}C and δ^{15}N in adults and nestlings of the six seabird species from the Grand Connétable Island, French Guiana. Significant correlation values are bolded. ND refers to the inability to carry out statistical testing due to a low sample size.

Species	Adults			Nestlings		
	n	*r*	*P*	*n*	*r*	*P*
Cayenne tern	20	0.63	**<0.01**	20	0.75	**<0.01**
Laughing gull	20	−0.52	**0.02**	20	−0.15	0.54
Brown noddy	20	0.43	0.06	20	0.71	**<0.01**
Frigatebirds	20	0.48	**0.03**	20	0.43	0.06
Royal tern	15	0.09	0.74	20	0.01	0.96
Sooty tern	6	0.96	**<0.01**	2	ND	ND
Overall	101	0.54	**<0.01**	102	0.88	**<0.01**

trace elements (As, Fe, Hg, Mn, Se, and Zn), the interaction between species and age was significant ($F > 5.60$; $P < 0.01$), and post-hoc comparison of contaminants within age classes are shown in Fig. 3. Average trace element concentrations, median along with standard deviation (SD) values and their range are shown in Table 2.

PCA on adults reduced the variation in the concentrations of eight trace elements to two principal components (PCs), explaining 55% of the total variance (Fig. 4a). The first axis indicated that adults with high PC1 scores are associated with high levels of As (loading = 0.497) and Se (0.441), and low levels of Cu (−0.396), Mn (−0.383), Pb (−0.371), Zn (−0.237), and Hg (−0.232), while the second axis indicated that adults with high PC2 scores are associated with low levels of Hg (−0.618) and high levels of Zn (0.448), Cu (0.397), Se (0.346), Pb (0.280), and As (0.223), as showed in Fig. 4a. The factor species significantly explained the variation of trace elements represented by both PC1 ($F = 75.70$; $P < 0.01$) and PC2 ($F = 47.40$; $P < 0.01$). Moreover, both PC1 and PC2 were significantly negatively correlated to δ^{13}C (PC1: $r = -0.61$; $P < 0.01$; PC2: $r = -0.57$; $P < 0.01$) and δ^{15}N (PC1: $r = -0.31$; $P < 0.01$ and PC2: $r = -0.63$; $P < 0.01$).

PCA performed on nestlings also reduced the variation of concentrations for the eight trace elements to two PCs, explaining 62% of the total variance (Fig. 4b). The first axis indicated that nestlings with high PC1 scores are associated with high levels of Se (loading = 0.458) and As (0.323) and low levels of Mn (−0.444), Fe (−0.380), Hg (−0.366), and Zn (−0.353), while the second axis indicated that nestlings with high PC2 scores are associated with high levels of Cu (0.629), Pb (0.438), and As (0.339), and low levels of Hg (−0.432), as showed in Fig. 4b. The factor species significantly explained the variation of trace elements represented by both PC1 ($F = 132.50$; $P < 0.01$) and PC2 ($F = 104.60$; $P < 0.01$). Moreover, PC1 was significantly negatively correlated to δ^{13}C ($r = -0.66$; $P < 0.01$) and δ^{15}N ($r = -0.75$; $P < 0.01$), while PC2 was not correlated with δ^{13}C nor δ^{15}N.

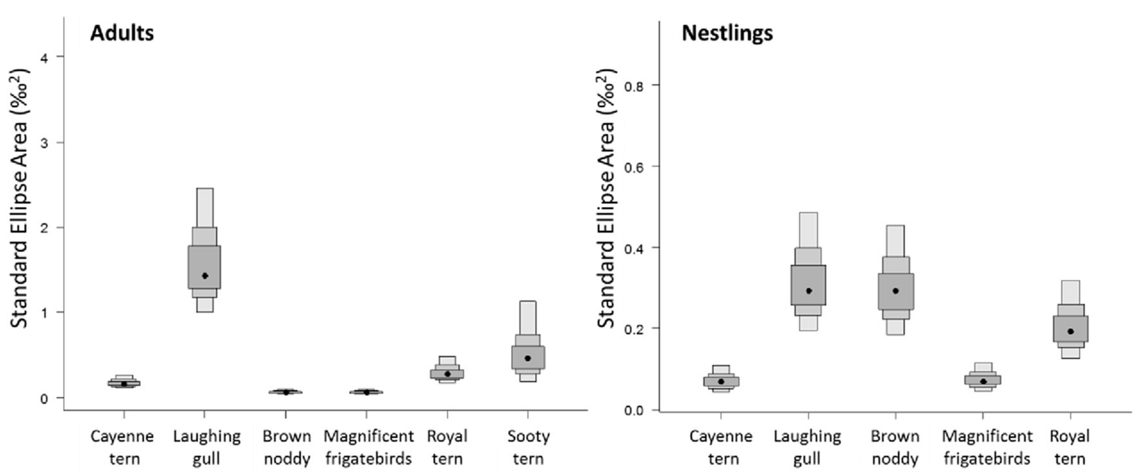

Fig. 2. Density plots of the standard ellipse area (SEA) based on δ^{15}N and δ^{13}C values (‰) in adults (left) and nestlings (right) of the six seabird species from the Grand Connétable Island, French Guiana. Black dots represent their mode, and the shaded boxes representing 50, 75 and 95% credible intervals from dark to light grey, respectively.

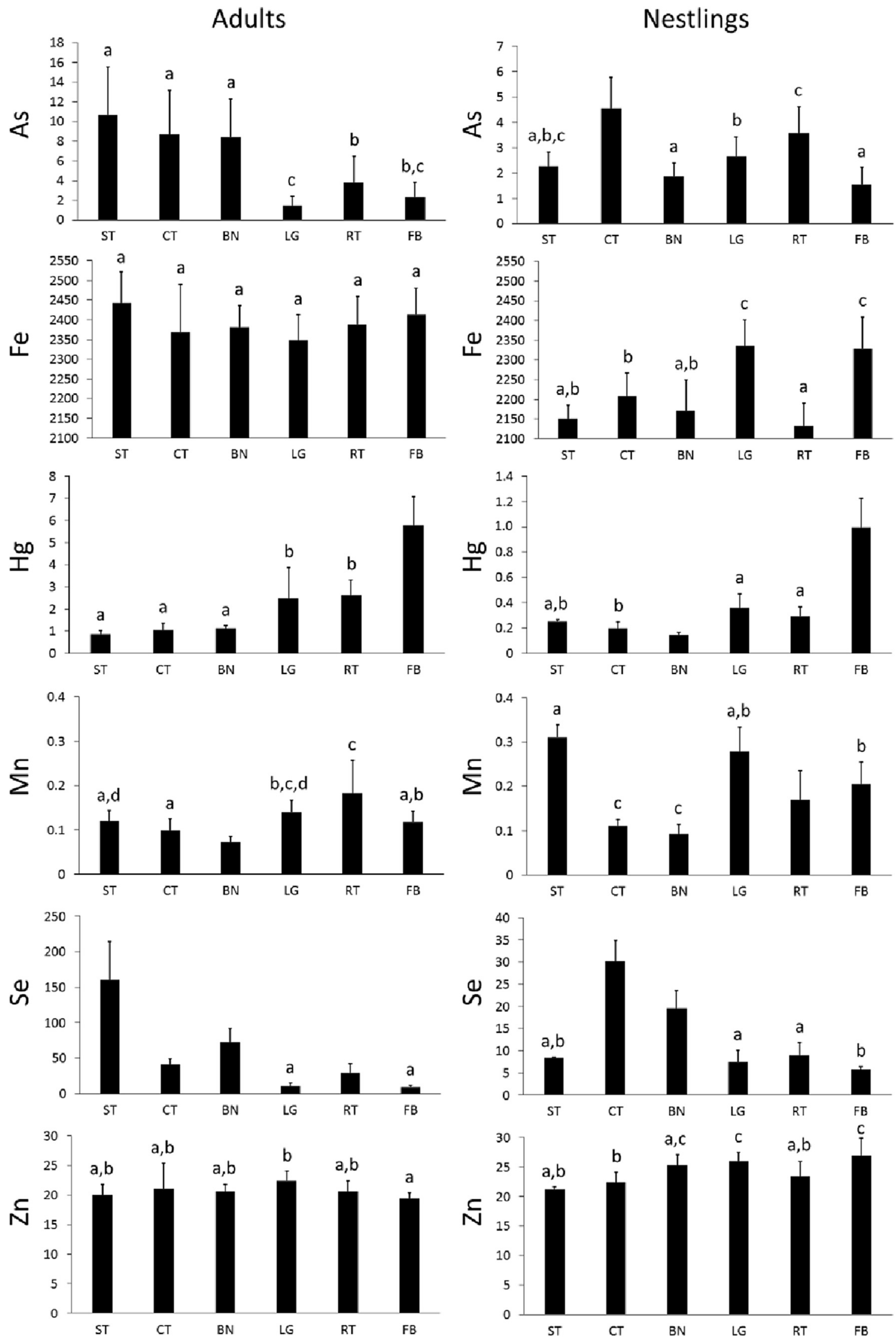

Fig. 3. Interspecific comparison for adult and nestling trace element concentrations (μg g^{-1} dw) in red blood cells of the six seabird species from the Grand Connétable Island, French Guiana. Values are mean ± SD. Species (arranged along increasing adult Hg levels) sharing the same letter have burdens that are not significantly different (Tukey HSD, $P > 0.05$). Nestlings' values of Hg and Se, and adults' values of As, Hg, Mn, Se, and Zn were *log₁₀*-transformed to achieve the requested normality for statistical tests. Species abbreviation: CT = Cayenne tern; LG = Laughing gull; BN = Brown noddy; FB = Magnificent frigatebird; RT = Royal tern; ST = Sooty tern.

Table 2
Red blood cell trace element concentrations (μg g^{-1} dw) in adults and nestlings of the six seabird species from the Grand Connétable Island, French Guiana. For each age class, first row values are mean ± SD and second row values represent the range (median). Detection frequencies were lower than 100% in the Ni content in Laughing gull nestlings (65%) and Brown noddy nestlings (55%). ND refers to non-detects. Frigatebirds data are earlier reported by (Sebastiano et al., 2016).

		Cayenne tern	Laughing gull	Brown noddy	Frigatebirds	Royal tern	Sooty tern
As	adults	8.7 ± 4.4	1.5 ± 1.0	8.4 ± 3.9	2.4 ± 1.4	3.9 ± 2.6	10.7 ± 4.9
		2.0–20.8 (9.1)	0.5–4.3 (1.1)	2.5–16.7 (7.8)	0.6–7.3 (2.1)	0.7–9.1 (3.0)	4.6–19.0 (9.2)
	nestlings	4.6 ± 1.2	2.7 ± 0.8	1.9 ± 0.5	1.6 ± 0.7	3.6 ± 1.0	2.3 ± 0.6
		2.2–6.2 (4.9)	1.3–3.9 (2.7)	1.1–2.8 (1.9)	0.7–3.6 (1.5)	2.0–6.3 (3.5)	1.9–2.7 (2.3)
Cd	adults	ND	ND	0.051 ± 0.021	ND	ND	0.026 ± 0.014
				0.034–0.118 (0.044)			0.015–0.050
	nestlings	ND	ND	0.015 ± 0.004	ND	ND	ND
				0.010–0.029 (0.015)			
Cu	adults	0.9 ± 0.1	1.3 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
		0.8–1.0 (0.9)	1.0–1.9 (1.3)	0.6–0.9 (0.8)	0.7–0.9 (0.8)	0.9–1.3 (1.1)	1.0–1.1 (1.1)
	nestlings	0.8 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	1.1±<0.1
		0.7–1.0 (0.8)	1.1–1.5 (1.3)	0.7–1.2 (0.8)	0.6–0.9 (0.7)	0.9–1.3 (1.1)	1.0–1.1 (1.1)
Fe	adults	2368 ± 123	2348 ± 65	2382 ± 54	2413 ± 68	2388 ± 71	2444 ± 78
		2000-2528 (2397)	2197-2432 (2363)	2269-2485 (2385)	2235-2503 (2411)	2226-2475 (2410)	2348-2582 (2441)
	nestlings	2207 ± 58	2337 ± 64	2171 ± 78	2330 ± 80	2133 ± 56	2151 ± 34
		2113-2326 (2205)	2178-2423 (2339)	2029-2302 (2164)	2146-2477 (2337)	2050-2286 (2123)	2127-2175 (2151)
Hg	adults	1.1 ± 0.3	2.5 ± 1.4	1.1 ± 0.1	5.8 ± 1.3	2.6 ± 0.7	0.9 ± 0.2
		0.6–1.7 (1.0)	0.5–5.8 (2.3)	0.9–1.4 (1.1)	3.8–7.8 (5.6)	1.5–3.8 (2.8)	0.6–1.1 (0.9)
	nestlings	<0.1 ± 0.1	0.4 ± 0.1	0.2±<0.1	1.0 ± 0.2	0.3 ± 0.1	0.3±<0.1
		0.1–0.3 (0.2)	0.2–0.6 (0.3)	0.1–0.2 (0.1)	0.7–1.7 (1.0)	0.2–0.4 (0.3)	0.2–0.2 (0.2)
Mn	adults	0.1±<0.1	0.1±<0.1	0.1±<0.1	0.1±<0.1	0.2 ± 0.1	0.1±<0.1
		0.1–0.2 (0.1)	0.1–0.2 (0.1)	0.1–0.1 (0.1)	0.1–0.2 (0.1)	0.1–0.3 (0.2)	0.2–0.2 (0.1)
	nestlings	0.1±<0.1	0.3 ± 0.1	0.1±<0.1	0.2 ± 0.1	0.2 ± 0.1	0.3±<0.1
		0.1–0.2 (0.1)	0.2–0.4 (0.3)	0.1–0.1 (0.1)	0.1–0.3 (0.2)	0.1–0.4 (0.2)	0.3–0.3 (0.3)
Ni	adults	ND	ND	ND	ND	ND	ND
	nestlings	ND	0.1 ± 0.1	0.1 ± 0.1	ND	ND	ND
			<0.1–0.2 (0.1)	<0.1–0.3 (0.1)			
Pb	adults	0.02±<0.01	0.06 ± 0.04	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.03	0.02 ± 0.00
		0.01–0.06 (0.02)	0.02–0.20 (0.04)	0.01–0.05 (0.01)	0.02–0.04 (0.02)	0.02–0.11 (0.02)	0.01–0.03 (0.01)
	nestlings	0.02±<0.01	0.04 ± 0.03	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
		0.01–0.06 (0.02)	0.02–0.11 (0.03)	0.01–0.03 (0.02)	0.01–0.03 (0.02)	0.01–0.05 (0.02)	0.01–0.02 (0.02)
Se	adults	41.6 ± 0.7.0	10.7 ± 3.8	72.7 ± 18.6	9.1 ± 1.9	28.8 ± 13.2	160.1 ± 54.1
		26.3–52.7 (41.7)	4.3–17.9 (10.3)	42.5–103.0 (71.7)	6.7–13.1 (8.7)	9.1–62.9 (27.9)	62.2–215.6 (173.1)
	nestlings	30.3 ± 4.6	7.4 ± 2.7	19.6 ± 3.9	5.8 ± 0.6	8.9 ± 2.9	8.4 ± 0.2
		22.2–39.0 (29.5)	5.0–15.6 (6.8)	12.4–27.7 (19.4)	4.6–6.6 (5.8)	4.8–16.8 (8.4)	8.3–8.5 (8.4)
Zn	adults	21.0 ± 4.3	22.4 ± 1.6	20.7 ± 1.1	19.4 ± 0.9	20.7 ± 1.7	20.0 ± 1.8
		17.0–37.4 (20.0)	18.5–24.4 (22.7)	18.9–22.8 (20.6)	18.3–22.1 (19.4)	19.0–24.8 (20.2)	18.5–23.1 (19.3)
	nestlings	22.4 ± 1.7	26.0 ± 1.4	25.3 ± 1.7	26.9 ± 3.0	23.5 ± 2.5	21.2 ± 0.4
		19.5–26.5 (22.1)	22.4–27.8 (26.1)	22.2–28.1 (25.1)	22.5–32.6 (26.8)	20.4–27.5 (22.9)	20.9–21.5 (21.2)

3.3. Mercury

Hg concentrations widely varied among species, ranging from a minimum in a Brown noddy nestling (0.1 μg g^{-1} dw, Table 2) and a maximum value in an adult Frigatebird (7.8 μg g^{-1} dw, Table 2). Concentrations in adults were highest in Frigatebirds (median: 5.6 μg g^{-1} dw; range: 3.8–7.8 μg g^{-1} dw) and lowest in Sooty terns (median: 0.9 μg g^{-1} dw; range: 0.6–1.1 μg g^{-1} dw). In nestlings, Hg concentrations were highest in Frigatebirds (median: 1.0 μg g^{-1} dw; range: 0.7–1.7 μg g^{-1} dw) and lowest in Brown noddy (median: 0.1 μg g^{-1} dw; range: 0.1–0.2 μg g^{-1} dw). Linear models showed a significant and positive association between Hg concentrations and $\delta^{15}N$ values in nestlings (Table 3, Fig. 5), and between Hg concentrations and $\delta^{13}C$ values both in adults and nestlings (Table 3).

In adult individuals, Hg increased with $\delta^{15}N$ in Frigatebirds, and Hg also increased with $\delta^{13}C$ in all species (Table 3). In nestlings, Hg increased with $\delta^{15}N$ in all species, while there was no association between Hg and $\delta^{13}C$ in any species (Table 3). Finally, the molar ratio Hg:Se did not exceed 1 in any sampled individual (both adults and nestlings). The Hg:Se ratio showed an average value of 0.053 ± 0.085 (median: 0.015), ranging from 0.001 in a Sooty tern adult to 0.407 in a Frigatebird adult.

3.4. POPs

POP measurements were only performed in the Cayenne tern, Frigatebird, and Brown noddy, and their concentrations widely varied across these three species in a compound-specific way. Since *p,p'*-DDT was not detected in more than half of the individuals per species, comparisons were made for *p,p'*-DDE only. Furthermore, statistics were not performed for POP compounds below the LOQ (Table 4). Average, median, ranges along with standard deviation (SD) values of POPs are shown in Table 4.

Among DDTs, *p,p'*-DDE was the most abundant POP in adult Cayenne tern (median: 1100 pg g^{-1} ww; range: 160–5100 pg g^{-1} ww), while PCBs were the most abundant POP class in adult Frigatebird (median: 640 pg g^{-1} ww; range: 330–2700 pg g^{-1} ww). Adult Brown noddy had very low levels of POPs in comparison with the other two species, while *p,p'*-DDE was the most abundant compound in this species (median: 200 pg g^{-1} ww; range: 5–600 pg g^{-1} ww). Further statistical analyses could be performed

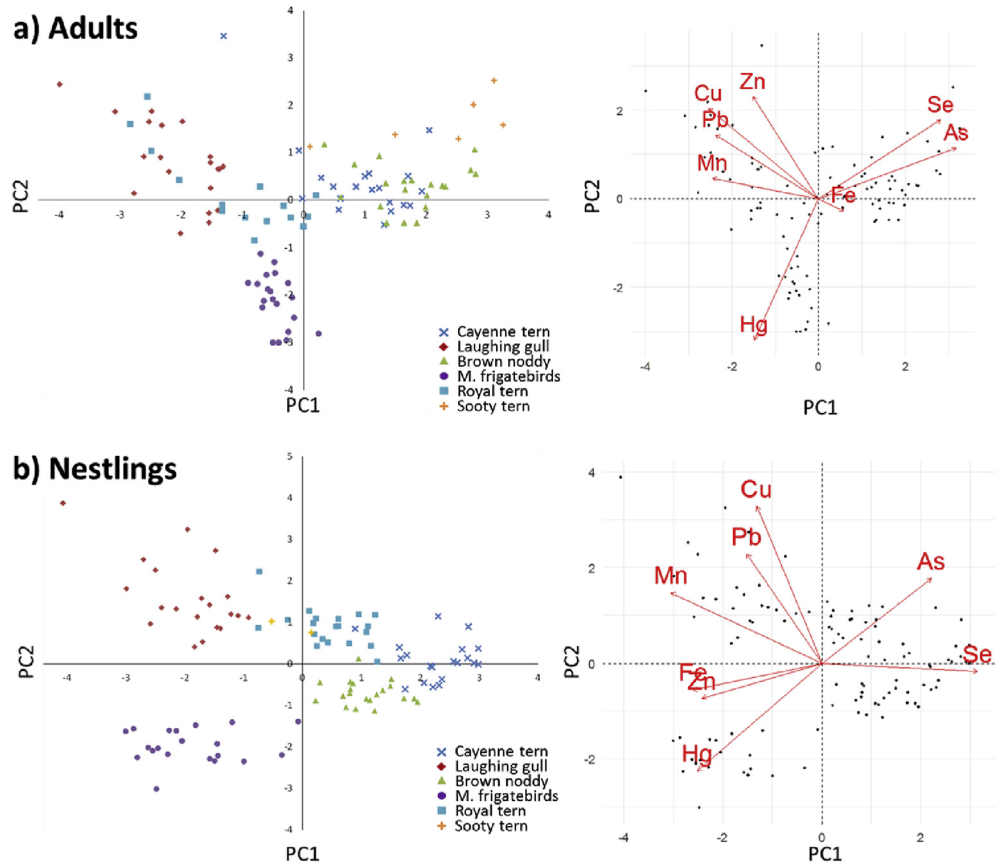

Fig. 4. Principal component analysis of trace element exposure in adult (a) and nestling (b) of the six seabird species from the Grand Connétable Island, French Guiana. The graphs on the left show species-specific PC values. On the right, each arrow represents a contaminant with the direction representing where the contaminants load in the principal component space. The length of the arrow represents the magnitude of the loading.

only for $\sum$PCBs and *p,p'*-DDE because all the other compounds were not detectable in all three species. Log-transformed $\sum$PCBs values showed significant differences among the three species ($F = 52.14$; $P < 0.01$), with Frigatebirds having higher PCB concentrations than both the Cayenne tern ($t = 2.78$; $P = 0.02$) and the Brown noddy ($t = 9.90$; $P < 0.01$), while the latter had lower PCB concentrations than Cayenne tern ($t = 7.12$; $P < 0.01$). Log-transformed *p,p'*-DDE values also showed significant differences among the three species ($F = 21.68$; $P < 0.01$), and concentrations in the Cayenne tern were higher than those in Frigatebirds ($t = -3.09$; $P < 0.01$) and Brown noddy ($t = 6.58$; $P < 0.01$), while the latter had lower *p,p'*-DDE concentrations than Frigatebirds ($t = 3.49$; $P < 0.01$). In adults, $\sum$PCBs did not increase with $\delta^{15}N$ and $\delta^{13}C$ (Table 3), while there was an association between *p,p'*-DDE and $\delta^{13}C$ (Table 3).

4. Discussion

The present study provides unique information on the occurrence and trophodynamics of trace elements and POPs in a seabird community from French Guiana on the east coast of South America. Our data showed the presence of high levels of Hg and strong associations with stable carbon and nitrogen isotopes, indicating that feeding is a driver of inter- and intra-specific variation in red blood cell levels of Hg. Furthermore, the strong interspecific variation in some trace elements was also explained by both the stable carbon and the nitrogen isotopes. Finally, our results confirmed that POPs in French Guiana marine seabirds occur at a very low concentrations.

4.1. Foraging ecology and trophic niche

Our study has found an overlap in the trophic niches of adults and nestlings for some species only (Fig. 1). Laughing gulls had a wide range of both $\delta^{13}C$ and $\delta^{15}N$ values, and was the only species to show a negative correlation between $\delta^{13}C$ and $\delta^{15}N$, likely due to their wide foraging area and opportunistic feeding (Fig. 1 and Supplementary Fig. S1). Furthermore, the Brown noddy and Sooty tern seem to adopt opposite feeding strategies for their nestlings. Specifically, Sooty tern adults showed significantly depleted $\delta^{15}N$ values compared to their nestlings (Supplementary Fig. S1), which are likely fed with higher trophic level food, a strategy to optimize foraging during the chick rearing period (Bugge et al., 2011). Brown noddy adults, instead, had much enriched $\delta^{15}N$ and $\delta^{13}C$ values compared to nestlings (Supplementary Fig. S1). This does not mean, however, that Brown noddy were feeding their nestlings with low quality diet. For instance, a previous study has found no support for the hypothesis that high quality diet contains a greater proportion of upper trophic level prey (Morrison et al., 2014). Conversely, this study found nestlings with a diet biased for lower trophic level prey to be in a better body condition than nestlings fed with higher trophic level prey (Morrison et al., 2014). In Frigatebirds, we would have expected depleted $\delta^{13}C$ values in comparison to other species, given their attitude to feed far from the breeding colony and possibly in southern latitudes (Sebastiano et al., 2016), but they were not, possibly because Frigatebirds also feed on the shrimp fishery discards, a strategy commonly used in this colony (Martinet and Blanchard, 2009).

Table 3
Linear models and linear mixed models (for overall comparison only) explaining the association between Hg concentration and stable isotopes and between persistent organic pollutants and stable isotopes. Significant P-values are bolded.

			Adults				Nestlings			
			Slope (SE)	*t*-value	*df*	*P*	Slope (SE)	*t*-value	*df*	*P*
Hg & $\delta^{15}N$	Overall	(intercept)	−0.77 (0.85)	−0.90	86.6	0.37	−4.78 (0.86)	−5.57	94.1	**<0.01**
		$\delta^{15}N$	0.11 (0.07)	1.65	98.8	0.10	0.29 (0.07)	4.27	100	**<0.01**
	Among species	(intercept)	−0.54 (0.82)	0.82	94	0.51	−4.39 (0.85)	−5.14	95	**<0.01**
		Cayenne tern	0.05 (0.07)	0.70	94	0.49	0.23 (0.07)	3.21	95	**<0.01**
		Laughing gull	0.11 (0.07)	1.58	94	0.12	0.26 (0.07)	3.90	95	**<0.01**
		Brown noddy	0.05 (0.07)	0.80	94	0.43	0.22 (0.07)	2.89	95	**<0.01**
		Frigatebird	0.17 (0.06)	2.78	94	**<0.01**	0.33 (0.06)	5.11	95	**<0.01**
		Royal tern	0.12 (0.06)	1.80	94	0.08	0.25 (0.07)	3.68	95	**<0.01**
		Sooty tern	0.03 (0.08)	0.44	94	0.66	0.26 (0.07)	3.46	95	**<0.01**
Hg & $\delta^{13}C$	Overall	(intercept)	8.79 (1.27)	6.91	90.3	**<0.01**	1.79 (1.49)	1.21	88.6	0.23
		$\delta^{13}C$	0.52 (0.08)	6.52	94.2	**<0.01**	0.19 (0.09)	2.05	93.5	**<0.05**
	Among species	(intercept)	8.13 (1.29)	6.29	94	**<0.01**	0.52 (1.60)	0.33	95	0.75
		Cayenne tern	0.50 (0.08)	6.26	94	**<0.01**	0.13 (0.10)	1.35	95	0.13
		Laughing gull	0.48 (0.08)	5.70	94	**<0.01**	0.10 (0.10)	0.99	95	0.18
		Brown noddy	0.50 (0.08)	6.19	94	**<0.01**	0.14 (0.09)	1.53	95	0.73
		Frigatebird	0.43 (0.09)	4.94	94	**<0.01**	0.04 (0.11)	0.34	95	0.32
		Royal tern	0.47 (0.08)	5.56	94	**<0.01**	0.11 (0.10)	1.10	95	0.27
		Sooty tern	0.49 (0.08)	6.41	94	**<0.01**	0.12 (0.10)	1.19	95	0.24
∑PCBs & $\delta^{15}N$	Overall	(intercept)	2.11 (4.04)	0.52	50.1	0.60	ND	ND		ND
		$\delta^{15}N$	0.28 (0.32)	0.88	54.4	0.39				
∑PCBs & $\delta^{13}C$	Overall	(intercept)	12.62 (10.67)	1.2	19.9	0.25	ND	ND		ND
		$\delta^{13}C$	0.45 (0.68)	0.66	20.2	0.52				
p,p'-DDE & $\delta^{15}N$	Overall	(intercept)	3.77 (6.92)	0.54	39.6	0.59	ND	ND		ND
		$\delta^{15}N$	0.14 (0.55)	0.26	42.3	0.80				
p,p'-DDE & $\delta^{13}C$	Overall	(intercept)	−34.38 (18.2)	−1.89	18.0	0.08	ND	ND		ND
		$\delta^{13}C$	−2.54 (1.16)	−2.20	18.35	**<0.05**				

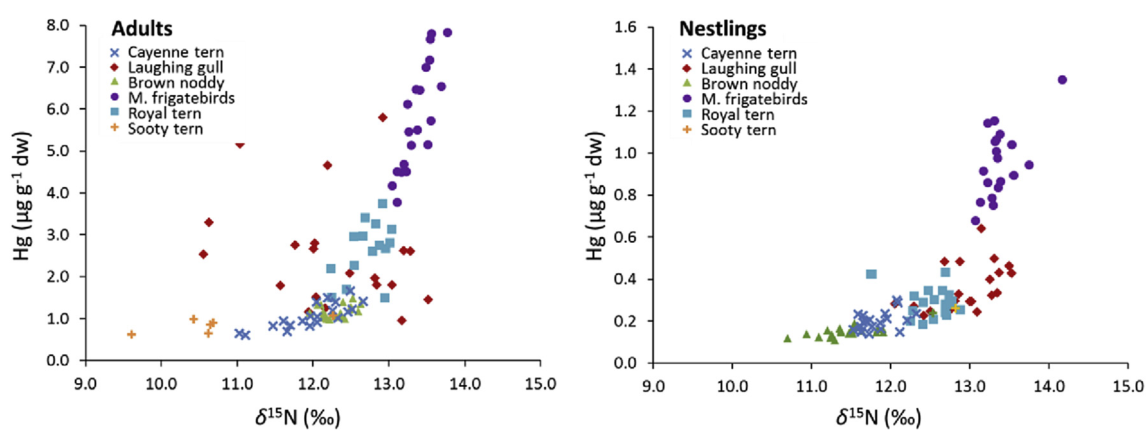

Fig. 5. Relationship between Hg concentrations (µg g^{-1} dw) and $\delta^{15}N$ (‰) in adults and nestlings of the six seabird species from the Grand Connétable Island, French Guiana.

Standard ellipse areas also confirmed a difference in the foraging ecology of the six species and provided some insight on their feeding habits. Fig. 2 clearly shows how Frigatebird, Brown noddy and Cayenne tern adults are specialized foragers, while the Royal tern, the Sooty tern and especially the Laughing gull adults are generalists. This scenario is, however, different for their nestlings. Most species (except Sooty tern nestlings for which the sample size is small) seem to have a more generalist foraging strategy, especially for Brown noddy and Frigatebirds, which might indicate, for instance, that during the reproductive period adults may catch prey items that are not generally present in their diet. However, this pattern was not found in Laughing gull nestlings, which showed a consistently smaller standard ellipse area than adults. This might indicate, for instance, that although the general diet of gulls includes a huge variety of prey items and might also include discards from the shrimp fishery and, in some populations, human waste products, adults might restrict the variety of food that is given to their nestlings during their development.

4.2. Trace element exposure

In addition to providing information on the foraging ecology of the entire community, $\delta^{15}N$ and $\delta^{13}C$ values explained interspecific variation in the exposure to trace elements. Indeed, trace element concentrations were significantly different among the investigated species, both in adults and in nestlings, especially for As, Hg, and Se (Fig. 3). Adult Sooty tern, Brown noddy, and partially the Cayenne tern were more associated with the first PC, while both Frigatebird and Sooty tern were more associated with the PC2. Both PC1 and PC2 values were significantly correlated to $\delta^{15}N$ and $\delta^{13}C$ values,

Table 4
Values of plasma levels of POPs across species and age classes of the three seabird species from the Grand Connétable Island, French Guiana. For each age class, first row values are mean ± SD and second row values represent the range (median). Concentrations are expressed as pg g^{-1} wet weight. ND refers to non-detects. Frigatebirds data are earlier reported by (Sebastiano et al., 2016).

	Adults			Nestlings		
	Cayenne tern	Brown noddy	Frigatebirds	Cayenne tern	Brown noddy	Frigatebirds
∑PCBs	510 ± 470 67-2200 (370)	91 ± 68 5-280 (79)	920 ± 640 330-2700 (640)	30 ± 30 3-110 (15)	ND	44 ± 39 14-200 (35)
∑CHLs	11 ± 9 4-37 (8)	ND	10 ± 3 5-16 (11)	ND	ND	4 ± 7 2-32 (3)
HCB	150 ± 140 3-480 (100)	ND	12 ± 11 2-41 (7)	ND	ND	11 ± 6 2-33 (11)
p,p'-DDE	1300 ± 1100 160-5100 (1100)	200 ± 200 5-600 (200)	430 ± 560 75-2300 (220)	ND	ND	40 ± 45 13-210 (25)
HCHs	34 ± 27 5-120 (29)	13 ± 13 3-45 (8)	ND	20 ± 14 3-55 (17)	11 ± 8 3-26 (9)	11 ± 7 2-20 (12)
∑PBDEs	ND	ND	ND	ND	ND	ND

indicating that inter-individual differences in trace element concentrations in adults can be explained by both the trophic level and the foraging areas of the species. This means, for instance, that the accumulation pattern of some trace elements might be more related to the foraging area of the species, while it might be more dependent on the trophic level of the species for other trace elements, and further studies are needed to clarify this aspect.

Regarding the nestlings, a similar pattern to that of adults was found (Fig. 4b). Most species, except the Sooty tern and the Royal tern, were associated with the first PC. Frigatebird, Laughing gull and Royal tern, instead, were more associated with the PC2. The variation in PC1 values was correlated to δ^{15}N and δ^{13}C values, while PC2 values were not.

To the best of our knowledge, the present study is the first to show this pattern between the levels of As, Hg, and Se in a seabird community. Species with high levels of Hg have also low As and Se. Studies have shown that As might be an essential trace element (Nielsen, 1998; Uthus, 2003), and a more recent study has highlighted that As might maintain optimal levels of S-adenosylmethionine (Uthus, 2003), a common co-substrate which is involved in diverse essential metabolic pathways (Lu, 2000).

Selenium, first known as a toxic element, is an essential element and the main component of both selenoproteins, a wide group of proteins with the role of antioxidant enzymes (e.g. glutathione peroxidase), and enzymes required to maintain an optimal thyroid functioning, as the iodothyronine deiodinases, and thioredoxin reductase (Tapiero et al., 2003). Over the past years, given its involvement in some crucial physiological functions (Rayman, 2012), the knowledge on the relationship between Se bioavailability and health status and on the important role that Se supplementation plays in diseases has radically increased in humans (Rayman, 2012; Tinggi, 2008). One of the aspects that has been well studied is the "protective effect" of Se against Hg toxicity (Ackerman et al., 2016; Polak-Juszczak and Robak, 2015; Sørmo et al., 2011). Indeed, because of the high affinity between these two elements, Hg binds to Se to produce insoluble tiemannite in the liver of many mammals and certain birds (Ikemoto et al., 2004). Despite this mechanism is essential for Hg detoxification, the formation of this insoluble compound compromises Se biological functions and availability, which might pose a threat when Se availability is reduced (Ralston and Raymond, 2010).

Hg varied widely within this seabird community, with the highest concentrations in Frigatebirds, Royal tern, and Laughing gull, while Brown noddy, Cayenne tern, and Sooty tern had lower concentrations (Table 2). Such a difference among species is related to their foraging ecology. The overall positive association between Hg and δ^{15}N in nestlings (Fig. 5) and the significant positive association between Hg and δ^{15}N in adult Frigatebirds (Table 3, Fig. 5) suggest that Hg is effectively bioaccumulated and biomagnified in this seabird community. Furthermore, the positive association between Hg and δ^{13}C suggests that variation in Hg concentrations within this community is also related to the foraging location, as it has been shown in previous studies (Bearhop et al., 2000; Blévin et al., 2013). However, the association between Hg concentrations and stable isotope values showed a different pattern among species. In adults, Hg is effectively bioaccumulated only in Frigatebirds, and Hg concentrations increased with δ^{13}C in all species. The relationship with the carbon stable isotope indicates that Hg exposure in oceanic habitats is lower than the exposure in costal habitats, likely due to important gold-mining activities in this region. In nestlings, Hg is effectively bioaccumulated in all species, while there was no association with δ^{13}C.

Finally, adult seabirds showed higher Hg concentrations than nestlings did (Table 2). Since seabirds are able to excrete Hg in feathers (Dauwe et al., 2003), blood Hg in adults reflects the short-term Hg contamination of individuals (Fort et al., 2015), and therefore provides information for the sampling period, usually the breeding season in seabirds (Goodale et al., 2008). In nestlings, concentrations usually reflect the Hg exposure since hatching and maternal transfer of Hg (Ackerman et al., 2016), even if the latter can be also excreted in the down.

4.3. Persistent organic pollutant exposure

Among environmental contaminants, POPs may act as disruptors of endocrine function and may stimulate or inhibit the secretion of both reproductive and pituitary hormones (Tartu et al., 2015a; Verboven et al., 2010; Verreault et al., 2008). POPs are of concern because they are known to bioaccumulate and biomagnify (Bustnes et al., 2013; Elliott et al., 2015; Mello et al., 2016). Generally, the concentrations we found are in agreement with the few studies that have been recently carried out in this region on sea turtles (De Andres et al., 2016; Guirlet et al., 2010).

4.4. Trace elements and POPs toxicity

Adults and nestlings of the 6 seabird species from French Guiana did not contain As concentrations exceeding 50 μg g^{-1} dw, a commonly used threshold for direct As toxicity in seabirds (Neff, 1997) and should therefore not represent a threat. Furthermore, despite we found an association between low concentrations of Se and high concentrations of Hg, the molar ratio Hg:Se was much below 1 in all individuals, indicating that Se is in excess compared to Hg and thus is not a limiting factor for the detoxification of Hg (Sørmo et al., 2011). Hg concentrations within the Grand Connétable avian community widely vary among species, and were high as compared to literature values for tropical regions. However, they are similar to high-contaminated seabirds in other areas whether from temperate, subpolar, or polar regions (Supplementary Fig. S2). Our study shows, for instance, that average Hg concentration was much higher in French Guiana compared to another tropical seabird community in Seychelles archipelago (Catry et al., 2008). Further, despite in Catry et al. (2008) Hg analyses were performed using whole blood, which reflects relatively similar concentrations than that of red blood cells, Hg concentrations in the Brown noddy were much lower than those of French Guiana (Catry et al., 2008). Similar Hg concentrations have been previously associated with deleterious effects (Costantini et al., 2014; Tartu et al., 2016) with consequences at the population level such as reduction of breeding, hatching and fledging success, and population decline (Goutte et al., 2014a, 2014b). Finally, as compared to other seabirds exposed to POPs, our results revealed concentrations of thousand times lower than those associated with deleterious effects (Erikstad et al., 2013; Goutte et al., 2015), which very likely do not pose a health threat to this seabird community.

5. Conclusions

Our study confirms that POPs are present in very low concentrations in French Guiana seabird species and likely represent a minor concern. However, it shows the presence of high levels of Hg, suggesting a possible health concern for some species, and that the concentrations of trace elements and POPs are associated with the feeding ecology of the species. Further efforts should be made to investigate the health impact of Hg exposure on these species and the community as a whole. Possibly, the analysis of Hg in other tissues and with other non-destructive tools (e.g. feathers), might also provide information on Hg exposure at different stages of the breeding period. These investigations should also clarify whether the negative relationship between high Hg and low As and Se concentrations might lead to side effects due to their potential deficiency, as it has been previously shown in captive animals (Fischer et al., 2008; Wang et al., 2009).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.04.040.

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