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High levels of mercury and low levels of persistent organic pollutants in a tropical seabird in French Guiana, the Magnificent frigatebird, *Fregata magnificens*

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Abstract

In the present study, trace elements and persistent organic pollutants (POPs) were quantified from Magnificent frigatebirds (*Fregata magnificens*) breeding at a southern Atlantic island.. Stable isotope ratio of carbon (δ^{13}C) and nitrogen (δ^{15}N) were also measured to infer the role of foraging habitat on the contamination. For another group from the same colony, GPS tracks were recorded to identify potential foraging areas where the birds may get contaminated. Fourteen trace elements were targeted as well as a total of 40 individual POPs, including organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). The concentration of Hg in the blood was up to 6 times higher in adults (5.81 ± 1.27 µg g^{-1} dw.) than in nestlings (0.99 ± 0.23 µg g^{-1} dw.). A similar pattern was found for POPs. ∑PCBs was the prevalent group both in adults (median 673, range 336 – 2801 pg g^{-1} ww.) and nestlings (median 41, range 19 – 232 pg g^{-1} ww.), followed by the sum of dichlorodiphenyltrichloroethanes and metabolities (∑DDTs), showing a median value of 220 (range 75 – 2342 pg g^{-1} ww.) in adults and 25 (range 13 – 206 pg g^{-1} ww.) in nestlings. The isotope data suggested that the accumulation of trace elements and POPs between adults and nestlings could be due to parental foraging in two different areas during incubation and chick rearing, respectively, or due to a shift in the feeding strategies along the breeding season. In conclusion, our work showed high Hg concentration in frigatebirds compared to non-contaminated seabird populations, while other trace elements showed lower values within the expected range in other seabird species. Finally, POP exposure was found generally lower than that previously measured in other seabird species.

Capsule abstract

In the present study we found high levels of mercury and low levels of persistent organic pollutants in a tropical seabird breeding in a protected area.

Keywords: trace elements, persistent organic pollutants, seabirds, contaminants, French Guiana.

Introduction

Since the last few decades, there has been a significant increase of trace element contamination of the environment and, among those trace elements, mercury (Hg) is a highly toxic non-essential metal. Overall, Hg derives from both natural and anthropogenic sources, but human activities have increased the global amount of circulating Hg. Once deposited in aquatic ecosystems, inorganic Hg is subject to biotic reactions (e.g. methylation) resulting in the production of methylmercury (Me-Hg). Me-Hg is the highly toxic form of Hg in organisms that assimilated it via food intake. Once incorporated in organisms, Me-Hg biomagnifies within food webs from lower to higher trophic levels. Hg has neurological and endocrinological effects and impacts reproduction, behaviour, development, and ultimately demography in humans and wildlife (Wolfe et al. 1998; Tan et al. 2009), especially in those species which occupy a high trophic level (e.g. seabirds), and are therefore potentially exposed to high contaminant loads (Frederick and Jayasena 2010; Tartu et al 2013; Goutte et al. 2014a). Birds are also vulnerable to other trace metals, particularly to non-essential trace elements such as silver (Ag), cadmium (Cd) and lead (Pb), which although much less studied (Burger 2008), have the potential to negatively affect reproduction, survival and growth (Scheuhammer 1987; Larison et al. 2000). High exposure to essential trace elements has been sometime associated with negative effects in birds (Sánchez-Virosta et al. 2015), but since wild birds are often exposed to a mixture of trace elements, it is generally difficult to demonstrate a causal link between environmental levels of specific compounds and health impairments (Burger 2008, Sánchez-Virosta et al. 2015). Similarly, several persistent organic pollutants (POPs), have been associated with many physiological, immune, endocrine, fitness and demographic consequences and with a decrease in the reproductive success (Bustnes et al. 2006; Verreault et al. 2010, Erikstad et al. 2013; Costantini et al. 2014). Although a long term study on the spatial and temporal trends of POPs revealed that these compounds are expected to decline in the Northern Hemisphere (Braune et al. 2005), they appear to still represent a potential threat to adult survival and thus for population dynamics (Goutte et al. 2015). Several studies on seabirds have focused their attention on the contamination in the polar regions (Goutte et al. 2014b; Goutte et al. 2015; Tartu et al. 2015; Bustnes et al. 2015), which are indeed considered a sink for Hg and organic pollutants (Gabrielsen and Henriksen 2001). Most of these contaminants, including Hg from coal burning sources and pesticides used in agriculture are primarily released from the industrialised areas, and their transport to the Arctic region occurs mainly via the atmosphere but also through large rivers and oceanic currents (Gabrielsen and Henriksen 2001). Compared to polar breeding sites, the level of knowledge is much less about contaminant exposure of seabirds in tropical regions. Moreover, since individual detection probabilities of seabirds at breeding colonies are generally high because of high overall site fidelity (Gauthier et al. 2012), and since long lived apex predators should be particularly exposed to persistent and biomagnifying contaminants (Rowe 2008), many seabirds species are ideal models to assess the physiological and behavioural effects of environmental pollution.

The main goal of this study was to investigate the presence of trace elements and POPs in a long-lived seabird, Magnificent frigatebirds (*Fregata magnificens*, hereafter frigatebirds) breeding at Grand Connétable Island, a small island of the coasts of French Guiana, which offers a unique situation to study contaminants in a multiple stressor framework. **Indeed, information on POPs and trace elements is missing in high trophic level species in this region, and an assessment of contaminant exposure has been**

previously focussed only on Hg accumulation in humans and fish. Based on previous studies which have shown the presence of important input of Hg in French Guiana (Roulet et al. 1999), and given the increasing urbanization in the country, we hypothesised **that a contamination is ongoing, and we aimed to clarify if the high levels of Hg (or other toxic substances) might be correlated with a decrease of essential trace elements in this top predator seabird.**. Moreover, since stable carbon and nitrogen isotope measurements have been successfully used to describe the trophodynamics of trace elements and POPs in marine ecosystems (Eulaers et al. 2014; Bearhop et al. 2000), stable isotopes were analysed to study the role of dietary contaminant pathway. Additionally, Global Positioning System (GPS) tracking was conducted on adult frigatebirds of this colony to identify the foraging areas and then the possible sources of the contamination during reproduction.

Materials and methods

2.1 Sample collection

The field sampling was carried out in 2013 on Grand Connétable island, a protected area located off the Atlantic coast of South America (French Guiana, 4°49'30N; 51°56'00W). This island hosts a unique colony of Magnificent frigatebird that is considered one of the most important in South America, and represents the only breeding site for this seabird species in French Guiana (Dujardin and Tostain 1990). Breeding adults (n = 20, 11 females and 9 males during the incubation/early brooding stage) and 30 days old nestlings (n = 20) were captured by hand or with a nose at the end of a fishing rod (Chastel et al. 2005) on May 27th - 28th and June 25th, respectively. Adults and nestlings were not related to each other. Within few minutes after capture, 2 mL of blood were collected from the brachial vein using a heparinized syringe and a 25G needle. **Samples were immediately put on ice and**

centrifuged in the field within around 1 hour to separate plasma (to be used for POPs) and red blood cells (to be used for trace elements and stable isotopes). After centrifugation, both plasma and red blood cells were kept in dry ice until the end of the field work and, when at the laboratory, were kept in a -20 ^{0}C freezer until laboratory analysis.

2.2 Stable isotope analysis

The isotopic niche of frigatebirds was used as a proxy of their ecological niche, with δ^{13}C values of seabirds indicating foraging habitats and δ^{15}N values indicating trophic level (Newsome et al. 2007). The stable isotopic method is based on time-integrated assimilated food, with different tissues recording trophic information over different time scales. In the present study, δ^{13}C and δ^{15}N values were measured in red blood cells, which provide trophic information on a few weeks before sampling (Hobson & Clark 1993). Analyses were performed on lyophilised red blood cells of which 0.30 ± 0.05 mg subsamples were weighed in tin cups for stable isotope analyses. Isotopic analyses were performed at the Littoral Environnement et Sociétés (LIENSs) laboratory at the University of La Rochelle (France) with a Thermo Scientific Delta V Advantage mass spectrometer coupled to a Thermo Scientific Flash EA1112 elemental analyser. The results are expressed in the usual δ (‰) notation relative to the deviation from international reference standards (Pee Dee Belemnite for δ^{13}C and atmospheric nitrogen for δ^{15}N). Based on replicate measurements of internal laboratory standards, the experimental precision did not exceed ± 0.15 and ± 0.20‰ for δ^{13}C and δ^{15}N, respectively.

2.3 Contaminant analysis

2.3.1 Trace elements

The analysis of trace element concentrations was carried out by the Littoral Environnement et Sociétés (LIENSs) laboratory at the University of La Rochelle (France). Fourteen trace elements were analysed on lyophilized red blood cells. Total Hg was quantified with an Altec Advanced Mercury Analyzer AMA 254 spectrophotometer. **Prior and after freeze-drying, blood samples were weighed to determine the percentage of water in blood, and aliquots ranging from 5 to 10 mg were analysed for quality assessment, as described in Bustamante et al. (2008).** Arsenic (As), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), selenium (Se), and zinc (Zn) were analyzed using a Varian Vista-Pro ICP-OES and silver (Ag), cadmium (Cd), cobalt (Co), nickel (Ni), lead (Pb), and vanadium (V) using a Series II Thermo Fisher Scientific ICP-MS (aliquots mass: 50 − 200 mg dw.) as described in Bustamante et al. (2008). Certified Reference Materials (CRM; dogfish liver DOLT-3, NRCC, and lobster hepatopancreas TORT-2, NRCC) were treated and analysed in the same way as the samples. Results were in good agreement with the certified values, and the standard deviations were low, proving good repeatability of the method. The results for CRMs displayed recoveries of the elements ranging from 88% to 116% (n = 10). All the results for trace elements are presented in absolute concentrations in $\mu g\ g^{-1}$ dry weight (dw.).

2.3.2 POPs

The analysis of POPs was performed at the Toxicological Centre of the University of Antwerp (Belgium). The analytical protocol was based on the methods described earlier by Eulaers et al. (2011) and consisted in the processing of 1 mL of plasma by solid-phase extraction and clean-up on silica acidified with sulfuric acid (44% w/w). The protocol allowed for the analysis for 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), cis-nonachlor (CN), trans-nonachlor (TN), oxychlordane (OxC), *β*- and *γ*-hexachlorocyclohexanes (HCHs), and 7 polybrominated diphenyl ethers (PBDEs: BDE 28, 47, 99, 100, 153, 154, and 183). Internal standards (CB 143, *ε*-HCH and BDE 77) were used to quantify the targeted compounds using gas chromatography (Agilent GC 6890, Palo Alto, CA, USA) coupled to mass spectrometry (Agilent MS 5973). Most PCB congeners, as well as *p,p'*-DDT and *p,p'*-DDE were separated using a HT-8 capillary column (30 m*0.22 mm*0.25 µm; SGE Analytical Science, Zulte, Belgium), with the mass spectrometer operated in electron impact ionization mode. The remaining PCB congeners, as well as HCB, CHLs (Chlordanes), HCHs, and PBDEs were separated using a DB-5 capillary column (30 m*0.25 mm*0.25 µm; J&W Scientific, Folsom, CA, USA) and the mass spectrometer was operated in electron capture negative ionization mode. Mean ± SD recoveries of the internal standards CB 143 and BDE 77 were 86 ± 6 % and 93 ± 10 %, respectively. Procedural blanks were analysed every 12th plasma sample, and plasma concentrations were corrected for average procedural blank values. The limit of quantification (LOQ) was compound-specifically set at 3*SD of the procedural blank concentration or, for compounds not detected in blanks, set at a 10:1 signal to noise ratio.

2.4 Global Positioning System (GPS) transmitters

During the breeding season of 2011, from July 5th to 7th, 12 brooding adults were equipped with GPS data loggers **(Gipsy, Technosmart, Rome, Italy)**, which recorded GPS locations per second for 32 up to 85 h. **GPS data loggers were taped to the back or tail feathers using Tesa© tape, and weighted ~20g, which represented <2% of the bird weight.** Since birds needed to be recaptured to recover the GPS, data were recovered from 7 GPS units only.

2.5 Statistical analysis

A principal component analysis (PCA) **based on correlation matrix with a direct oblimin factor rotation (i.e., oblique) solution** was used to **reduce the number of variables into a few representative variables explaining variability in metal accumulation**. This approach was preferred instead of examining each metal or POP separately, because (i) concentrations are usually correlated with each other and (ii) this enabled us to reduce the number of statistical models because running many models may increase the chance for type II error. Trace elements and POPs with concentrations below the LOQ were replaced with a value equal to ½*LOQ. Ag, Cd, Co, Cr, Ni, and V had a concentration below the LOQ in all individuals and therefore were not included in the PCA on trace elements, while the other trace elements were quantified in all individuals. Among POPs, PBDEs group was not included in the PCA, since concentrations were below the LOQ for each individual. Then, compounds from the same class were grouped (PCBs, DDTs, and CHLs), and the PCA was applied. In addition, the suitability of the use of PCA to reduce data was tested through the Kaiser-Mayer-Olkin measure of sampling adequacy (K-M-O = 0.70 for trace elements and K-M-O = 0.60 for POPs) and the Bartlett's test of sphericity ($p < 0.01$ for both PCAs), showing the appropriate power of the PCA. After examination of the scree plot, **the number of significant principal components was selected on the basis of the Kaiser criterion** with eigenvalue higher than 1 **(Kaiser 1960). According to Frontier (1976), eigenvalues are considered interpretable if they exceed eigenvalues generated by the broken-stick model, so** the Broken Stick model performed with the PAST software (3.08 version) was utilized to underline which axes significantly explained variance in our data-set. To compare differences among adults and nestlings in the content of POP and trace elements, a parametric test was used when data were normally distributed, and non-parametric test were utilized when data were not normally distributed. The Spearman's rho test was used to test correlations among different POPs. Data on POPs have been reported as median value since they showed a wide range among samples, hence the mean value would have been an overestimation. Finally, the correlation among trace elements and stable isotope values and among POP groups and stable isotope values were also estimated, respectively, using the Spearman's rho correlation. Since in order to decrease Hg toxicity there should be an amount of Se available equal or higher than that of Hg so that the molar ratio of Se:Hg is greater than 1 (Raymond and Ralston 2009), the molar ratio Se:Hg was calculated using the formula "molar concentration (mol g^{-1} of dw.) = concentration (µg g^{-1} dw.) * 1000 / atomic weight (g mol^{-1})". All statistical analyses were performed using SPSS (22.0.0 version).

Results

3.1 Trace elements

Of the fourteen trace elements analysed, six had a concentration below the LOQ (Ag, Cd, Co, Cr, Ni and V) both in adults and nestlings while the remaining eight were quantifiable in all individuals, including both essential (As, Cu, Fe, Mn, Se, and Zn) and non-essential (Hg and Pb) elements (Table 1). Fe and Zn reported the highest concentrations among essential elements (2413 ± 68 in adults and 2330 ± 80 in nestlings for Fe, and 19.44 ± 0.90 in adults and 26.93 ± 2.95 in nestlings for Zn expressed as µg g^{-1} dw.). Notably, Hg had a quantifiable concentration in all individuals and showed the highest concentration among non-essential elements (5.81 ± 1.27 in adults and 0.99 ± 0.23 µg g^{-1} dw. in nestlings; Table 1 and S1). Blood concentrations of As, Fe, Pb and Se were significantly higher in adults than in nestlings ($p < 0.05$), while Mn and Zn were significantly higher in nestlings ($p < 0.01$), and Cu was similar between adults and nestlings ($p = 0.06$, Table 1). In particular, Hg showed significantly higher concentrations among the two groups, with adults showing a mean concentration of six times higher than the one for nestlings ($p < 0.01$). The Se:Hg molar ratio in adults (3.9) was almost 4 times lower than the one in nestlings (15). PCA reduced the targeted eight trace elements to three components (explaining 46.22%, 18.15% and 13.58% of the total variance, respectively), while the Broken Stick model suggested to focus on PC1 only. As, Hg, Fe, Mn, and Zn were associated with the first axis (Figure 1), and according to the t-test, the age of the individuals (nestlings or adults) was a significant variable explaining the variation of trace elements along PC1 ($t = -10.70$, $p < 0.01$; Figure 2). In this scenario, 46.22 % of the total variance was explained by the differences in trace element concentrations between adults and nestlings. **Adult females and males differed only for Mn (higher in females, $p = 0.03$) and Se (higher in males, $p = 0.03$) concentration.**

3.2 POPs

Of the 40 POP compounds targeted, 15 were not detected in both adults and nestlings, and some congeners were below the LOQ for one group only (either adults or nestlings, Table 2). On average, ΣPCBs, ΣCHLs, ΣDDTs were higher in adults than in nestlings ($p < 0.01$), while HCBs ($p = 0.383$) and ΣHCHs ($p = 0.718$) were similar between adults and nestlings. PBDEs were not detected in any sample (Table 2). ∑PCBs was the most important group based in terms of concentration, showing a median (range) of 673 pg g^{-1} ww. (336 - 2801 pg g^{-1} ww.) in adults and 41 pg g^{-1} ww. (19 - 232 pg g^{-1} ww.) in nestlings, followed by ∑DDTs at 220 pg g^{-1} ww. (75 - 2342 pg g^{-1} ww.) in adults and 25 pg g^{-1} ww. (13 - 206 pg g^{-1} ww.) in nestlings. Among adults, the congeners CB 153, 268 pg g^{-1} ww. (114 - 869 pg g^{-1} ww.) and CB 180, 165 pg g^{-1} ww. (78 - 879 pg g^{-1} ww.), contributed most to the ΣPCBs (34% and 24%, respectively), while among nestlings, CB 153, 17 pg g^{-1} ww. (< 1.0 - 84 pg g^{-1} ww.) and CB 180, 8 pg g^{-1} ww. (3.0 - 40 pg g^{-1} ww.) contributed most to ∑PCBs (22 % and 11 %, respectively) (Table 2). Finally, there was a prevalence of heptaCBs (40 ± 9 % in adults and 25 ± 4 % in nestlings) and hexaCBs (46 ± 14 % in adults and 34 ± 9 % in nestlings; Figure S1). **DDE was the only congener to differ significantly between adult males and females, with higher values in males ($p < 0.01$).**

Spearman's rho correlation coefficients among POP groups were positive between PCBs and CHLs ($r = 0.91$, $p < 0.01$), PCBs and DDTs ($r = 0.90$, $p < 0.01$), CHLs and DDTs ($r = 0.88$, $p < 0.01$), HCB and HCHs ($r = 0.44$, $p < 0.01$). Finally, the PCA reduced the targeted POPs to a number of two components (explaining 45.72% and 24.76% of the total variance, respectively), while Broken Stick model suggested PC1 as the only significant axis. The results of the PCA for the POP profiles are presented in Figure 3. The POPs profile significantly differed between adults and nestlings along the PC1 ($t = -11.13$, $p < 0.01$; Figure 4).

3.3 Stable isotopes and GPS

Differences between adults and nestlings for stable isotope values were significant for $\delta^{13}C$ (adults = –15.01 ± 0.11, nestlings = –15.19 ± 0.09; $p < 0.01$), while they were not different for $\delta^{15}N$ (adults = 13.37 ± 0.20, nestlings = 13.41 ± 0.28; $p = 0.99$; Figure 5).

Hg was significantly positively correlated to $\delta^{15}N$ in both adults ($r = 0.84$, $p < 0.01$) and nestlings ($r = 0.52$, $p = 0.02$). Moreover, in adults only, there was a significant positive correlation between $\delta^{15}N$ and As ($r = 0.66$, $p = 0.01$), and significant negative correlations between $\delta^{15}N$ and other trace elements were limited to Pb ($r = -0.52$, $p = 0.02$) and Zn ($r = -0.66$, $p < 0.02$). Adults also showed a positive correlation between $\delta^{13}C$ and Hg ($r = 0.51$, $p = 0.02$), while there were no significant correlations among stable isotope values and POPs both in adults and in nestlings.

Finally, GPS tracks showed that 6 out of 7 adults alternated long trips toward Brazilian coasts, south of the Grand Connétable colony, with short trips near the island (Figure 6). They showed a wide variance in the trips and, overall, covered an average distance per foraging **round**trip **(one way and return)** of 219.3 km with a standard deviation of 173.1 km, with the longest trip being 513.9 km and the shortest 5 km.

Discussion

4.1 Trace elements

Our results showed the presence of a high blood level of Hg in Magnificent frigatebirds breeding in French Guiana. **In 2009, the National Forestry Office estimated that in French Guiana 1,333 km of watercourses and 12,000 hectares of tropical forest were directly affected by gold mining (Mansillon et al. 2009), and that the number of illegal mining sites was recently estimated between 500 and 900 (Tudesque et al. 2012). In addition,** the changing geomorphology of the Amazon soil **is an additional source of Hg** (de Oliveira 2001), **so that** Hg has become a primary pollutant in the Amazonian basin (Roulet et al. 1999) and is a matter of great concern in French Guiana (Fujimura et al. 2011). Even so, an evaluation of its impact on local wildlife is, however, still missing. A previous study has shown how frigatebirds may move up to 1,400km away from the breeding colony outside the breeding season (Weimerskirch et al. 2006), and may therefore be contaminated far from French Guiana. However, the high Hg levels found in **both adult and** nestling frigatebirds suggest a contamination **in the lower trophic levels from the Guianas coasts up to the upper Brazilian coasts, which includes the foraging areas of our study population during the breeding season (Figure 6).** Since Hg biomagnifies within food webs (Lavoie et al. 2013), adults usually show higher concentrations than nestlings (Carravieri et al. 2014). Consistently, Hg was around six times higher in adults than nestlings (Table 1), and our results showed French Guiana frigatebirds to have values of Hg similar to highly Hg-contaminated species (e.g., *Diomedea exulans, Stercorarius skua*) (see Table S1). Such blood Hg concentrations have been associated with both a reduction of parental commitment (Tartu et al. 2016) and of the breeding success (Goutte et al. 2014a). In addition, similar Hg concentrations have been shown to interfere with several endocrine mechanisms (Tartu et al. 2013, 2014) and to increase oxidative stress (Costantini et al. 2014), a condition that may decrease reproductive success (Costantini 2014) and facilitate herpes infection (Sebastiano et al. 2016). In this scenario, it is important to take into consideration that Hg in the nestlings' red blood cells reflects **Hg** exposure since hatching as well as maternal Hg transfer through the eggs **(Lewis et al. 1993)**, while adult Hg concentrations reflect the exposure since the last moult (Dauwe et al. 2003). So, the Hg content **found** in the blood of adults **might be lower than actually is in other tissues, since birds are able to excrete Hg in feathers (Dauwe et al. 2003).**

The PCA showed that the high Hg concentration is coupled with high levels of As, Fe, and Se and low levels of Mn and Zn, while Cu and Pb did not show a related pattern (Figure 1). However, for some trace elements such as Cu, Fe, Mn, and Pb, concentrations were very low as compared to literature values in other seabird species (Summers et al. 2014; Carravieri et al. 2014). Interestingly, among non-essential trace elements, Ag and Cd were below the LOQ for every sample, Pb concentrations were very low, while Hg was the only non-essential trace element with high concentrations.

A previous study has underlined that As concentrations varied **widely among different tissues**, being higher in liver and muscle tissues, and **varied** with the age of the organism, geographic location, and proximity to anthropogenic activities (Eisler 1988). In birds, inorganic As is considered highly toxic in comparison with organic compounds of this element and may disrupt reproduction, and trigger sub-lethal effects or even induce individual's death (Eisler 1994; Kunito et al. 2008). However, marine animals have only a limited ability to bioaccumulate inorganic arsenic from solution (Neff 1997), so As concentrations in living organisms are generally low (Braune and Noble 2009), and concentrations of As in frigatebirds are much lower than the threshold levels of other seabirds, and therefore should not represent a threat for this population (Eisler 1994).

In contrast to non-essential elements, Zn is an essential micronutrient and its deficiency has been associated to an increase in oxidative stress and DNA damage, and a decrease in antioxidant defences (Song et al. 2009). Zn is also one of the main component of metallothioneins, a group of proteins which play an essential role in heavy metal detoxification (Siscar et al. 2013). A comparison among tissues and different species is difficult to interpret, but Zn content showed concentrations similar to other seabird species (Carvalho et al. 2013; Fromant et al. 2016). **However, the PCA has underlined a strong lowering of the Zn content in the individuals with higher levels of Hg. As a result, since Zn has a stimulatory action on the immune response, further studies are warranted in order to clarify if the decrease in Zn content with the increase in Hg might reduce the immune competence of this seabird.**

In a different way, Se, besides being an essential constituent of selenoproteins utilised as a cofactor for reduction of glutathione peroxidases, (Beckett and Arthur 2005) is also important for the detoxification of Hg exposure. In fact, previous studies have emphasized the "protective effect" of Se on Hg toxicity (Raymond and Ralston 2009). Its protective effect was initially presumed to involve Se sequestration of Hg, thereby preventing its harmful effects. However, as more has become understood about Se physiology, the mechanism of MeHg/Hg toxicity and the mechanism of Se protective effect have also become clear. The high affinity between Hg and Se results in Hg binding to Se (Ralston and Raymond 2010), with the consequent generation of mercuric selenide (HgSe), which is well known to be a non-toxic form in marine mammals and birds (Nigro and Leonzion 1996; Ikemoto et al. 2004). In order to be able to decrease Hg toxicity, there should be an amount of Se available higher than that of Hg so that the molar ratio of Se:Hg is greater than 1 (Raymond and Ralston 2009). Since the molar ratio was 3.9 for adults and 15 for nestlings, **and since the PCA has shown that individuals with high levels of Hg tend to have higher levels of Se,** it is likely that Se is contributing to the detoxification of Hg (Sørmo et al. 2011), preventing from Hg toxic effects more in nestlings than in adults. However, Se in blood**, which was higher in males,** was lower compared to other seabirds (Fromant et al. 2016), and further investigation is needed to understand if the amount of this essential trace element is adequate to contribute to the organisms' physiological functions.

4.2 POPs

To the best of our knowledge, no studies have previously described plasma POP levels in Magnificent frigatebirds or more generally in French Guiana seabirds. In Mexico, a study of Magnificent frigatebirds eggs detected low levels of OCPs and PCBs (Trefry et al. 2013). In French Guiana only one study has investigated whole blood levels of OCPs and PCBs in the eggs of leatherback turtles *Dermochelys coriacea*, and found low levels (Girlet et al. 2010). As mentioned earlier, most seabird POP studies have been conducted on polar and especially Arctic species. In the present study, POP concentrations were generally much lower than what has been found previously in polar seabirds (e.g. Tartu et al. 2015a). The contamination with organochlorine compounds and their metabolites can lead to lethal as well as sub-lethal effects in wildlife (Beyer et al. 1996). In particular, DDTs are highly relevant for apex seabirds, since they are associated with eggshell thinning and thus reduced reproductive success (Beyer et al. 1996). Our results pointed out that the *p,p'*-DDT content was below the LOQ, while *p,p'*-DDE showed a median value of 220 pg g^{-1} ww. in adults **(being higher in males)**, and 25 pg g^{-1} ww. in nestlings, respectively, which are much lower than other top predator seabirds (Bustnes et al. 2006). POPs have never been measured in frigatebird plasma and a reliable comparison with other tissues cannot be made since different tissues show different toxicodynamics.

Comparisons with other species showed PCB 153 and other PCBs, HCB and *p,p'*-DDE to be similar to low contaminated populations of common eider *Somateria mollissima* in the sub-Arctic and high Arctic regions (Bustnes et al. 2012; Fenstad et al. 2014), but much lower than in moderately POPs-contaminated Antarctic seabirds, like the snow petrel (*Pagodroma nivea*; Tartu et al. 2015a). Although ∑PCBs show higher concentrations than the other chemical classes, with a median of 673 pg g^{-1} ww. in adults and 41 pg g^{-1} ww. in nestlings, these concentrations are much lower than those reported in the blood of 7 polar seabirds among which the extremely contaminated Glaucous gull *Larus hyperboreus* (Tartu et al. 2015b). In polar seabirds specifically, high PCB contamination is associated with concentration from dozens (mean 47,000 pg g^{-1} ww., Tartu et al. 2015a) up to hundreds of times higher (mean 448,700 pg g^{-1} ww., Bustnes et al. 2006) than those we found in frigatebirds from French Guiana.

4.3 Stable isotopes and GPS

Differences and similarities in trace elements and POPs between nestlings and adults may be explained by trophic ecology. For example, nestlings may differ from adults in δ^{15}N if they are fed on a different diet or a different trophic level (Overman and Parrish 2001). This explanation is supported by several studies on seabirds (Hobson 1993; Schmutz and Hobson 1998), which found that adults provide to their offspring a food different from that they feed on. One way to increase energy gain per unit time of nestlings would be to increase the size of the fish caught for the nestlings, a strategy that has been recorded in other seabirds (Bugge et al. 2011). However, our results do not support this hypothesis. The similar stable nitrogen isotope values between nestlings and adults suggest that they feed on similar trophic level prey.

On the other hand, the stable carbon isotope values in the present study showed adults to have significantly higher δ^{13}C values than nestlings (Figure 5). A latitudinal decline in δ^{13}C values has been documented in marine mammals and seabirds (Kelly 2000), and studies have shown patterns which might suggest a decreasing δ^{13}C from the coast to the open sea (Eulaers et al. 2014), but information of such stratification in French Guiana is not available. Since at this stage of development, frigatebird nestlings are not able to fly, the stable isotope values in nestlings reflect the prey provided by the adults. Hence, the different carbon stable isotope values between adults and nestlings might be explained in two different ways: (a) adults may get their food in a different feeding area than where they forage for their nestlings (GPS tracks of the breeding season 2011 showed how most adults alternated short trips, mostly to the north, with more long trips in the direction of the Brazilian coasts) (Figure 6); (b) adults may have changed their feeding strategies between the incubation stage and the chick rearing period. In fact, since δ^{13}C in seabird red blood cells reflects up to three-four weeks before the blood sampling (Hobson and Clark 1992), δ^{13}C in adults might have reflected the foraging habitat during the incubation period. In addition, δ^{13}C in nestlings might have reflected the foraging habitat during the beginning of the chick rearing, since nestlings were around 30 days of age. However, differences in the carbon composition are significant from the statistical point of view, but studies are needed to clarify if such difference can be ecologically significant, and if it can be related to the differences in the trace element concentrations.

Conclusions

Although our study provided the first evidence of the presence of POPs in French Guiana frigatebirds, PCB and DDT concentrations were generally lower compared to those found in other seabird species, especially in polar seabirds, and they are not likely to be a threat for this population. However, even if concentrations of these pollutants are low, they may have a combined effect with trace elements and especially Hg. Our study clearly shows that this frigatebird population is bearing high Hg burden, and there is an urgent need to evaluate whether increased blood Hg concentrations may affect endocrine and fitness aspects in this top predator bird, as has been documented in other seabird species. Other essential and non-essential trace elements showed different accumulation in adults and nestlings, but values were in the range of previous studies on other seabirds. Since the trophic position did not differ between adults and nestlings (same nitrogen isotope value), an explanation for the different POPs and metal profiles between adults and nestlings might lie with the foraging area of adults (carbon isotope values), which appeared to change over the breeding season. Furthermore, **in our study population, a previous study has reported the occurrence of herpes virus outbreaks in this colony (De Thoisy et al. 2009), which is causing high mortality of nestlings. These herpes virus outbreaks make this population a highly relevant biological model for investigating the interactions between pollutant exposure and impact of virus activity of population viability. Indeed, previous studies have underlined that there might be a strong relation among exposure to trace elements and virus infections (Koller 1975; Gainer 1977), and, more specifically,** Hg is highly suspected to aggravate herpes simplex virus-2 infection in mice (Christensen et al. 1996). Our data indicate that future studies may be warranted to better understand if the herpes virus outbreaks in this population are favoured by the high Hg contamination.

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Figure captions

Figure 1. Plot of the PC1 correlation coefficients on trace elements. **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Figure 2. Scatter plot of the principal component analysis for trace elements for adult individuals (black squares) and nestlings (red circles).

Figure 3. Plot of the PC1 correlation coefficients on POPs. **Correlation is significant at the 0.01 level (2-tailed).

Figure 4. Scatter plot of the principal component analysis for POPs for adult individuals (black squares) and nestlings (red circles).

Figure 5. Stable carbon and nitrogen isotope values (mean ± SD) of red blood cells of adults and nestlings of the Magnificent frigatebird from French Guiana.

Figure 6. GPS tracks of seven Magnificent frigatebirds adults recorded during the breeding season of 2011. The white dotted line represents the political border between French Guiana and Brazil.

Figures

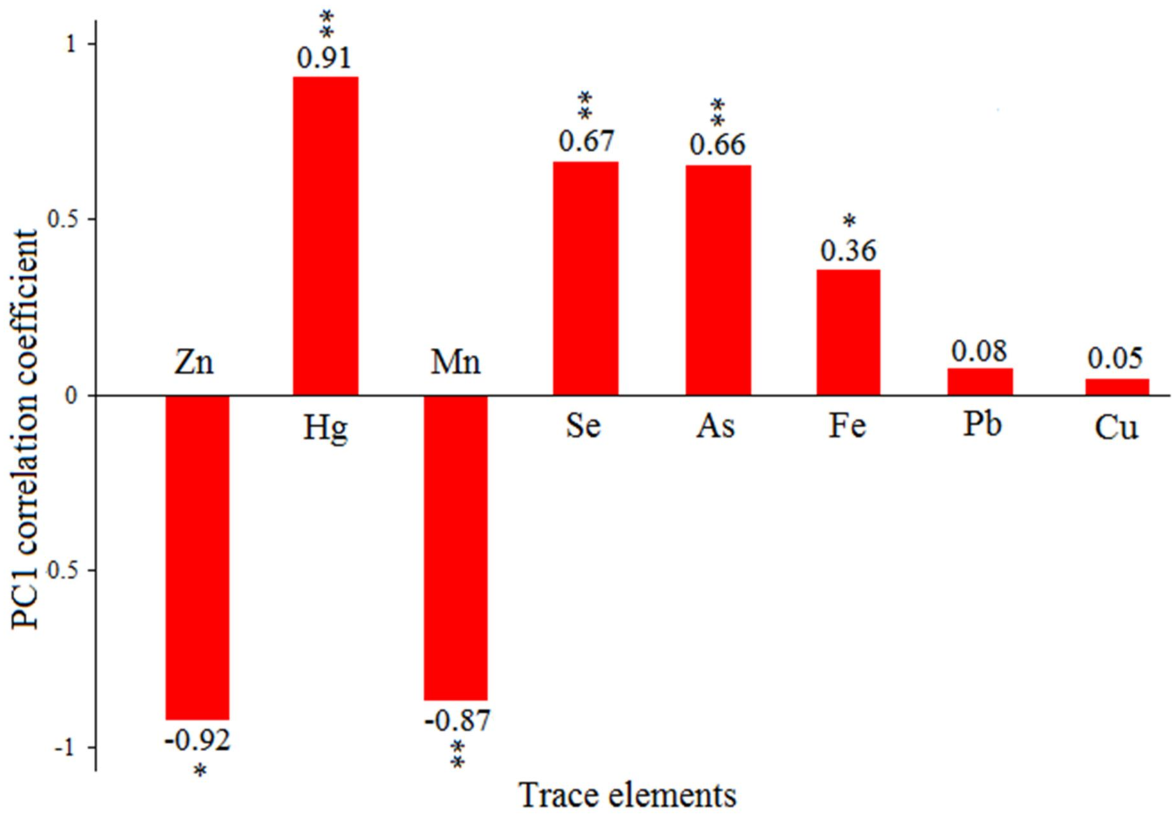
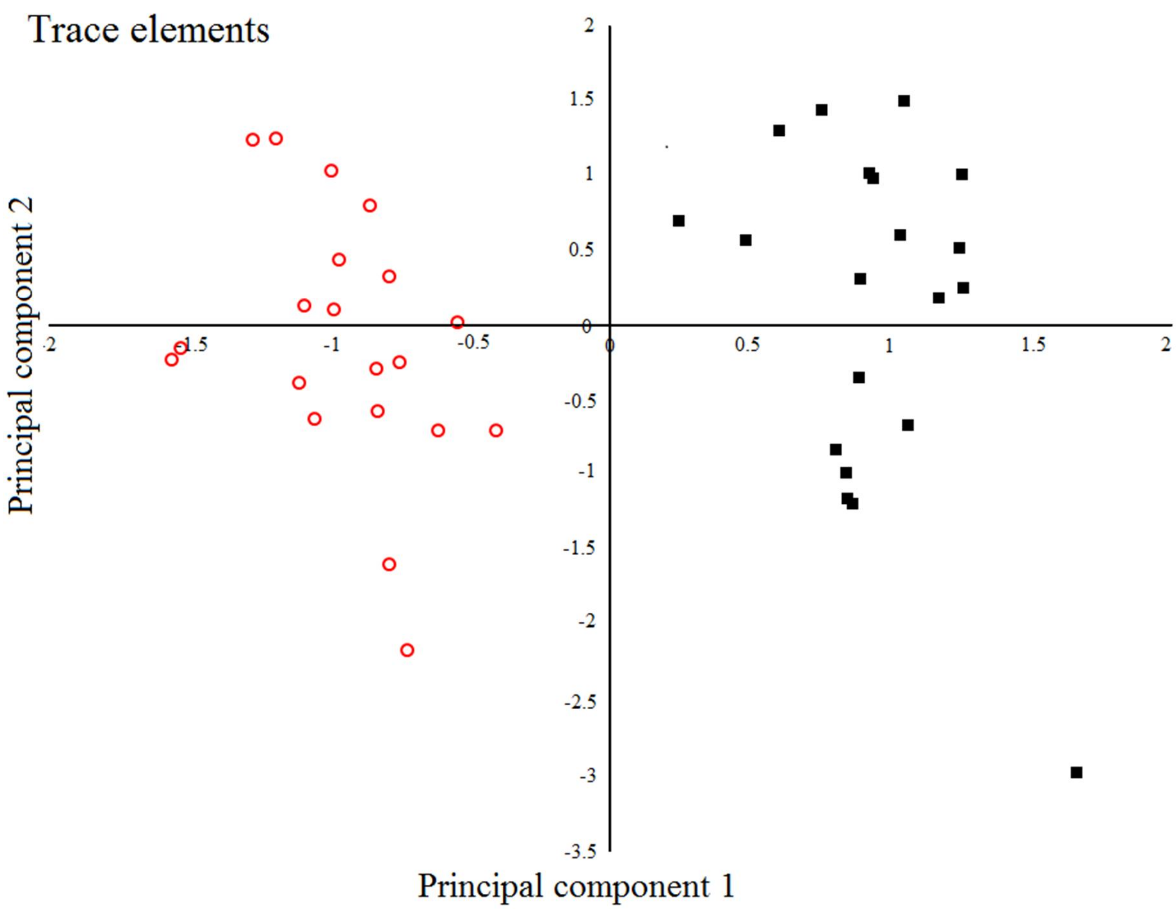


Figure 1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-1.5
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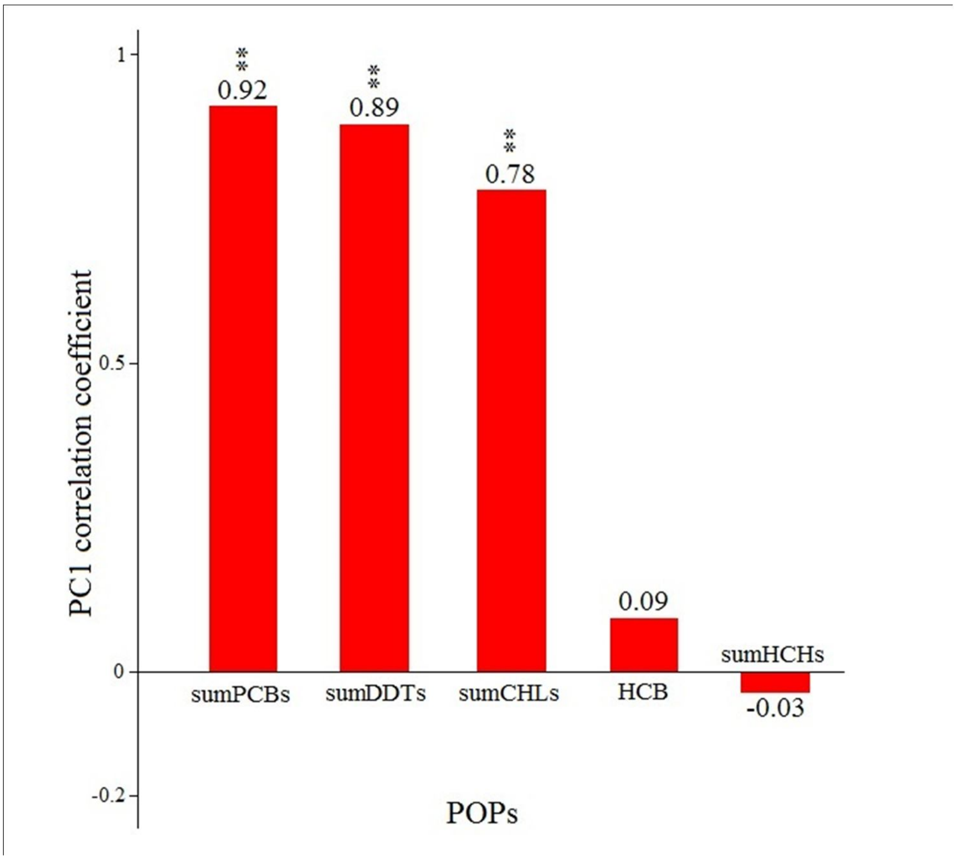
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2

Figure 2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**
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**
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**
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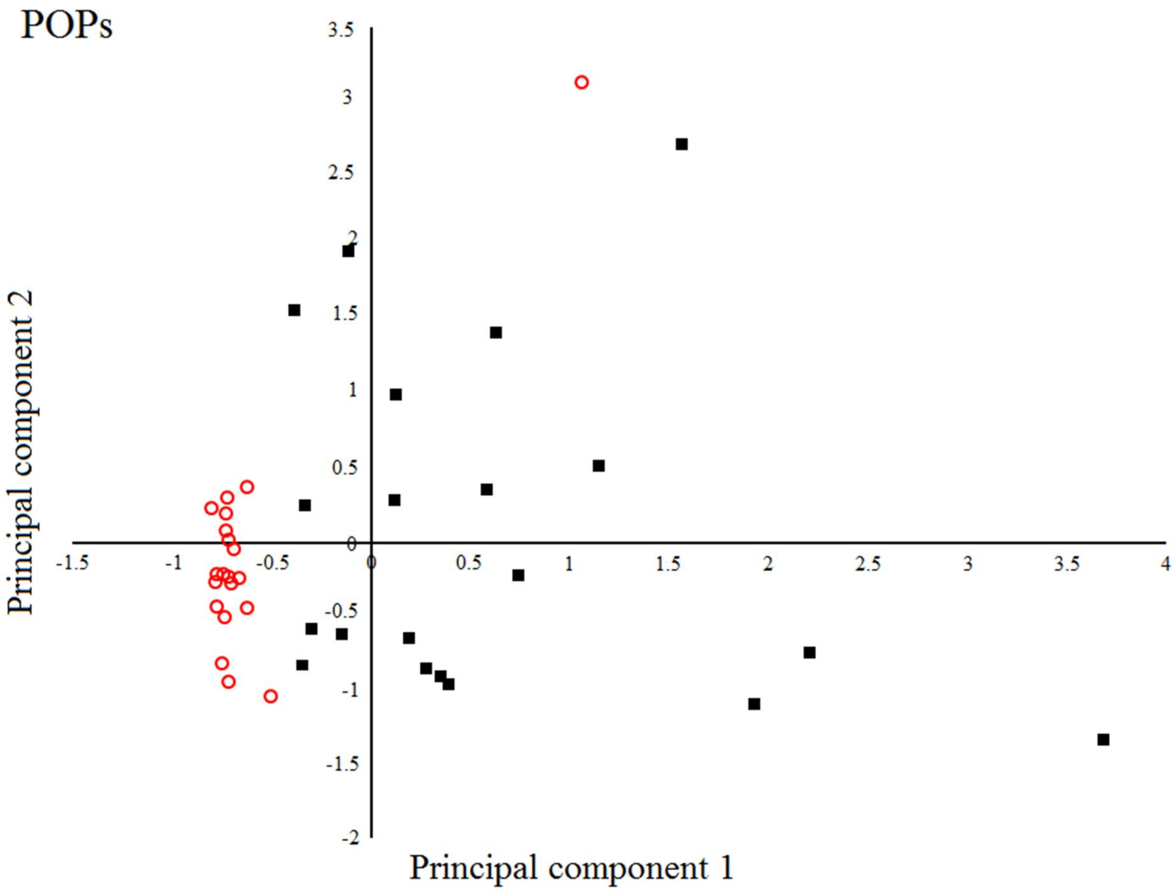
sumDDTs
sumCHLs
HCB
sumHCHs
POPs

Figure 3


POPs
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Principal component 1
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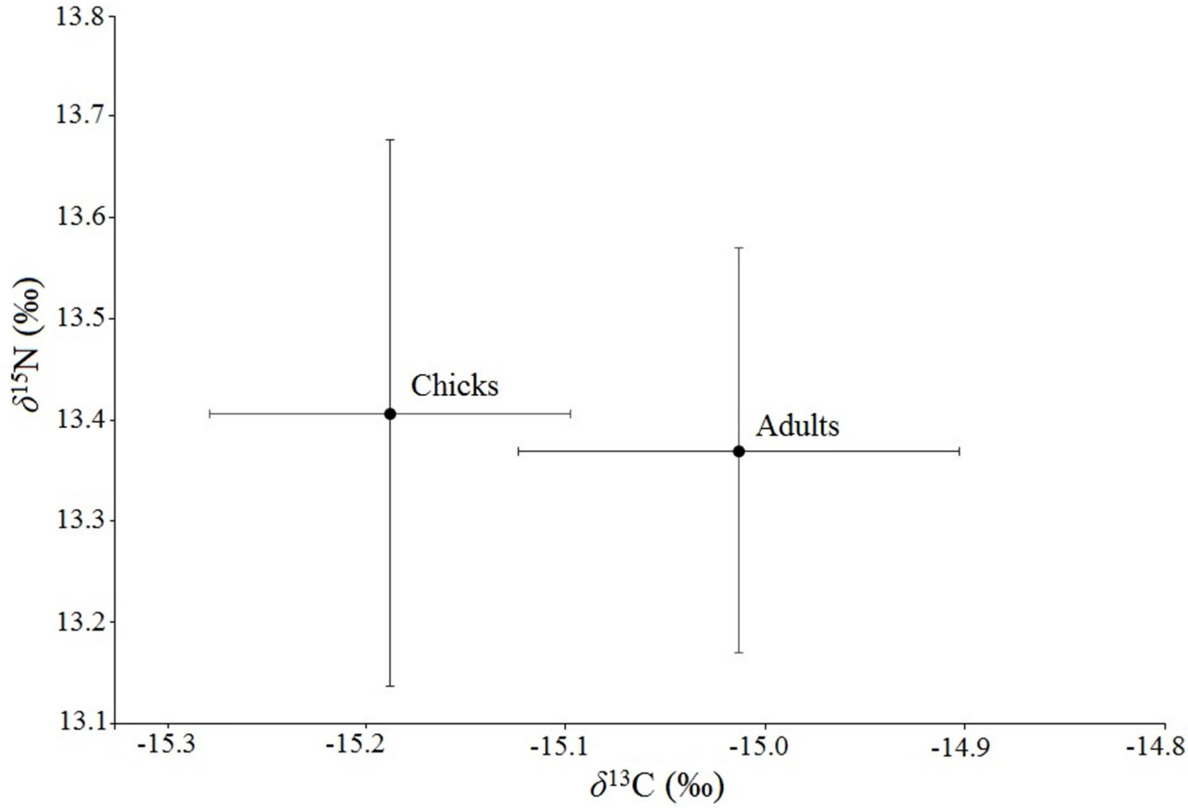
3.5
4

Figure 4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13.5
13.4
13.3
13.2
13.1
δ15N (‰)
Chicks
Adults
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-15.2
-15.1
-15.0
-14.9
-14.8
δ13C (‰)

Figure 5

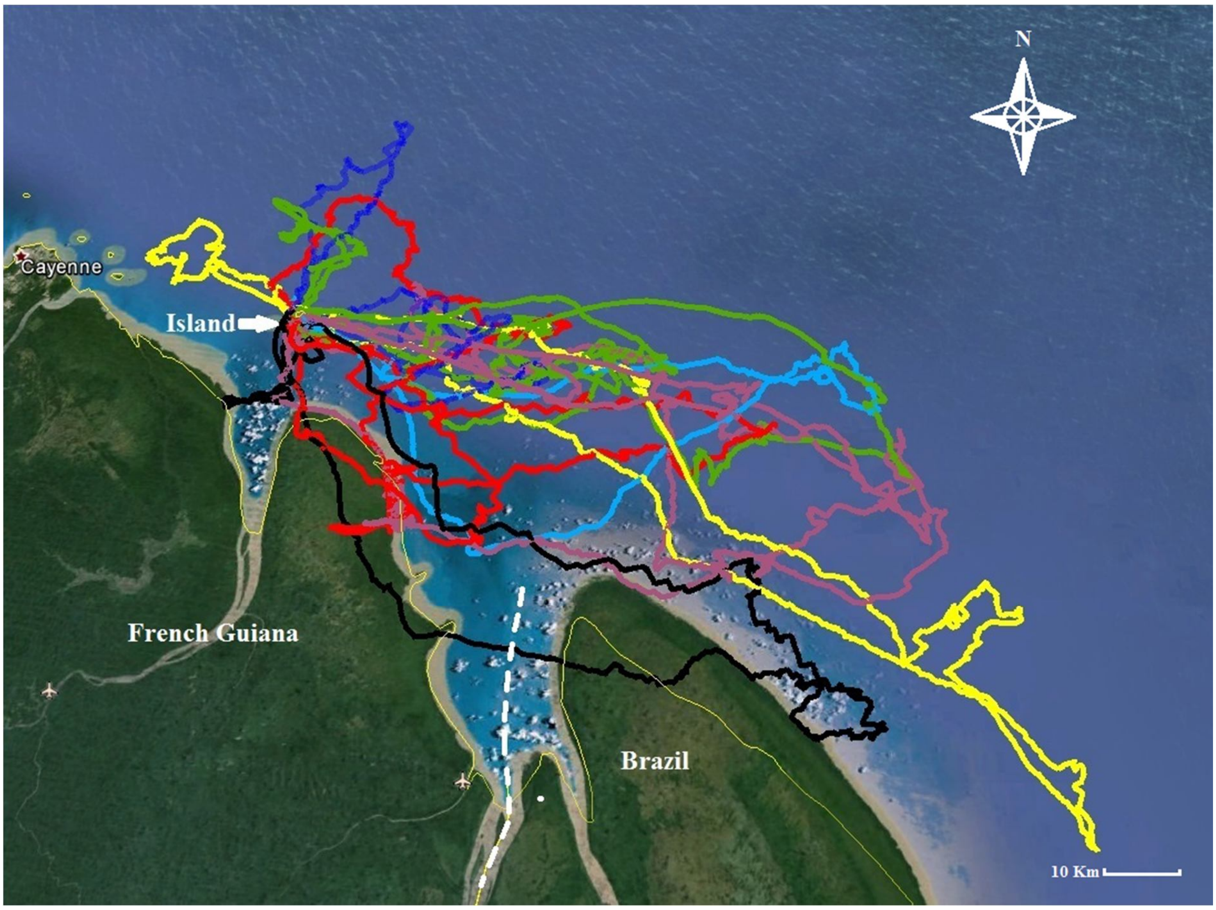

Figure 6

Tables

Table 1. Concentrations (µg g^{-1} dw.) of trace elements in red blood cells of adult and nestling Magnificent frigatebirds. df = detection frequency.

	Adults			**Nestlings**			***P***
	mean ± SD	median (range)	df (%)	mean ± SD	median (range)	df (%)	
Non-essential trace elements							
Ag	-	-	0	-	-	0	-
Cd	-	-	0	-	-	0	-
Hg	5.81 ± 1.27	5.62 (3.78 – 7.83)	100	0.99 ± 0.23	0.96 (0.68 – 1.68)	100	**< 0.01**
Pb	0.02 ± 0.01	0.02 (0.02 – 0.04)	100	0.02 ± 0.005	0.02 (0.01 – 0.03)	100	**< 0.01**
Essential trace elements							
As	2.35 ± 1.44	2.15 (0.58 – 7.33)	100	1.55 ± 0.67	1.51 (0.67 – 3.61)	100	**0.04**
Co	-	-	0	-	-	0	-
Cr	-	-	0	-	-	0	-
Cu	0.78 ± 0.07	0.80 (0.65 – 0.90)	100	0.74 ± 0.07	0.73 (0.60 – 0.86)	100	0.06
Fe	2413 ± 68	2411 (2235 – 2503)	100	2330 ± 80	2337 (2146– 2477)	100	**< 0.01**
Mn	0.12 ± 0.03	0.11 (0.09 – 0.19)	100	0.21 ± 0.05	0.19 (0.13 – 0.19)	100	**< 0.01**
Ni	-	-	0	-	-	0	-
Se	9.09 ± 1.91	8.74 (6.67 – 13.09)	100	5.75 ± 0.63	5.82 (4.57 – 6.57)	100	**< 0.01**
Zn	19.44 ± 0.90	19.36 (18.29 – 22.08)	100	26.93 ± 2.95	26.80 (22.49 – 32.62)	100	**< 0.01**
V	-	-	0	-	-	0	-

Table 2. POP concentrations in adults and nestlings of Magnificent frigatebirds for all congeners analysed. Concentrations are expressed as pg g^{-1} of wet weight. ND = not detected.

Congener	Adults median (range)	Adults mean ± SD	Nestlings median (range)	Nestlings mean ± SD	P
CB 28	ND	ND	ND	ND	ND
CB 52	ND	ND	ND	ND	ND
CB 49	ND	ND	ND	ND	ND
CB 74	ND	ND	ND	ND	ND
CB 101	ND	ND	ND	ND	ND
CB 99	<4 (<4 - 68)	7 ± 16	ND	ND	ND
CB 105	<2 (<2 - 29)	45 ± 6	ND	ND	ND
CB 118	22 (6 - 122)	29 ± 25	<1 (<1 - 8)	2.15 ± 1.88	<0.01
CB 128	<1 (<1 - 4)	1 ± 1	ND	ND	ND
CB 138	56 (26 - 277)	78 ± 60	6 (2 - 38)	7 ± 7	<0.01
CB 146	28 (<1 - 134)	35 ± 38	ND	ND	ND
CB 153	268 (114 - 869)	333 ± 211	17 (<1 - 84)	19 ± 16	<0.01
CB 156	7 (<1 - 21)	9 ± 5	ND	ND	ND
CB 170	47 (23 - 217)	68 ± 50	<3 (<1 - 13)	3 ± 3	<0.01
CB 171	<1 (<1 - 2)	1 ± <1	ND	ND	ND
CB 174	ND	ND	ND	ND	ND
CB 177	<1 (<1 - 4)	1 ± <1	ND	ND	ND
CB 180	165 (78 - 879)	240 ± 189	8 (3 - 40)	10 ± 8	<0.01
CB 183	32 (14 - 122)	42 ± 31	<1 (<1 - 11)	2 ± 2	<0.01
CB 187	34 (15 - 141)	43 ± 33	3 (<2 - 25)	4 ± 5	<0.01
CB 194	21 (7 - 154)	32 ± 32	<1 (<1 - 3)	1 ± <1	<0.01
CB 196/203	23 (7 - 108)	30 ± 25	<1 (<1 - 6)	1 ± 1	<0.01
CB 199	9 (<4 - 30)	11 ± 8	<1 (<1 - 3)	1 ± <1	<0.01
CB 206	<1 (<1 - 14)	<3 ± 3	ND	ND	ND
CB 209	ND	ND	ND	ND	ND
ΣPCBs	673 (336 - 2801)	967 ± 688	41 (19 - 232)	51 ± 44	<0.01
OxC	<1 (<1 - 7)	<2 ± <2	ND	ND	ND
TN	11 (5 - 16)	10 ± 3	<3 (<2 - 32)	4 ± 7	<0.01
CN	<1 (<1 - 5)	<2 ± 1	<1 (<1 - 5)	1 ± <1	0.10
ΣCHLs	14 (7 - 22)	14 ± 5	4 (3 - 37)	6 ± 7	<0.01
HCB	7 (2 - 41)	12 ± 11	11 (<2 - 33)	11 ± 6	0.38

***p,p'*-DDE**	220 (75 - 2342)	426 ± 561	25 (13 - 206)	40 ± 45	<0.01
***p,p'*-DDT**	ND	ND	ND	ND	ND
ΣDDTs	220 (75 - 2342)	426 ± 561	25 (13 - 206)	40 ± 45	<0.01
***β*-HCH**	2 (2 - 19)	8 ± 6	2 (2 - 11)	3 ± 2	0.38
***γ*-HCH**	2 (2 - 82)	8 ± 18	12 (2 - 20)	11 ± 7	0.01
ΣHCHs	14 (5 - 84)	16 ± 18	14 (5- 23)	14 ± 7	0.72
					ND
BDE 28	ND	ND	ND	ND	ND
BDE 47	ND	ND	ND	ND	ND
BDE 100	ND	ND	ND	ND	ND
BDE 99	ND	ND	ND	ND	ND
BDE 154	ND	ND	ND	ND	ND
BDE 153	ND	ND	ND	ND	ND
BDE 183	ND	ND	ND	ND	ND
ΣPBDEs	ND	ND	ND	ND	ND