Trace elements and persistent organic pollutants in chicks of 13 seabird species from Antarctica to the subtropics

Alice Carravieri, Paco Bustamante, Pierre Labadie, Hélène Budzinski, Olivier Chastel, Yves Cherel

ABSTRACT

Human activities have disrupted the natural cycles of both essential and non-essential metals (Outridge et al., 2018; Sen and Peucker-Ehrenbrink, 2012). In addition, synthetic compounds including legacy and emerging organic pollutants are still intentionally and non-intentionally released into the environment (van den Berg et al., 2017). Trends and effects of environmental contaminants in abiotic and biotic matrices have been extensively studied in the Northern Hemisphere, while data is much scarcer in the Southern Hemisphere (Outridge et al., 2018; UNEP, 2019; van den Berg et al., 2017). Southern Hemisphere countries have recently experienced increases in mercury (Hg) emissions from artisanal and small-scale gold mining (Eagles-Smith et al., 2018; UNEP, 2019), and are exempt from some Stockholm Convention restrictions to the use of persistent organic pollutants (POPs) such as DDT for malaria fighting (van den Berg et al., 2017). This could influence large-scale transport and deposition of contaminants in oceanic waters, which cover a great portion of this hemisphere. Yet, ecotoxicological and biogeochemical studies in Southern Hemisphere oceanic waters are dramatically lacking. Apart from localised point pollution from large research stations (Wild et al., 2015), oceanic waters surrounding far-removed archipelagos, such as those found in the southern Indian Ocean, are free from significant, local sources of chemical compounds. Quantifying contaminant

Keywords: Stable isotopes, Selenium, Petrels, Penguins, Albatrosses

1. Introduction

Seabirds from remote regions are mainly exposed to environmental contaminants from non-point contamination of their food webs. Pre-fledging seabird chicks are fed by their parents with marine prey captured in the vicinity of breeding colonies. Contaminant concentrations in tissues of pre-fledging chicks can thus be mostly related to local dietary sources, and have the potential to unravel spatial patterns of environmental contamination in marine ecosystems. Here, mercury (Hg), 13 other trace elements, and 18 persistent organic pollutants (POPs) were quantified in blood of chicks across four breeding locations that encompass a large latitudinal range in the southern Indian Ocean (from Antarctica, through subantarctic areas, to the subtropics), over a single breeding season. Thirteen species of penguins, albatrosses and petrels were studied, including endangered and near-threatened species, such as Amsterdam albatrosses and emperor penguins. Blood Hg burdens varied widely between species, with a factor of ~50 between the lowest and highest concentrations (mean ± SD, 0.05 ± 0.01 and 2.66 ± 0.81 µg g⁻¹ dry weight, in thin-billed prions and Amsterdam albatrosses, respectively). Species relying on Antarctic waters for feeding had low Hg exposure. Concentrations of POPs were low in chicks, with the exception of hexachlorobenzene. Contaminant concentrations were mainly explained by species differences, but feeding habitat (inferred from δ¹³C values) and chicks’ body mass also contributed to explain variation. Collectively, our findings call for further toxicological investigations in Amsterdam albatrosses and small petrel species, because they were exposed to high and diverse sources of contaminants, and in macaroni penguins, which specifically showed very high selenium concentrations.

Capsule: Seabird chicks from four distant sites in the southern Indian Ocean had contrasted blood metallic and organic contaminant patterns depending on species, feeding habitat and body mass.
exposure to living organisms from these areas can thus substantially contribute to filling the gap in our understanding of spatiotemporal trends of environmental contamination in the Southern Hemisphere.

Seabirds are particularly suitable to study spatiotemporal trends of contamination in remote marine environments because they are easily accessible during reproduction on land, and display high fidelity to breeding and feeding sites (Burger and Gochfeld, 2004; Elliott and Elliott, 2013). Seabirds are mainly exposed to environmental contaminants through food ingestion, and thus integrate the contamination of their food webs (Borgå et al., 2004; Lavoie et al., 2013). In the last decades there has been an increased interest in the bioaccumulation of metallic and organic compounds in Southern Hemisphere seabirds (Becker et al., 2016; Brasso et al., 2015; Finger et al., 2016). While taking into account the dietary drivers of exposure, some studies on emblematic species have revealed previously ignored geographical patterns of contaminant abundance in Southern Ocean food webs (Carravieri et al., 2014b; Roscales et al., 2016), providing guidance for biogeochemical efforts. Multi-specific studies of contaminant exposure over large oceanic regions are very powerful (Becker et al., 2016; Polito et al., 2016). First, they have the potential to give an integrative picture of contaminant patterns across different trophic levels (Anderson et al., 2010; Becker et al., 2016; Carravieri et al., 2014c). Second, they are necessary to quantify natural variation in essential element concentrations, which is drastically understudied (Anderson et al., 2010; Finger et al., 2016). Third, they can help us identify good bioindicator species for spatiotemporal monitoring of concentrations, and insights into the biogeochemical behaviour of specific contaminants (Hg stable isotopes, Renedo et al., 2018). Finally, they can reveal risk of potential toxic effects in particular species. However, carrying out multi-specific investigations over large spatial scales in remote environments is logistically challenging. In addition, data collection over different time periods may result in a temporal bias in the measured contaminant concentrations. Multi-specific studies in seabirds have so far focused mainly on adult individuals (Anderson et al., 2010, 2009; Becker et al., 2016; Roscales et al., 2016; but see Blévin et al., 2013; Sebastiano et al., 2017), where tissue contaminant concentrations are governed by a complex mixture of intrinsic (age, sex, breeding status, Borgå et al., 2004; Lerma et al., 2016) and extrinsic (migration over large distances or season, e.g., Finger et al., 2016; Leat et al., 2013) factors that can limit our capacity to identify specific sources of exposure. By contrast, pre-fledging seabird chicks (hereafter chicks) are fed by their parents with marine prey captured in the vicinity of breeding colonies. In fully-grown chicks, influence of in ovo exposure after maternal transfer of contaminants into the egg is negligible (Bourgeon et al., 2013), because of the growth dilution effect or/and excretion into feathers (Ackerman et al., 2011). Concentrations in their tissues are thus mainly representative of local exposure over a limited time window (the chick-rearing period). In addition, flightless chicks are more accessible than adults, and tissues such as blood and feathers can be easily and quickly sampled at the nest (e.g., during ringing sessions, Carravieri et al., 2014a). Therefore, chicks are excellent candidates to evaluate contaminant exposure in several species across multiple breeding sites.

The present study focusses on chicks of 13 seabird species (three Eudyptes sp.; two Aptenodytes and Pygoscelis sp.; one Diomedea, Halobaena, Pachyptila, Pagodroma, Procellaria, and Thalassarche sp., Table S1) breeding at four sites over a large latitudinal gradient in the southern Indian Ocean, from Antarctic to subtropical waters (Fig. S1). At these sites, chick diet has been relatively well-studied (Table S1), and includes marine prey ranging from crustaceans to fish and cephalopods, encompassing multiple trophic levels. This work complements a recent investigation in chicks of Catharacta species (Carravieri et al., 2017) and its specific objectives were (1) to quantify blood contaminant concentrations (14 essential and non-essential trace elements and 18 legacy POPs) in the 13 species, including previously undocumented and endangered ones, (2) to unravel species- and dietary-related differences in exposure, by using the isotopic niche as a proxy of the trophic niche (Newsome et al., 2007), (3) to confirm previously shown geographic patterns of contamination, and (4) to identify good bioindicator species for long-term monitoring. We expected (i) stronger inter-specific differences in non-essential trace element and POP patterns, than in essential trace elements that are physiologically regulated (Walker et al., 2012); (ii) chicks to have increasing blood Hg concentrations from Antarctic to subtropical environments (Carravieri et al., 2017, 2014c, 2014b; Cherel et al., 2018); and (iii) all chicks to bear low concentrations of POPs when compared to Northern Hemisphere species, with the exception of HCB (Bengtson Nash et al., 2007; Carravieri et al., 2014b).

2. Material and methods

2.1. Study sites and sampling procedure

Fieldwork was conducted at four sites that lie in different water masses of the southern Indian Ocean (Fig. S1): Adélie land (66°40’S, 140°01’E) in high-Antarctica, Kerguelen (49°21’S, 70°18’E) and Crozet Archipelago (46°26’S, 51°45’E) in the subtropical zone sensus lato (between the Polar Front and the Subtropical Front), and Amsterdam Island (37°50’S, 77°31’E) in the subtropics (north of the Subtropical Front). Well-feathered chicks were captured by hand before fledging during the 2011–2012 breeding season at all sites (N = 139 in total, n = 9–14 individuals per species, Table 1). Blood (1.5–6 mL depending on species size) was sampled from the brachial vein with heparinized syringes. Whole blood was centrifuged less than 2 h after sampling, and blood cells and plasma were stored at −20 °C until laboratory analyses in France.

2.2. Trace element, POP and stable isotope analyses

Blood is increasingly used to quantify contaminant burdens in seabirds. Trace elements partition preferentially in red blood cells (Friborg et al., 1985), while POPs are mainly associated with plasma lipids (Keller et al., 2004; Matthews et al., 1984). Trace elements were measured at the laboratory LIENSs, La Rochelle, France, from lyophilized red blood cells. Total Hg was quantified with an Altex AMA 254 spectrophotometer (limit of detection, LOD, 0.005 μg g −1 dry weight, dw; aliquot mass: 5–10 mg dw) following Bustamante et al. (2006). Arsenic (As), chromium (Cr), copper (Cu), iron (Fe), manganes (Mn), nickel (Ni), selenium (Se) and zinc (Zn) were analysed using a Varian Vista-Pro ICP-OES (LOD, ranging from 0.02 (Cr) to 3.3 (Fe, Zn) μg g −1 dw) and silver (Ag), cadmium (Cd), cobalt (Co), lead (Pb) and vanadium (V) using a Thermo Fisher Scientific X Series 2 ICP-MS (LOD ranging from 0.015 (Cd) to 0.3 (V) μg g −1 dw), following Kojadinovic et al. (2011) (aliquots mass: 20–200 mg dw). POPs were measured in plasma (300 μl aliquots) at the laboratory EPOC-LPTC, Bordeaux, France by gas chromatography coupled with electron capture detection (GC-EC/GC) (Tapie et al., 2011). Seven PCBs (CB-28, −52, −101, −118, −138, −153 and −180; limit of quantification, LOQ, ranging from 0.1 (CB-180) to 1.8 (CB-52) ng g −1 wet weight, ww) and 11 OCs (HCB, γ-HCH, Heptachlor, 2,4′-DDE, 4,4′-DDE, cis-chlordane, trans-nonachlor, 4,4′-DDD, 2,4′-DDT, 4,4′-DDT, Mirex; LOQ ranging from 0.05 (Heptachlor, trans-nonachlor) to 0.6 (HCB) ng g −1 ww) were targeted. CB-28 and CB-50 co-eluted in all samples, and are reported as CB-28/50. Quality assurance and quality control, as well as LODs and LOQs for all trace elements and POPs are described in the Supplementary Information.

Blood δ13C and δ15N stable isotope ratios were determined at the laboratory LIENSs in red blood cells with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyser (Thermo Scientific Flash EA 1112) (aliquots mass: ~0.3 mg dw). Results are in δ notation relative to Vienna PeeDee Belemnite and atmospheric N2 for δ13C and δ15N, respectively. Measurement errors were < 0.15‰ for both δ13C and δ15N values.

Results are given as mean ± SD in μg g −1 dw for trace elements, in ng g −1 ww for POPs, and in % for stable isotope values. “Blood” within
Table 1
Blood δ¹³C and δ¹⁵N values (‰), Cu, Fe, Hg, Se concentrations (µg g⁻¹ dw), Se:Hg molar ratio, and HCB concentrations (ng g⁻¹ ww) in chicks of penguins, albatrosses and petrels from the southern Indian Ocean. Total and quantifiable sample sizes are given in parenthesis next to species names and element values, respectively. Values are mean ± SD.

<table>
<thead>
<tr>
<th>Species</th>
<th>δ¹³C</th>
<th>δ¹⁵N</th>
<th>Cu</th>
<th>Fe</th>
<th>Hg</th>
<th>Se</th>
<th>Se:Hg</th>
<th>Zn</th>
<th>HCB</th>
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<tbody>
<tr>
<td>Adélie Land, Antarctica</td>
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<tr>
<td>Emperor penguin (10)</td>
<td>−25.73 ± 0.21 (10)</td>
<td>12.72 ± 0.16 (10)</td>
<td>0.49 ± 0.05 (10)</td>
<td>2373 ± 80.3 (10)</td>
<td>0.37 ± 0.07 (10)</td>
<td>3.14 ± 0.41 (10)</td>
<td>22.4 ± 5.23 (10)</td>
<td>24.6 ± 2.30 (10)</td>
<td>0.22 ± 0.07 (9)</td>
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<tr>
<td>Adélie penguin (14**)</td>
<td>−24.47 ± 0.39 (14)</td>
<td>10.91 ± 0.24 (14)</td>
<td>0.95 ± 0.27 (14)</td>
<td>2517 ± 58.7 (14)</td>
<td>0.20 ± 0.04 (14)</td>
<td>4.07 ± 0.56 (14)</td>
<td>53.8 ± 13.6 (14)</td>
<td>31.4 ± 19.7 (14)</td>
<td>0.16 ± 0.03 (10)</td>
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<tr>
<td>Snow petrel (10)</td>
<td>−26.15 ± 0.09 (10)</td>
<td>12.29 ± 0.19 (10)</td>
<td>0.41 ± 0.07 (10)</td>
<td>2250 ± 102 (10)</td>
<td>0.10 ± 0.03 (10)</td>
<td>14.3 ± 4.04 (10)</td>
<td>400 ± 165 (10)</td>
<td>27.7 ± 2.54 (10)</td>
<td>0.51 ± 0.16 (10)</td>
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<td>Snow petrel (14)</td>
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<td>Mayès Island, Kerguelen archipelago</td>
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<tr>
<td>Blue petrel (11)</td>
<td>−23.49 ± 0.48 (11)</td>
<td>8.75 ± 0.32 (11)</td>
<td>0.47 ± 0.05 (11)</td>
<td>2175 ± 94.7 (11)</td>
<td>0.13 ± 0.05 (11)</td>
<td>32.9 ± 8.37 (11)</td>
<td>710 ± 283 (11)</td>
<td>30.4 ± 4.02 (11)</td>
<td>0.86 ± 0.56 (11)</td>
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<tr>
<td>Thin-billed prion (12*)</td>
<td>−23.79 ± 0.36 (12)</td>
<td>7.80 ± 0.25 (12)</td>
<td>0.64 ± 0.47 (12)</td>
<td>2177 ± 85.4 (12)</td>
<td>0.05 ± 0.01 (12)</td>
<td>33.9 ± 7.57 (12)</td>
<td>1845 ± 538 (12)</td>
<td>30.0 ± 1.98 (12)</td>
<td>0.87 ± 0.30 (10)</td>
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<td>Possession Island, Crozet archipelago</td>
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<tr>
<td>King penguin (10)</td>
<td>−21.82 ± 0.19 (10)</td>
<td>10.72 ± 0.22 (10)</td>
<td>1.30 ± 0.20 (10)</td>
<td>2337 ± 148 (10)</td>
<td>0.80 ± 0.11 (10)</td>
<td>4.24 ± 0.62 (10)</td>
<td>13.6 ± 2.41 (10)</td>
<td>21.4 ± 1.83 (10)</td>
<td>0.42 ± 0.29 (10)</td>
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<tr>
<td>Gentoo penguin (10)</td>
<td>−19.11 ± 0.32 (10)</td>
<td>8.67 ± 0.95 (10)</td>
<td>1.16 ± 0.12 (10)</td>
<td>2437 ± 114 (10)</td>
<td>0.80 ± 0.39 (10)</td>
<td>4.16 ± 0.72 (10)</td>
<td>20.1 ± 9.61 (10)</td>
<td>26.1 ± 3.51 (10)</td>
<td>0.12 ± 0.02 (8)</td>
</tr>
<tr>
<td>Macaroni penguin (11)</td>
<td>−21.75 ± 0.20 (11)</td>
<td>8.89 ± 0.29 (11)</td>
<td>1.18 ± 0.31 (11)</td>
<td>2395 ± 247 (11)</td>
<td>0.94 ± 0.17 (11)</td>
<td>172 ± 47.8 (11)</td>
<td>468 ± 120 (11)</td>
<td>20.8 ± 1.58 (11)</td>
<td>0.29 ± 0.08 (10)</td>
</tr>
<tr>
<td>Southern rockhopper penguin (9)</td>
<td>−20.32 ± 0.10 (9)</td>
<td>7.14 ± 0.19 (9)</td>
<td>0.78 ± 0.13 (9)</td>
<td>2228 ± 155 (9)</td>
<td>0.19 ± 0.03 (9)</td>
<td>5.41 ± 0.85 (9)</td>
<td>73.3 ± 10.0 (9)</td>
<td>28.2 ± 2.61 (9)</td>
<td>0.14 ± 0.06 (9)</td>
</tr>
<tr>
<td>White-chinned petrel (11*)</td>
<td>−21.59 ± 0.67 (11)</td>
<td>12.21 ± 0.78 (11)</td>
<td>0.90 ± 0.14 (11)</td>
<td>2367 ± 72.0 (11)</td>
<td>1.12 ± 0.39 (11)</td>
<td>46.0 ± 13.6 (11)</td>
<td>113 ± 443 (11)</td>
<td>25.2 ± 2.03 (11)</td>
<td>0.24 ± 0.09 (10)</td>
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<tr>
<td>Amsterdam Island</td>
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<tr>
<td>Northern rockhopper penguin (10)</td>
<td>−19.61 ± 0.07 (10)</td>
<td>8.69 ± 0.17 (10)</td>
<td>1.08 ± 0.31 (10)</td>
<td>2357 ± 135 (10)</td>
<td>0.23 ± 0.04 (10)</td>
<td>7.89 ± 2.87 (10)</td>
<td>90.9 ± 33.3 (10)</td>
<td>31.6 ± 5.36 (10)</td>
<td>All &lt; LOQ</td>
</tr>
<tr>
<td>Indian yellow-nosed albatross (10)</td>
<td>−19.18 ± 0.15 (10)</td>
<td>15.99 ± 0.26 (10)</td>
<td>1.21 ± 0.13 (10)</td>
<td>2057 ± 169 (10)</td>
<td>0.73 ± 0.45 (10)</td>
<td>62.8 ± 11.6 (10)</td>
<td>283 ± 136 (10)</td>
<td>24.6 ± 1.80 (10)</td>
<td>0.17 ± 0.10 (7)</td>
</tr>
<tr>
<td>Amsterdam albatross (11*)</td>
<td>−18.69 ± 0.16 (11)</td>
<td>15.12 ± 0.18 (11)</td>
<td>1.17 ± 0.07 (11)</td>
<td>1823 ± 184 (11)</td>
<td>2.66 ± 0.81 (11)</td>
<td>26.9 ± 7.72 (11)</td>
<td>27.5 ± 10.5 (11)</td>
<td>22.7 ± 2.08 (11)</td>
<td>0.86 ± 0.41 (10)</td>
</tr>
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</table>

| Factor | 3 | 1.4 | 53 | 57 | 132 | 1.5 | 15 |

* N = 10 for HCB determination.
** N = 11 for HCB determination.
* Factor between the species with the highest and lowest mean concentrations of all contaminants and the Se:Hg ratio.
the best models. The e
ff
formation Criterion corrected for small sample sizes (AICc) to identify
ments and POPs. We used forward selection and the Akaike’s In-
centrations we applied post-hoc Tukey’s honestly signi
2009). To evaluate between-species variation in contaminant con-
ci
titudinally from Antarctic to subantarctic through to subtropical

2.3. Statistical analyses

All statistical analyses were performed in the R environment (R Core
Team, 2018). First, in order to explore contaminant co-variance and
between-specific differences in concentrations, a principal component
analysis (PCA) was carried out on log-transformed, scaled trace ele-
ments and POPs with detection frequencies (DF) > 70%. Observations
below the LOQ were substituted with values drawn randomly from the
range [0; LOQ]. Second, generalised linear (GLM) multifactorial models
were used to quantify the effect of species, feeding habitat (inferred
from \( \delta^{13} \)C values) and their interaction (species: \( \delta^{13} \)C), on trace ele-
ments and POPs. We used forward selection and the Akaike’s Infor-
mation Criterion corrected for small sample sizes (AICc) to identify
the best models. The effect of explanatory variables was inferred
through Akaike’s weights (Burnham and Anderson, 2002). Model spec
ification and validation were based on residual analysis (Zuur et al.,
2009). To evaluate between-species variation in contaminant con-
centrations we applied post-hoc Tukey’s honestly significant difference (HSD) tests on the selected models. Some trace elements (Cd, Mn) had
DF below 70% overall, but were quantified in most individuals of some
species. For those elements, between-species differences were quantify-
ed through a linear model (Cd/Mn ~ Species) on log-transformed
values to meet normality and homoscedasticity of residuals, followed
by Tukey’s HSD. Since different species were sampled at each location,
the site effect on contaminant concentrations is confounded by the
species effect. Site was thus not included as an explanatory variable,
and its effect on contaminant concentrations was described qualita-
tively. Nevertheless, \( \delta^{13} \)C values already function as a reliable proxy of
feeding habitat in the southern Indian Ocean, where they increase la-
titudinally from Antarctic to subantarctic through to subtropical

3. Results

3.1. Trace element, POP and stable isotope values

Among essential elements, Cu (overall range: 0.29–2.11 µg g\(^{-1}\) dw),
Fe (1575–2662 µg g\(^{-1}\) dw), Se (2.71–237 µg g\(^{-1}\) dw), and Zn
(18–46 µg g\(^{-1}\) dw) were quantified in all individuals (Table 1),
while Co, Cr, Ni, V were detected in a very few chicks or none (Table S2).
Mn had a relatively high occurrence (DF = 55%, overall range:
0.10–0.78 µg g\(^{-1}\) dw, Table S2), but some species had no detectable
levels, notably emperor penguins and Amsterdam albatrosses. As
was quantifiable in white-chinned and snow petrels (Table S2) and in few
individuals of other species. Among non-essential elements, only Hg
had a DF of 100% (overall range: 0.03–4.40 µg g\(^{-1}\) dw). Cd was notably
abundant in subantarctic petrel species, with the highest concentra-
tions found in blue petrels (0.41 ± 0.19 µg g\(^{-1}\) dw, Table S2). Ag and Pb
were detected in no or very few individuals, respectively (Table S2).

Of the 14 targeted POPs, only HCB was quantified in more than 70%
individuals (overall range: < 0.06–2.07 ng g\(^{-1}\) ww, Table 1, S3). All
PCBs, chlordanes, Mirex and DDT metabolites had DF below 15%, ex-
cept 4′,4′-DDE (DF = 36%), which was quantified in most individuals
of blue and white-chinned petrels, thin-billed prions and yellow-nosed
and Amsterdam albatrosses, being highest in the latter (0.82 ± 1.10 ng g\(^{-1}\)
ww, Table S3). Blue petrels, thin-billed prions and Amsterdam alba-
trrosses also had quantifiable levels of trans-nonachlor (Table S3).
The Amsterdam albatross was also the only species where most individuals
had quantifiable levels of 4′,4′-DDT (0.46 ± 0.38 ng g\(^{-1}\) ww, Table
S3), and of CB-180 (Table S3). CB-28/50 was quantified in some em-
peror, king and gentoo penguins (Table S3). The Amsterdam albatross
was the only species where most individuals had quantifiable levels of 4′,4′-DDT (0.46 ± 0.38 ng g\(^{-1}\) ww, Table S3), and of CB-180 (Table S3).

Blood \( \delta^{13} \)C and \( \delta^{15} \)N values varied widely among chicks (ANCOVA,
\( F_{12,126} = 678 \) and 508 for \( \delta^{13} \)C and \( \delta^{15} \)N raw values separately to test for between-species differences. In addition, we used the Stable Isotope Bayesian Ellipses in R package (SIBER, Jackson et al.,
2011) on centred and rescaled isotopic values to draw species-specific
bivariate ellipses of isotopic niches.

3.2. Inter-contaminant patterns

The contaminants included in the PCA were Cu, Fe, Hg, Se, Zn, and
HCB. Cu, Hg and Zn contributed most to PC1, while PC2 was governed
mainly by Co, Cr, Ni, V. Nevertheless, \( \delta^{13} \)C values already function as a reliable proxy of
feeding habitat in the southern Indian Ocean, where they increase la-
titudinally from Antarctic to subantarctic through to subtropical

Fig. 1. Standard ellipse areas corrected for small sample size of red blood cell
\( \delta^{13} \)C and \( \delta^{15} \)N (%o) values in chicks of penguins, albatrosses and petrels
from the southern Indian Ocean. Abbreviations: AA, Amsterdam albatrosses; AP,
Adélie penguins; BP, blue petrels; EP, emperor penguins; GP, gentoo penguins;
KP, king penguins; MP, macaroni penguins; NRP, northern rockhopper pens-
Guins; SP, snow petrels; SRP, southern rockhopper penguins; TBP, thin-billed
prions; WCP, white-chinned petrels; YNA, Indian yellow-nosed albatrosses. (For
interpretation of the references to colour in this figure legend, the reader is
referred to the web version of this article.)

Oceanic waters (Cherel and Hobson, 2007; Jaeger et al., 2010). Finally,
the \( \delta^{15} \)N proxy was not included as an explanatory variable in multi-
factorial analyses because of the large variation in baseline \( \delta^{15} \)N values
in food webs found across the latitudinal range of the four sites (Jaeger
et al., 2010). However, blood \( \delta^{15} \)N values were used in conjunction
with \( \delta^{13} \)C values to quantify the isotopic niches of the 13 species and
confirm that they corresponded to previously published results (Table S1).
ANOVA F tests were applied to blood \( \delta^{13} \)C and \( \delta^{15} \)N raw values separately to test for between-species differences. In addition, we used the Stable Isotope Bayesian Ellipses in R package (SIBER, Jackson et al.,
2011) on centred and rescaled isotopic values to draw species-specific
bivariate ellipses of isotopic niches.
3.3. Intrinsic and extrinsic drivers of Hg, other trace element and HCB concentrations

Multifactorial analyses indicated that species was a strong predictor of trace element and HCB concentrations in chicks of penguins, albatrosses and petrels. Blood δ13C values and body mass of chicks were also important drivers of element patterns, since models that included them in addition to species had high support in the selection procedure (ΔAICc < 4, Table S5). Namely, the model including species, δ13C values, and their interaction was the best in predicting blood Hg concentrations, and explained a very high proportion of deviance (93%, Table 3). Vertical lines delimitate the water masses (Antarctic, subantarctic, subtropics) where parents capture prey for their chicks according to stable isotope results (Fig. S1) and previous work on the same populations (Table S1). Abbreviations: AA, Amsterdam albatrosses; AP, Adélie penguins; BP, blue petrels; EP, emperor penguins; GP, gentoo penguins; KP, king penguins; MP, macaroni penguins; NRP, northern rockhopper penguins; SP, snow petrels; SRP, southern rockhopper penguins; TBP, thin-billed prions; WCP, white-chinned petrels; YNA, Indian yellow-nosed albatrosses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Relationship between red blood cell Hg concentrations (µg g⁻¹ dw) and δ13C (‰) values in chicks of penguins, albatrosses and petrels from the southern Indian Ocean. Fitted lines represent the best selected model (Hg ~ species + δ13C + species:δ13C, Table 3). Vertical lines delimitate the water masses (Antarctic, subantarctic, subtropics) where parents capture prey for their chicks according to stable isotope results (Fig. S1) and previous work on the same populations (Table S1). Abbreviations: AA, Amsterdam albatrosses; AP, Adélie penguins; BP, blue petrels; EP, emperor penguins; GP, gentoo penguins; KP, king penguins; MP, macaroni penguins; NRP, northern rockhopper penguins; SP, snow petrels; SRP, southern rockhopper penguins; TBP, thin-billed prions; WCP, white-chinned petrels; YNA, Indian yellow-nosed albatrosses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
chicks had surprisingly higher $\delta^{15}N$ values than Amsterdam albatross chicks, but the diet of the latter is unknown (probably large fish and cephalopods; Cherel et al., 2017); (4) blue petrels and thin-billed prions from the subantarctic Kerguelen Islands foraged in both subantarctic and Antarctic waters when feeding their chicks, thus explaining their low blood $\delta^{13}C$ values and why they were classed among Antarctic feeding species in our figures.

4.1. Comparison to previous work on trace elements and POPs in seabird blood

Most ecotoxicological studies in seabirds focus on adults, where concentrations are usually higher than in chicks (Sebastiano et al., 2017, 2016; Renedo et al., 2018). This prevents direct comparison between age-classes, so here we focus on chicks’ comparisons. The range of Hg concentrations of the 13 focal species was extremely wide. Namely, small petrels and rockhopper penguins had low blood Hg concentrations ($< 0.10 \mu g \cdot g^{-1} dw$) comparable to those found in chicks of most Northern Hemisphere species (Bond and Diamond, 2009; Kahle and Becker, 1999; Lerma et al., 2016). Chicks of larger penguins, petrels and yellow-nosed albatrosses had intermediate concentrations (0.80–1.00 $\mu g \cdot g^{-1} dw$), comparable to Magnificent frigatebirds *Fregata magnificens* (Sebastiano et al., 2016) and great skuas *Stercorarius skua* (Bearhop et al., 2000). Finally, Amsterdam albatross chicks had very high Hg concentrations similar to those found in the sympatric brown skua *Catharacta lombergi*, which has the highest known concentration documented so far in wild bird chicks (Carravieri et al., 2017). Non-essential elements other than Hg were overall under detection limits. Yet, Cd was quantified in blue, thin-billed and white-chinned petrels at relatively high concentrations when compared to other studies in chicks and adults (Anderson et al., 2010; Sebastiano et al., 2016, but see Carvalho et al., 2013). This is likely related to Cd-enriched prey such as the hyperiid amphipod *Themisto gaudichaudii*, which is used to feed the chicks (Table S1).

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Fig. 4. Concentrations of Hg, Cu, Fe, Se, and Zn ($\mu g \cdot g^{-1} dw$) in red blood cells and HCB ($ng \cdot g^{-1} ww$) in plasma of chicks of penguins, albatrosses and petrels from the southern Indian Ocean. Species order representation is based on latitude of feeding grounds where parents capture prey for their chicks, and their trophic positions, according to stable isotope results (Fig. 1) and previous work on the same populations (Table S1). Vertical lines delimitate the southern Indian Ocean water masses (Antarctic, subantarctic, subtropics). Letters indicate significant between-species differences (Tukey HSD, $p < 0.05$). Abbreviations: AA, Amsterdam albatrosses; AP, Adélie penguins; BP, blue petrels; EP, emperor penguins; GP, gentoo penguins; KP, king penguins; MP, macaroni penguins; NRP, northern rockhopper penguins; SP, snow petrels; SRP, southern rockhopper penguins; TBP, thin-billed prions; WCP, white-chinned petrels; YNA, Indian yellow-nosed albatrosses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Overall, concentrations of essential elements were in the same range of those found in seabirds worldwide, in both adults and chicks (Cu: range 0.5–1.0 µg g \(^{-1}\) dw; Fe: range 2200–2300 µg g \(^{-1}\) dw; Zn: range 20–35 µg g \(^{-1}\) dw; Finger et al., 2016; Lucia et al., 2016; Sebastiano et al., 2017, 2016, but see Carvalho et al., 2013; Osborn et al., 1979), because they are maintained in a narrow range of concentrations as a consequence of homeostatic processes. White-chinned and snow petrels had higher Mn concentrations than those of other seabirds (adults, Anderson et al., 2010; Finger et al., 2016). Most penguin species had Se concentrations in the range of other seabird chicks (< 10 µg g \(^{-1}\) dw, Burger and Gochfeld, 1997; Sebastiano et al., 2016), while petrels and albatrosses had higher burdens (10–60 µg g \(^{-1}\) dw). Surprisingly, macaroni penguin chicks had very high Se concentrations, similar to those of adults of some procellariiformes species (100–200 µg g \(^{-1}\) dw, Anderson et al., 2010; González-Solís et al., 2002), but lower than those of brown skua chicks from Amsterdam Island (up to 650 µg g \(^{-1}\) dw on average, highest burden reported so far in wild bird chicks, Carravieri et al., 2017). This could stem from concurring trophic and physiological factors that are discussed below (Section 4.2.1).

Only a handful of studies have reported blood POP concentrations in seabird chicks worldwide (Bourgeon et al., 2013; Carravieri et al., 2017, 2016; Colabuono et al., 2016; Sebastiano et al., 2017, 2016), because low concentrations, and a limitation in the volume of blood that can be sampled, complicate POP quantification in this age class. Chicks from the present study had overall low burdens of POPs, in particular PCBs but also most organochlorine pesticides (chlorodanes, Mirex, DDT metabolites), which were detected in a few very individuals. As expected, the only exception to this general pattern was HCB, a POP known to strongly bioaccumulate and biomagnify in Southern Ocean food webs (Sengston Nash et al., 2007). HCB was detected in almost all individuals at concentrations (i) comparable to other Southern Hemisphere seabird chicks (Carravieri et al., 2017; Colabuono et al., 2016), and (ii) higher than those of Northern Hemisphere species (Bourgeon et al., 2013; Sebastiano et al., 2017, 2016). Blood POP concentrations in adults of other Antarctic and subantarctic species were overall higher than those found in chicks of the present study (Corsiolini et al., 2007; Dehnbard et al., 2017; Tartu et al., 2015), likely as a consequence of exposure of the adults during longer periods over distant wintering areas (Weimerskirch et al., 2015).

4.2. Intrinsic and extrinsic drivers of contaminant concentrations

4.2.1. Hg and Se

Since Hg is efficiently biomagnified under its methylated form (Me-Hg), high trophic level prey, such as fish and cephalopods show higher Hg concentrations than planktonic organisms (Anderson et al., 2009; Lavoie et al., 2013). Hence, inter-specific variability in seabird Hg exposure is often attributed to their trophic level (Anderson et al., 2009; Blévin et al., 2013). Yet in the present study, spatial factors seemed to overcome trophic drivers to a certain extent. As previously shown at the individual (Carravieri et al., 2014b), population (Carravieri et al., 2017), species (Cherel et al., 2018) and community levels (Blévin et al., 2013; Carravieri et al., 2014c), inter-specific differences in Hg concentrations in blood were strongly driven by feeding habitat (\(\delta^{13}C\) values). Specifically, all chicks fed with Antarctic prey, from both neritic (emperor and Adélie penguins) and oceanic waters (small petrels), had low blood Hg concentrations irrespective of their trophic position, at both the inter- and intra-specific scale. This confirms the homogeneity of Hg transfer to upper predators in waters south of the Polar Front (Carravieri et al., 2017, 2014b; Cherel et al., 2018), in both neritic and oceanic environments. High Me-Hg concentrations in Antarctic coastal waters, particularly in association with sea-ice (Gossa et al., 2011), and relatively high Hg burdens in key prey species such as Antarctic krill Euphausia superba (Seco et al., 2019) contrast with low Hg concentrations in Antarctic upper predators. This suggests that the relative simplicity of Antarctic food webs, where upper predators rely on a few key mid-trophic species, prevents strong biomagnification of Hg in Antarctic waters.

There was a net increase in blood Hg concentrations in species feeding north of the Polar Front (Fig. 3, Cherel et al., 2018), since most chicks at the subantarctic Crozet Islands displayed relatively high Hg concentrations. At the subtropical site, the micronekton-eater northern rockhopper penguin had 3- and 12-fold lower blood Hg concentrations than yellow-nosed and Amsterdam albatrosses, respectively, which rely on high trophic level prey (Cherel et al., 2013). Yet, northern rockhopper penguin Hg concentrations were similar to those of southern rockhopper penguins at Crozet archipelago, suggesting comparable Hg content in subtropical and subantarctic micronektonic species. In addition, the within-population slopes of the relationship between blood Hg and \(\delta^{13}C\) values were weak in Antarctic waters, and progressively steeper from subantarctic to subtropical environments (Fig. 3), emphasising a strong Hg transfer variation in different water masses. Contrary to findings at Kerguelen Islands (Carravieri et al., 2013), gentoo penguins feeding on pelagic prey (low \(\delta^{13}C\) values) had higher blood Hg concentrations than benthic feeders (high \(\delta^{13}C\) values). This could be related to the birds’ trophic positions, because \(\delta^{13}N\) values were exceptionally high in those individuals with low \(\delta^{13}C\) values (Fig. S3). Collectively, our results indicate a stronger biomagnification of Hg in subtropical than subantarctic and Antarctic waters. This inverted latitudinal pattern in Hg biomagnification strength contradicts previous large-scale results in aquatic food webs (Lavoie et al., 2013) and needs further investigation.

Hg dynamics in organisms can also be influenced by Se. Specifically, at high concentrations Hg and Se are known to interact and mitigate mutually their toxic effects (Cuvin-Aralar and Furness, 1991). Hg and Se concentrations often correlate in seabird tissues, and the Se:Hg ratio is used as an indicator of the balance between the two elements, and thus to infer potential risk of toxicity (Scheuhammer et al., 2015). Se concentrations did not show obvious geographical patterns, and were not correlated to Hg burdens. Yet, between-species differences were substantial. Specifically, blue petrels and thin-billed prions had high blood Se concentrations, likely as a result of feeding on Se-rich crustacean species such as T. gaudichaudii (Anderson et al., 2010). Macaroni penguin chicks are also fed extensively with crustaceans, in particular the euphausid Euphausia vallentini and T. gaudichaudii (Table S1), being likely exposed to large quantities of dietary Se. Yet, such a strikingly high Se concentrations in blood compared to the other crustacean consumers could also be the result of specific physiological mechanisms that need further investigation. Hg concentrations in macaroni penguin chick feathers (authors’ unpublished data) were unexpectedly low given their Hg burdens in blood. Indeed, their feather to blood ratio was 0.5, while it was in the range 1.7–6.1 in all other species. This could suggest that macaroni penguin chicks have a reduced capacity of Hg excretion into feathers that might be compensated by an increased, selective absorption and accumulation of Se from the diet, in order to achieve protection against Hg toxicity.

4.2.2. Other trace elements

Even though variation in essential trace elements was lower than in non-essential ones, clear differences were highlighted among species. Chick body mass was shown to be a significant predictor of essential element concentrations, in particular of Zn. These results are consistent with optimal concentrations of essential elements being species-specific (Walker et al., 2012). The clear decrease of Zn concentrations with body mass was unexpected and could suggest either that (i) smaller species have higher requirements of Zn, or (ii) Zn requirements are similar across species, but circulating concentrations are subject to a dilution effect, or other body mass-dependent physiological mechanisms. Feeding habitat (\(\delta^{13}C\) values) also explained partially the variation in essential trace element concentrations. Blood Cu and Fe concentrations were high in neritic species such as Adélie and gentoo penguins, suggesting high bioavailability of these elements in...
association with shelves. Conversely, blood Cu concentrations were low in chicks fed with pelagic prey from Antarctic waters, which is consistent with low essential element concentrations in Southern Ocean waters (Petri and Zauke, 1993). Finally, by considering results in the 13 species, and comparison with the literature (see Section 4.1 above), yellow-nosed and Amsterdam albatross chicks seem to be Fe-deficient, although the rationale is unclear. Certainly there is a need for a better understanding of the factors that govern essentiality ranges in wild birds.

4.2.3. POPs

Contrary to previous studies (Carravieri et al., 2014b; Roscales et al., 2016), here there was no clear geographical pattern in blood POP concentrations in seabird chicks. Instead, chicks fed with prey captured in open oceanic waters had higher concentrations than those relying on neritic prey, irrespective of the water mass. Specifically, blue petrels and thin-billed prions, which feed on small crustaceans captured at the surface in high-productivity Antarctic oceanic waters (Cherel et al., 2014), had the most varied patterns (HCB but also 4,4′-DDE, Heptachlor and trans-nonachlor) and higher POP concentrations of all species. This is consistent with results in tissues of Antarctic prions Pachyptila desolata, which showed a remarkable diversity of organic compounds (Fromant et al., 2016). Conversely, northern rockhopper penguins, which feed on micronecton in subtropical waters, had the lowest abundance of POPs in blood. This suggests that feeding on low trophic level prey from surface, productive Antarctic oceanic waters lead to high exposure from ready integration of POPs into food webs (biological pump, Galbán-Malagón et al., 2012). Amsterdam albatross chicks had similarly high and diverse POP burdens (HCB but also 2,4′-DDE, 4,4′-DDE, 4,4′-DDT, Heptachlor and trans-nonachlor), likely as a result of feeding at a very high trophic level.

4.3. Species that raise ecotoxicological concern

Strong evidence from laboratory and fieldwork studies indicates that non-essential elements such as Cd, Hg, and Pb, and POPs have several deleterious effects on seabird physiology (Eagles-Smith et al., 2018). Specifically, they can disrupt key neurological and endocrine mechanisms, with consequences on development, reproduction, and survival (Goutte et al., 2018; Tan et al., 2009; Tartu et al., 2015). These contaminants can also cause immunosuppression; they increase susceptibility to disease, the abundance of intestinal parasitic nematodes, and can alter the immune response (Letcher et al., 2010; Sagerup et al., 2000). On the other hand, concentrations of essential elements that fall outside the window of essentiality can also have negative impacts (deficiency/toxicity, Walker et al., 2012). Here, Amsterdam albatross chicks (i) had high burdens of Hg (see also Cherel et al., 2018) and HCB, (ii) were recently exposed to DDT (unlike most other species), and (iii) had relatively low concentrations of essential elements such as Fe, Se, and Zn, which are critical for blood production (Fe), protection against Hg toxicity and oxidative stress (Se), and immune and growth (Zn) (Smith, 2003; Walker et al., 2012). A very similar pattern of contamination was found in yellow-nosed albatross chicks, although Hg concentrations were not as high. These results raise toxicological concern for these two endangered species, in particular in relation to outbreaks of avian cholora, which impacts the yellow-nosed albatross population with demographic consequences (Rolland et al., 2009; Weimerskirch, 2004). Although POP concentrations were overall low relative to other seabirds worldwide, the diversity and concentrations found in small petrel species using surface-feeding strategies deserves further investigation, also in relation to their vulnerability to plastic debris ingestion (Fromant et al., 2016). Finally, the extremely high Se:Hg molar ratios in macaroni penguins and small petrels (~300–1800) indicate a disproportional excess of Se over Hg, with potential toxicological consequences.

5. Conclusion

This work is a significant contribution to our understanding of contaminant exposure and accumulation in seabirds from remote sites of the Southern Hemisphere. A key finding was the large variation in contaminant concentrations among species, including of some essential trace elements, as a result of a combination of intrinsic (species, body mass) and extrinsic (diet and habitat) drivers. Hg transfer to seabirds was confirmed to increase from Antarctic to subtropical latitudes, while POP transfer seemed to be stronger to small pelagic petrels feeding in subantarctic and Antarctic waters. Yet, quantification of Hg and POP burdens in prey species from Antarctic, to subantarctic, up to subtropical waters is necessary to give a better picture of the spatial versus trophic factors that drive transfer to seabirds. Our results raise toxicological concern for Amsterdam and yellow-nosed albatrosses, as well as small petrel species and macaroni penguins, which should be further investigated. In order to fully quantify exposure risk at populations’ and species’ level, contaminant concentrations should be measured in adults too, over both the breeding and non-breeding period. Representative species with contrasted feeding strategies that could be used as bioindicators for long-term monitoring, in addition to those previously identified (Blévin et al., 2013; Carravieri et al., 2014c, 2013), are emperor penguins and snow petrels in Antarctica, and northern rockhopper penguins and yellow-nosed albatrosses in the sub tropics.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary material

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References
